Organic Flame Retardants in the Indoor Environment

by

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ABSTRACT

Flame retardants (FRs) have become ubiquitous contaminants found in humans, animals, various outdoor environments, e.g. air, soil, sediment, etc., and indoor environments particularly, homes, automobiles, classrooms and workplaces all over the world. These chemicals are global contaminants of concern as they are persistent, can bioaccumulate, biomagnify and have potential for long-range atmospheric transport. Most FRs are toxicants to human health since they affect thyroid hormones, endocrine systems and neurobehavioural development and are possibly carcinogenic. The overall hypothesis of this study is that the use of FRs in consumer goods and materials is leading to contamination of indoor environment at levels that may be detrimental to human health.

In this study, analytical methods based on gas chromatography-electron impact/mass spectrometry and liquid chromatography electrospray ionization/mass spectrometry were developed and/or validated for the separation, identification and quantitation of various classes of FRs. The FRs investigated included polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), tetrabromobisphenol A (TBBPA) and organophosphate esters [tris(1,3-dichloro-2-propyl) phosphate (TDCPP), tris(2-chloroethyl) phosphate (TCEP), tris(1-chloro-2-propyl) phosphate (TCPP) and triphenyl phosphate (TPP)]. These were measured in indoor dust from a wide range of microenvironments, including homes, offices, classrooms, automobiles, three workplaces – an e-waste recycling site, a polyurethane factory and a textile industry – and an *in vitro* human gastro-intestinal tract (GIT).

The measured concentrations of the FRs were used to estimate the exposure of toddlers, teenagers and adults to the FRs of interest via dust ingestion and in some cases dermal absorption of dust by using various exposure scenarios. The relative importance of each exposure route was assessed for the studied population groups. The potential sources of the FRs in the different microenvironments were established by using various advanced parametric and non-parametric statistical tests. Causes of variability in indoor dust concentrations of FRs were elucidated.

Two types of *in vitro* GIT models mimicking the enzymatic and physiochemistry preponderant for a FED and a FASTED state were developed, validated and applied for the first time to study the oral bioaccessibility of organophosphate esters and also to study the bioaccessibility of PBDEs. Strong relationships were found for the bioaccessibility of OPEs and their water solubilities as well as the log K_{ow} of PBDEs.

PREFACE

The experimental work described in this thesis was carried out in the School of Chemistry and Physics, University of KwaZulu-Natal, Durban, from January 2012 to December 2014, under the supervision of Professor BS Martincigh.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

DECLARATION 1 - PLAGIARISM

I, Ovokeroye Akpojevwe Abafe, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledge as being sourced from other researchers. Where other written sources have been quoted, then:
   a. Their words have been re-written but the general information attributed to them has been referenced
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5. This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledge, and the source being detailed in the thesis and in the references sections.

Signed……

DECLARATION 2 – PUBLICATIONS

Details of contributions to publications that form part and/or include research presented in this thesis.

List of manuscripts/ publications

1. **Ovokeroye A. Abafe** and Bice S. Martincigh (2015). Polybrominated diphenyl ethers and polychlorinated biphenyls in the indoor dust of e-waste recycling facilities in South Africa: Implications for occupational exposure. *Article in press, Environmental Science and Pollution Research. DOI.10.1007/s11356-015-4627-z.*
   (Contribution: OAA designed the study protocol, carried out the experiment, interpreted the results and wrote the initial manuscript draft, BSM closely supervised all stages of the work.)
2. **Ovokeroye A. Abafe** and Bice S. Martincigh (2014). Polybrominated diphenyl ethers and polychlorinated biphenyls in indoor dust in Durban, South Africa. *Article in press, Indoor Air.* **http://dx.doi.org/10.1111/ina.12168**:
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3. **Ovokeroye A. Abafe** and Bice S. Martincigh (2014). Modification and Application of an *in vitro* Human Gastrointestinal Tract for the Determination of Oral Bioaccessibility of Polybrominated Diphenyl Ethers. *Organohalogen Compounds. **76**, 277 – 280.*
   (Contribution: OAA designed the study protocol, carried out the experiment, interpreted the results and wrote the initial manuscript draft. BSM closely supervised all stages of the work.)
4. **Ovokeroye A. Abafe** and Bice S. Martincigh (2014). Organophosphorus Flame Retardants and Plasticizers in Dust from Automobiles, Homes, Offices and University Classrooms: Implications for Personal Exposure via Inadvertent Dust Ingestion. *Organohalogen Compounds. **76**, 273 – 276.*
   (Contribution: OAA designed the study protocol, carried out the experiment, interpreted the results and wrote the initial manuscript draft, BSM closely supervised all stages of the work.)
5. **Ovokeroye A. Abafe** and Bice S. Martincigh* (2014). Modifications of Unified Bioaccessibility Method for the determination of oral bioaccessibility of polybrominated diphenyl ethers in contaminated dust of e-waste recycling sites and standard reference materials. *Manuscript awaiting submission.*

(Contribution: OAA designed the study protocol, carried out the experiment, interpreted the results and wrote the initial manuscript draft, BSM closely supervised all stages of the work.)

6. **Ovokeroye A. Abafe** and Bice S. Martincigh (2014). Determination of tetrabromobisphenol A in dust samples from electronic waste recycling sites by gas chromatography mass-spectrometry and liquid chromatography tandem mass spectrometry. *Manuscript awaiting submission.*
   (Contribution: OAA designed the study protocol, carried out the experiment, interpreted the results and wrote the initial manuscript draft, BSM closely supervised all stages of the work.)
7. **Ovokeroye A. Abafe** and Bice S. Martincigh (2014). Assessment of concentrations and human exposure magnitude of tetrabromobisphenol A in dust from multi microenvironment in Durban, South Africa. *Manuscript awaiting submission.*
   (Contribution: OAA designed the study protocol, carried out the experiment, interpreted the results and wrote the initial manuscript draft, BSM closely supervised all stages of the work.)
8. **Ovokeroye A. Abafe** and Bice S. Martincigh (2014). Simultaneous determination of tri- to deca- polybrominated diphenyl ethers (PBDEs) in automobile dust by gas chromatography/ electron impact ionization-mass spectrometry. *Manuscript awaiting submission.*
   (Contribution: OAA designed the study protocol, carried out the experiment, interpreted the results and wrote the initial manuscript draft, BSM closely supervised all stages of the work.)
9. **Ovokeroye A. Abafe** and Bice S. Martincigh (2014). Organophosphate flame retardants and plasticizer in indoor dust from South Africa: implications for personal exposure via inadvertent dust ingestion. *Manuscript awaiting submission.*
   (Contribution: OAA designed the study protocol, out experiment, interpreted the results and wrote the initial manuscript draft. BSM closely supervised all stages of the work).
10. **Ovokeroye A. Abafe** and Bice S. Martincigh (2014). Concentrations and oral bioaccessibility of phosphorus flame retardants in indoor dust of textile and polyurethane industries. *Manuscript awaiting submission.*
    (Contribution: OAA designed the study protocol, carried out the experiment, interpreted the results and wrote the initial manuscript draft, BSM closely supervised all stages of the work.)

LIST OF CONFERENCE PRESENTATIONS

1. **Ovokeroye A. Abafe** and Bice S. Martincigh. Development of an *in vitro* human gastrointestinal tract for the determination of oral bioaccessibility of polybrominated diphenyl ethers in contaminated sites. *Analitika 2014*, **Parys, South Africa, 7th – 11th September, 2014**. ***Oral presentation***
2. **Ovokeroye A. Abafe** and Bice S. Martincigh. Organophosphorus Flame Retardants and Plasticizers in Dust from Automobiles, Homes, Offices and University Classrooms: Implications for Personal Exposure via Inadvertent Dust Ingestion. *34th International Symposium on Halogenated Persistent Organic Pollutants,* (DIOXIN 2014)**. 31st August – 5th September, 2014, Madrid, Spain**. ***Poster presentation***
3. **Ovokeroye A. Abafe** and Bice S. Martincigh. Modification and Application of an i*n vitro* Human Gastrointestinal Tract for the Determination of Oral Bioaccessibility of Polybrominated Diphenyl Ethers. *34th International Symposium on Halogenated Persistent Organic Pollutants*, (DIOXIN 2014) **31st August – 5th September, 2014, , Madrid, Spain**. ***Poster presentation***
4. **Ovokeroye A. Abafe** and Bice S. Martincigh. Determination of Tetrabromobisphenol-A in indoor dust. *41st National Convention of the South African Chemical Institute,* **East London, South Africa, 1st - 6th December, 2013.** ***Poster presentation.***
5. **Ovokeroye A. Abafe** and Bice S. Martincigh. Endocrine Disrupters in the South African Indoor Environment: Implications for Personal Exposure. *University of KwaZulu-Natal, College of Agriculture, Engineering and Science Postgraduate Research Symposium*, **Howard College, South Africa, 1th November 2013.** ***Oral presentation.***
6. **Ovokeroye A. Abafe** and Bice S. Martincigh. Determination and Occupational Exposure Assessment of Polybrominated Diphenyl Ethers and Polychlorinated Biphenyls in Dust from Electronic Waste (e-waste) Recycling Sites in South Africa. *South African Chemical Institute Postgraduate Research Colloquium,* **Durban, South Africa, 26th September, 2013.** ***Oral presentation***
7. **Ovokeroye A. Abafe** and Bice S. Martincigh. Concentrations and human exposure of polybrominated diphenyl ethers (PBDEs) via indoor dust in South Africa. *International Chemistry Conference in Africa (ICCA),* **Pretoria, South Africa, 8th -12th July, 2013.** ***Poster presentation***
8. **Ovokeroye A. Abafe** and Bice S. Martincigh. Determination and Occupational Exposure Assessment of Polybrominated Diphenyl Ethers (PBDEs) and Polychlorinated Biphenyls (PCBs) in Dusts from E-Waste Recycling Sites. *International Chemistry Conference in Africa (ICCA),* **Pretoria, South Africa, 8th -12th July, 2013**. ***Oral presentation***

9. **Ovokeroye A. Abafe** and Bice S. Martincigh. Concentrations and human exposure of polybrominated diphenyl ethers (PBDEs) via indoor dust in South Africa. *Sixth International Symposium on Brominated Flame Retardants*, (BFR 2013). **San Francisco, California, USA, 6-10 April 2013.** ***Poster presentation.***
10. **Ovokeroye A. Abafe** and Bice S. Martincigh. Determination of Human Exposure to Persistent Polyhalogenated Compounds via an Indoor Environment. *University of KwaZulu-Natal, College of Agriculture, Engineering and Science Postgraduate Research Symposium,* ***Pietermaritzburg*****, South Africa, 29th October 2012.** ***Poster Presentation.***

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Chapter 1

Introduction

Annually, fire kills over 4000 people, injures more than 20000 people and causes property damage in excess of an estimated $ 11 billion in the USA [4]. Similarly, in 2005, over 4000 Europeans lost their lives with fire losses estimated to be more than 15 billion euros in Europe [4]. However, in the last quarter of a century, fire incidence has reduced partly due to the fire prevention regulations requiring the use of flame retardant chemicals in various industrial products such as polymers, plastics, textiles, electric and electronic equipment, building materials, etc. To meet fire safety regulations, such as The Underwriters' Laboratories 94 (UL 94), the Standard for Safety of Flammability of Plastic Materials for Parts in Devices and Appliances testing and the California Technical Bulletin (TB) 117 for furniture [1], flame retardants are used in large volume. While flame resistant products save lives and reduce the economic cost of fires, there are increasing concerns over the environmental and health effects of flame retardants. This chapter discusses the background and provides statements of the problem and the research questions answered in this thesis.

1.1 Background

Fire safety is a primary part of fire safety measures. Fire safety aims to minimize the number of fires and damage that arises from them. The measures include hampering initiation of fires, controlling their spread and possibly excluding flash-over [2]. Avoiding fires, or limiting them, makes escape possible over a longer period of time. Hence, life, health and property are efficiently safeguarded. A better means of protecting combustible materials against initiating fires is the use of flame retardants (FRs). These enhance the fire safety level of combustible materials, for example plastics, and accomplish the respective conditions for fire sources of small and higher energies up to 40 kW m^{-2} [2].

Flame retardants are chemical additives used in consumer products to reduce their flammability [3]. Conventional applications include building materials, vehicles, textiles, furniture, foams, and electrical and electronic products. Previously, the most widely used brominated flame retardants were polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) and tetrabromobisphenol A (TBBP-A) [3].

On August 2nd 2005, an Air France jet overshot the runway at Toronto's Pearson International Airport and burst into flame. Surprisingly, all 309 crew and passengers survived. Ensuing reports by fire safety officials, credited fire-resistant materials which hindered the spread of flames and smoke, and hence allowed ample time for escape [4].

The use of FRs has been estimated to reduce fire deaths in Europe by as much as 20 % and to save as many as 280 lives annually in the USA [4]. This is beneficial to society considering that in the year 2005, fire took the lives of over 3000 people, injured up to 17000 and destroyed properties worth approximately 10 billion US dollars in the USA alone [4].

Fire retardant materials are built by integrating chemicals designed to prevent ignition or hinder the initial phase of fire. The knowledge of utilizing additives to reduce a material's flammability dates back to over 2000 years ago when the Egyptians soaked and dried paper and wood to render them fireproof [4]. Later in 1820, Gay-Lussac, on request from Louis XVIII of France, found that certain ammonium salts were effective for protection of precious textiles, a routine that continues today. A replacement of the traditional building materials, such as wood and metals, by various plastics has occurred since the twentieth century [4]. The previously used inorganic salts could not be incorporated onto these plastics because of the considerable reduction of the material's thermal stability [4]. The development of halogen-based organic FRs was a key innovation as they could be incorporated into plastics. Hence, the use of modern FRs increased proportionally with the greater use of synthetic polymers [4].

Interest in FRs began in the 1970s, due to the poisoning that arose in Michigan ascribed to the unintentional mixing of a bag of Firemaster FF-1, a commercial polybrominated biphenyl (PBB) mixture, into the feed of dairy cattle, livestock and poultry [5].

The world demand for FR additives is predicted to be 2.2 million metric tonnes in 2014 [6]. The elemental composition of FRs varies. They may contain halogens (bromine and chlorine), phosphorus, nitrogen, metals, minerals based on borax, antimony trioxide, molybdenum, or even a nanocomposite [1]. Some halogenated flame retardants, such as some brominated flame retardants (BFRs) and polychlorinated biphenyls (PCBs), are proven to be persistent, bioaccumulative and/or toxic in the environment, and to animals and humans. For over 40 years, halogenated flame retardants have been of concern for public health, with a subsequent ban in the production of PCBs in 1973 [1]. Recently, the production and use of BFRs has been more strictly restricted by the European Union (EU) and has been voluntarily phased out in the USA [1]. The production of pentaBDE has been forbidden in the EU since 2003, and the frequently used decabromodiphenyl ether (decaBDE) in electrical and electronic equipment has been forbidden in Europe [1]. The conference of the parties of the Stockholm Convention on Persistent Organic Pollutants (POPs) of the United Nations Environment Programme officially labelled and included the pentaBDE and the octaBDE as POPs in 2009 (decision SC-4/14, SC-4/18 [1]). These developments have subsequently necessitated the use of alternatives such as the phosphorus flame retardants (PFRs) for these BFRs.

PFRs have been in use for over 15 decades and are considered as appropriate alternatives for BFRs. Not only are several BFRs being replaced by PFRs, but also the halogen

containing PFRs may need to be substituted by non-halogenated PFRs. For instance, tris(2-chloroethyl)phosphate (TCEP) and tris(chloropropyl)phosphate (TCPP), with boiling points of 351 °C and 342 °C respectively, have been substituted by resorcinol-bis(diphenylphosphate) (RDP) with a boiling point 587 °C, which is less volatile and therefore less likely to be released into the environment [1]. Human and environmental effects of PFRs differ from one PFR to another. Like the BFRs, PFRs have been associated with several deleterious effects similar to those impacted by BFRs [1]. In their review, van der Veen and de Boer [1] stated that "if PFRs would be used as an alternative for PBDEs, it is important to avoid compounds, which are more persistent, bioaccumulative and toxic to humans and the environment than BFRs".

1.2 Research Overview

Flame retardants have become ubiquitous contaminants found in humans, animals, various outdoor environments, e.g. air, soil, sediment, etc., and indoor environments, particularly homes, automobiles, classrooms and workplaces, all over the world. These chemicals are global contaminants of concern as they are persistent, can bioaccumulate, biomagnify and have the potential for long-range atmospheric transport. Most FRs are toxicants of particular concern to human health as they affect thyroid hormones, endocrine systems and neurobehavioural development, and are possibly carcinogenic. Since flame retardants have been used widely in indoor applications, their levels in indoor environments greatly surpass those outdoors [7, 8]. Indoor environments have been recognised recently as significant sources of human exposure to FRs [8-15], with toddlers and young children facing higher exposures than adults owing to their greater ingestion of dust. People working in jobs like electronic waste recycling, textile and polyurethane foam (PUF) manufacturing, and the disposal of goods containing FRs, are also at significant risk of FR exposure. With these concerns in mind, a number of research gaps are evident with regards to certain facets of the environmental fate and behaviour, and the routes and magnitude of human exposure to BFRs, PCBs and PFRs.

The general hypothesis established in this thesis is that the use of FRs in consumer goods and materials is leading to the contamination of the indoor environment at levels that may be detrimental to human health. In order to verify this hypothesis, the present study aimed to:

- Develop and/or modify and validate analytical methodologies for the determination of polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), tetrabromobisphenol A (TBBPA), tris(2-chloroethyl)phosphate (TCEP), tris(chloropropyl)phosphate (TCPP), tris(1,3-dichloro-2-propyl)phosphate (TDCPP), and triphenylphosphate (TPP) in environmental matrices.
- Modify and validate a simple, quick and cost effective derivatization method for TBBPA followed by GC-EI/MS analysis in comparison with the conventional LC-MS/MS detection.

- Compare the efficiencies of different analytical sample preparation methods, namely, ultrasonic- assisted extraction and soxhlet extraction techniques, for the recovery of PBDEs and PCBs in indoor dust.
- Elucidate potential emission sources of the target organic flame retardants present in homes, offices, classrooms, e-waste recycling facilities, textile and polyurethane manufacturing industries, and to clarify the factors influencing levels of FRs in indoor environments.
- Estimate human exposure to the target FRs via dust ingestion and in some cases dermal absorption of dust in Durban, South Africa, and to assess the relevance of each exposure route to the overall exposure of adults, teenagers and toddlers by using diverse exposure scenarios.
- Assess the absolute magnitude and relative significance of occupational exposure to PBDEs and PCBs via dust ingestion and dermal absorption of dust within a South African e-waste recycling facility.
- Evaluate the influence of routine e-waste recycling site maintenance on the levels and profiles of PBDEs and PCBs indoors of the recycling environment.
- Compare the comprehensive data of the target FRs in this study with levels reported from other parts of the world to give a clear picture of the usage pattern of organic FRs in South Africa.
- Develop and/or modify and validate an i*n vitro* human gastrointestinal tract (GIT) model to study the oral bioaccessibility of eight environmentally relevant PBDE congeners, TCEP, TCPP, TDCPP and TPP, following dust ingestion.
- Study some factors responsible for the bioaccessibility of PBDEs in the i*n vitro* GIT model.

1.3. Structure of the Thesis

This thesis is written in manuscript format as a series of stand-alone chapters. Chapter 2 deals with relevant literature on the production volume and usage, physiochemistry, environmental levels, human exposure, and regulatory aspects of the target FRs. Chapter 3 describes two modified and validated sample preparation techniques, namely, ultrasonic–assisted extraction and Soxhlet extraction, followed by simultaneous detection of eight environmentally relevant tri- to deca-BDE congeners in indoor dust from automobiles. Human exposure to PBDEs via automobile dust ingestion and dermal absorption of dust were estimated for toddlers, young children, occupationally exposed adults such as taxi drivers and non-occupationally exposed adults. Chapter 4 reports a simple derivatization method for TBBPA in dust from e-waste dismantling/recycling sites followed by GC-EI/MS analysis compared with LC-MS/MS analysis of TBBPA. In Chapter 5, the levels and human exposure magnitude of TBBPA from three different microenvironments namely, homes, offices and automobiles in comparison with levels from other countries is reported. Chapter 6 reports the levels of PBDEs and PCBs in indoor dust from homes, offices and computer laboratories in comparison with data from other part of the world. Also, sources of PBDEs and PCBs indoors were established and

the relationship between home characteristics such as electronics, foams and furniture, age of buildings and type of floor, with indoor PBDE and PCB concentrations were elucidated in Chapter 6. Levels and causes of variability in PBDE and PCB profiles, and the occupational exposure magnitude of PBDEs and PCBs are highlighted in Chapter 7. In Chapter 8, the levels and profiles of TCEP, TCPP, TDCPP and TPP are reported in indoor dust of four different microenvironments, namely, homes, offices, computer laboratories and automobiles. Sources and causes of variability of these organophosphate FRs were elucidated. Similarly, the relationships between indoor characteristics and levels of PFRs are highlighted. The human exposure magnitude of PFRs for different population groups in South Africa was assessed in Chapter 8. Similarly, levels of PFRs in South Africa were compared with levels reported for several locales worldwide. In Chapter 9, a novel *in vitro* GIT model focusing on the 'fasted' and 'fed' state conditions was developed and validated to study the oral bioaccessibility of PBDEs in e-waste dust samples and in standard reference materials. Some factors responsible for the bioaccessibility of PBDEs are also elucidated in Chapter 9. Levels and profiles of TCEP, TCPP, TDCPP and TPP in dust from the indoor environment of a textile industry and a polyurethane industry are reported in Chapter 10. Also, the human oral bioaccessibility of these PFRs was assessed by modifying the *in vitro* GIT model of Chapter 9 for PFRs. Chapter 11 contains the summary of the findings of this thesis, and also provides insight on research gaps and future perspectives.

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Chapter 2

Literature Review

This chapter is focuses on the processes of combustion and the mechanisms of action of the different classes of flame retardants during combustion processes. Past and current literature on production volume and use, emission sources, environmental levels and human body burdens, as well as regulatory aspects and pathways of human exposure to polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), organophosphate esters (OPEs) and tetrabromobisphenol A (TBBPA) are reviewed.

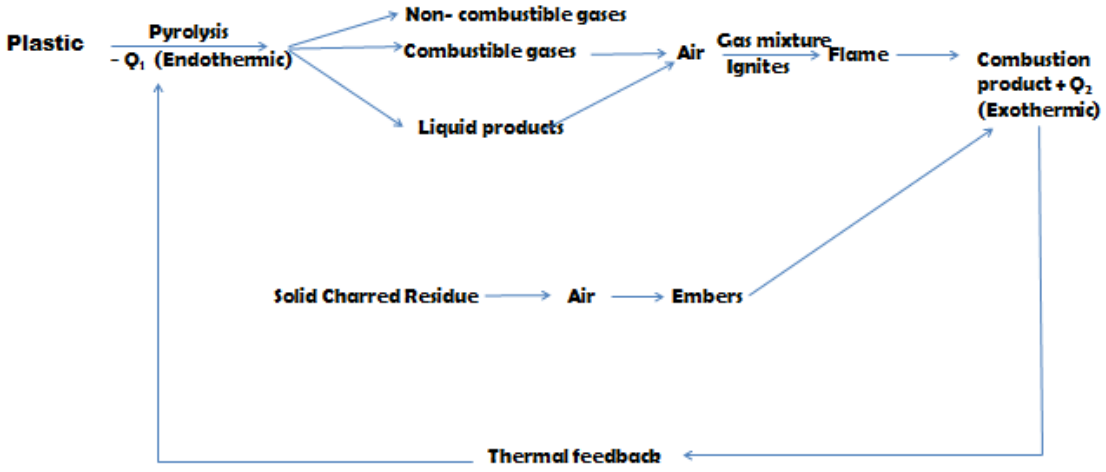
2.1 Combustion Processes

Combustion is a process comprising a host of single steps and is basically initiated by endothermic heating and decomposition. The flammable gases formed mix with atmospheric oxygen and ignite, resulting in an exothermic process of flame propagation and heat release (1). Through heating and pyrolysis, endothermic processes take place by overcoming the high bond binding energies (typically 200 to 400 kJ mol^{-1}) between individual atoms.

Ignition begins in the exothermic part of the process which supersedes the endothermic pyrolytic reaction. Pyrolysis is strengthened by thermal feedback which fuels the flame at an increasing level. The flame disseminates over the decomposed material surface (Fig. 2.1). The dispersion flame is sustained by extremely high energy hydrogen (H) and hydroxyl (OH) radicals, which presents a high velocity on the flame front (1).

2.2 Mode of Action of Flame Retardants

Figure 2.1 Combustion process (1).



A FR should impede, or even subdue, the combustion process. Subject to their nature, FRs can act chemically and/or physically in the solid, liquid or gas phase. They meddle with combustion during certain stages of this process, for example, during heating, decomposition, ignition or flame spread.

2.2.1 Physical Action

Combustion process can be delayed through various physical actions. These are:

Cooling

Endothermic processes initiated by additives cool the substrates to a temperature below that required for sustaining the combustion process, e.g. aluminum hydroxide.

Coating (Formation of a protective layer)

The condensed combustible layer can be protected from the gaseous phase with a solid or gaseous protective layer. The condensed phase is consequently cooled. Smaller quantities of pyrolysis gases are evolved, and the oxygen essential for the combustion process is excluded, and heat transfer is hindered, e.g. phosphorus and boron compounds.

Dilution

The integration of inert substances (e.g. fillers) and additives, which evolve inert gases on decomposition, dilutes the fuel in the solid and gaseous phases so that the lower ignition limit of the gas mixture is not surpassed, e.g. aluminum hydroxide [2].

2.2.2 Chemical Action

The most substantial chemical reactions interfering with the combustion process occur in the solid and gas phases:

Reaction in the gas phase

The free radical mechanism of the combustion process, which takes place in the gas phase, is disrupted by the FR. The exothermic processes are hence stopped, the system cools down, and the distribution of flammable gases is reduced and finally completely suppressed, e.g. halogenated FRs [2].

Reaction in the solid state

The FR can cause a layer of carbon to form on the surface of the material. This may occur through the dehydrating action of the FR, generating double bonds in the material. This forms a carbonaceous layer by cyclizing and cross-linking, e.g. phosphorus compounds [2].

2.3 Categories of Flame Retardants

There are four main families of FR chemicals and several types of design changes that can afford fire resistance, namely,

(a) halogenated FRs,
(b) inorganic FRs,

(c) nitrogen-based FRs, and
(d) phosphorus FRs.

The halogenated flame retardants are primarily based on chlorine and bromine.

2.4 Mechanism of Action of Halogenated FRs

For a solid substance to burn, it has to be preheated by an external source. The consequence is thermal decomposition (pyrolysis phase) of the material with attendant release of flammable gases, which subsequently react with atmospheric oxygen to produce visible flames, and more heats is generated (burning phase). When the generated heat is adequate, the material will continue decomposing and the burning process becomes self-propagating. Halogenated flame retardants act by reducing or preventing the burning phase through the reduction of heat generation and production of more flammable gases. When exposed to high temperature, the halogen radicals $X^{\cdot}$ (Cl or Br) of the flame retardant are released and react with the hydrocarbon molecules of flammable gases to produce HX. This product reacts with $OH^{\cdot}$ and/or $H^{\cdot}$ radicals to form H_2O, H_2 and $X^{\cdot}$ radicals which can restart the cycle.

The halogen-containing flame retardants act by interfering with the radical chain mechanism occurring in the gas phase. The high-energy $\cdot OH$ and $H\cdot$ radicals formed by chain branching:

$$H^{\cdot} + O_2 \rightarrow OH^{\cdot} + O$$

$$O + H_2 \rightarrow OH^{\cdot} + H^{\cdot}$$

are removed by the halogen-containing flame retardant. Initially, the flame retardant breaks down to

$$RX \rightarrow R^{\cdot} + X^{\cdot}$$

where X is either chlorine or bromine. The hydrogen halide is formed by the reaction of the halogen radical

$$X^{\cdot} + RH \rightarrow R^{\cdot} + HX$$

This subsequently interferes with the radical chain mechanism:

$$HX + H^{\cdot} \rightarrow H_2 + X^{\cdot}$$

$$HX + OH^{\cdot} \rightarrow H_2O + X^{\cdot}$$

The high-energy $H^{\cdot}$ and $OH^{\cdot}$ radicals are removed by reaction with HX and replaced with low-energy $X^{\cdot}$ radicals. The real flame retardant effect is thus produced by HX. The reaction with the hydrocarbon regenerates the hydrogen halides consumed:

$$X^{\cdot} + RH \rightarrow R^{\cdot} + HX.$$

Hence, HX ultimately acts as a catalyst.

2.5 Brominated Flame Retardants

Brominated flame retardants (BFRs), a subgroup of the halogenated organic flame retardants, are ubiquitous chemicals with large and global industrial use because of their low cost and high performance efficiency. The three classes of BFRs produced in high volumes are: polybrominated diphenyl ethers (PBDEs), tetrabromobisphenol-A (TBBP-A) and hexabromocyclododecane (HBCD). The concentrations of BFRs in products may range from 5 – 30 %. The most widely used BFRs are TBBP-A with a global demand of 170000 metric tons in 2004, alongside decabromodiphenyl ether (decaBDE), HBCD, pentabromodiphenyl ether (pentaBDE) and octabromodiphenyl ether (octaBDE), for which the worldwide market demands in 2001 were 56100, 16700, 7500 and 3790 metric tons respectively (2). These chemicals are used as flame retardants in various applications including: plastic housings for electronic devices and printed circuit boards, construction materials and upholstery (3). Whilst TBBP-A is used majorly as a "reactive" FR, chemically bound to the polymer matrix, PBDEs are used as "additive" FRs physically incorporated into the polymer matrix during manufacturing, and hence, they are believed to be more easily released to the environment from products (4).

2.6 Polybrominated Diphenyl Ethers

Polybrominated diphenyl ethers are a subgroup of BFRs consisting of 209 structurally related chemicals or congeners. Some members of the subgroup have been widely employed as flame retardants to increase the fire safety of plastics and other materials in homes, cars and offices. PBDEs can be subdivided based on the number of bromine atoms in the molecule, which varies from one to ten. By analogy with the PCBs, they are numbered using the same system of Ballschmiter and Zell (1980) (5) (see Fig. 2). However, there appear to be many fewer actual PBDE congeners in the commercial mixtures than the theoretical number possible, largely because many of the congeners lack stability and tend to debrominate.

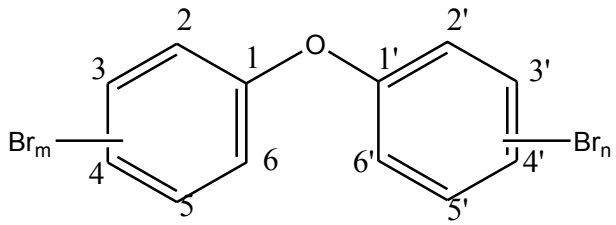


Figure 2.2 General structural formula of PBDEs.

2.6.1 Production and Use of PBDEs

PBDEs are major industrial products with a total worldwide production of approximately 67,400 metric tons/year (2). Nevertheless, their use is not squarely dispersed over the industrialized world. The Americas account for slightly more than 50%, whereas all of Europe accounts for 12% (2).

Structurally, PBDEs consist of a diphenyl ether unit, with one to ten of the hydrogen atoms substituted by bromine atoms. PBDEs have a general molecular formula of $C_{10}H_{10-x}Br_xO$, where x ranges from 1 to 10. All of the three commercially available mixtures of PBDEs consist of mixtures of congeners with different degrees of bromination.

The three dominant mixtures of PBDEs in commerce are named in accordance with the average degree of bromination as decabromodiphenyl ether (decaBDE), octabromodiphenyl ether (octaBDE) and pentabromodiphenyl ether (pentaBDE). There are reports of a fourth mixture, tetrabromodiphenyl ether (tetraBDE), having been used in the past.

Although the production of PBDEs has continued to increase in the United States and Canada, voluntary bans have resulted in their declining use in Europe. DecaBDE represents the major product in all markets, accounting for approximately 80% of the total PBDE production worldwide (2). Unlike the other commercial products, decaBDE is a relatively pure mixture composed of ≥ 97 % decabromodiphenyl ether (decaBDE or BDE-209), < 3% nonabromodiphenyl ether (nonaBDE), and small amounts of octaBDE. DecaBDE is used as an additive flame retardant primarily in electric and electronic equipment, as well as textiles, where it is applied as a polymer backcoat to the fabric.

Commercial octaBDE is a more complicated mixture with several congeners present. Typically, these are approximately 10–12% hexabrominated diphenyl ethers (hexaBDE), 44% heptabrominated diphenyl ethers (heptaBDE), 31–35% octaBDE, 10–11% nonaBDE, and < 1% decaBDE (2). It is not clear, if any pentaBDE is present in the commercial octaBDE product. OctaBDE is a minor PBDE product, used as an additive in polymers for application in plastic housings and smaller components, such as office equipment.

A third commercial PBDE product, pentaBDE, or “pentabrom” is a viscous liquid used primarily in textiles as an additive in polyurethane foams, where up to 30% of the weight of the foam can be accounted for by this flame retardant (2). The composition of commercial pentaBDE covers the bromination range of tetrabrominated to hexabrominated, with small amounts of tribrominated congeners. Penta mixtures are generally composed of 24-38% tetraBDE, 50-60% pentaBDE and 4-8% hexaBDE (2) (Table 2.1). The major PBDE congeners are IUPAC numbers 47 (tetraBDE), 99 and 100 (pentaBDEs), and 153 and 154 (hexaBDEs). BDEs 47 and 99 are the major congeners in the mixture, accounting for approximately 75% of the total mass. There is roughly twice as much BDE 99 as BDE 47 in the commercial mixtures, and approximately equal amounts of BDEs 153 and 154 (2). The use of pentaBDE has decreased in response to a voluntary ban in Europe, as well as its inclusion on the list of the “nasty nine” persistent organic pollutants (2).

PBDEs are used only for flame retardant purposes. The rationale for using brominated compounds as flame retardants is based on the ability of halogen atoms, generated from the thermal decomposition of the bromo organic compound, to chemically produce and retard the development of fire (6). Factors favouring the use of PBDEs are therefore the high bromine content (which means good fire-retardant properties), thermal stability, and relatively low cost. They are used as additive flame retardants at concentrations of 5-30% in many different polymers, resins and substrates, and common plastics, including acrylonitrile butadiene styrene and high impact polystyrene (6). Additive flame retardants leach and escape from the finished polymer product more easily than reactive flame retardants. Examples of products containing flame retardants, and especially PBDEs, include many components of electronic devices, e.g. cabinets for and circuit boards in personal computers and television (TV) sets and various other products (electrical cables, switches and capacitors, building materials, and textiles). The physicochemical properties of PBDEs are presented in Table 2.2.

Table 2.1 Compositions of commercial PBDEs (6).

	Commercial Product			
Congener group	tetraBDE (%)	pentaBDE (%)	octaBDE (%)	decaBDE (%)
Unknown	7.6			
TriBDE		0 – 1		
tetraBDE	41 – 41.7	24 – 38		
pentaBDE	44.4 – 45	50 – 62		
hexaBDE	6 – 7	4 – 8	10 – 12	
heptaBDE			43- 44	
octaBDE			31 – 35	
nonaBDE			9 – 11	0.3 – 3
decaBDE			0 – 1	97 – 98

Table 2.2 Physical properties of some PBDE congener groups (6).

	tetraBDE	pentaBDE	octaBDE	decaBDE
Chemical formula	$C_{12}H_6Br_4O$	$C_{12}H_5Br_5O$	$C_{12}H_2Br_8O$	$C_{12}Br_{10}O$
Molecular mass	485.8	564.8	801.5	959.2
Vapour pressure/ Pa	$2.7\text{-}3.3 \times 10^{-4}$ (20 °C)	$2.9–7.3 \times 10^{-5}$ (20 °C)	$1.2–2.7 \times 10^{-7}$ (20 °C)	$<1 \times 10^{-4}$ (25 °C) 670 (306 °C)
Melting point/ °C	79 – 82	92-98	~200	290 – 306
Boiling point/ °C	—	>300 (decomposition)	—	Decomposition
Water solubility/ $\mu g\ dm^{-3}$	—	0.0009 (20 °C)	—	20 – 30
log K_{ow}	5.9 – 6.2	6.5 – 7.0	8.4 – 8.9	10

2.6.2 Emission Sources of PBDEs

PBDEs are used as additives in polymers during polymer manufacture, and are not chemically bonded to the polymers, hence they can readily transfer from these materials to the surface (a process known as blooming) (7) and ultimately into the surrounding environment during the product's useful life (8, 9).

PBDEs are released into the environment via diverse pathways including volatilization or product abrasion throughout the useful life of treated products in indoor environments such as homes, offices, automobile interiors, classrooms and workplaces (10). Similarly, PBDEs can be emitted during the manufacture of PBDEs or PBDE-containing products, subsequent waste disposal including processing of waste at incineration plants (11, 12), landfills (13-15), sewage treatment plants (16, 17), and spraying of treated wastewater for irrigation (18), and also during PBDE-treated product recycling (19-24). It has also been suggested that debromination of constituents of the decaBDE formulation may also be a possible source for less brominated PBDEs in the environment (25, 26).

2.6.3 Environmental Levels and Behaviour of PBDEs in the Environment

2.6.3.1 Air and Dust

A better part of our daily life is spent indoors, in homes, offices, schools, etc., availing us of many opportunities for lengthy exposure to chemical contaminants from commercial products and residential settings in the indoor environment. Takigami et al.

(27) reported elevated concentrations of 240 and 730 ng g^{-1} PBDEs, in indoor dusts of two Japanese homes. DecaBDE was the major congener followed by nona- and octaBDE. The authors also reported total BDE concentrations of 17 – 39 and 33 – 55 pg m^{-3} in indoor air of both homes. The authors showed a low air/dust partition ratio (K_{ad}) (i.e. log $K_{ad} \leq 0$) for both homes for highly brominated PBDEs (penta to decaBDEs), which implies a preferential adsorption to dust by this compounds (27). Estimates for the rate of total PBDE (mainly decaBDE) exposure in the study were 1.2 – 74 ng day^{-1} for adults and 13 – 150 ng day^{-1} for children in the two homes. Dust ingestion accounted for more than 83% of the total exposure estimates, irrespective of the assumed dust ingestion rate for adults and children (27). Iacovidou et al. (28) reported average concentrations of 3.9 ± 2.1 pg m^{-3} and 0.9 ± 0.4 pg m^{-3} for gas phase and particle- bound PBDEs respectively, accounting for 20 ± 7% of the total atmospheric concentration of the eastern Mediterranean. This indicates that air mass is an important factor controlling the variation of PBDE levels in the background atmosphere of the south eastern Mediterranean (28).

PBDEs has been detected in indoor and outdoor dusts and air samples in both urban and remote areas across the United Kingdom (170 – 400 ng g^{-1}) (29), Sweden (1.6 – 3200 pg m^{-3} and 6.1 – 1400 ng g^{-1} in both air and dust, respectively) (30, 31), Germany (37900 ng g^{-1}, dust) (32), Japan (19 – 25 pg m^{-3} and 17 – 33 pg m^{-3} for outdoor and indoor air, respectively and 240 – 730 ng g^{-1} for dust) (27) and the United States (1780 – 25,200 ng g^{-1}, dust) (33), insinuating the long-range atmospheric transport of PBDEs. In Africa, PBDEs have been reported in several indoor environments (34-36) at levels generally lower than concentrations reported for most developed countries.

2.6.3.2 Soil, sediment and sewage sludge

The organic carbon contents of sediments and soils make them a sink for most organic contaminants including PBDEs. Eljarrat et al. (37) reported the levels of PBDEs in agricultural soil after application of sewage sludge. The study reported 1.2 - 45 fold higher ∑BDEs in soil following the amendment of sewage sludge in five different sites in Spain. Similarly, sewage sludge amendment of soil in two Swedish farms resulted in 2-13 fold increased ∑BDEs (38), with BDE-209 having the highest concentration in both the Swedish and Spanish studies. Generally, BDE-209 increases in importance in soil, sediment and sewage sludge (39-42). In South Africa, PBDEs has been detected in sediment (43, 44), landfills (45) and sewage sludge (46). The behaviour of PBDEs in different environmental media are associated with the partition coefficients of individual PBDE congeners. Table 2.3 summarizes reported levels of PBDEs in soil, sediments and sewage sludge from different locations in the world.

Table 2.3 Reported levels of PBDEs in soil, sediment and sewage sludge from different locations.

Matrix	Country	Concentration/ng g^{-1}	Reference
Urban area soil	China	0.0013 – 2.7	(47)
Farmland soil	China	40 – 95	(48)
Soil around e-waste	China	21 – 179	(48)
Background soil	China	0.080 – 35	(49)
Soil around e-waste	China	>3.8 – 390000	(50)
Sediment impacted by e-waste shredding	China	>3.8 – 220000	(50)
Industrial road soil	Indonesia	6.2 – 13	(51)
Urban road soil	Indonesia	0.96 – 4.7	(51)
Municipal dumpsite	Indonesia	0.29 – 21	(51)
Rural road soils	Indonesia	0.36 – 3.7	(51)
Agricultural soils	Indonesia	0.037 – 0.24	(51)
Sediment core	Belgium	1.4 – 8413	(52)
Sediment	Belgium	<0.01 – 1200	(53)
Sediment	Netherlands	<0.10 – 1664	(54)
Sediment	Netherlands	<0.1 – 510	(17)
Sediment	Spain	2 – 132	(55)
Sediment	USA	56 – 4890	(56)
Sediment	Sweden	1.1 – 275	(57)
Sediment	Kuwait	0.04 – 1595	(58)
Sediment	Korea	1.16 – 43.6	(59)

2.6.3.3 Aquatic invertebrates, fish and marine mammals

PBDEs have been reported in various aquatic organisms. Andersson and Blomkvist (60) reported PBDEs as high as 27 mg kg^{-1} and 110 mg kg^{-1} in lipids in muscle and lipids in liver respectively of pike from the Swedish River Viskan, which was a recipient of effluent water from nearby textile factories. However, Sellström et al. (61) reported lower PBDE levels in the River Viskan. High levels of PBDEs have been found in fish from waters with suspected or known sources of contamination compared with fish from other sampling spots which contained measurable PBDE levels. Evidently, herring and seal from the Baltic Sea contain higher PBDE levels than the same or similar species from other waters (6, 62-64). PBDEs in the range 8.4 – 100 µg BDE-47 kg^{-1} lipid have been reported in herring collected from three North Sea regions from the straits of Dover. Similarly, lake eels from Dutch freshwater contained < 20 to 1700 µg BDE-47 kg^{-1} lipid (65). PBDE concentrations ranging from 0.1 – 14.6 µg kg^{-1} weight have been observed in fish and shellfish samples from Osaka Bay, Japan. Bottlenose dolphins have been reported to contain PBDEs as high as 8 mg kg^{-1} lipid (66). Similarly, blubber of pilot whales caught off the coast of the Faroe Islands from 1994 – 1996 were found to contain PBDEs (67). PBDE levels in the pilot whales were shown to be sex and age dependent. Strandman et al. (68) reported PBDE levels in Baltic herring to range from 13 – 24 µg kg^{-1} lipid weight while the concentration of PBDEs in Baltic sprats ranged from 22 – 149 µg kg^{-1} lipid weight. The highest concentrations of PBDEs in the aquatic environment available in the literature have been reported in the American continent. PBDE levels in crab, fish and marine mammals from British Columbian waters have been documented (69). The highest level of 1400 µg BDE-47 kg^{-1} lipid was reported in porpoise from the Canadian study. In lake trout from the Great lakes, Alaee et al. (70) reported PBDEs in the range 135 – 545 µg kg^{-1} lipid weight. PBDEs in Lake Michigan fish were reported to contain six–fold higher PBDE concentrations than the Baltic salmon (71). Different levels of PBDE concentrations have been documented in aquatic organisms in different locales worldwide (72-74).

2.6.3.4 PBDEs in birds and terrestrial mammals

PBDEs have been reported in various terrestrial/avian organisms. Peak PBDE concentrations have been reported in Guillemot eggs collected in the mid-1980s in the study of Sellström et al. (75) in which Guillemot eggs were sampled annually between 1969 – 2001. In that study, the concentrations of BDE-47 and BDE-99 increased about 15 – 20 fold from the early 1970s up to the late 1980s, when the peak maximum was observed. The maximum level was followed by a rapid decrease in concentrations during the remainder of the study period (75). The Osprey (*Pandion haliaetus*) has been reported to contain relatively high PBDE concentrations (62), unlike many other non-aquatic avian or terrestrial wild-living species, such as moose and reindeer, which have been reported to contain low or non-detectable PBDE levels (6). Generally, BDE-209 has been detected with greater detection frequency in bird tissues and eggs than in aquatic biota. Jimenez et al. (76) reported the highly brominated PBDE congeners, such as BDE-

183, BDE-197, BDE-196, BDE-207, BDE-206 and BDE-209, and several other unidentified octa- and nona-BDE congeners in almost all samples of Peregrine falcon eggs bred in Spain. Their result suggested the influence of habitat and feeding habits on the PBDE congener distribution patterns in birds of prey, hence indicating that birds feeding in terrestrial habitats and on other birds are likely to be more exposed to the higher brominated BDE congeners compared to marine species (76). Similar to this hypothesis, Jaspers et al. (77) detected only BDE-209 in the range 52 – 85 ng g^{-1} lw in the muscle and liver of terrestrial birds out of seven species of aquatic and terrestrial predatory birds from Flanders, Belgium (77); further suggesting that terrestrial species may be more exposed to higher brominated BDE congeners than aquatic birds (77). The accumulation and tissue specific distribution of BDE-209 in terrestrial songbird species has been studied (78). Muscle concentrations of BDE-209 were shown to be about two-fold those in liver, with the highest accumulation in blood of exposed starlings (78). PBDEs have been detected in 100% of samples of bird eggs in South Africa, with the highest PBDE concentration of 396 ng g^{-1} lipid weight found in the African sacred ibis (79). The distribution pattern of PBDE congeners was reflective of differences in trophic levels, migratory behaviour, and distance to the contaminant source, and exposure to different PBDE mixtures among the different bird species (79). Similarly, PBDEs have been detected in all 162 samples of chicken egg yolks in Canada (80). Whilst BDE-209 was recognized as the major contributor to ∑PBDE in egg yolk, the total PBDE concentrations exhibited large differences in variability between combinations of regions and category of egg yolk (80).

Soil has been shown to be an important source of PBDEs in eggs laid by free-foraging chicken (81). BDE-209 has been reported in 6 of 44 liver samples, and 19 of 25 serum samples, but not in any other tissues of birds of prey from Belgium (82). In the UK, BDE-209 in terrestrial bird eggs (2 – 108 ng g^{-1} lw), muscles (13 – 563 ng g^{-1} lw) and livers (6 – 200 ng g^{-1}) have been reported (361). The concentrations of BDE-209 in eggs of 3 different peregrine falcon populations from Sweden were reported to range between 26 – 370 ng g^{-1} (83). However, very high concentrations of the higher brominated PBDEs (up to 4.1 µg g^{-1} lw for BDE-209) have been detected in 95 Californian peregrine falcon eggs (84).

Herzke et al. (85) showed BDE-153 as the most abundant congener in eggs of peregrine falcons, golden eagles and merlin in Norway. The concentration of PBDEs was shown to largely depend on the species (85). The concentrations of BDE-209 ranged from <0.1 – 160 and <0.1 – 11 ng g^{-1} lw in liver and adipose tissues respectively of raccoon dogs from Japan (86). The bioaccumulation of BDE-209 ranged from 3.7 – 200 ng g^{-1} lw (adipose tissue), 9.1 – 760 ng g^{-1} lw (liver) and 3.9 – 290 ng g^{-1} (muscles) of 33 red foxes from Belgium (82).

2.6.3.5 Human body burdens of PBDEs

PBDEs have been widely reported in various human matrices implying the bioavailability of PBDEs to humans, although the extent of such bioavailability needs to be assessed further. Table 2.4 summarizes levels of PBDEs reported in different human samples. Higher concentrations of $\sum_{n=7}$ PBDE congeners have been reported in cord blood compared to maternal blood or breast milk in South Korea, indicating prenatal exposure to PBDEs (87). Huang et al. (88) showed the relationship of BDE-99 and BDE-209 in humans and triiodothyronin (T_3); and also BDE-17, 28, 47 and 183 with thyroid stimulating hormone (TSH). Generally, BDE-47, 153 and 209 are most abundant PBDE congeners reported in humans (87-90). Fängström et al. (91) reported higher BDE-209 blood concentrations in 7-year-old children compared to their mothers. The measured human levels of BDE-209 could be indicative of current exposure because of its low bioaccumulation potential caused by its relatively short half-life (approximately 1 week) compared with the lower brominated congeners such as BDE-153 with a half-life of 11.5 years (92). The high concentrations may be related to the exposure of children via unintentional dust ingestion and/or dust dermal absorption (93).

Table 2.4 Reported levels of PBDEs (in ng g^{-1}) in various human samples.

Matrix	Country	∑PBDEs (range)	Reference
Serum	China	(ND – 6.08)	(89)
Serum	China	7.2	(88)
Breast milk	China	1.12**	(94)
Serum	Korea	8.06**	(90)
Plasma	Hong Kong	5.4*	(95)
Breast milk	Taiwan	3.93**	(96)
Breast milk	Sweden	4.0**	(97)
Adipose tissue	Czech Republic	(0.2 – 54.3)	(98)
Breast milk	Indonesia	(0.49 – 13)	(99)
Breast milk	Italy	(1.6 – 4.1)	(100)
Breast milk	Vietnam	0.57 -84	(101)
Breast milk	Spain	5.5**	(102)
Breast milk	Spain	2.2** & 2.5**	(103)
Breast milk	Ghana	4.5*	(104)
Breast milk	India	1.4**	(105)
Breast milk	India	2.2 – 7.4	(105)
Breast milk	US	29.2	(106)
Fetal blood	US	41.3	(107)
Maternal blood	US	41.1	(107)
Bile	Japan	2.54	(108)
Blood	Japan	3.52	(108)
Adipose tissue	Japan	1.8 – 46	(109)

Adipose tissue	Belgium	4.70	(110)
Adipose tissue	Finland	10.34	(68)
Breast milk	South Africa	1.3*	(111)
Breast milk	Philippines	3.9*	(112)
Breast milk	Philippines	2.2*	(112)

*median concentration, **mean concentration, ND - not detected

2.6.4 Regulatory Aspects of PBDEs

The ban of PBDEs was first proposed in the early and mid–1980s and early 1990s by Germany, Sweden and the Netherlands, before the final prohibition of penta- and octaBDE formulations in the European Union in the summer of 2004 (113). The decaBDE formulation was originally included in the list of chemicals to be prohibited in electric and electronic equipment as of July 2006, by The Restriction of Hazardous Substances in Electrical and Electronic Equipment Directives (RoHs directive). However, in October 2005, an exemption was granted by the European Commission for this mixture. The annulment of this exemption by the European Court of Justice went into force as of July 1, 2008. The Swedish government restricted the use of decaBDE in textiles, furniture and cables since 2007. Similarly, as of April 1, 2008, the Norwegian government introduced a ban on decaBDE in preparations and products such as cellular rubber, textiles and upholstery. In the United States, different regulations have been put in place at the state level with some states banning the manufacture or distribution of the penta- and octa-BDE formulations. The states of Maine and Washington, partially restricted decaBDE since 2008. The producers of the commercial penta- and octaBDE formulations in the US voluntarily discontinued their production since the end of 2004 (113). Whilst regulations for the lower brominated congeners are in place, continued exposure to these substances will occur for a long time due to their long half-lives and the continued use of PBDE-containing products. Similarly, the continued production of decaBDE products in some parts of the world will continually add to the existing environmental burden and also serve as a repository for the lower brominated congeners. In South Africa, there is no special legislation or regulations on PBDEs, although South Africa is a signatory to the Stockholm Convention on persistent organic pollutants, which has restricted the production and use of commercial penta- and octaBDE formulations since 2009.

2.7 Tetrabromobisphenol A

Tetrabromobisphenol A (TBBPA) is the most widely used brominated flame retardant (114), with a production volume covering almost 60 % of the total BFR market (115). TBBPA is majorly used as a reactive flame retardant, in which it is covalently bonded to the host material, for example in epoxyl and polycarbonate resins used in printed circuit boards and electronic equipment (116). Approximately 18 % of TBBPA is used as an additive flame retardant in which TBBPA is mixed with the host material, for example in the manufacture of high impact polystyrene (HIPs) and acrylonitrile-butadiene-styrene

(ABS) resins (115). Apart from additively added TBBPA, excessive non-polymerized TBBPA is always present and can be emitted from a product in which TBBPA has been used as a reactive flame retardant (114); hence, contaminating the environment.

2.7.1 Production, Application and Uses

Tetrabromobisphenol A is produced in the USA, Israel and Japan, but not in the European Union (115). The production process of TBBPA involves the bromination of bisphenol-A with bromine in the presence of solvent, e.g. methanol or a halocarbon, aqueous monoethers or hydrobromic acid. Owing to the nature of the production process and the consequent by-product (methyl bromide and hydrobromic acid) that may be produced, the production of TBBPA is largely carried out in closed systems (115). TBBPA is reportedly the brominated flame retardant with the highest production volume, covering around 60% of the total BFR market (3, 115), with an estimated production of 170000 tons in 2004 (114). Of the total TBBPA produced, 84.62 % is used in Asia, and the total market demand for TBBPA in America and Europe is 9.38% and 6% respectively (117). The European BFR Industry Panel (EBFRIP) stated that the TBBPA market is increasing with a shift in the consumption volume observed towards Asia (115).

The importation of TBBPA into a country can follow various formats, either as finished or partially finished products or as a primary product or raw material. For example, plastics, printed circuit boards and electronic equipment may contain TBBPA. These imports may be an important source of TBBPA in Africa, but limited or no information is currently available. For example, in the EU, where TBBPA is not produced, TBBPA risk assessment estimated the amount of TBBPA imported as a primary product to the EU as 13800 tons/year; and as partially finished products such as epoxy resins and polymers to be 6000 tons/year; and as finished products to be 20200 tons/year (115).

Fifty-eight percent of TBBPA is used as a reactive flame retardant in polycarbonate resins, phenolic resins, epoxy resins and in printed circuit boards, whilst 18 % is used for the production of TBBPA derivatives and oligomers, and another 18 % is used as an additive flame retardant in the manufacture of acrylonitrile-butadiene-styrene (ABS) resins or high impact polystyrene (HIPs). Although, the spokespersons of the BFR industry claim “because it was not effective, TBBPA was never used as an additive flame retardant in HIPs” (115, 118), it has been indicated by the European FR Association that TBBPA is possibly used in HIPs.

TBBPA is used chiefly as an intermediate in the production of epoxy and polycarbonate resins, where it becomes covalently bound in the polymer and is hence an essential part of the product. The exposure that remains originates from unreacted TBBPA, if it has been added in excess during the production process. Polycarbonate resins are generally used in electronic appliances, communication and electronic equipment, transportation devices, recreation and sport equipment, signs and lighting fixtures. While, the unsaturated polyesters find uses in simulated marble floor tiles, furniture parts, bowling

balls, sewer pipes, coupling compounds, buttons, automotive patching compounds and for encapsulating electrical devices. The commercial FR epoxy resins comprise up to almost 20% bromine. These resins are primarily used in the manufacture of rigid epoxy laminated printed circuits.

As an additive FR, TBBPA is used with antimony oxide for optimal performance (115). TBBPA is generally thought as an alternative additive flame retardant to the octaBDE mixture in acrylonitrile butadiene styrene (ABS) resins. Due to the ban on octaBDE, it is possible that the amount of TBBPA used in this particular application could increase in the future. As an additive FR, TBBPA does not react chemically with the other component of the polymer and hence may leach out of the polymer matrix after incorporation, with significant implications for human exposure. TBBPA concentrations usually around 10% and 20% by weight are commonly found in these applications depending on the polymer. ABS resins are used in automotive parts, pipes and fittings, business machines, telephones and refrigerators. While HIP resins are majorly used in electrical and electronic equipment, consumer products, packaging, furniture, and building and construction materials (119). The major additive use of TBBPA is found in television casings (115, 118). The use of TBBPA as an additive FR also includes PC monitor casings, fax machines and photocopiers, and components in printers, vacuum cleaners, coffee machines and sockets/plugs. TBBPA is used in the production of derivatives, some of which find uses as FR. Many organobromine compounds including bromophenol are naturally produced in the environment, mostly by marine organisms (115, 120). Bis(3,5-dibromo-4-hydroxylphenyl)methane, which is structurally similar to TBBPA, is produced by the segmented marine worm *Thelepus setosus* (115). However, TBBPA has not been identified as being naturally produced (115).

2.7.2 Physicochemical Properties

At 20 °C, TBBPA is a white crystalline powder. The physicochemical properties of TBBPA are summarized in Table 2.5.

Table 2.5 Physicochemical properties of tetrabromobisphenol A (115).

Property	Value
Molecular formula	$C_{15}H_{12}Br_4O_2$
Molecular mass	543.9 g mol^{-1}
Density	2.12 g cm^{-3}
Vapour pressure	6.24 x 10^{-1}Pa
Melting point	181 – 182 °C
Boiling point	316 °C (decomposes at 200 – 300 °C)
Acid dissociation	pKa_1 = 7.5
constant	pKa_2 = 8.5
log K_{ow}	5.90
Water solubility	Pure water = 4.4 x10^{-7} mol dm^{-3} at 25 °C

pH 5 = 2.7 x 10^{-7} mol dm^{-3} at 25 °C
pH 7 =23.2 x 10^{-7} mol dm^{-3} at 25 °C
pH 9 = 43.0 10^{-7} mol dm^{-3} at 25 °C

2.7.3 Toxicology and Human Health Effects

Low acute toxicity has been reported for TBBPA in rats and mice following oral administration. An LD_{50} of > 5 and > 4 g kg^{-1} has been shown for rats and mice, respectively (115). The main concern regarding TBBPA is its potential to act as an endocrine disruptor due to its structural resemblance to the thyroid hormone thyroxin (T_4) and bisphenol A, a suspected endocrine disruptor (115). Rat pituitary GH3 cell lines have been used to examine the thyroid hormonal activity of TBBPA, whereby the release of growth hormone is thyroid hormone-dependent (121). The authors showed that TBBPA stimulated the production of growth hormone and enhanced the proliferation of GH3 cells. In the same light, TBBPA enhanced the proliferation of the rat pituitary MtT/E-2 cell lines, in which the growth is estrogen-dependent; thereby suggesting TBBPA acts both as a thyroid hormone and estrogen agonist (115, 121). Similar to these findings, a growth of GH3 cells, which could not be counteracted by the inhibiting growth of the anti-estrogen fulvestrant, has been observed (122), thus suggesting the effect of TBBPA is thyroid hormone-like and estrogen receptor-mediated (122). Whilst TBBPA produced a thyreomimetic effect on the GH3 pituitary cell line, an antithyroidal effect has been observed on Chinese hamster ovary cells ephemerally transfected with T_3 receptors, likewise an inhibition of the binding of triiodothyronine (T_3) to thyroid hormone receptors (123). Furthermore, *in vitro* studies have shown TBBPA to be a potent inhibitor for the binding of T_4 to transthyretin, the thyroid hormone-binding transport protein in plasma. TBBPA binds 10 times more strongly than the natural ligand T_4 (115, 124-126).

TBBPA has been shown to be a rather potent inhibitor of the sulfation of estradiol by estrogen sulfotransferase, an important inactivation route of estradiol (127). An increased bioavailability of estradiol *in vivo* may result from the inhibition of this enzyme. Other studies have confirmed the resultant weak estrogen-like properties (124, 125). *In vitro* inhibition of the expression of CD 25, a receptor necessary for the propagation of activated T-cells, has demonstrated TBBPA to be immunotoxic (128). Similarly, TBBPA has been reported to interfere with cellular signalling pathways (129). The neurotoxicity of TBBPA was established *in vitro* by the inhibition of neurotransmitter uptake into synaptosomes and uptake of dopamine into synaptic vesicles and the generation of free radicals (130, 131). A partial life-cycle test with *Danio rerio* (Zebrafish) exposed to environmentally relevant concentrations of water-borne TBBPA by Kuiper, et al. (132) resulted in toxicological effects including decreased egg production, decreased success with reproduction, severe disorientation and lethargy (132).

2.7.4 Environmental Levels and Behaviour

2.7.4.1 Air and dust

Limited data are available for TBBPA in abiotic matrices despite its widespread use. Elevated concentrations of TBBPA have been reported in indoor air of offices, homes, etc., compared to outdoor air concentrations of TBBPA. The higher indoor levels of TBBPA are presumed to arise from the use of TBBPA in HIP enclosures for electronic products and in printed circuit boards. Table 2.7 shows TBBPA concentrations reported in various abiotic matrices worldwide.

Very high TBBPA concentrations have been detected in air samples from an electronic waste recycling plant and in offices equipped with computers, while TBBPA was not detected in outdoor air samples from Sweden (133). In a Swedish study, mean TBBPA concentrations of 0.036 ng m^{-3} in offices (n = 6) containing computers, 0.093 ng m^{-3} in classrooms (n = 2) and 0.035 ng m^{-3} in computer repair facilities (n = 2) were observed, however, in outdoor air samples, TBBPA was not detected in the same study (24). Similarly, average TBBPA concentrations of 30 ng m^{-3} and 140 ng m^{-3} have been reported in air from a dismantling hall and a shredder respectively from an electronic waste recycling plant (24). The observation showed that TBBPA is primarily present in the particulate phase rather than in the vapour phase (24). Alaee et al. (4) reported TBBPA concentration of 70 pg m^{-3} in archived filter samples collected in the Arctic region, Dunai, Russia. Similarly, Xie et al. (117) showed that atmospheric concentrations of TBBPA ranged from below the detection limit to 0.85 pg m^{-3} in a rural forest in northern Germany. TBBPA concentrations in the northeast Atlantic Ocean ranged from <0.04 – 0.17 pg m^{-3} (24). The higher concentration was found in a sample collected off the west Norwegian coast, thus suggesting an input source from land to ocean transport (117). The relevance of electronic products as an emission source of TBBPA has been reported by Tollbäck et al. (116). In their study, a TBBPA concentration in indoor air of a dismantling hall within a Swedish electronic recycling plant was 13.8 ng m^{-3}. The indoor air concentration of TBBPA in Kanagawa and Tokyo, Japan was reported in 14 out of 26 analysed samples, with a mean concentration of 0.2 ng m^{-3} (134). Indoor and outdoor air TBBPA concentrations ranging from 7.1 – 9.5 pg m^{-3} and 9 – 16 pg m^{-3} respectively have been reported in Hokkaido, Japan (27).

Unlike other BFRs, such as PBDEs, reports on indoor dust concentrations of TBBPA are scarce and appear to constitute a research gap (115). Available data indicate that TBBPA concentrations are at the low end of those reported for PBDEs (29, 135). This is in line with the fact that TBBPA is primarily used as a reactive flame retardant hence its release from treated products is expected to be less simple than for compounds used as additive flame retardants. In the UK, Abdallah et al. (135) reported median dust concentrations of TBBPA in various microenvironments to be 62 ng g^{-1} (homes, n = 45), 230 ng g^{-1} (public microenvironments, n = 4), 36 ng g^{-1} (offices, n = 28) and 2 ng g^{-1} (cars, n = 20).

These values were lower than TBBPA concentrations in the range of 190 – 340 ng g^{-1} reported in UK domestic dust (9). These latter data were similar to TBBPA concentrations ranging from 490 – 520 ng g^{-1} reported in two domestic dust samples from Hokkaido, Japan (27). TBBPA concentrations in domestic dust from Belgium ranged from 1 – 1480 ng g^{-1} and 45 – 100 ng g^{-1} in office dust (136). TBBPA dust concentrations of 1728 ng g^{-1} have been observed in homes from the Philippines (137). A continuous increase in TBBPA concentrations from 0.4 to 2.0 ng g^{-1} was observed over the period of a year in a newly constructed building in Michigan, USA (138). Yu and Hu (139) reported TBBPA concentration in the range of 18.9 and 39.6 μg g^{-1} in dust samples from computers in Chinese offices. Table 2.7 shows TBBPA concentrations in dust from several locations worldwide.

2.7.4.2 Soil, sediment, sewage sludge and water

A TBBPA concentration of 0.12 ng g^{-1} in soil has been documented in samples collected outside a TBBPA production plant in China (140). In a Chinese soil near a garbage discharge site, the concentration of TBBPA ranged between 1.4 and 1.8 μg g^{-1} (139). From the TBBPA partition propensity to the atmospheric particulate phase and its octanol-water partition coefficient, it should be anticipated that soil would constitute a major sink for TBBPA. This is, however, influenced by the rate of degradation in soil and the subsequent atmospheric transport and deposition (115).

Available data suggest that sewage sludge and sediments are an important sink for TBBPA due to its physicochemical properties. The available data also reflect the release of TBBPA to these matrices from industrial plants that either manufacture or use TBBPA. The first study of TBBPA in sediment from the Neya River in Japan, reported a concentration of 20 ng g^{-1} dry weight (141).

TBBPA has been detected in river and estuarine sediment samples from Belgium, the Netherlands and the UK. TBBPA concentrations ranged from 0.1 – 67 ng g^{-1} dry weight, 0.1 – 3.2 ng g^{-1} dry weight, 0.1 – 6.9 ng g^{-1}dry weight and 2.5 ng g^{-1}dry weight in sediments from the Scheldt basin, the Western Scheldt, Dutch rivers and UK rivers respectively. The highest TBBPA concentration of 9.8 μg g^{-1} dry weight was reported in freshwater sediments from the UK river Skerne, close to a BFR manufacturing site (142). TBBPA concentrations of 0.04 – 0.13 ng g^{-1}dw have been reported in sediments from Norwegian lakes (143). Similarly, a TBBPA concentration of 0.51 ng g^{-1} has been reported in surface sediment from Lake Erie, Canada (144).

Several studies have reported TBBPA in sewage sludges worldwide. Chu et al. (144) reported TBBPA concentrations of 2.1 – 28.3 ng g^{-1} dry weight in sludge samples in wastewater treatment and pollution control plants in Ontario, Canada. The mean concentration of TBBPA in 50 samples (TBBPA not detected in 12 samples) from a Swedish sewage treatment plant was 32 ng g^{-1} dry weight (145). Other studies have reported TBBPA in sludges from various locations (146). The concentration of TBBPA

in influent and effluent wastewater were 130 and 7.7 ng dm^{-3} respectively, while the concentrations ranged from 0.3 – 540 ng dm^{-3} in landfill leachates from Industrial waste sites in Japan (146). TBBPA concentrations of 620 ng dm^{-3} and 11 ng dm^{-3} have also been reported in raw and treated leachates from Japan (15) respectively. Reported concentrations of TBBPA in various abiotic matrices are presented in Table 2.7.

2.7.4.3 Biotic samples

In spite of the widespread use of TBBPA, very scarce data of TBBPA in biotas are available for use in environmental assessments. TBBPA has been examined in the muscles of six bird species with median concentrations in the range of 28 to 173 ng g^{-1} lipid weight (147). Morris et al. (142) reported TBBPA concentration of <2.9 ng g^{-1} lw in the eggs of common tern sampled from Western Scheldt. TBBPA in the liver sample of five cormorants (*Phalacrocorax carbo*) from England ranged between 2.5 to 14 ng g^{-1}. Similarly, TBBPA concentration ranging from <3 – 13 pg g^{-1} wet weight have been reported in eggs from four different Norwegian bird-of-prey species (85). In their study, Vorkamp et al. (148) did not detect TBBPA in any of the 33 egg samples of peregrine falcon from south Greenland. Berger et al. (149) reported a TBBPA concentration of 13 pg g^{-1} wet weight in different species of predatory birds in Norway. TBBPA concentrations in the range of 6 – 35 ng g^{-1} wet weight has been reported in 18 of 68 blubber samples of porpoises (*Phocoena phocoena*) stranded in UK waters between 1994 and 2003 (150). The concentration of TBBPA in Mysid shrimp (*Neomysis interger*) was 0.8 and 0.9 ng g^{-1} lipid weight in two sites in the Scheldt estuary (54). Mean TBBPA concentrations in three marine top predators : bottlenose dolphin blubber (n = 15), bull shark muscle (n = 13) and Atlantic sharp nose shark muscle (n = 3) from the coastal waters of Florida, USA, were 1.2 ± 3.0 ng g^{-1}, 9.5 ± 12.0 ng g^{-1} lw and 0.87 ± 0.50 ng g^{-1} lw, respectively (151). Table 2.6 shows reported levels of TBBPA in different biological matrices worldwide.

2.7.4.3 Human body burdens

Reports of TBBPA in human samples are generally limited. This could partly be as a result of the short biological half-life of TBBPA, estimated at 2 days (152). This is in line with the phenolic structure of TBBPA that can rapidly form conjugates and subsequently be excreted (153). Notwithstanding, TBBPA may accumulate in humans due to prolonged exposure.

TBBPA in serum of Norwegians working at an electronic dismantling facility have been reported to range from 0.34 – 1.3 ng g^{-1} (154). In a Norwegian time trend study of TBBPA in serum for the period 1977 – 1999; TBBPA was not detected in pooled sera in the period 1977 – 1981, while there was a slight increase in serum concentrations (0.44 to 0.65 ng g^{-1} lw) observed for the period 1986 to 1999 (154). Highest TBBPA concentrations have been reported in serum pooled from the age group 0 – 4 years in a study were several age groups from 0 – 60 years were examined (154). Nagayama et al. (155) have reported a mean TBBPA concentration of 1.35 ng g^{-1} lw in blood samples of

14 out of 24 Japanese adults. While the measured mean concentration of TBBPA in 20 adipose tissues from New York was 0.048 ng g^{-1} (151). Sellström and Jansson (156) found TBBPA in human hair in the vicinity of TBBPA manufacturing sites in Arkansas, USA. Several other reports of TBBPA in human samples of occupationally exposed adults have been documented (92, 154, 157).

Table 2.6 Concentrations (ng g^{-1}) lipid weight of TBBPA) in biological matrices.

Species	Tissues	Location	Concentration	Reference
Birds				
Cormorant	Liver	England & Wales	2.5 – 14	(142)
Peregrine falcon, white tailed sea eagle,Osprey, Golden eagle	Egg	Norway	<0.003 – 0.013	(142)
Cormorant tern	Egg	Western Scheldt	<2.9	(142)
Fish				
Atlantic sharpnose shark	Muscle	USA	0.87	(151)
Bull shark	Muscle	USA	9.5	(151)
Yellow eel	Muscle	Scheldt basin	<0.1 -2.1	(142)
Yellow eel	Muscle	Dutch rivers	<0.1 – 1.01	(142)
Eel	Muscle	Scheldt estuary	<0.1 – 13	(142)
Eel	Muscle	Dutch rivers	< 0.1 – 1.3	(142)
Whiting	Muscle	North Sea	<97 – 245	(142)
Cod	Liver	North Sea	<0.3 – 1.8	(142)
Hake	Liver	Atlantic	<0.2	(142)
Marine mammals				
Bottlenose dolpin	Blubber	USA	1.2	(151)
Harbor seal	Blubber	Wadden Sea	<14	(142)
Harbor porpoise	Blubber	UK	6 – 35	(150)
Harbour porpoise	Blubber	North Sea	<11	(142)
Harbour porpoise	Blubber	North Sea	0.1 – 418	(142)

Harbour porpoise	Blubber	Tyne/ Tees	0.31	(142)
Invertebrates				
Mysid	Whole	Scheldt estuary	0.8 – 0.9	(54)
Hermit crab	Whole	North Sea	<1 – 35	(142)
Common whelk	Whole	North Sea	5.0 -96	(142)
Sea star	Whole	Scheldt	< 1- 2	(142)
Sea star	Whole	Tees estuary	205	(142)
Humans				
General population	Serum	Norway	0.34 – 0.71	(154)
Circuit board producers	Serum	Norway	0.54	(154)
Laboratory personnel	Serum	Norway	O.54	(154)
Electronic dismantlers	Serum	Norway	1.3	(154)
Computer technicians	Serum	Sweden	0.55 – 1.84	(157)
Electronic dismantling plant	Serum	Sweden	1.1 – 4.0	(152)
General population	Adipose tissue	USA	0.048	(158)

2.7.5 Regulatory Aspects of TBBPA

There are currently no regulations restricting the production and use of TBBPA or its derivatives. However, the EU directive of 2003 on the handling of waste electrical and electronic equipment (WEEE) (directive 2002/ 96/EC, 2003) (159) was adopted and includes a requirement for the selective treatment of plastics containing TBBPA and other brominated flame retardants.

TBBPA is on the 4th list of priority chemicals (Regulation 2364\2000\EC, 2000) (160) was anticipated under the European Council Regulation (EEC) No. 793/93 of March, 23rd 1993, with respect to the evaluation and control of the risks of existing substances.

REACH is a recently implemented European Union regulation on the safe use of chemicals, which specifically deals with the registration, evaluation, authorization and restriction of chemical substances (161). REACH entered into force on the 1st June, 2007. Under the REACH framework, TBBPA is likely one of the first substances to go through the procedure of registration as a result of its high volume production (3).

Environmental risk assessment for TBBPA in the EU has confirmed a risk in some circumstances for surface water, soil and sediment when TBBPA is used as an additive FR in acrylonitrile butadiene styrene (ABS) plastics (118). The Bromine Science and Environment Forum (BSEF) showed that risks from the additive application of TBBPA are manageable through a Voluntary Emissions Control Action Programme (VECAMP). Eight-nine % of TBBPA additive customers in Europe have signed up VECAMP and have already begun reducing their emissions (3). In Japan, TBBPA has been included in the Environmental Surveillance Program since 2003 (115). China is currently preparing legislation on WEEE, similar to the directive of the EU concerning WEEE 2002/96/EC (115). The Australian Ministry of Health and Ageing declared TBBPA as a "Priority Existing Chemical" in June 2005 (115). Whilst Canada is in the process of assessing the human and environmental risks of TBBPA and its derivatives (115). No regulatory actions are currently available for TBBPA or any BFR in South Africa.

Table 2.7 Reported levels of TBBPA in various abiotic matrices.

Matrix	Location	Concentration	Reference
Air			
Outdoor air	Wadden Sea	0.31 – 0.69 pg m^{-3}	(117)
Outdoor air	Northeast Atlantic	<0.04 – 0.17 pg m^{-3}	(117)
Outdoor air	Artic, Russia	70 pg m^{-3}	(4)
Outdoor air rural site	Northern, Germany	<0.04 – 0.85 pg m^{-3}	(4)
Outdoor air	Japan	7.1 – 9.5 pg m^{-3}	(27)
Indoor air	Japan	9 – 16 pg m^{-3}	(27)
Indoor air	Japan	0.2 ng m^{-3}	(134)
Computer office	Sweden	0.035 ng m^{-3}	(133)
Computer office	Sweden	0.036 ng m^{-3}	(24)
Classroom	Sweden	0.093 ng m^{-3}	(24)
Recycling plant	Sweden	29.7 ng m^{-3}	(133)
Computer repair facility	Sweden	0.035 ng m^{-3}	(24)
E-waste dismantling hall	Sweden	30 ng m^{-3}	(24)
Electronic recycling plant	Sweden	13.8 ng m^{-3}	(116)
E-waste Shredder	Sweden	140 ng m^{-3}	(24)
Dust			
Domestic dust	Hokkaido, Japan	490 – 520 ng g^{-1}	(27)
Newly constructed building	Michigan, USA	0.4 – 2 ng g^{-1}	(138)
Dust inside computer	China	8.9 -39.6 μg g^{-1}	(139)
Personal homes	Germany	ND – 470 ng g^{-1}	(32)

Personal homes	Philippines	ND – 1728 ng g^{-1}	(137)
Personal homes	Belgium	11.7 ng g^{-1}	(162)
Offices	Belgium	70.4 ng g^{-1}	(162)
Personal homes	Belgium	1 – 1480 ng g^{-1}	(136)
Offices	Belgium	45 – 100	(136)
Personal homes	UK	62 ng g^{-1}	(135)
Public microenvironment	UK	230 ng g^{-1}	(135)
Cars	UK	2 ng g^{-1}	(135)
Offices	UK	36 ng g^{-1}	(135)
Newly constructed building	USA	0.2 – 520 ng g^{-1}	(163)
Primary & Nursery classroom	UK	110 ng g^{-1}	(164)
Personal homes	Japan	490 – 520 ng g^{-1}	(27)
Soil			
Near-garbage discharge site	China	1.4 – 1.8 μg g^{-1}	(139)
Soil	China	25.2 ng g^{-1}	(165)
Outside production plant	China	0.12 ng g^{-1}	(150)
Sediment			
Sediment	Scheldt basin	0.1 -67 ng g^{-1}dw	(142)
Sediment	Western Scheldt	0.1 – 3.2 ng g^{-1}dw	(142)
Sediment	Neya River, Japan	20 ng g^{-1}dw	(141)
Sediment	Lakes mjosa	0.04 – 0.13 ng g^{-1}dw	(143)
Sediment	Dutch Rivers	0.1 – 6.9 ng g^{-1}dw	(142)
Sediment	River Tees, UK	25 ng g^{-1}dw	(142)
Sediment	Asia	<0.2 – 1.6 ng g^{-1}	(146)
Sediment	UK rivers	2 – 5 ng g^{-1}dw	(142)
Sediment downstream plastic factory	Sweden	270 ng g^{-1}	(156)
Sediment upstream plastic factory	Sweden	34 ng g^{-1}	(156)
Sediment near BFR production site	River Skerne, UK	9.8 μg g^{-1}	(142)
Sewage sludge			
Influent sewage sludge	UK	7.5 ng g^{-1}dw	(142)
Effluent sewage sludge	UK	<3.9 ng g^{-1}	(142)
Sewage sludge	UK	57 ng g^{-1}	(142)

Sewage sludge	The Netherlands	79 ng g^{-1}	(142)
Influent sewage sludge	The Netherlands	<6.9 ng g^{-1}	(142)
Effluent sewage sludge	The Netherlands	42 ng g^{-1}	(142)
Sewage sludge	Sweden	<0.3 – 220 ng g^{-1} dw	(166)
Sewage sludge	Sweden	32 ng g^{-1}	(145)
Treated sludge	Cork,Ireland	192 ng g^{-1}	(142)
Composite and digestate	Switzerland	510 ng g^{-1} dw	(167)
Sewage sludge from wastewater treatment plant	Canada	2.1 – 28.3 ng g^{-1}	(144)
Sewage sludge from wastewater	Montreal, Canada	300 ng g^{-1} dw	(168)
Sewage sludge from treatment plants	Canada	<1 – 46.2 ng g^{-1}	(169)
Sewage sludge	Canada	14.3 – 43.8 ng g^{-1} dw	(169)

2.8 Polychlorinated Biphenyls

Polychlorinated biphenyls (PCBs) are a family comprising of 209 man-made structurally related chemicals that were widely used more than 36 years ago in a host of industrial applications owing to their insulating and flame-retardant properties. PCBs have a general chemical formula of $C_{12}H_{10-n}Cl_n$, where n = 1 to 10. There are 209 theoretically possible PCB congeners; however, only approximately 130 of these are present in commercial products. PCB numbering and nomenclature is the same as for the PBDEs. The general structure of PCBs is shown in Fig. 2.3.

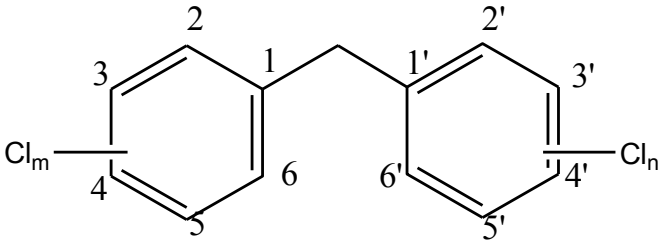


Figure 2.3 General structure of PCBs.

Due to chlorination, PCBs possess excellent thermal stability, dielectric properties, and resistance to oxidation, acids and bases (170). Because of these properties, PCBs have been used in enormous quantities for various applications such as dielectric fluids in transformers and capacitors, hydraulic fluids, heat transfer fluids, lubrication oils and as additives in pesticides, adhesives, plastics, copying papers, etc. (171). Between World

War II and prior to their ban, PCBs were used in buildings, as additives to caulking, grouts, paints and sealants (172). The sources of PCBs can be grouped into closed and open. The majority of PCBs were used in closed sources such as capacitors, electric transformers, etc. (172). Most closed sources of PCBs are subject to inspections and regulations in developed countries and recently in South Africa. For instance, in South Africa, PCBs in closed sources are inspected by government agents and the volume must be reported and listed in the South African inventory of PCB bulk materials, those in current use and stored PCBs (173). Studies have reported that PCBs in open sources such as caulking and building sealants can impact the environment, making it important to ensure measures deal with these sources (172, 174, 175).

2.8.1 Production and Use of PCBs

PCBs were manufactured as mixtures of numerous PCB congeners, through progressive chlorination of batches of biphenyl until a target percentage of chlorine by mass was obtained (176). The most commonly used name for PCB technical mixtures is the Aroclor series. These mixtures are named with a four digit distinctive suffix number that indicates the degree of chlorination (177). The first two digits depict the number of carbon atoms in the biphenyl ring (i.e. 12 for PCBs); the last two numbers refer to the percentage of chlorine by mass in the mixture. For example, Aroclor 1260 indicates that the mixture contains approximately 60 % chlorine by weight (176).

The production and commercial use of PCBs began in 1929. Because of their remarkable electrical insulating properties and their flame resistance, PCBs gained widespread use as insulators and coolants in transformers and other electrical equipment where these properties are essential. Combustible insulating fluids were replaced by PCBs, hence reducing the risk of fires in factories, hospitals, office buildings, schools, homes, and other public microenvironments. The code of some cities requires that all capacitors and transformers be of PCB-type. PCBs do not only make capacitors flame resistant, they also allowed capacitors to be made smaller, hence reducing equipment costs. In reality, some insurance companies require PCB equipment in many locations (173). It has been estimated that approximately 1.7 million tons of PCBs were produced between 1929 and 1989 worldwide (178).

PCBs were never produced in South Africa, however, PCB oils and equipment containing PCB oils was imported for mainly electricity generation (173). PCBs belong to group II hazardous substances in South Africa and have a unique tariff code in the South African tariff book. PCBs in excess of 50 ppm have been inventoried in 17086 pieces of equipment owned by the South African Electricity Supply Commission (ESKOM) in 2010. This equipment includes transformers, auxiliary equipment and capacitor cans. The inventory implicated 4% of the equipment to contain PCBs in excess of 500 ppm; 62% contained PCBs in the range 50 – 499 ppm; 2% contained PCBs between 20 – 49 ppm and 32% contained PCBs ranging between 1 – 19 ppm (173). In the same light, the steel industry reported PCB levels in excess of 51 – 500 ppm in 33%

of 30 pieces of equipment inventoried in the Vanderbylpark works in South Africa. Between 2003 and 2008, Evraz Highveld Steel carried out an inventory and reported PCB levels between 70 – 215 ppm in equipment owned by the company (173). It is evident from Table 2.8 that PCBs are still being circulated in various sectors of the South African economy.

Table 2.8 PCB destroyed between 2005 – 2010 (kg/sector) in South Africa (173).

Sector	PCB oils	PCB equipment
Mining	4918.5	-
Electricity	119244.5	828179.5
Cement manufacturing	3989.2	-
Chemicals	8889	10380
Petrochemicals	5928.5	-
Transport	5928.5	37657
Total	148898.2	876216.5

2.8.2 Nomenclature and Physicochemical Properties of PCBs

PCBs are a family of chemical compounds in which chlorine atoms replace some or all of the hydrogen atoms on the biphenyl molecule (Fig. 2.4). PCBs were produced and sold under different trade names including Aroclor, Pyranol, Pyroclor (USA), Phenoclor, Pyralene (France), Clophen, Elaol (Germany), Kaneclor, Santotherm (Japan), Fenclor, Apirolio (Italy) and Sovol (USSR) (179). In South Africa, the PCB trade names include Askarel, Chlorectol, Elemex and Inerteen (180).

Two correlated but different nomenclature systems are currently used for PCBs:

(i) The International Union of Pure and Applied Chemistry (IUPAC) names that recognize the number of the carbon to which chlorines are attached and lists the numbers sequentially, for example, the PCB congener with chlorines on carbon atoms 2, 3, 4 and 3' is identified as 233'4.

(ii) The second but most widely used system was established by Ballschmiter and Zell (5) as a simplified reference to specific congeners. The structural arrangements of the PCB congeners are correlated in an ascending order of the number of chlorine substitutions within each sequential homologue. An unprimed number in a specific PCB congener structure is considered to be of higher priority than the same number when primed, thus resulting in the congeners being numbered from PCB 1 to PCB 209.

The predominant congeners in biotic and abiotic matrices are PCB 28, 52, 101, 138, 153 and 180 (Table 2.9). These PCBs were the focus of initial environmental analyses. The magnitude of these six congeners represent the total PCB concentration in the environment, and depends on the original commercial mixtures used, e.g. Aroclor 1242, Aroclor 1260, Aroclor 1254, Clophen A 30, Clophen A50, and possible changes in PCB

composition owing to differences in environmental behaviour of the individual congeners. The sum of the PCB concentrations are estimated by assuming that the total of the six congeners represent 20% of the total PCB concentrations (181), which is the average value found in several PCB mixtures and environmental samples.

Table 2.9 Numbering and chemical structure of six frequently reported PCB congeners

IUPAC numbering	Structure		CAS No.
*PCB 28	2,4,4'	trichlorobiphenyl	7012-37-5
PCB 52	2,2',5,5'	tetrachlorobiphenyl	35693- 99 – 3
PCB 101	2,2', 4,5,5'	pentachlorobiphenyl	37680-73-2
PCB 138	2,2',3,4,4',5'	hexachlorobiphenyl	35065-28-2
*PCB 153	2,2', 4,4,5,5'	hexachlorobiphenyl	35065-27-1
*PCB 180	2,2',3,4,4',5,5'	heptachlorobiphenyl	35065-29-3

*Most abundant PCB congeners reported in various biotic and abiotic matrices in South Africa. (182-185)

The physicochemical properties of individual PCB congeners have a strong influence on the environmental fate and behaviour of the congener. In particular, the environmental fate and behaviour of an individual PCB congener is strongly influenced by its volatility, lipophilicity and aqueous solubility. The lower chlorinated PCB congeners such as PCB 28 and PCB 52 possess somewhat greater vapour pressures and water solubilities than higher-chlorinated congeners such as PCB 153 and PCB 180, while the higher-chlorinated congeners are more lipophilic. These differences have a significant effect on the partitioning of individual congener among different environmental compartments. The normalized organic carbon:water partition coefficient (K_{oc}) is a measure of the affinity of PCBs for organic carbon in sediment and soil (Table 2.10). Though K_{oc} values are generally presumed to be constant, the values may be dependent on environmental conditions and contact times between solid matrix and contaminant (186).

Table 2.10 Physicochemical properties of some selected PCB congeners (values at 25 °C).

Congener IUPAC number	Vapour pressure/Pa (187)	Water solubility/mol m^{-3} (187)	Henry's law constant (log HLC/atm m^{-3} mol^{-1}) (188)	Octanol:water partition coefficient ($logK_{ow}$) (189)	Organic carbon:water partition coefficient ($logK_{oc}$) (186)
28	2.77×10^{-2}	1.21×10^{-3}	-3.544	5.67	6.31
52	1.93×10^{-2}	3.62×10^{-4}	-3.496	5.84	6.56
101	3.58×10^{-3}	1.09×10^{-4}	-3.610	6.38	7.34
138	4.87×10^{-4}	4.40×10^{-5}	-3.886	6.83	8.00

153	6.63×10^{-4}	3.70×10^{-5}	-3.783	6.92	8.13
180	5.06×10^{-4}	1.66×10^{-5}	-3.969	7.36	8.78

2.8.3 Toxicology and Health Effects of PCBs

PCBs are among the twelve chemicals nominated as persistent organic pollutants (POPs) targeted initially by the United Nations Stockholm Convention of May 2001, when several nations including South Africa, and the European Community decided to reduce or eliminate PCB production, use, and/or release. POPs are extremely stable toxic organic compounds that persist in the environment, and accumulate in fat tissues. PCBs are among the several truly global environmental pollutants. They have been found in measurable but low levels in nearly every marine animal and plant specimens, fish, mammals, bird eggs and humans. Human exposure to PCBs occurs primarily through low-level food contamination as well as indoor dust contamination. PCBs possess dioxin-like toxicity. Reported toxic effects of PCBs include dermal toxicity, immunotoxicity, teratotoxicity, reproductive effects, carcinogenicity and endocrine disruption (190-192). The binding of PCBs to the aryl hydrocarbon (Ah) cellular receptor initiates the toxicity mechanism of PCBs (193, 194). PCB tumorigenecity in rodents was first reported in mice (195, 196). These studies were preceded by those of Schaeffer et al. (197) who showed that PCBs induced tumours in rats. PCB absorption by humans and animals is through the skin, lungs and the gastrointestinal tract. When in the body, PCBs are transported via the blood stream to the liver and other muscles and adipose tissue where they accumulate. The toxicity of PCBs in humans is congener specific; in which the structural specificity of PCBs for enzyme induction is a well understood indicator of potential toxicity (183). Neighbouring hydrogen atoms present in the meta and para positions increase the predisposition of the PCB molecule to metabolism via enzymatic activity of the P450 system (183, 198). The mixed-function oxidase (MFO), which is a group of microsomal cytochrome P450–dependent enzyme systems, is responsible for catalyzing the biotransformation process of xenobiotics (183, 199). The cytochrome P450-enzymes determine an organism's ability to metabolize compounds such as PCBs, hence influencing the occurrence of these chemicals in biological tissues (200). PCBs are metabolized through the MFO system, typically the CYP1A and CYP2B subfamilies, which are sensitive to the level and position of chlorination (183, 201).

PCB congeners lacking ortho, but containing para- and meta-chlorines with adjacent, unsubstituted ortho-meta carbons are metabolized by CYP1A, whilst CYP2B metabolizes congeners with unsubstituted meta-para carbons with chlorines at the ortho positions. PCBs can be categorized into three groups, each inducing a separate MFO:

(a) Phenorbarbital–type (PB-type) PCBs: These induce P450 – CYP 2B including 2B1 and 2B2. They are generally less toxic and more readily excreted.

(b) 3-methylchloranthene-type PCBs (3-MC-type) are inducers of P450 – CYP1A; 3-MC-type PCBs are planar molecules with conformational hindrance at the site for oxygen. This leads to increased stability and decreased detoxification potential.
(c) PCBs that induce both PB and 3-MC, are known as mixed-type PCBs (183, 201).

One of the most unswerving effects of PCB exposure in neonatal and adult rodents is a decrease in thyroxine (T_4) concentrations. PCBs induce hepatic microsomal enzymes UDP-glucuronosyltransferases (UGTs), which conjugate triiodothyronine (T_3) and T4 prior to biliary excretion. Morse et al. (202) have reported an increased glucuronidation activity for T_4 in hepatic microsomes from pregnant rats treated with PCB 169 and PCB 77 on gestational day (GD) 1 and GD 2 to 18, respectively, as well as their fetuses and offsprings throughout the lactation period. Also, pregnant and weaning rats exposed to Aroclor 1254 from GD 10 to 16, exhibited similar effects (203). However, circulating concentrations of thyroid stimulating hormone (TSH), that controls thyroid hormone synthesis, have not been shown to increase under PCB exposure conditions. A decreased plasma level of thyroid hormones in adults exposed to PCBs via their diet has been reported. In a cohort study of North Carolina neonates born between 1978 and 1982, no relationship was found between cord blood T_4 and PCB levels (204). Evidence suggests that PCB effects are also mediated by PCB metabolites. The major metabolites of tetra- and penta-chlorinated biphenyls – methylsulfonyl metabolites found in human milk and tissue have been reported to decrease T_3 and T_4 serum levels in adult rats (205). Regrettably, comparing the relative potencies of PCBs and their metabolites *in vitro* poses difficulties owing to the extensive adsorption of these substances to the plasticware and glassware used in such experiments. T_4 is bound to transthyretin (prealbumin) in the plasma of rodents, which together with thyroxin transports the complex of retinol-binding protein and retinol (206).

PCBs have been reported to induce estrogenic and antiestrogenic effects; this is due partly to the generation of hydroxylated PCB metabolites (207). The antiestrogenecity of PCBs is likely not only by the interaction with estrogen receptors (ER) but also by a cross-talk of the aryl hydrocarbon receptor (AhR) with ER routes after activation by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-like PCBs. Three environmentally persistent PCB congeners – PCB 138, PCB 153 and PCB 180 – have been shown to possess receptor-mediated antiandrogenic effects (208). PCBs have been reported to decrease and also increase male anogenital distance (AGD), which is an indication of prenatal androgenisation (206). The androgenic effects of PCBs have been reported in female offspring after intraperitoneal exposure of dams to PCB 77 or PCB 47 from GD 7 to GD 18. The treatment resulted in an increased female AGD, but had no obvious effect on male AGD (209). Effects of PCBs on serum steroid concentrations have been reported (210-212). Studies have also reported vitamin A depletion by PCB exposure (191, 213). Behavioural effects resulting from exposure to PCB 28 and PCB 153 among other PCB congeners are well documented (214-221).

2.8.4 Emission Sources of PCBs

There are no documented naturally occurring PCBs; hence all PCBs present in nature can be attributed to man-made materials. The major sources of emission of PCBs into the environment can be divided into four groups:

(a) Production of PCBs and products (equipment) containing PCBs,

(b) Use of PCBs and materials containing PCBs,

(c) Emission from PCB-polluted reservoirs, and

(d) Thermal processes.

Although commercial mixtures of PCBs and PCB-containing products are no longer produced, it is known that during the production of capacitors, PCB losses reached up to 10 – 20% of the dielectrics used for filling. In South Africa and some other parts of the world, PCBs had and still have various applications in closed systems such as dielectric liquids in transformers and capacitors, hydraulic and cooling equipment, cables, etc., and also as peptizers for paper impregnation, paint manufacture, and caulking and joint sealing materials, from which they may seep into the surrounding environment (173). Another major source of PCB emission includes various PCB-containing wastes, such as electronic waste, worked out equipment and materials that are eventually recycled or removed into dumps. Also of importance are contaminated dust, soil, sediment, water, etc. that may act as a secondary source of PCB emissions. Thermal processes are other important sources of PCBs in the environment. In these processes, PCBs are synthesized like dioxins: the formation of PCBs as a by-product is very likely in any chemical process involving chloride and organic carbons, or emitted as a result of the incomplete combustion of a PCB impurity in the raw material (fuel).

Berdowski et al. (222) highlighted coal combustion, steel smelting (open-hearth, converter, electric), waste incineration, sintering, and electrical equipment such as capacitors and transformers, in the European PCB emission inventory for 1990. PCBs in electrical equipment are hypothetically the greatest source of environmental pollution of PCBs in several emission inventories (222). For example, in the UK, over 90% of PCB emissions originated from transformer and capacitors leaks and also fragmentizing operations. The study of Berdowski et al. (222) showed that as much as 94% of PCB emissions in the UK originate from this source. The case may not be different in South Africa as 80.1% of PCB oils and PCB equipment destroyed in South Africa between 2005 and 2010 was from the energy sector (173). Other contributors were 3.3% (mining sector), 2.6% (cement manufacturing), 6.0% (chemicals), 4.0% (petrochemicals) and 4.0% (transport). A recent inventory undertaken by ESKOM for PCB oils and equipment in South Africa revealed that 17086 pieces of equipment owned by ESKOM contained PCBs with a content in excess of 50 ppm. The equipment included transformers, capacitor cans and auxiliary equipment. Four % of this equipment had PCB contents

well above 500 ppm, the levels of PCBs in 62% of the equipment ranged between 50 – 499 ppm; 2% contained PCBs in the range 20 – 49 ppm and 42% contained PCBs ranging from 1 – 19 ppm (173).

2.8.5 Environmental Levels and Behaviours of PCBs

2.8.5.1 Air and dust

On a global scale, PCBs have been studied and detected in air samples from various countries. In the USA, Eisenreich et al. (223) reported PCB concentrations in the range 1-10 ng m^{-3} in air of urban areas, whilst the mean concentrations of PCBs in rural Ontario, Canada and Adirondack, New York, USA (two rural areas) were 0.2 and 0.95 ng m^{-3} respectively (224, 225). These values are in line with the 0.2 – 1.5 ng m^{-3} of PCBs in continental areas (223). Means PCB concentrations of 0.2 ng m^{-3} (range 0.02 – 0.5 ng m^{-3}) have been reported in the Arctic and Antarctic (226, 227). For the eastern Arctic, Harner et al. (228) reported an air concentration of 0.074 ng m^{-3}. In marine and coastal areas, PCB concentrations ranging from 0.01 – 0.7 ng m^{-3} have been observed. Eisenreich et al. (223) reported the concentrations of PCBs in the Great Lakes to range from 0.2 – 4.0 ng/ m^3. Indoor air concentrations of PCBs have been shown by various authors to be at least one order of magnitude higher than those from the surrounding ambient outdoor atmosphere (229). Mean PCB concentrations of 100 ng m^{-3} in an industrial research building, and 210 ng m^{-3} inside the laboratories compared to PCB concentrations of 20 ng m^{-3} in air outside the facility, have been documented. An average PCB indoor air concentration of 310 ng m^{-3} in a home, and an average outdoor air concentration of 4 ng m^{-3} have been reported in samples collected on the same day (230). Various electrical appliances and devices such as fluorescent lighting ballasts and building materials (e.g. caulking, elastic sealants), which may have PCB-entrenched components, may be an emission source of PCBs in indoor air, hence significantly elevating indoor air levels of PCBs above the outdoor background levels (231).

Wilson et al. (232) reported higher indoor air PCB concentrations (ranging from not detected (ND) – 18.4 ng m^{-3}) than outdoor air (ranging from not detected – 2.07 ng m^{-3}). Similarly, Rudel et al. (233) reported PCB concentrations in the range ND – 25 ng m^{-3} in indoor air of homes in the Boston, USA area. PCB concentrations in excess of the Swiss tentative guideline value of 6 μg m^{-3} for PCBs in indoor air have been documented (175). PCB concentrations in the range 111 to 393 ng m^{-3} have been reported in the indoor of a university building in the USA (172). The US EPA instructed a clean-up program in the case of the PCB–contaminated university building. The clean-up program included removal of window components from the building, caulking residues from window opening abatement, removal of PCB- entrenched caulking from window frames, ventilator unit removal and replacement, destruction of PCB-entrenched foam board, space and duct cleaning and restoration. The EPA clearance criteria for declaring the decontamination complete included air samples with <1000 ng m^{-3} PCBs, surface wipe

samples with <10000 ng/100 cm^2, and remaining porous building constituents, such as concrete and brick block, with <1 ppm PCB by mass (172). In South Africa, PCBs have been documented in the outdoor air of an industrialized and an urban area of KwaZulu-Natal (185).

The concentration of PCBs in indoor dust remain elevated in literature data. In a study, the sum of three PCB congeners (PCB 52, 105, 153) ranged from 21 to 190 μg g^{-1} in indoor dust of two houses (234). Hwang et al. (33) reported indoor dust concentrations of PCBs in the range <10 to 227 ng g^{-1}. In the indoor environment, were dissipation is particularly slow due to lack of sunlight, moisture, and microbial activity, PCBs will likely persist much longer and, consequently, pose a health risk for a prolonged period. Colt et al. (235) reported PCB concentrations in the range ND – 10200 ng g^{-1}. In the study, an increased risk of non-Hodgkin lymphoma was associated with PCB exposure, with evidence of higher effects for PCB 180 (235). In a more recent study, PCB concentrations in Californian dust samples ranged from below the detection limit to 270 ng g^{-1}. Concentrations of PCBs ranging from <1 ppm to 81 ppm have been reported in dust collected from the ventilation system of a building (172). Studies have associated higher PCB concentrations in dust with older residence/buildings (235-237). Correlations of PCB concentrations in dust with floors and carpet age is well documented (237, 238), implying that PCBs can accumulate on these surfaces over time. Occupational PCB exposure has been shown to enter the residential environment via dusty skin, clothing and shoes (237). In their study, Vorhees et al. (238) established that the two highest PCB concentrations were found in homes with residents reporting prior occupational exposure to PCBs. This finding was supported by Whitehead et al. (237), remarking that a resident's employment in a manufacturing, assembly, or industrial operation, conferred an increased risk for residential PCB-138 detection, whilst, an employment as an electrician, lineman, or cable puller was associated with higher PCB-118 loadings. A strong positive correlation between PCB concentrations in house dust and entryway dust from 8 homes have been documented (239), implying that PCB-contaminated dust can be tracked inside from the outdoor environment. In their study, Whitehead et al. (237) observed that residents who usually removed shoes when entering the home had lower PCB loadings than residents who did not often remove their shoes upon entering the home; since shoes can transport dust (240) into the home and other indoor environments, hence removal of shoes is one simple strategy to reduce residential PCB contamination (237). The authors have also shown that residents owning a pet dog or cat that lived inside were less likely to have detectable concentrations of PCBs, and hence have lower PCB loadings (237). Dog ownership has been associated with lower PCB levels in residential dust; this is in line with studies that showed animals may constitute an important "sink" for higher chlorinated PCBs in industrialized environments (241). Because pets are frequently in contact with carpets and floors, they are more likely to be in contact with dust than their human counterparts (237). Hence, PCBs can be taken up by household pets and may remain stored in their tissues (242). The concentrations of PCBs in dust from the Northern California Childhood Leukemia

Study (NCCLS) residences were all below the 1 ppm action level for PCB remediation in bulk materials such as contaminated soil required by the US EPA (243). This reference value is, however, not intended for residential dust and might not be a suitable standard, especially in homes with young children as they are more vulnerable to dust contaminated with PCBs due to the fact that children ingest more dust than adults (174).

2.8.5.2 PCBs in soil, sediments and sewage sludge

Historic PCB concentration profiles in sediments of the lower Passaic River, New Jersey, USA have been studied by determining PCB concentrations at different depths. The total PCB concentration in the sediment peaked at 4.7 mg kg^{-1} dry weight in the 1970s and consistently decreased to 1.1 mg kg^{-1} dry weight in the 1990s (244). In dated sediments from Newark Bay Estuary including the Passaic River, the highest PCB concentrations were reported in buried sediments from the Passaic River and Newark Bay, in line with historic deposition during the 1960s and 1970s (245). Coplanar PCB concentrations peaked from 1967 to 1972 and decreased rapidly from 1972 to 1977 and slowly levelled-off to almost one-third of the peak level in sediment core samples attributable to the period from 1935 to 1993 in Tokyo Bay, Japan (246). PCB (majorly congeners containing 4, 5 or 6 chlorine atoms per biphenyl molecule) concentrations have been reported to range between 1.1 – 141 ng g^{-1} dw in sediments from the lower Nakdong River of Korea (247); while in the Baltic Sea, the concentrations of PCBs was reported to range from ~1 to 149 ng g^{-1} dw (248). In the Greak Lakes, Li et al. (249) reported total PCB accumulation in sediments to be approximately 300 ± 50 tonnes. Evidence of *in situ* PCB degradation in sediment was found with an estimated $t_{1/2}$ of 11 and 17 years, at two open water locations in Lake Ontario (249). PCB concentrations in the range 0.0050 to 101.0 µg kg^{-1} dw have been reported in sediment from Germany (250). In surficial sediments from the riverine coastal waters of Surubaya, Indonesia, PCB concentrations varied from less than the detection limit (<DL) to 420 ng g^{-1} dw (51). The concentration of PCBs in the Egyptian Mediterranean Sea ranged from 7.06 – 75.17 ng g^{-1} (251). Nieuwoudt et al. (182) reported total PCB concentrations in the range of 120 to 4700 ng g^{-1} dw in sediment from residential and industrial areas of central South Africa. PCBs have been reported in the Hartbeespoort Dam, North West Province and Voëlvlei dams of the Western Cape Province, South Africa (252) and also from the Northern and Southern Indian Ocean coastline of South Africa (253). The concentrations of PCBs measured in sediment from different water bodies in South Africa were 0.06 mg kg^{-1} dw in Voëlvlei dam, Western Cape Province and 0.32 mg kg^{-1} dw in Hartbeespoort dam, Northwest Province (252), unlike the study of Grobler (254) where no detectable levels of PCBs (Aroclor 1254 and 1260) were found in sediment and other aquatic life of the Olifants River in Mpumalanga Province, South Africa.

PCB concentrations in the range 357 to 3820 pg g^{-1} dw have been reported in soil samples from a major UK conurbation (255). PCB concentrations in surface soil were found to range from 26 to 97000 pg g^{-1} dw in samples from Greenland and mainland, Europe

(256). The background soil PCB concentrations were reportedly influenced by proximity to source region and soil organic matter content. Total PCB concentrations in the range 1.5 to 2.6 μg g^{-1} have been reported in soils from storage dumpsters in Manitoba Hydro in Winnepeg, Canada (257). The concentrations of PCBs in Swiss soil were reported to range from 1.1 to 12 μg kg^{-1} (258). In South Africa, surface soil PCB concentrations for most PCB congeners ranged from 1 to 10 ng g^{-1}, with site to site differences (185). Soil PCB contaminations ranging from 3.3 to 34 mg kg^{-1} have been reported around buildings with undisturbed caulking that contained 10000 to 36200 mg kg^{-1} PCBs (259). The elevated PCB concentration in soil could be associated in part to the fact that PCBs in caulking may be mobilized as complexes with dissolved organic matter which also leach off the caulking material (259). Several studies have documented PCB contaminations in sewage sludge (260-262).

2.8.5.3 Biotic samples

Since PCBs are lipophilic they can accumulate in fatty tissues. In South Africa, mean PCB concentrations of 104 μg kg^{-1} have been reported in the blubber of seals (263). PCB levels of 20 μg g^{-1}, 13μg g^{-1} and 8.4 μg g^{-1} have been reported in blubber from North Coast, Central Coast and the South Coast bottlenose dolphins off the coast of KwaZulu-Natal, South Africa respectively (264). In common dolphins, Cockcroft et al. (265) reported a mean PCB concentration of 4.04 μg g^{-1} blubber wet weight in dolphins caught during the "sardine run" off the coast of KwaZulu-Natal (265). de Kock et al. (266) reported PCB loads in the range 0.01 – 15.51 μg g^{-1} in the blubber of seals and small whales caught in the period 1977 to 1987 from the east and west coasts of Southern Africa. The concentrations of total PCBs measured in fish species from the Isipingo Estuary, KwaZulu-Natal, ranged from 5.7 to 869 μg kg^{-1} wet weight across all species (184). PCB concentrations in eggs of coastal birds have been reported to range from 0.05 – 0.89 μg g^{-1} (267). Recently, Quinn et al. (183) reported PCB concentrations ranging from 0.9 to 296 ng g^{-1} wet weight in wild bird eggs from an industrialized area in South Africa. Similarly, Batterman et al. (185) reported elevated PCB levels in South African cow milk. PCB concentrations in several biotic samples are well documented worldwide (268-270) at comparable levels with reported values in South Africa.

2.8.5.4 Human body burden of PCBs

Human milk fat has been reported to contain PCBs in the range of 0.5 to 4.0 mg kg^{-1} (271). The average PCB concentrations in whole breast milk in the Canadian population increased steadily from 6 μg kg^{-1} in 1970, to 12 μg kg^{-1} in 1975 and 26 μg kg^{-1} in 1982, prior to a decline to 6 μg kg^{-1} in 1986 (272). PCB concentrations in breast milk of Swedish women decreased steadily from 910 to 324 ng g^{-1} lipid weight for the period 1967 – 1997 (273). In a yearly trend study of PCB concentrations in the breast milk of a Japanese population, the mean PCB content increased from 1.302 μg g^{-1} fat basis in 1972 to its peak of 1.514 μg g^{-1} fat basis in 1974 and decreased to 0.200 μg g^{-1} fat basis in 1998 (179). The concentrations of PCBs in human breast milk samples from women

living in Vietnamese e-waste recycling sites ranged from 28 to 59 ng g^{-1} lipid weight (101). The authors documented a specific accumulation, unrelated to diet of low-chlorinated PCBs in e-waste recyclers, suggesting extensive exposure to these compounds during e-waste recycling activities; likely via inhalation and ingestion of contaminated dust (101). PCB concentrations ranging from 28.70 to 1044.26 ng g^{-1} lipid weight and 26.88 to 218.80 ng g^{-1} lipid weight have been reported in blood of residents of two e-waste recycling areas in Southern, China (274). Average PCB concentrations of 69 ng g^{-1} lipid weight have been reported in blood of the Guiyu population, China (275). The median concentrations of five PCB congeners in the maternal blood plasma of a Stockholm population study was 134 ng g^{-1} lipid weight (276); in the UK general population, Thomas et al. (277) reported median PCB concentrations of 118 ng g^{-1} lipid weight in human blood. Other authors have reported elevated PCB concentrations in blood of different populations (278-281). PCB concentrations as high as 181.99 ng g^{-1} dw have been documented in human hair samples of residents around e-waste disassembly sites in Zhejiang Province, China (282). The concentrations of the sum of 23 PCB congeners ranged from 70 - 1130 ng g^{-1} and 90 – 1140 ng g^{-1} lipid weight in human adipose tissues and liver samples respectively from Belgium (283). A report has shown that 66.4% of Americans studied had PCB concentrations less than 1 mg kg^{-1} and 5.1% had PCB concentrations in excess of 3 mg kg^{-1} in human adipose tissues collected between 1970 and 1983 (284).

2.8.6 Regulatory Aspects of PCBs

Polychlorinated biphenyls have been labelled as hazardous substances pursuant to the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) of 1980 (285) and as toxic chemicals under Section 313 of the Title III of the Superfund Amendments and Reauthorization Act (SARA) of 1986 (285). Title III of SARA is also known as "The Emergency Planning and Community Right-to-Know Act (EPCRA) of 1986". The statutory sources for labelling PCBs as CERCLA hazardous substances are Section 311(b)(4) and 307 (a) of the Clean Water Act (CWA) and section 112 of the Clean Air Act (CAA) in the USA.

The South African legal framework on persistent organic pollutants (POPs) is supported by the South African National Code of Practice. The codes are representative of voluntary technical standards and become legally binding if incorporated into law. The South African National Standards (SANs) which are relevant to POPs management include:

- ✓ SANs 10219 – Labelling and Packaging
- ✓ SANs 10228 – Identification and Classification of Dangerous Goods for Transport
- ✓ SANs 10263 – Classification of Pesticide for Sale and Handling
- ✓ SANs 10206 – Handling, Storage and Disposal of Pesticides

- ✓ SANs 10229 – Packaging for Transport
- ✓ SANs 290 – Mineral Insulating Oils – management of PCBs

Aside the national legislation that is applicable to environmental management in South Africa, the country also participates actively in international organizations and agreements on the management of chemicals and wastes. The country has signed and ratified a handful of international environmental conventions and agreements for which the Department of Environmental Affairs (DEA) is the national central point. South Africa is a party to four other international chemical–related conventions and agreements, which in line with the Stockholm Convention provides an international framework governing the environmentally sound management of hazardous chemicals and wastes all through their life cycle. The conventions include the Basel Convention on transboundary of hazardous wastes; the Rotterdam Convention on prior informed consent; the Montreal protocol and the Strategic Approach to International Chemical Management (SAICM) (173).

The Stockholm Convention prohibits the production of PCBs, but gives parties to the convention until 2025 to take action to phase out the use of PCB oils and equipment contaminated with PCBs. All recovered PCBs must be treated and eliminated by 2028 (173).

Though PCBs were never produced in South Africa, PCB oils and equipment containing PCB oils were imported mainly for use in electricity generation. PCBs are listed as a Group II hazardous substance in South Africa and have been assigned a unique tariff code in the South African tariff book. This enables them to be identified specifically on import and PCBs are placed on the Custom and Excise list of "prohibited and restricted" imports and exports. Hence, customs will retain any PCBs entering the country with no future imports of PCBs expected to be received into the country. To manage existing PCB oils and contaminated equipment, a national standard on Mineral Insulating Oil Management referred to as SANs 290: 2007 has been developed (173). The standard identifies materials containing between 51 – 500 ppm as PCB contaminated, and materials having in excess of 500 ppm are referred to as PCB-containing materials (173). This standard administers certain inspection, labelling, retrofilling and management measures to mitigate the risk related with these materials. At present, there is no national inventory of PCBs in South Africa and no phase-out plan to certify that the phase-out time-frame for PCB oils and contaminated equipment enacted by the Stockholm Convention will be met (173).

The government notice (GN) R549 in the South African Government *Gazette* 378181 of 10th July, 2014, as published by the Honourable Minister of Environmental Affairs, entitled "Regulations to phase-out the use of PCB materials and PCB contaminated materials" (286). The PCB regulation prohibits the use, production, import, export and sale of PCB materials or PCB contaminated material in South Africa, except the holders

of PCB materials or PCB contaminated materials who must register the PCB article with the Director-General of the Department of Environmental Affairs within 90 days from 10th July, 2014 and develop a phase-out plan within one year of registration. These obligations are in terms of the Stockholm Convention which requires that signatories are to phase-out the use of PCBs by 2025 (286).

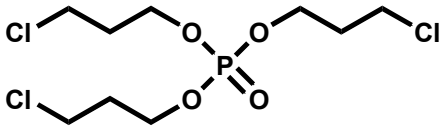
2.9 Phosphorus Flame Retardants

Phosphorus flame retardants (PFRs), which have been in use for over one and a half centuries (287), are believed as appropriate alternatives for BFRs. Due to the demand for vapour-phase activity, a number of volatile PFRs: tributylphosphate (TBP), triphenylphosphate (TPP) and triphenylphosphine oxide (TPPO), have been recognized as likely substitutes for bromine-containing formulations used in textile back-coatings (287). PFRs are compatible with other processing chemicals and are easy to use. Some of the PFRs are known to facilitate the recyclability of printed circuit boards, as it is more realistic and cost-effective to recover copper from halogen free circuit boards. van der Veen and de Boer (287) stated "If PFRs would be used as alternative for PBDEs, it is important to avoid compounds, which are more persistent, bioaccumulative and toxic to humans and the environment than BFRs (287)".

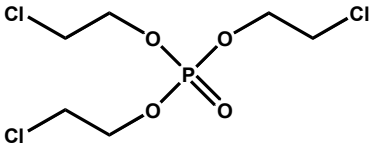
2.9.1 Production, Application and Uses of PFRs

The global consumption of PFRs amounted to 186000 tons in 2001, however, there has been a sharp increase in consumption of PFRs in recent years (287). For example, in the year 2006, European consumption was estimated to amount to 93000 tons compared to 83000 tons in 2001 (287, 288). Increasing fire resistance standards coupled with legal restrictions on sister products, such as PBDEs and PCBs, has led to an increase in the production and application of PFRs. PFRs are additives to polymeric materials that usually make up 1 – 30% of the composition with an average of 5 – 15% (289).

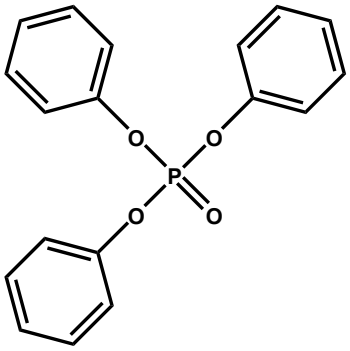
Organophosphate esters (OPEs) compose the very commonly used group among the PFRs, which includes phosphonates, phosphites and phosphines (287). The industrial production of OPEs involves the reaction of phosphorus oxychloride ($POCl_3$) with various reactants. OPEs are structural derivatives of phosphoric acid that can be grouped into three, namely; trialkyl-, alkyldiaryl-, and triaryl phosphates. Additionally, the alkyl phosphates can be halogenated or non-halogenated. Generally, OPEs are semi-volatile compounds with low to moderate solubility in water and relatively high affinity to particles. Though, due to variation in their substituent characteristics, they have strongly differing chemical and physical properties. These disparities in properties make them useful in diverse applications. The structure of the four OPEs studied in this thesis are shown in Fig 2.5.



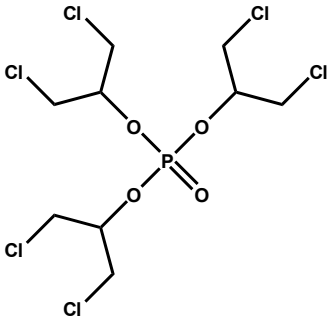
Tris[chloropropyl]phosphate (TCPP)



Tris[chloroethyl]phosphate (TCEP)



Triphenyl phosphate (TPP)



Tris[1,3-dichloropropan-2-yl]phosphate (TDCPP)

Figure 2.4 Phosphorus flame retardants examined in this thesis

OPEs are used for two purposes: the halogenated ones as flame retardants (FRs), while the non-halogenated ones are basically used as plasticizers (290). The non-derivatized alkyl phosphates such as triphenyl phosphate (TPP) are majorly used as plasticizer lubricants and pore-size regulators, although in some instances, they are also used as FRs (290). PFRs such as TPP are also used in combination with halogenated and non-halogenated FRs in different commercial mixtures regularly added to polyurethane foam (287).

Various OPEs are used as additives for different materials depending on the properties desired. Trialkyl phosphates are less thermally stable than triaryl phosphates, and hence are less effective as flame retardants. However, trialkyl phosphates have better plasticizing properties and enhance the low temperature flexibility of plastics and synthetic rubber (291). Therefore, OPEs are used as FRs and/or plasticizers in a wide range of products such as polyurethane foams (PUF), polyvinyl chloride (PVC), thermoset resins, thermoplastic materials, textile finishes, polyesters and celluloses. The flame retardant properties of PVC, cellulose acetate and other plastic materials are enhanced with triaryl phosphate (292). Computer housings made of acrylonitrile-butadiene-styrene (ABS) and polycarbonate (PC) blends are regularly flame retarded with TPP. The chlorinated OPEs such as TCEP, TCPP and TDCPP are used to flame retard both flexible and rigid polyurethane foam (PUF), rubber and textile coatings (293). Whilst flexible PUFs are commonly found in products such as upholstered furniture and mattresses, rigid PUFs are used primarily for thermal insulations. Hospital and prison mattresses are mostly treated with TDCPP (294). TCEP, TCPP and TDCPP have also been found in products such as sound-and shock–absorbing materials, foam fillers, and

wood preservation coatings (295, 296). Aside from being used as FRs, some OPEs, such as TPP, are used as extreme pressure additives and antiwear agents in hydraulic fluids, lubricants, transmission oils and motor oils to prevent surface damage. TPP is usually added at levels between 1 – 5% in several aircraft hydraulic fluids (297).

2.9.2 Physicochemical Properties of PFRs

There is a great distinction in the physicochemical properties of PFRs. The solubility of PFRs decreases by increasing the molecular mass. When their hydrolysis half-lifes are equal, the PFRs with lower masses are more likely to be found in the aquatic environment than those with higher molecular masses, which is confirmed by the log K_{ow} values of the PFRs (287). Henry's law constants at 25 °C for most PFRs vary between 2.8×10^{-4} and 1.7×10^{-23} atm m^{-3} mol^{-1}. The extensive range of Henry's law constant values of PFRs shows that the distribution of PFRs over air and environmental waters such as oceans is highly variable (287). Similarly, the vapour pressure of PFRs at 25 °C ranges from 1.9 to 9.5×10^{-21} mmHg and the bioconcentration factors (BCF) ranged from 1.37 to 10^6 (287).

Table 2.11 Physicochemical properties of the studied PFRs (287).

Name	Abbreviation	log K_{ow}	Flash point/°C	Reactive or additive FR	Boiling point/°C	Melting point /°C	Solubility in water at 25^{0}C/ mg dm^{-3}	Vapour pressure at 25°C/ mmHg	Henry law constant at 25 °C/ atm m^{-3} mol^{-1}	Soil adsorption coefficient (log k_{oc})	Bioaccumulation/ bioconcentration factor (BCF)
tris(2-chloroethyl) phosphate	TCEP	1.44	202	Additive	351	-55	7.0×10^3	1.1×10^{-4}	3.3×10^{-6}	2.48	1.37
tris(chloropropy) phosphate	TCPP	2.59	312	Additive	342	-40	1.60×10^3	1.9×10^{-6}	6.0×10^{-8}	2.71	42.4
tris(1,3-dichloro-2-propyl)phosphate	TDCPP	3.8	378	Additive	457	88	1.50	7.4×10^{-8}	2.6×10^{-9}	2.35	13.5
triphenyl phosphate	TPP	4.59	220	Additive	370	49	1.90	1.2×10^{-6}	3.3×10^{-6}	3.72	113

2.9.2.1 Tris(2-chloroethyl) phosphate

Tris(2-chloroethyl)phosphate (TCEP) is an additive FR (298), which when there is fire, the phosphorus is active in the solid phase. However, TCEP also has a gas phase mechanism of action through the chlorine (287). The boiling point of TCEP is 351 °C and it is stable on short term exposure at 150 °C. TCEP decomposes rapidly at temperatures above 220 °C to form carbon monoxide, hydrogen chloride, 2-chloroethane and dichloroethane (287). There is a decrease in the hydrolytic stability of TCEP with increasing temperature and pressure or very high pH (293).

2.9.2.2 Tris (chloropropyl) phosphate

Tris(chloropropyl) phosphate (TCPP), a halogen-containing PFR, is a clear and colourless liquid (293), used as an additive FR (299). The commercial product contains a mixture of halogenated phosphoric acid esters of which the main components are 75% tris(chloroiso-propyl) phosphate and 15 – 30% bis(1-chloro-2-propyl)-2-chloropropyl phosphate (300). TCPP has both a solid and a gas phase fire performance mechanism, whereby the phosphorus is active in the solid phase and the chlorine in the gas phase. TCPP decomposes at temperatures above 150 °C to form carbon monoxide, carbon dioxide, phosphorus compounds and hydrochloric acid. In the presence of acids and bases, TCPP decomposes to form phosphoric acid and chloropropanol (287).

2.9.2.3 Tris (1,3-dichloro-2-propyl) phosphate

Tris(1,3-dichloro-2-propyl)phosphate (TDCPP), a halogen-containing PFR, is a viscous colourless liquid used as an additive FR in resins, latexes and foams (292, 293). The majority of the foams are used in the automotive industries and some are used in furniture (287). TDCPP has the same product applications as TCPP, however, due to the high cost of TDCPP, it is only used in applications were a more effective FR is required (287). TDCPP is the most often detected PFR in foams and baby products (301).

2.9.2.4 Triphenyl phosphate

Triphenyl phosphate (TPP), an aryl phosphate, is used as an additive FR (298). TPP is active only in the gas phase (302). TPP is one of the most effective FRs for various polymers. TPP decomposes to form phosphoric acid, which reacts to form pyro phosphoric acid, and acts as heat transfer barrier in the condensed phase (169).

2.9.3 Toxicology and Health Effects of PFRs

2.9.3.1 Tris(2-chloroethyl) phosphate

Tris(2-chloroethyl)phosphate is a toxicant to aquatic organisms and may cause chronic adverse effects (287). It is carcinogenic to animals (293), a neurotoxin in rats and mice (303, 304) and has been reported to induce adverse reproductive effects in rats (305).

Antagonistic biological effects related to humans, such as hemolytic and reproductive effects, reduced fertility, longer estrous cycle length, and reduced sperm motility, and density have been documented (305). The LC_{50} values reported for fish varied from 6.5 – 250 mg dm^{-3} (306). There is a paucity of data on the toxic effects of TCEP in animals and humans, however, Lehner et al. (307) associated the acute death of dogs after ingestion of car seat cushions containing large amount of TCEP to its toxicity. However, potential interaction among different compounds may not be excluded (287). Other toxic effects of TCEP are well documented (290). TCEP is not regulated by legislation, but has been replaced in some products under consumer pressure (287). Enormous quantities of TCEP have been used in buildings, and these may remain active sources for several years (290). The Scientific Committee on Health and Environmental Risks (SCHER's) predicted environmental concentrations and the predicted no effect concentration (PEC/PNEC) ratios of TCEP are below 1 for all compartments, but mostly above 0.1 for both the terrestrial and aquatic compartments (308); meaning that no adverse effects are expected. The conclusion from the PEC/PNEC ratio reveals that TCEP can be used but not recommended as a substitute for BFRs (287). If environmental concentrations of TCEP increase, the PEC/PNEC ratio will also increase and may be higher than 1, meaning it is unsafe to use TCEP (287).

2.9.3.2 Tris (chloropropyl) phosphate

Tris(chloropropyl)phosphate (TCPP) is a potential carcinogen (309). The acute oral, inhalation and dermal toxicity of TCPP have been reported in rats (300). LD_{50} values for TCPP ranged from 500 – 4200 mg kg^{-1}, higher than 4.6 mg dm^{-3} to higher than 17.8 mg dm^{-3} and 1230 - 5000 mg kg^{-1} bw (287). TCPP is not acutely toxic and for the chronic toxicity, a NOEL of 36 mg kg^{-1} bw was reported (300). TCPP has been reported to decrease cell numbers and alter neurodifferentiation (310). TCPP accumulates in the kidney and liver, while it is metabolized in hydroxides of phosphoric acid in animal studies (300). The findings from the PEC/PNEC ratio suggest that TCPP can be used but not recommended as a substitute for BFRs, due to its accumulation in the liver and kidney and its potential carcinogenicity (287).

2.9.3.3 Tris(1,3-dichloro-2-propyl)phosphate

Tris(1,3-dichloro-2-propyl)phosphate (TDCPP) is harmful on inhalation (287). When in the body, it can easily enter the blood stream (311). In rats fed with TDCPP for 2 years, tumors were observed in their livers, kidneys and testes (311). In the ASTDR report, no significant relationship was found between exposure to TDCPP and cancer (311), although, the WHO (293) and Andresen et al. (290) reported TDCPP as carcinogenic. Data from TDCPP mutagenicity showed that the chemical is not genetotoxic (293). TDCPP has been shown to exhibit concentration-dependent neurotoxicity, inhibition of DNA synthesis, and decreased cell numbers and altered neurodifferentiation (310). TDCPP showed elevated oxidative stress; but no adverse effect on cell viability growth

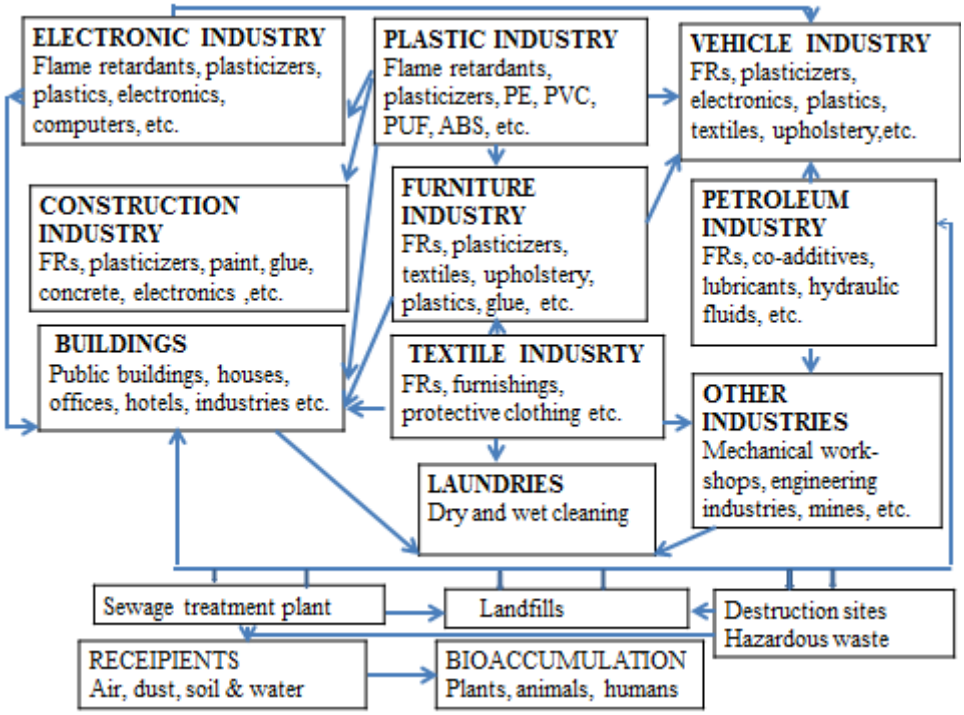
was detected (310). TDCPP is reported to be more neurotoxic than TCEP and TCPP, which only promoted the cholinergic phenotype. The LD_{50} of TDCPP in rats by the oral and dermal route are 2300 and > 2000 mg kg^{-1} bw respectively (293). The toxicity of TDCPP in fish was determined to be 1.1 mg dm^{-3} (96 hrs LC_{50}) (306). For daphnia, acute toxicity data determined were 3.8 mg dm^{-3} and 4.6 mg dm^{-3} (48 hrs, EC_{50}) (312). For mice, a no observed effect level (NOEL) of 15.3 mg kg^{-1} bw d^{-1} and the lowest observed effect levels (LOEL) for increased liver weight was 62 mg kg^{-1} bw d^{-1} (293). TDCPP is also a skin irritant (287).

2.9.3.4 Triphenyl phosphate

Several toxicity studies of TPP has been conducted with various conclusions (287). TPP has been associated with delayed neurotoxicity (309) and is possibly neurotoxic (290). However, a low neurotoxicity was observed by Pakalin et al. (313). Contact dermatitis has been reported with TPP (298). TPP inhibits human blood monocyte carboxylesterase, which affects the immunologic defense system (314). TPP is acutely toxic to aquatic organisms (315, 316), and is the most toxic triaryl phosphate to shrimps, fish and daphnia (300). Algae growth is completely inhibited at TPP concentrations of 1 mg dm^{-3} or more (287). Lassen and Lokke (317) reported acute toxicity of TPP in fish (96 hrs LC_{50}) to range from 0.36 mg dm^{-3} in rainbow trout to 290 mg dm^{-3} in bluegills (317). Altered hormone levels and decreased sperm concentrations have been observed for TPP in house dust (318). A NOEL of 690 mg kg^{-1} d^{-1} has been reported for developmental and birth defect effects of TPP in rats (319). For algal inhibition, an EC_{50} of 0.26 – 2.0 mg dm^{-3} has been reported, and for acute toxicity in rats LD_{50} values of 3500 – 10800 mg kg^{-1} (287). In studies of chronic toxicities of TPP, an estimated no observed effect concentration (NOEC) for *daphnia* of 0.1 mg dm^{-3} has been reported and a NOEC of 0.0014 mg dm^{-3} was reported for survival and growth of fish (319). The USEPA stated that this level is of high concern (319).

2.9.4 Emission Sources of PFRs

TCEP, TCPP, TDCPP and TPP are used mainly as additive FRs, i.e. they are not chemically bound to the products in which they are added. Hence, they may diffuse out of the products during production and use, thus reaching the environment via leaching, volatilization and abrasion throughout the lifetime of the product (Fig. 2.6). The general application of PFRs increases the risk that they may end up in different environmental compartments through volatilization, leaching or abrasion.



Source: Modified from Marklund et al. (320)

Figure 2.5 Flow chart of emission sources of organophosphorus flame retardants.

2.9.5 Environmental Levels and Behaviour of PFRs

Organophosphate esters are not known to occur naturally in the environment, but only due to anthropogenic activity (293, 297). OPEs have been measured in various indoor and outdoor environments (Table 2.12). In the indoor environment, OPEs have been reported in air and dust from microenvironments such as homes, offices, public places, cars, etc., at concentrations normally at mg kg^{-1} levels (for dust samples) and ng m^{-3} levels (for air samples) (289, 320-325). In the outdoor environment, PFRs have been detected in surface and drinking waters and sediments (290, 326-328); soil (329, 330); and groundwater and wastewater (290, 327, 331-334); leachates from disposal sites (335, 336); and particulates from the Antarctica (337). OPEs have also been reported in biota (338, 339) and in human adipose tissues and seminal plasma (340-343). There are currently no data on organophosphate flame retardants in South Africa; however, Abdallah and Covaci (321) recently reported levels of organophosphate esters in indoor dust from Egypt.

Table 2.12 Reported levels of PFRs in environmental matrices.

Matrix	PFR	Microenvironment	Reported Concentrations	Location	Reference
Indoor air	TCEP	Lecture hall	ND		(344)
		Office	730 mg m^{-3}		(345)
	TCPP	1 year old car	< 0.12 ng m^{-3}	Sweden	(289)
		Computer hall	1080 ng m^{-3}		(344)
	TDCPP		< 0.11 ng m^{-3}	Sweden	(289)
		Hospital ward	150 ng m^{-3}		(345)
	TPP		<0.05 ng m^{-3}	Norway	(292)
Dust	TCEP		< 0.08 µg g^{-1}		(346)
			5.64 µg g^{-1}		(346)
	TCPP		140 ng g^{-1}	Boston, MA	(347)
			14 mg kg^{-1}		(300)
	TDCPP		< 80 ng g^{-1}		(346)
			67 mg kg^{-1}		(320)
	TPP		< 150 ng g^{-1}	Boston, MA	(347)
			1.8 mg g^{-1}	Boston, MA	(347)
Drinking water	TCEP		4 ng dm^{-3}		(348)
			99 ng dm^{-3}		(348)
	TCPP				
	TDCPP		0.25 µg dm^{-3}		(348)
	TPP		< 500 ng dm^{-3}		(348)
Influent	TCEP		<0.025 ng dm^{-3}	Spain	(349)
			2500 ng dm^{-3}	Norway	(292)
	TCPP		0.32 ng dm^{-3}	Spain	(349)
			18 µg dm^{-3}	Sweden	(288)
	TDCPP		210 ng dm^{-3}	Sweden	(288)
			820 ng dm^{-3}	Norway	(292)
	TPP		< 0.015 ng dm^{-3}	Spain	(349)
			14 µg dm^{-3}	Norway	(292)
Effluent	TCEP		<0.025 ng dm^{-3}	Spain	(349)
			2200 ng dm^{-3}	Norway	(292)
	TCPP		0.31 ng dm^{-3}	Spain	(349)
			24 µg dm^{-3}	Sweden	(288)
	TDCPP		20 ng dm^{-3}	Germany	(290)

			740 ng dm^{-3}	Norway	(292)
	TPP		<0.015 ng dm^{-3}	Spain	(349)
			3.5 µg dm^{-3}	Norway	(292)
		Car demolishing			
Sediment	TCEP	site	5500 µg kg^{-1}	Norway	(292)
	TCPP		0.15 µg kg^{-1}	Norway	(350)
			24000 µg kg^{-1}	Norway	(292)
	TDCPP		<0.09 µg kg^{-1}	Norway	(350)
		Car demolishing			
		site	8800 µg kg^{-1}	Norway	(292)
	TPP		<0.10 µg kg^{-1}	Norway	(350)
				Liesing,	
			160 µg kg^{-1}	Austria	(328)
Biota	TCEP	Blue mussel	<0.06 µg kg^{-1}	Norway	(350)
		Perch	160 µg kg^{-1}	Sweden	(339)
	TCPP	Blue mussel	< 0.2 µg kg^{-1}	Norway	(350)
		Burbot liver	17 µg kg^{-1}	Norway	(350)
	TDCPP	Beach crab	< 0.025 µg kg^{-1}	Norway	(350)
		Perch	140 µg kg^{-1}	Sweden	(339)

2.9.6 Regulatory Aspects of PFRs

The phosphorus flame retardants are subjected to continuous scrutiny. The chlorinated organophosphate esters, in particular, TCEP, TCPP, and TDCPP, are under risk assessment in the European Union. TCEP is restricted under the EU RoHs Directive Commission Decision 2010/571/EU (351). TCEP has since been substituted with TCPP in most products (287). Although, there is currently no legislation regulating TPP, it is recently been substituted with resorcinol-bis(diphenyl phosphate) (RDP) and bisphenol-A diphenyl phosphate (BADP) in most products (302).

2.10 Human Exposure to BFRs, PCBs and PFRs

Several routes of human exposure to BFRs, PCBs and PFRs including dust ingestion, diet, dermal contact and inhalation have been suggested (figure 2.6). Whilst little information is available on the magnitude of dermal absorption of BFRs via skin contact, it appears to be a very minor pathway of exposure (294).

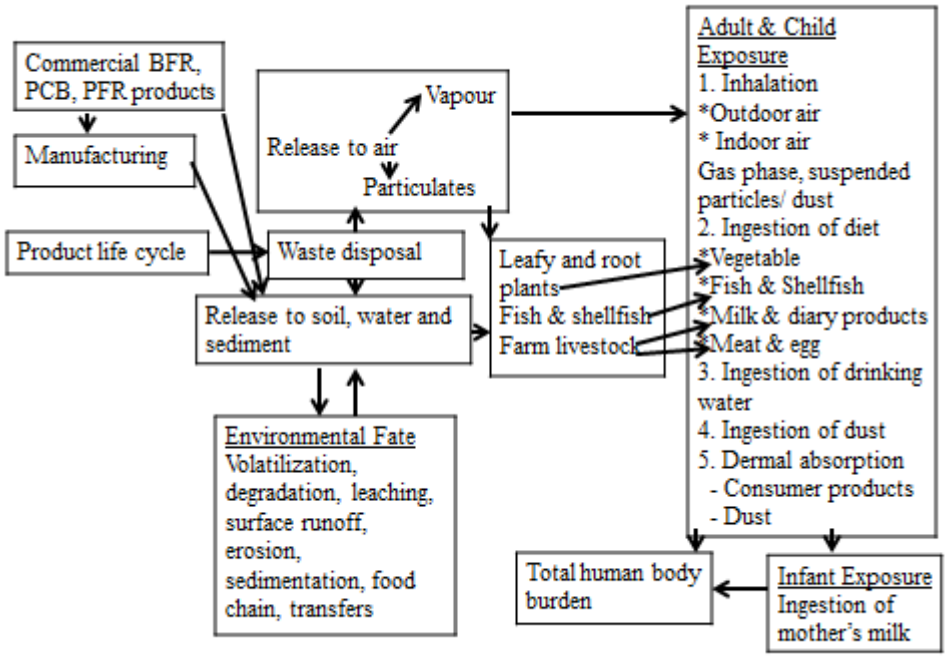


Figure 2.6 Pathways of human exposure to flame retardants. Source: Modified from (93, 352).

Inhalation of contaminated air can be a major exposure pathway of the studied FRs for occupationally exposed individuals with concentrations as high as 650000 pg m^{-3} (353); 393 ng m^{-3} (172); 140 ng m^{-3} (24) and 950 ng m^{-3} (320, 345) reported for ∑PBDEs, ∑PCBs, TBBPA and ∑PFRs respectively, in indoor air from different electronic dismantling/recycling facilities and other public microenvironments. However, non-

occupational exposure to the listed FRs via indoor air inhalation is unlikely to exert a significant contribution to overall exposure to these flame retardants owing to the low levels of these compounds reported so far in both outdoor and indoor air (81, 93). Hence, for non-occupationally exposed individuals; the significant intake of PBDEs, TBBPA, PCBs and PFRs are likely from food and indoor dust ingestion.

Diet was believed for a long time as the most important source of human exposure to organic flame retardants as was the case for many other POPs (354). However, diet could not account for the differences in internal human exposure of PBDEs observed between Europe and North America. Levels of PBDEs in comparable food items were the same on both continents (with the exception of meat products which contained slightly higher levels in North America). Hence, the discrepancy could not be explained from the concentration differences in human matrices exclusively on the basis of dietary exposure (even when considering the differences in dietary habits). Recently, exposure from house dust was proposed as the "missing" pathway to internal human exposure that can explain such differences. Enormous variations in PBDE levels in indoor dust have been documented across the Atlantic similar to the human internal exposure pattern (93). Existing data on human exposure to PBDEs, with greater stress on external exposure routes (air, diet and dust) and internal exposure pathways (i.e. human milk and blood) were reviewed recently by Frederiksen et al. (93). The authors proposed that the use of diverse consumer products may contribute to exposure in domestic environments. Dust seems to be an additional source of indoor PBDE exposure, and its ingestion bears the greatest intake of BDE-209 of all sources; possibly this is also true for other congeners. Exposure to PBDE through dust is important for toddlers as they ingest, or are in contact with, more dust than adults. Human milk is another important source of infant exposure to PBDEs.

Human exposure to PFRs through the diet, i.e. ingestion of fish and meat, seems to be of minor significance compared to exposure through inhalation of air and ingestion of dust (339). The exposure to PFRs by consumption of fish, on the basis of the average concentrations of TCPP, TPP, TCEP, TBP, EHDPP, TBEP, TDCPP and TCP, was 20 ng kg^{-1} day^{-1} for an adult of 70 kg, while on consumption of eelpout, with a total PFR concentration of 15 µg g^{-1} lw resulted in an exposure of 180 ng kg^{-1} day^{-1} (339). The finding showed that people who consume fish daily are not at risk compared to the suggested guideline value of 40 µg kg^{-1} day^{-1} (339). Likewise, a breastfed baby of 5 kg who consumes 1 litre of mother's milk per day is exposed to 64 ng kg^{-1} day^{-1} of PFRs; a level drastically lower than the guideline value. However, calculated exposures to PFRs through inhalation and ingestion of dust and air have been reported at levels as high as 5.8 µg kg^{-1} day^{-1} (345). For occupational settings, exposure to TPP has been reported to be as high as 750 to 12800 ng day^{-1} in a circuit board factory and electronic waste dismantling factory (344, 355, 356). Other exposure pathways need to be considered, such as dermal contact and intake through water and food via migration of plasticizers in

plastic packaging to food. Also, children can be orally exposed to PFR-treated fabrics (339).

For PCBs, available data suggests inhalation and diet are the main exposure pathways. Exposure to PCBs via inhalation has been reported to be 0.1 μg kg^{-1} and 0.6 – 1.2 ng kg^{-1} (357). In Finland, a PCB daily dose via the diet of 0.24 μg kg^{-1} for an adult weighing 60 kg has been reported (358). Similarly, Darnerud et al. (359) reported a daily dietary exposure to PCBs to be 0.05 μg kg^{-1} body weight. In Vietnam, diet accounted for 69% of the daily intake of PCBs, although, the contribution of inhalation was high for di- to tetraPCB. The importance of inhalation as a PCB exposure pathway is in line with the studies of Harrad et al. (360), in which inhalation was estimated to contribute 4.2 – 63% of the total PCB exposure (360). Exposure to PCBs via dust ingestion has received limited attention.

In this work some identified research gaps with respect to aspects of the environmental fate and behaviour, routes and the magnitude of human exposure to BFRs, PCBs and PFRs were addressed.

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Chapter 3

Simultaneous determination of tri- to deca-polybrominated diphenyl ethers (PBDEs) in automobile dust by gas chromatography/ electron impact ionization-mass spectrometry

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ABSTRACT

An accurate and precise assessment of polybrominated diphenyl ethers (PBDEs) is of major importance particularly in a complex matrix such as indoor dust. However, several setbacks ranging from a lack of sensitivity to thermal degradation, as well as long analysis time, have been experienced particularly for larger PBDE (hepta- through decaBDE) congeners. In this study, an analytical method was developed and validated for the simultaneous determination of tri- through to decaBDEs. In addition, the efficiencies of two extraction techniques: ultrasonic assisted extraction (UAE) and Soxhlet extraction (SE), were compared. A gas chromatographic separation effected on Restek RTX 1614® capillary column coupled with low resolution mass spectral detection was successfully applied to determine all PBDE congeners in EPA method 1614 native PAR stock solution. The method was validated with a standard reference material (SRM 2585) and was subsequently used to determine BDE-28, 47, 99, 100, 153, 154, 183, and 209 in dust from 19 personal and previously owned automobiles. The $\sum_{n=8}$ PBDE in samples ranged from 573 to 11833 ng g^{-1}. BDE-209 accounted for approximately 42% of $\sum_{n=8}$ PBDE in the samples. The results show that automobiles provide ample opportunity for human exposure to PBDEs via dust ingestion, particularly for toddlers and occupationally exposed adults.

Keywords*: Automobile dust, Polybrominated diphenyl ethers, Gas chromatography, Mass spectrometry, Extraction techniques, Exposure assessment.*

3.1 Introduction

Polybrominated diphenyl ethers (PBDEs) are omnipresent environmental contaminants that have been largely used as flame retardants in consumer products. PBDEs have been produced commercially in three major formulations: penta (consisting of 38-49% BDE-47 and BDE-99 each alongside other tri- to heptaBDEs, octa (a mixture of hexa- to deca-BDEs, the exact congener composition varied between the two principal formulations marketed), and deca (92-97% BDE-209, in addition to various nona- and octa-BDEs) (1). The main applications of these commercial formulations are as follows: the penta-mix to flame retard carpet paddings, furniture upholstery, printed circuit boards and microprocessor packing in computers; the octa-mix to treat high impact polystyrene (HIPs) and acrylonitrile-butadiene-styrene copolymers (ABS); and the deca–mix in HIPs applied in plastic casings for electrical products like computers and televisions, and also in textiles (1, 2).

Since commercial PBDE mixtures are physically incorporated into polymers and other substrates, they are more easily released into the environment during initial manufacture, incorporation into products, application of the products, repair/recycling of products and product disposal (3). PBDEs have been reported to be present in both biotic and abiotic matrices (4-8). They are of concern due to their "grasshopper effect" and because of their persistent, bioaccumulative and toxic characteristics. The major components in the penta and octa commercial BDE products were listed as persistent organic pollutants (POPs) at the 4th meeting of the Conference of the Parties (COP4) of the Stockholm Convention in 2009 (3, 9). Since indoor dust has been implicated as an important exposure route to PBDEs (10-13), an accurate assessment of PBDEs in the indoor environment is of major importance.

Different analytical strategies have been reported in the literature for the detection of PBDEs in various media (1, 3, 14-17). A LC/NI-APPI/MS/MS method is reported to be suitable for the analysis of the non-volatile BDE-209 due to its selectivity and the unique parent and fragment ions of this congener, however, coelution of isobaric BDE congeners is a peculiar possibility, and hence may likely overestimate the values of the lower BDE congeners. Similarly, the gas chromatographic analysis of larger PBDE congeners (hepta- to decaBDEs) suffers several drawbacks including a lack of sensitivity with gas chromatography-electron impact ionization/mass spectrometry (GC-EI/MS) analysis and a lack of mass discrimination between native and ^{13}C-labelled isomers, hence prevents the use of ^{13}C-labelled isomers as internal standards in gas chromatography-negative chemical ionization/mass spectrometry (GC-NCI/MS) analysis (9). Similarly, both GC-EI/MS and GC-NCI/MS analysis of BDE-209 is fraught by thermal degradation and long analysis time, and thus necessitates the use of shorter columns with higher temperature limits and higher phase ratios to minimize on-column degradation (9). As a result, separate analysis of tri- to heptaBDEs and the larger BDE congeners such as BDE-209 are often reported in the literature. Thus, from the foregoing, the aim of the present study

was to develop and validate a GC-EI/MS method for the simultaneous analysis of tri- through to decaBDEs. The analytical method devised was applied to the analysis of dust from automobile interiors, and to compare the efficiencies of two PBDE extraction techniques. The PBDE content of the dust was used to assess the implication of dust ingestion as a pathway for PBDE ingestion for both occupationally and non-occupationally exposed individuals.

3.2 Materials and methods

3.2.1 Chemicals

Method 1614 PAR PBDE stock solution [1 µg mL^{-1} each of 2,4,4'-tribromodiphenyl ether, BDE 28; 2,2',4,4'-tetrabromodiphenyl ether, BDE 47; 2,2',4,4',5-pentabromodiphenyl ether, BDE 99; 2,2',4,4',6-pentabromodiphenyl ether, BDE 100; 2,2',4,4',5,5'-hexabromodiphenyl ether, BDE-153; 2,2',4,4',5,6'-hexabromodiphenyl ether, BDE 154; 2,2',3,4,4', 5',6-heptabromodiphenyl ether, BDE-183 and 10 µg mL^{-1} 2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether, BDE-209] was received as a kind donation from Cambridge Isotope Laboratories, Andover, MA, USA. $^{13}C_{12}$ labelled decachlorobiphenyl ($^{13}C_{12}$ PCB-209), used as an internal standard, was obtained from Wellington Laboratories, Guelph, Ontario, Canada. Silica gel 90 was from Sigma Aldrich, Switzerland. The silica gel was baked at 130 ºC for 16 hours prior to use. Standard reference material (SRM 2585: Organic Contaminants in house dust) was obtained from the National Institute of Standards and Technology (NIST), Gaithersburg, Maryland, USA. A Restek RTX – 1614® fused silica (5% diphenyl, 95% dimethyl polysiloxane) capillary column was obtained as a generous gift from RESTEK Corporation, Bellefonte, Pennsylvania, USA.

3.2.2 Sampling

Dust samples were collected from 10 personal and 9 previously-owned automobiles available for resale. The previously-owned vehicles were sampled at a dealership in Durban, South Africa. All automobiles from the dealership had been through a thorough cleaning process prior to display for resale. The personal automobiles had not undergone any form of cleaning at least three days before sampling. Dust samples were collected from the entire car interior with a MoTo Quip super wet and dry auto vacuum cleaner. the vacuum cleaner contained a dust unit which could easily be removed and emptied after each collection. Between each sampling, it was cleaned with a disposable cloth wetted with isopropanol. Samples were stored in amber bottles -10 °C until analysis. Details for each automobile, such as manufacturer, model year and interior characteristics, were obtained via questionnaires (see Supplementary Material Table 3.1).

Prior to extraction, non-dust particles were hand-picked from samples, and then the samples were homogenized by sieving through a 212 µm stainless steel sieve.

3.2.3 Extraction

PBDEs were extracted from dust samples by means of ultrasonic-assisted extraction (UAE) and Soxhlet extraction (SE). For UAE, both extraction temperature and time were optimized. Different solvent combinations were tested for the simultaneous extraction of PBDE congeners and the internal standard $^{13}C_{12}$ PCB-209.

The following solvent combinations 1:3 and 3:1 (v/v) n-hexane:toluene mixture, 1:3 and 3:1 (v/v) n-hexane:methanol mixture, 1:3 hexane-acetone and 1:3 hexane:diethyl ether mixture, were tested. Two ultrasonication temperatures: 25 °C and 40 °C were optimized for the analytes. Similarly, extraction times of 10, 15, 30, 45 and 60 min were evaluated for the recovery of the analytes (Fig 3.1).

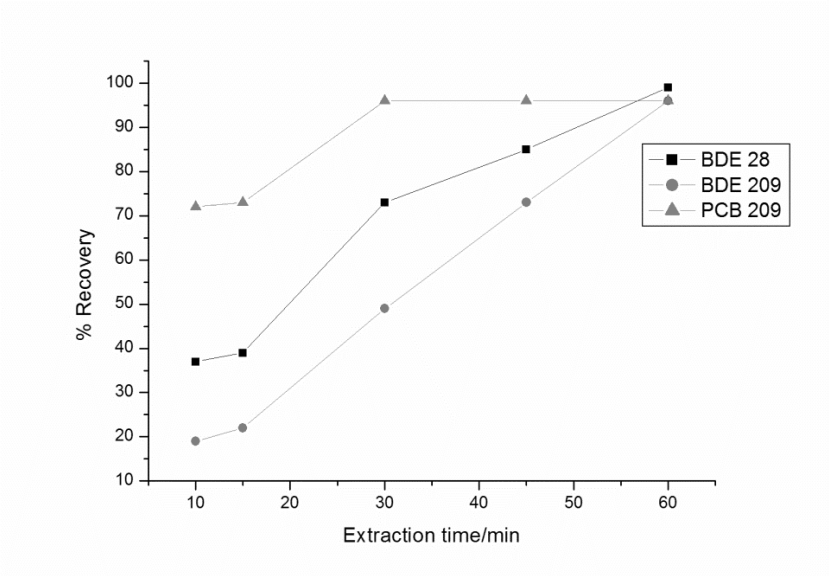

Figure 3.1 Effects of extraction time on the recovery of BDE 28, 209 and PCB 209 in 1:3 n-hexane: methanol.

A 1:3 n-hexane:methanol mixture was found effective at an ultrasonication temperature of 40 °C and 60 min extraction time.

For UAE, approximately 0.12 – 1.0 g dust was placed in a 20 mL glass test tube and spiked with 50 ng $^{13}C_{12}$ PCB-209 internal standard. Samples were extracted with 10 mL of 1:3 (v/v) n-hexane:methanol in an ultrasonic bath at 40 °C for 30 minutes. Before ultrasonication, samples were shaken in an orbital shaker for 10 minutes. Two extraction cycles were carried out without the addition of fresh solvent. Solvent was separated from solute by centrifugation at 3500 rpm for 10 minutes. Supernants were stored at -4 ℃ until fractionation and clean-up.

For Soxhlet extraction, 1.0 g dust was soxhlet extracted with 100 mL 1:3 (v/v) n - hexane: methanol at 70 °C for 8 hours. Extracts were reduced to approximately 10 mL prior to clean-up. No statistical significant difference ($p = 0.793$) was obtained for the result of the UAE and the SE. However, the UAE method was adopted throughout the study, since one of the objectives of the study was to develop a simple and quick method for the simultaneous determination of tri-through to decaBDE.

3.2.4 Clean-up

Silica gel 90 was activated for 16 hours at 130 °C prior to use. Anhydrous sodium sulfate was baked at 450 °C for 5 hours before use. Silica gel and anhydrous sodium sulphate were cooled in a dessicator. Different mass ratios of silica gel to sodium sulphate were tested during method development. A mass of 3 g silica gel to 0.8 g sodium sulfate was found sufficient for clean-up of extracts from UAE. A mass of 8 g of silica gel topped with 3 g sodium sulfate was found sufficient for SE extracts. A 30 cm × 1 cm glass column was packed with the appropriate amount of adsorbent stated above and topped with the corresponding quantity of anhydrous sodium sulfate. The columns were solvent wet with 30 mL of the extraction solvents. PBDEs were eluted from the silica columns with 25 mL n-hexane, which was kept as fraction one and contained essentially BDE 209. Columns were further eluted with 30 mL 50/50 (v/v) diethyl ether/n- hexane, and kept as fraction 2. The column flow rate were maintained at 0.5 mL min^{-1}. All fractions were reduced to approximately 250 µL in a rotavap evaporator set at 55 °C. Extracts were stored in 1.5 mL amber GC-MS vials and then subjected to chromatographic analysis.

3.2.5 Chromatographic Analysis

Two chromatographic columns, namely, a Restek 5MS 30.0 m × 250 µm × 0.25 µm and a Restek RTX 1614 15 m × 250 µm × 0.1 µm were evaluated for the simultaneous analysis of PBDEs.PBDE analysis was performed on an Agilent 6890 (Palo Alto, CA, USA) gas chromatograph (GC), coupled to a 5973N series mass spectrometer (MS) operated in electron impact ionization (EI) mode. A Restek RTX-1614 capillary column (15 m × 250 µm × 0.1 µm) was used to effect separation and the MS was used in the selected ion monitoring (SIM) mode. The GC oven temperature programme was 90 °C held for 2 mins, then increased at 20 ºC min^{-1} to 270 ºC, followed by an increase of 10 °C min^{-1} to 325 ºC and held there for 5 min. Helium was employed as carrier gas at a flow rate of 1.2 mL min^{-1} and a constant linear velocity of 58 cm s^{-1}. The ion source and transfer line temperatures were 230 ºC and 350 ºC, respectively. The ionization energy was 70 eV. A volume of 1 µL of sample was injected in the pulsed splitless mode with an injector temperature of 285 ºC. The molecular ions $[M]^+$ or $[M+2],^+$ and the fragment ions $[M\text{-}Br_2]^+$ or $[M\text{-}Br_2+2]^+$, were monitored for tri- through to hepta-BDEs. M/Z 400 and 800 were monitored for BDE-209. The monitored M/Z for each PBDE congener was obtained by full scan mass spectrometric analysis of the pure PAR EPA method 1614 PBDE standard mixture (see Supplementary Material Figure 3.1B – I for

the total ion chromatogram of the each PBDE congener). Data were acquired with ChemStation software (Agilent Technologies, Santa Clara, CA, USA).

To evaluate the Restek 5MS column, the same GC and mass spectrometer were used as above. The GC was operated in a pulsed splitless mode with helium as carrier gas. The injection temperature was 285 °C. The oven temperature programme was an initial temperature of 70 °C held for 1 min and then increased at 20 °C min^{-1} to 295 °C, and held at 295 °C for 20 minutes. The MSD and interphase temperatures were 350 °C and 280 °C respectively. The GC column pressure was set at 36.6 kPa and the total column flow was 43.6 mL min^{-1}. The GC-MS was operated in the selected ion monitoring (SIM) mode. The molecular ions $[M]^+$ and $[M - Br_2]+$ were monitored for all BDE congeners as they were the most abundant.

Quantitation was carried out by means of a multiple point internal standard method. The $^{13}C_{12}$ labelled PCB-209 was employed as the internal standard for all PBDE congeners on the GC-MS system. The response factors were determined from the slope of a plot of the ratio of peak areas against the ratio of the concentrations. The values for the plots were obtained from a 4 – 6 point triplicate analysis of the PBDE standard solution diluted to fall within a concentration range of 5 – 500 ng mL^{-1} and 0.1 to 4 μg mL^{-1} for tri- to octa-BDEs and BDE-209, respectively.

3.2.6 Quality Control/Quality Assurance

Analysis of solvent, method and field blank samples were carried out simultaneously with all sample batches. Simulated laboratory dusts were used for field blanks to check the effects of the sampling technique on analytes in the samples. In this case, anhydrous sodium sulfate was spread on a precleaned bare floor with no electric or other possible sources of PBDEs. The samples (n = 3) were sampled by employing the sampling protocol for real dust samples. Samples were then passed through all the analytical procedure as for real samples. Analytical method blanks (n = 10) were prepared by taking the same mass of anhydrous sodium sulfate as the mass of real samples and passed through extraction, clean-up and chromatographic analysis steps to check the influence of solvents and daily laboratory practices on analytes. The complete method was validated with a standard reference material (SRM 2585: Organic Contaminants in House Dusts) that was extracted by both extraction techniques.

3.2.7 Statistics

The distribution fitting of the concentrations of PBDE congeners was tested with the Kolmogorov-Smirnov test of normality by using XLSTAT 2014 software (Addinsoft, New York, NY, USA). The difference in results from UAE and SE was tested with the Mann-Whitney non-parametric test by using XLSTAT 2014. Descriptive statistics such as sum, mean, median, minimum, maximum, t-test and analysis of variance (ANOVA) were calculated using Microsoft Excel 2010 and XLSTAT 2014. The confidence intervals were calculated as described by Bunhu (30). Limits of detection (LOD) and

quantitation (LOQ) were estimated following Thomsen et al.(31). Samples below the detection limit (< dl) were treated as zero throughout the statistical analysis.

3.3 Results and Discussion

Simultaneous determination of all eight PBDE congeners was achieved on the Restek RTX-1614 column. Excellent peak resolution was obtained for all PBDE congeners especially for BDE-99 and BDE-209 (see Fig. 3.2). Good accuracy and precision was obtained for all BDE congeners.

3.3.1 Chromatographic Analysis

The efficiency of the two analytical columns employed in this study was tested for the simultaneous determination of tri- to deca-BDE congeners. Scanty reports exist in the literature on the simultaneous determination of BDE 28 through to BDE 209 in a single gas chromatographic column. The response of both a Restek 5MS (30.0 m × 250 μm × 0.25 μm) and a Restek RTX 1614 (15 m × 250 μm × 0.1 μm) capillary column for tri- to deca-BDEs was observed. On the Restek 5MS capillary column, the LOD and LOQ for BDE-28 were 0.10 ng g^{-1} and 0.25 ng g^{-1} and for BDE-153 they were 0.33 ng g^{-1}, 0.81 ng g^{-1} respectively. Furthermore, the sensitivity of BDE 183 was poor and the response not reproducible, while BDE 209 was unresolved on this column. However, all studied BDE congeners in this study, where sufficiently resolved (see Fig 3.2) and quantified on the low-bleed Restek RTX 1614 capillary column with excellent LOD and LOQ for BDE-209, and likewise for the other BDE congeners (see Table 3.1).

A complete separation of BDE-28, 47, 99, 100, 153, 154, 183 and 209 was achieved under 18 minutes with outstanding peak symmetries, including for BDE-99, without peak tailing, which was a major challenge on the Restek 5MS column. Overall, the high response and excellent symmetry for all the studied BDE congeners particularly BDE-209 and BDE-99 on the Restek RTX-1614 column enhanced the accuracy of the determination of the analytes in samples, and the overwhelming short retention time, permitted the analysis of more samples per hour. Thus, the results reported for samples were those resolved on the RTX 1614 capillary column.

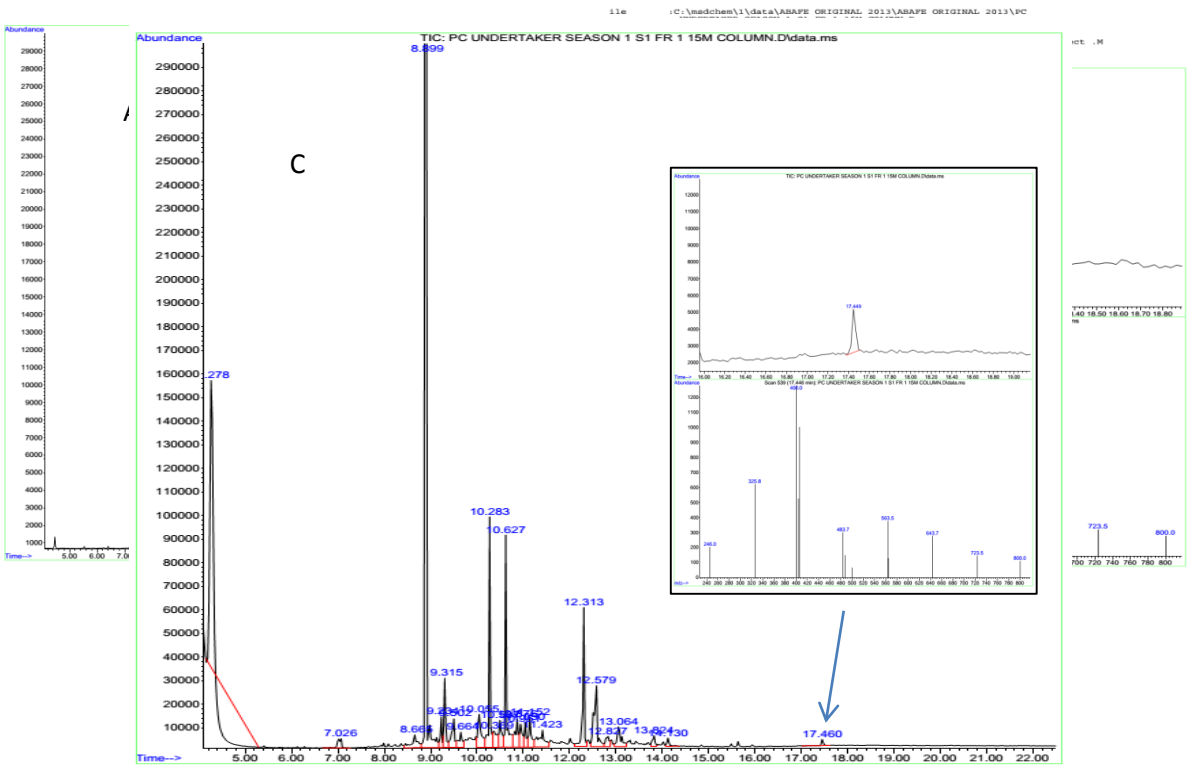

Figure 3.1 GC chromatogram and total ion chromatogram obtained on the Restek RTX-1614 capillary column for (A) PAR Mix PBDE standard solution and the internal standard, (B) expanded BDE-209 chromatogram and TIC elucidated in the mix standard solution, and (C) chromatogram of a typical dust sample (insert is elucidated BDE-209 peak in dust sample).

3.3.2 Validation Parameters for the Analytical Determination

The linearity of the analytical method was studied with calibration solutions. Excellent linearity $R^2 \geq 0.99$ was obtained for all analytes. All analytes were detected at levels of < 3 % of the values present in the samples in all method and field blank samples, and consequently results presented here were not blank corrected. The LOD and LOQ values ranged from 0.05 – 0.16 ng g^{-1} and 0.18 – 0.54 ng g^{-1}, respectively (Table 3.1).

The accuracy of the method was determined from the agreement of the measured values with the certified values of SRM 2585. On average, the difference between the certified values and the measured values of the eight PBDE congeners was 5.3% and 4.7% for the results measured with UAE and SE, respectively. This was determined as $[(\text{Measured - Certified}/\text{Certified}) \times 100]$. The method showed good accuracy with recovery of PBDE congeners ranging between 96 – 112% and 95 – 108% for UAE and SE respectively. The excellent low values of the relative standard deviations (% RSD) obtained for the PBDE congeners on both UAE and SE [on average 0.2 % for UAE and SE extracted PBDEs (Table 3.2)] indicates the good precision of the method.

Table 3.1 Limits of detection (LOD) and quantitation (LOQ) for PBDE congeners and the slope and correlation coefficients for the calibration lines

Congener	LOD/ ng g^{-1}	LOQ/ ng g^{-1}	Slope	R^2
BDE 28	0.05	0.18	0.6386	0.998
BDE 47	0.14	0.45	0.6833	0.991
BDE 99	0.15	0.49	0.7477	0.998
BDE 100	0.14	0.46	0.7677	0.990
BDE 153	0.09	0.31	0.3552	0.993
BDE 154	0.16	0.53	0.7677	0.990
BDE 183	0.11	0.36	0.3044	0.993
BDE 209	0.16	0.54	0.0294	0.990

Table 3.2 Analysis of SRM 2585 (Organic Contaminants in House Dust) for QC/QA

PBDE congener	Ultrasonic Assisted Extraction (n = 4)	Soxhlet Extraction (n = 3)		Ultrasonic/ Soxhlet Extraction	Ultrasonic/ Soxhlet Extraction	Ultrasonic / Soxhlet Extraction
	Measured concentrations/μg kg^{-1}		Certified values for PBDE congeners in SRM 2585/μg kg^{-1}	Percent recovery/%	Accuracy/%	Precision/%
BDE 28	48.1 ± 0.1	45.8 ± 0.1	46.9 ± 4.4	103 / 98	-2.6 / 2.3	0.2 / 0.2
BDE 47	480 ± 2.4	471 ± 2.3	497 ± 46	97/ 95	3.4 / 5.3	0.5 / 0.5
BDE 99	854 ±1.0	878 ± 1.4	892 ± 53	96 / 98	4.3 / 1.6	0.1 / 0.2
BDE 100	152 ± 0.2	130 ± 0.3	145 ± 11	105 / 90	-5.0 / 10.5	0.1 / 0.2
BDE 153	121.2 ± 0.1	118 ± 0.1	119 ± 1	102 / 100	-1.9 / 0.4	0.1 / 0.1
BDE 154	92.7 ± 0.2	90.2 ± 0.1	83.5 ± 2	111 / 108	-11.1 / -8.0	0.2 / 0.1
BDE 183	44.0 ± 0.04	41.5 ± 0.1	43.0 ± 3.5	102 / 96	-2.3 / 3.6	0.1 / 0.2
BDE 209	2806 ± 0.6	2650 ± 0.1	2510 ± 190	112 /106	-11.8 / -5.6	0.02 /0.004

3.3.3 PBDE Concentrations in Automobile Dust

PBDEs were detected in all 19 vehicles sampled. The presence of PBDEs in dust from these automobiles was expected since automobile interiors contain high levels of acrylonitrile butadiene styrene and polypropylene moulded parts in the instrument panels, and textiles and polyurethane foams in their interior upholstery as well as vehicle electronics (18). The levels of the individual PBDE congeners in each of the automobiles are presented in Supplementary Material Table 3.1. The sum of the concentrations of the eight PBDE congeners ($\sum_{n=8}$ PBDEs) ranged between 573 – 11833 ng g^{-1} (mean of 3319 and a median of 2769 ng g^{-1}). The descriptive statistics for PBDEs in these automobile is presented in Table 3.3. BDE 47, 99 and 209 were detected in all samples with BDE-209 being the most abundant. BDE-209 accounted for 42 % of all PBDEs in samples. The distribution pattern of PBDEs in this study was BDE-28 < BDE-154 < BDE-100 < BDE-47 < BDE-153 < BDE-183 < BDE-99 < BDE- 209. The levels of PBDEs in this study were generally low compared with levels as high as 33728 µg kg^{-1} for $\sum_{n=16}$ PBDEs reported in the Czech Republic (18) and higher levels reported in the USA (19, 20) and the UK (21). However, our results were similar to those reported in vehicle dust from Kuwait and Pakistan (22) and Portugal (23) (see Table 3.4). The observed difference can be attributed to sampling design, number of samples analysed and different car characteristics, such as ventilation system, age and manufacturer, etc. The similarity in sampling design, which involved vacuum cleaning of the entire vehicle interior contrary to the restricted sampling of seat surfaces, vehicle cabin and trunks reported by Legalante et al. and Harrad and Abdallah (19, 21), could possibly explain the resemblance of our results to those of Cunha et al. (23).

Table 3.3 Descriptive statistics for PBDE concentrations (ng g^{-1}) in automobile dust samples

	BDE 28	BDE 47	BDE 99	BDE 100	BDE 153	BDE 154	BDE 183	BDE 209	∑PBDEs
Mean	49	269	502	185	375	168	392	1379	3319
SD	73	248	521	271	501	250	489	1500	2813
Median	32	188	325	134	255	76	247	845	2769
Min	< DL	37	63	< DL	< DL	< DL	< DL	128	573
Max	325	1127	2046	1218	2101	1017	2269	5173	11833
5th percentile	< DL	47	120	< DL	< DL	< DL	< DL	154	725
95th percentile	168	645	1724	526	1070	551	922	4741	8106

<DL is below the detection limit

Table 3.4 Reported concentrations (in ng g^{-1}) of PBDEs in automobiles from selected studies

City	Number of PBDE congeners	Number of samples	Median	Mean	Range	Reference
South Africa	8	19	2769	3319	573 – 11833	This study
USA	21	66			<0.1 – 322000	(20)
USA					5 – 3570000	(19)
UK		20	57000	340000	140 – 2600000	(24)
Portugal	16	9			193 – 22955	(23)
Pakistan		15	650	30150	30 – 260800	(22)
Kuwait		15	700	11950	165 – 137000	(22)
Kuwait	14	19	531	2065	68 – 17200	(25)

The concentrations of penta-, hexa- and BDE-183 (Fig. 3.3) in this study were particularly high considering the percentage contribution of each of these congeners in commercial formulations. For example, commercial penta-BDE contains 52-60 % BDE-99 and BDE-100 and 24-38 % BDE-47; while the commercial octa-BDE formulation contains 62 % BDE-153 and 154 and 34 % congeners containing eight bromine atoms (23). One possible explanation is that these congeners do not arise as a result of their original presence in the commercial formulation but as a result of the enhanced photodegradation of BDE-209 (18, 23), as a consequence of the intense solar irradiances received in Durban, South Africa. The major degradation product of BDE-209 is BDE-183 (19). Support for this deduction is obtained from the strong positive correlations (r = 0.77, 0.57, 0.78 and 0.56) of the BDE-209 concentrations with those of BDE-183, BDE-99, BDE-47 and BDE-100, respectively.

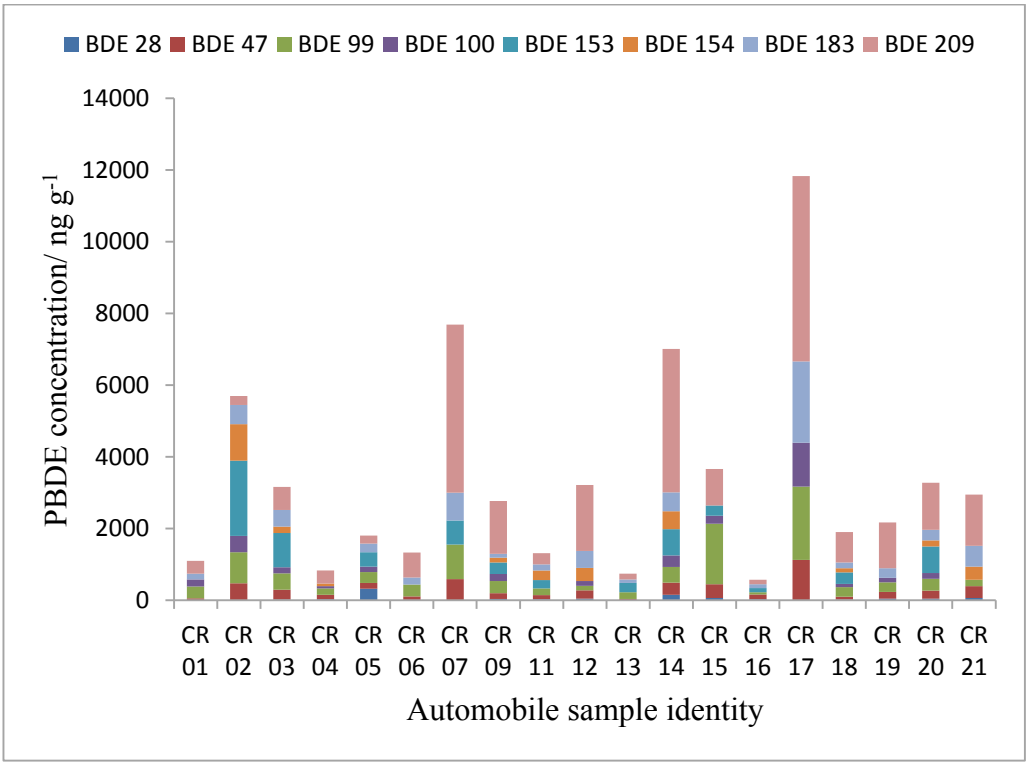

Figure 3.2 Distribution of PBDE congeners in automobile dust samples.

Automobiles from different manufacturers were sampled in this study. The correlations among concentrations of PBDEs in cars from the same manufacturer were studied in other to further investigate within-manufacturer relationships of PBDE concentrations. Strong positive correlations r = 0.63, 0.76 and 0.98 were obtained among automobiles manufactured by Honda (n = 2), Audi (n = 3) and Toyota (n = 4), respectively. From this observation similar sources of PBDEs in automobiles from each of the manufacturers can be assumed.

Boxplot of $\sum_{n=8}$ PBDEs levels grouped by vehicle manufacturer and boxplot of BDE-209 by vehicle model year are presented in Fig. 3.4 and Fig. 3.3 respectively. The data indicate close similarity among BDE congeners in automobiles from the same manufacturer; however, slight variations of about one to two orders of magnitude were observed for BDE-153 and BDE-209. This could be related to factors other than vehicle information obtained that are responsible for the difference in BDE-153 and BDE-209 levels (19). This is in line with a report that shows that BDE congeners in vehicle interior air are highly variable among automobile manufacturers, makes and replicate models of vehicles (26). For vehicle model, no relationship was observed between BDE-209 concentrations and the year of vehicle manufacture. However, a substantial decrease in BDE-209 concentrations in vehicles manufatured after the year 2009 was observed. PCA loadings and biplots (Fig 3.5) show three groupings of the distribution of BDE-209

concentrations with respect to vehicle manufacture year. The close distribution of BDE-209 levels were 1989 – 1998; 1999 – 2008 and 2010 – 2012 in these vehicles. However, this observation is limited by the number of vehicles of the same model as well as differences in vehicle interior temperature, use-pattern and ventilation system, which can have a major influence on the fate of BDE-209 in the indoor environment (27).

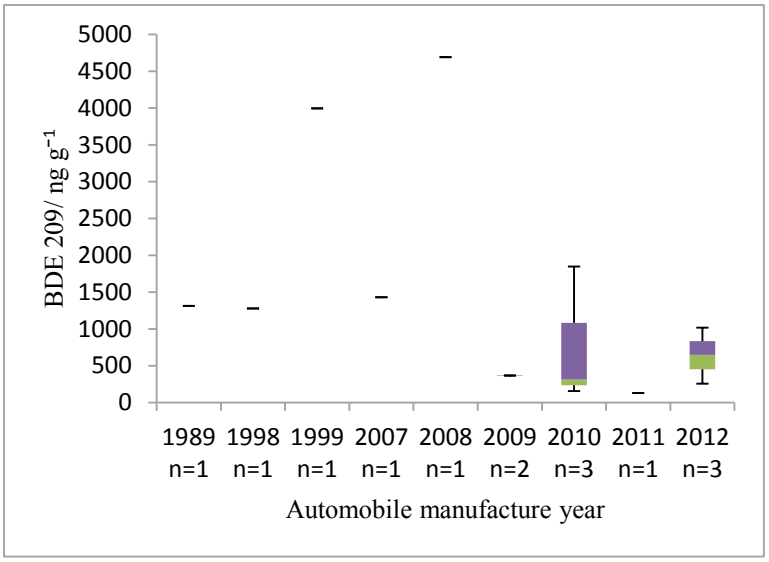

Figure 3.3 Boxplot of BDE-209 concentrations (in ng g^{-1}) measured in automobile dust grouped by vehicle model year. The lines of the box represent the interquartile ranges (25^{th}, 50^{th}, and 75^{th} percentiles) and the bars represent the minimum and maximum.

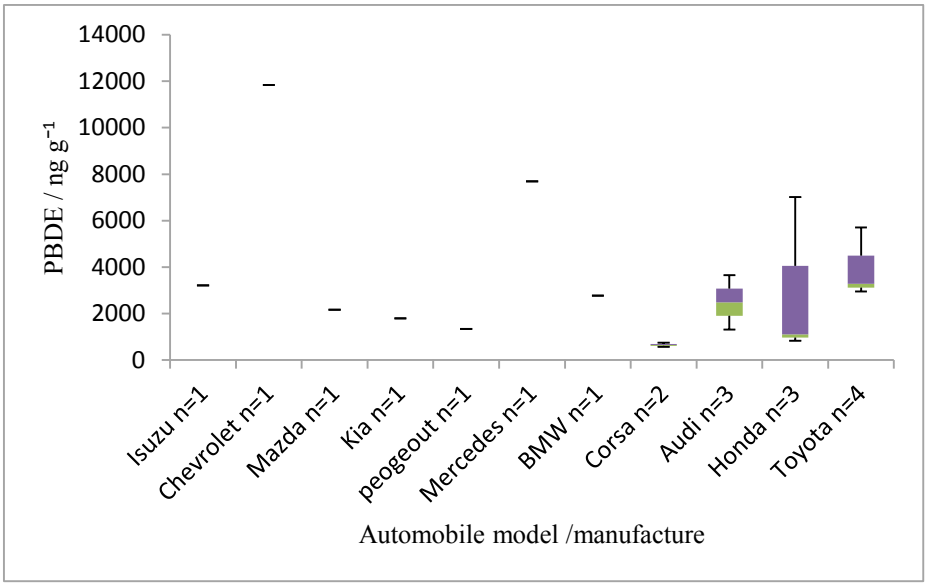

Figure 3. 4 Boxplot of $\sum_{n=8}$ PBDE concentrations (in ng g^{-1}) measured in the automobile dust grouped by vehicle manufacturer. The lines of the box represent the

interquartile ranges (25th, 50th, and 75th percentiles) and the bars represent the minimum and maximum.

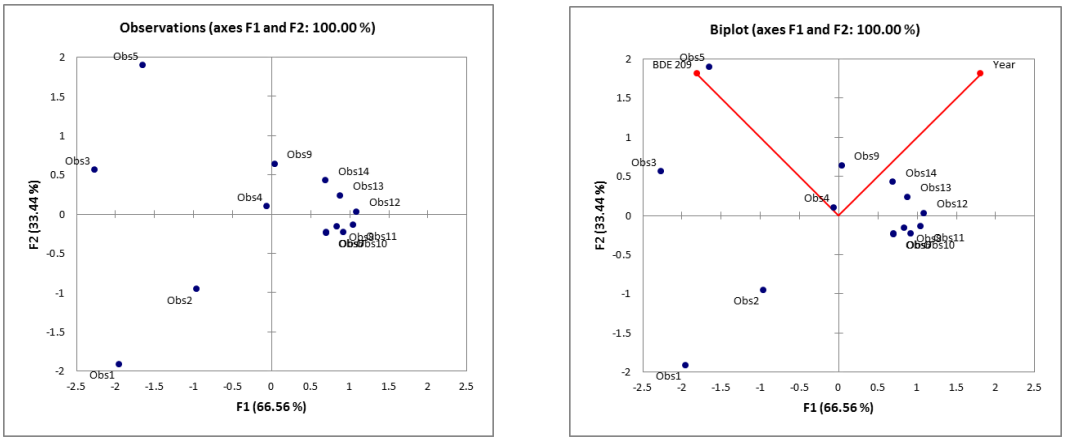

Figure 3.5 PCA loading and biplot for BDE-209 concentrations and automobile year (see Supplementary Material for PCA data).

3.3.4 Consequences for Human Exposure via Dust Ingestion

Exposure assessment owing to ingestion of PBDEs in automobiles dust rely on two approximated values: the time spent in vehicles and the dust intake rates (19). In this work, the dust exposure factors reported by Ali et al. (2013) (22) were employed. Briefly, 100 % PBDE absorption from ingested dust was assumed. Average intake rates of 20 and 50 mg d^{-1} and high dust intake rates of 50 and 200 mg d^{-1} for adults and toddlers respectively were used (see Table 3.5). Similarly, a value of 27.9 % for the time spent by taxi drivers (occupationally exposed adults) was used. Detailed questionnaires were used (Supplementary Material 3.1) to obtain the average time spent by adults and toddlers in personal cars. On average, South Africans spend 4.2 % of their daily time in cars.

Table 3.5 shows the daily exposure estimates for South African adults and toddlers to PBDEs via vehicle occupancy with different exposure scenarios. The estimated daily intakes of $\sum_{n=8}$ PBDEs in this study were several orders of magnitude lower than the corresponding EPA reference values (RfD) of 100, 100, 200 and 7000 ng kg^{-1} bw day^{-1} for BDE-47, 99, 153 and 209 respectively; and 2000 and 3000 ng kg^{-1} bw day^{-1} for penta and octa-BDE commercial mixtures respectively (18). However, the worst-case exposure scenario (i.e. 95th percentile concentration) for toddlers and taxi drivers should be a concern (Fig 3.6), considering the cumulative daily exposure that might arise from other microenvironments such as homes and schools, as well as other exposure pathways. Our results are in agreement with in-vehicle exposure to BDE-99 for adults and toddlers reported by Harrad and Abdallah (21); as well as the levels reported for both non- and occupationally-exposed adults and toddlers in Kuwait and Pakistan (22). However, our

exposure estimates were about two orders of magnitude higher than $\sum_{n=10}$PBDEs reported in the Czech Republic (18).

Table 3.5 Estimated daily exposure doses of PBDEs (in ng kg^{-1} bw day^{-1}) via dust ingestion from automobiles for three population groups. A value of 100% dust absorption was assumed and two dust intake rates were used (12, 24, 28).

Intake rate	Toddlers				Non-occupationally exposed adults				Occupationally exposed adults			
	5th percentile	Median	Mean	95th percentile	5th percentile	Median	Mean	95th percentile	5th percentile	Median	Mean	95th percentile
Average dust intake[a]	0.02	0.08	0.1	0.23	0.01	0.03	0.04	0.1	0.06	0.22	0.26	0.65
High dust intake[b]	0.48	1.85	2.27	5.4	0.02	0.08	0.1	0.23	0.14	0.55	0.66	1.62

[a]Assuming average dust intake rate of 20 mg d^{-1} and 50 mg d^{-1} for adults and toddlers, respectively.
[b]Assuming high dust intake rate of 50 mg d^{-1} and 200 mg d^{-1} for adults and toddlers, respectively.

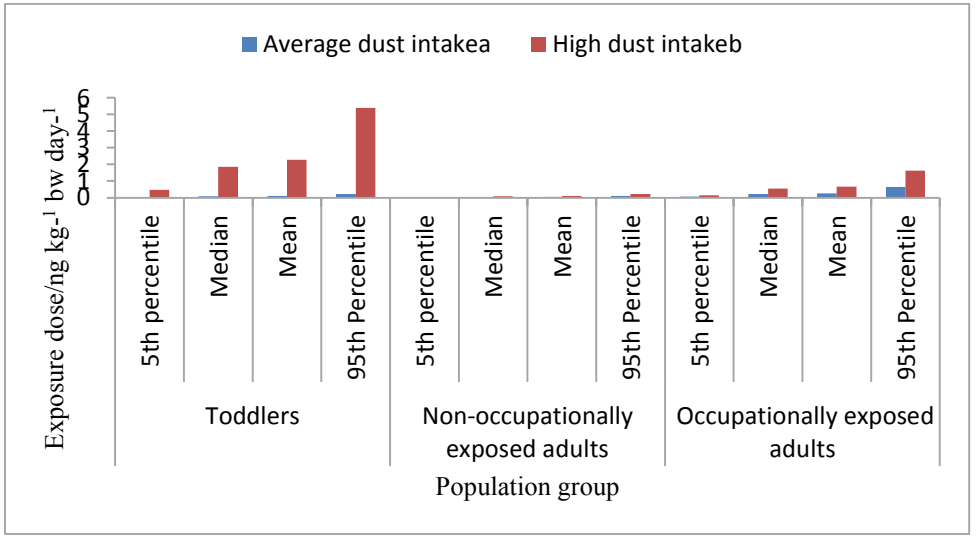

Figure 3.6 PBDE exposure estimates for toddlers, non-occupationally exposed and occupationally exposed adults via dust ingestion in automobiles in South Africa.

The total daily intake and related health risk is difficult to determine accurately, since potential additive effects of PBDEs are not well documented (18). Similarly, there are multiple human exposure pathways to PBDEs and much of the information required (e.g. levels of PBDEs in indoor vehicle air and data on typical South African dietary exposure to PBDEs) is not available. The contribution of individual pathways of exposure increased in the order: dust ingestion > food ingestion > indoor air inhalation for all population groups except for breastfed infants, for whom diet is the major source of PBDE exposure (18). In this work dermal absorption of PBDEs from automobile dust was considered as an insignificant exposure pathway akin to its classification as a minor exposure route in other indoor microenvironments (29).

3.4 Conclusions

The analytical method devised showed that all eight BDE congeners could be qualitatively and quantitatively determined with a Restek RTX-1614 capillary gas chromatographic column and a low resolution mass spectrometer. Separation of all congeners was achieved in less than 18 min for tri through to deca-BDEs in automobile dust samples. The levels of ∑PBDEs measured were low and similar to amounts measured in Kuwait, Pakistan and Portugal. To the best of our knowledge this is the first time that levels of PBDEs in automobiles from the African continent are reported. Furthermore, dust from automobiles is implicated as a source of human exposure to PBDEs through inadvertent dust ingestion. More studies are required to further understand the fate of PBDEs in automobiles.

Acknowledgements

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Supplementary Materials

3.1 Total ion chromatogram for PBDE congeners

```
File       :C:\msdchem\1\data\ABAFE ORIGINAL 2013\500PPB MIX BDE IS 50NG
...          CB 209 RUN 3 5UL.D
Operator   : Abafe
Instrument :   5973N
Acquired   : 12 May 2013  16:39     using AcqMethod ABAFE  BDE 2013 manaual inject .M
Sample Name: 500ppb mix bde IS 50NG CB 209 RUN 3 5UL
Misc Info  :
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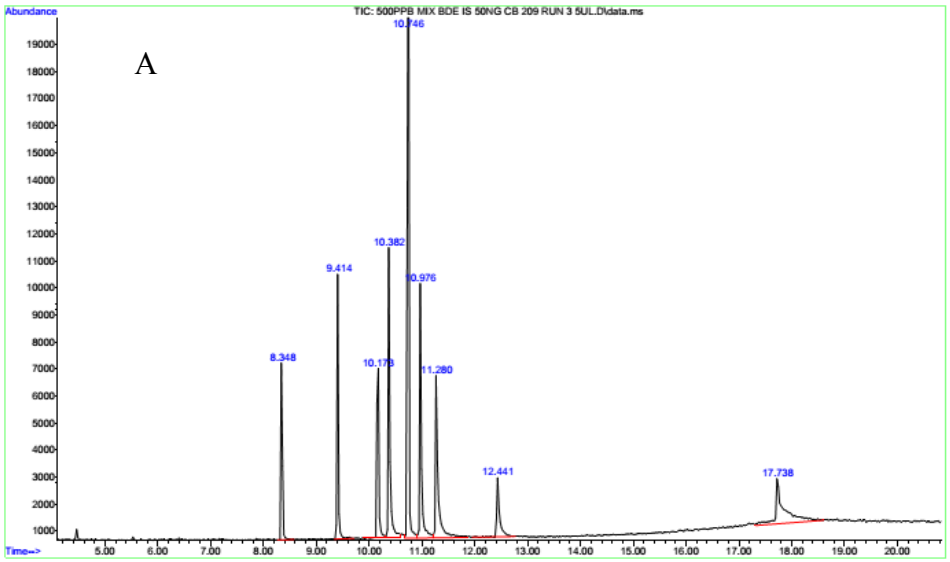

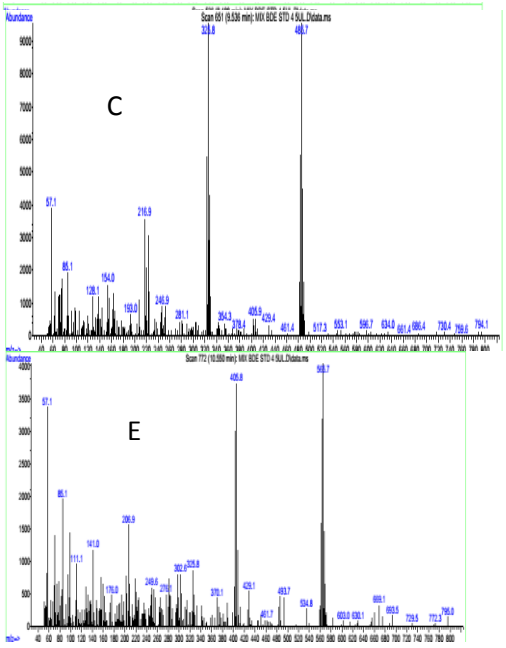

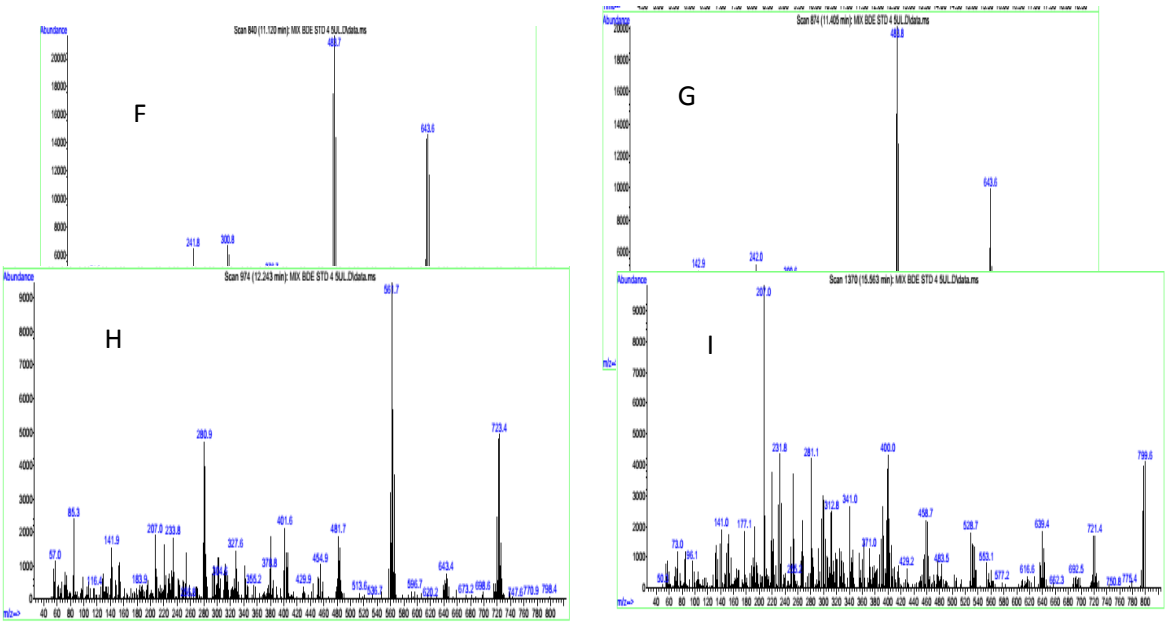

Figure S3.1: GC-EI/MS chromatogram (A) and Full Scan Mass Spectra (B-I) of the eight PBDE congeners studied.

3.2. Information on automobiles sampled.

Tables S3.1 Information on automobiles sampled and levels of PBDEs (in ng g^{-1}) measured in dust from their interiors.

Sample ID	Model and Manufacturer	Model year	Automobile condition	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-209
CR 01	Honda Jazz	2009	Personal	8	48	328	197	<DL	<DL	158	366
CR 02	Toyota Corolla	2012	Personal	<DL	475	870	449	2101	1017	533	256
CR 03	Audi A4	2012	Personal	23	275	452	173	956	181	457	648
CR 04	Honda Jazz	2009	Personal	32	117	182	61	<DL	76	<DL	363
CR 05	Kia Rio	2011	Personal	325	160	305	152	396	<DL	247	217
CR 06	Peugeot 406	2010	Resale	15	98	325	<DL	<DL	<DL	200	697
CR 07	Mercedes C180	2008	Resale	<DL	592	959	<DL	<DL	648	773	4693
CR 09	BMW X5	2011	Resale	38	157	346	198	319	130	114	1469
CR 11	Audi A6	2010	Resale	26	122	187	<DL	228	266	173	312
CR 12	Isuzu KB 250	2010	Resale	50	231	126	134	<DL	361	471	1847
CR 13	Opel Turbo Corsa	2010	Resale	<DL	37	191	<DL	255	<DL	103	157
CR 14	Honda Prelude	1999	Resale	150	342	434	328	726	499	535	3995
CR 15	Audi Q7	2012	Resale	63	381	1688	230	280	<DL	<DL	1018

CR 16	Opel Corsa Utility	2011	Resale	33	127	63	<DL	115	<DL	107	128
CR 17	Chevrolet Aveo	2008	Personal	BDL	1127	2046	1218	<DL	<DL	1914	5173
CR 18	Toyota Starlet		Personal	19	84	263	88	323	117	164	845
CR 19	Mazda 63	1998	Personal	42	188	267	128	<DL	<DL	268	1277
CR 20	Toyota Corolla	1989	Personal	47	219	337	158	742	171	296	1311
CR 21	Toyota Corolla	2007	Personal	66	330	173	<DL	<DL	372	579	1428

3.3 Questionnaires for car dust samples

Questionnaire : Car Dust Samples

Sample code:

Date:

Model and car manufacturer:

Year of manufacture:

No. of seats:

Car ventilation: ☐ **Natural** ☐ **Air conditioned**

Type of seat cover: ☐ **Fabric** ☐ **Leather**

Electronics inside the car (please tick box if appropriate):

Stereo ☐

Speakers ☐ **No.:**

GPRS ☐ **No.:**

DVD Player (Built-in) ☐ **No.:**

Radio ☐ **No:**

Other electronic devices either built-in or used regularly (i.e., portable DVD player)
(please specify):

Approximate time since vehicle last vacuumed:

Manufacturer, model number and date of manufacture (if known) of child seat (s). (If more than one, please give details of each):

Approximate time (hours per week) spent in the car by:

(a) Adults

(b) Children

S3.4 PCA for BDE 209 and automobile manufacture year

XLSTAT 2014.5.03 - Principal Component Analysis (PCA) - on 11/19/2014 at 3:44:40 PM

PCA Data	
Year	BDE 209
1989	1311.24
1998	1276.59
1999	3994.8
2007	1428.09
2008	4693.37
2009	365.7
2009	362.94
2010	312.43
2010	1846.56
2010	156.71
2011	128.11
2012	255.46
2012	648.1
2012	1017.62

Observations/variables table: Workbook = Book2 / Sheet = Sheet1 / Range = Sheet1!$A$1: $B$15 / 14 rows and 2 columns
PCA type: Pearson (n)
Type of biplot: Distance biplot / Coefficient = Automatic

Summary statistics:

	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
Year	14	0	14	1989	2012	2006.857	6.769
BDE 209	14	0	Variable	128.110	4693.370	1271.266	1416.487

Contribution of the observations (%):

	F1	F2
Obs1	20.537	39.170
Obs2	4.975	9.789
Obs3	27.469	3.340
Obs4	0.023	0.100
Obs5	14.588	38.423
Obs6	2.640	0.599
Obs7	2.650	0.606
Obs8	3.763	0.260
Obs9	0.010	4.357
Obs10	4.522	0.598
Obs11	5.818	0.219
Obs12	6.301	0.010
Obs13	4.158	0.588
Obs14	2.546	1.939

Squared cosines of the observations:

	F1	F2
Obs1	**0.511**	0.489
Obs2	**0.503**	0.497
Obs3	**0.942**	0.058
Obs4	0.316	**0.684**
Obs5	0.430	**0.570**
Obs6	**0.898**	0.102
Obs7	**0.897**	0.103
Obs8	**0.966**	0.034
Obs9	0.004	**0.996**
Obs10	**0.938**	0.062
Obs11	**0.981**	0.019
Obs12	**0.999**	0.001
Obs13	**0.934**	0.066
Obs14	**0.723**	0.277

Values in bold correspond for each observation to the factor for which the squared cosine is the largest

Contribution of the observations (%):

	F1	F2
Obs1	20.537	39.170
Obs2	4.975	9.789
Obs3	27.469	3.340
Obs4	0.023	0.100
Obs5	14.588	38.423
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Obs11	5.818	0.219
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Obs14	2.546	1.939

Squared cosines of the observations:

	F1	F2
Obs1	**0.511**	0.489
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Obs3	**0.942**	0.058
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Obs8	**0.966**	0.034
Obs9	0.004	**0.996**
Obs10	**0.938**	0.062
Obs11	**0.981**	0.019
Obs12	**0.999**	0.001
Obs13	**0.934**	0.066
Obs14	**0.723**	0.277

Values in bold correspond for each observation to the factor for which the squared cosine is the largest

S3.5 Distribution fitting for all PBDE congeners

XLSTAT 2014.5.03 - Distribution fitting - on 11/23/2014 at 5:21:14 AM
Data: Workbook = Statistical Analysis for flame retardants in automobiles.xlsx / Sheet = Sheet1 / Range = Sheet1!$B$1:$I$20 / 19 rows and 8 columns
Significance level (%): 5
Distribution: Normal
Estimation method: Moments

Summary statistics:

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
BDE 28	19	0	19	0.000	325.070	49.328	75.383
BDE 47	19	0	19	36.610	1126.830	268.843	254.903
BDE 99	19	0	19	62.750	2046.480	502.287	534.808
BDE 100	19	0	19	0.000	1218.400	185.016	278.722
BDE 153	19	0	19	0.000	2101.080	374.512	514.663
BDE 154	19	0	19	0.000	1017.250	167.913	256.721
BDE 183	19	0	19	0.000	2268.900	391.877	502.218
BDE 209	19	0	19	128.110	5172.760	1378.857	1541.066

Distribution fitting (BDE 28):

Estimated parameters (BDE 28):

Parameter	Value
μ	49.328
sigma	75.383

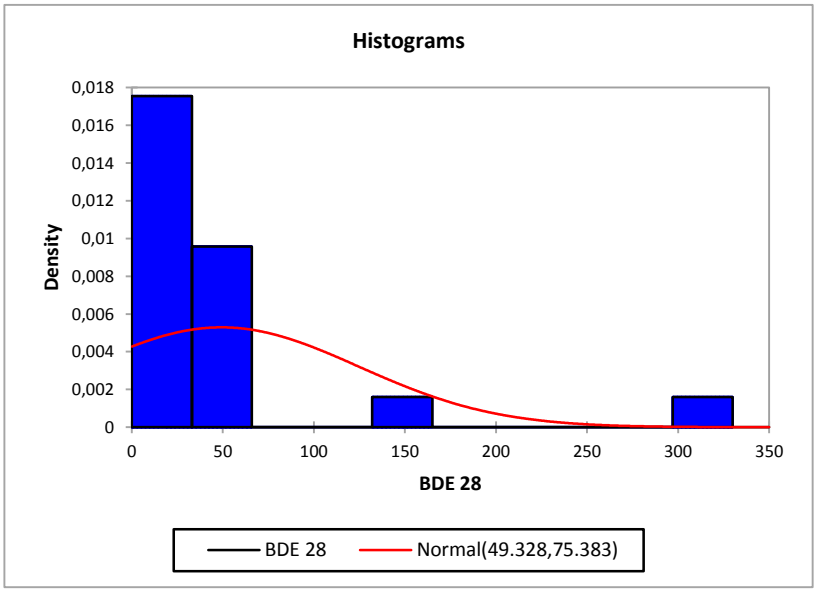
Statistics estimated on the input data and computed using
the estimated parameters of the Normal distribution (BDE 28):

Statistic	Data	Parameters
Mean	49.328	49.328
Variance	5682.620	5682.620
Skewness (Pearson)	2.622	0.000
Kurtosis (Pearson)	6.641	0.000

Kolmogorov-Smirnov test (BDE 28):

D	0.308
p-value	0.043
alpha	0.05

Test interpretation:
H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution
As the computed p-value is lower than the significance level alpha=0.05,
one should reject the null hypothesis H0, and accept the alternative hypothesis Ha.
The risk to reject the null hypothesis H0 while it is true is lower than 4.31%.



Descriptive statistics for the intervals (BDE 28):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	33	11	0.579	0.018	0.158
33	66	6	0.316	0.010	0.173
66	99	0	0.000	0.000	0.158
99	132	0	0.000	0.000	0.119
132	165	1	0.053	0.002	0.074
165	198	0	0.000	0.000	0.038
198	231	0	0.000	0.000	0.016
231	264	0	0.000	0.000	0.006
264	297	0	0.000	0.000	0.002
297	330	1	0.053	0.002	0.000

Distribution fitting (BDE 47):

Estimated parameters (BDE 47):

Value
268.843
254.903

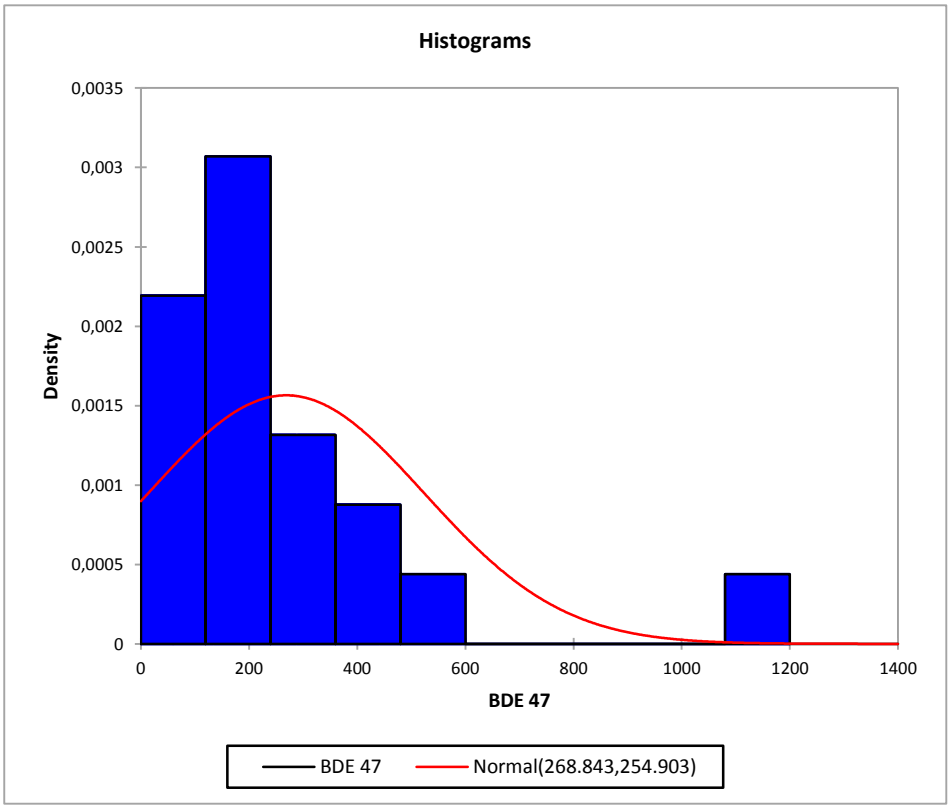
Statistics estimated on the input data and computed using the estimated parameters of the Normal distribution (BDE 47):

Statistic	Data	Parameters
Mean	268.843	268.843
Variance	64975.370	64975.370
Skewness (Pearson)	1.997	0.000
Kurtosis (Pearson)	4.028	0.000

Kolmogorov-Smirnov test (BDE 47):

D	0.191
p-value	0.449
alpha	0.05

Test interpretation:
H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution
As the computed p-value is greater than the significance level alpha=0.05, one cannot reject the null hypothesis H0.
The risk to reject the null hypothesis H0 while it is true is 44.93%.



Descriptive statistics for the intervals (BDE 47):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	120	5	0.263	0.002	0.134
120	240	7	0.368	0.003	0.175
240	360	3	0.158	0.001	0.185
360	480	2	0.105	0.001	0.157
480	600	1	0.053	0.000	0.107
600	720	0	0.000	0.000	0.059
720	840	0	0.000	0.000	0.026
840	960	0	0.000	0.000	0.009
960	1080	0	0.000	0.000	0.003
1080	1200	1	0.053	0.000	0.001

Distribution fitting (BDE 99):

Estimated parameters (BDE 99):

Parameter	Value
μ	502.287
sigma	534.808

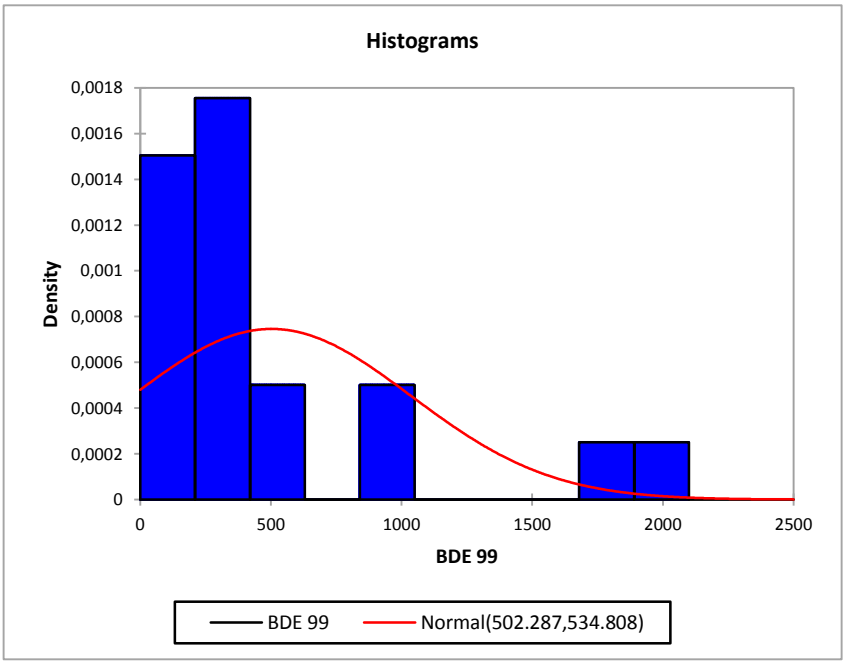
Statistics estimated on the input data and computed using the estimated parameters of the Normal distribution (BDE 99):

Statistic	Data	Parameters
Mean	502.287	502.287
Variance	286019.916	286019.916
Skewness (Pearson)	1.780	0.000
Kurtosis (Pearson)	2.041	0.000

Kolmogorov-Smirnov test (BDE 99):

D	0.327
p-value	0.026
alpha	0.05

Test interpretation:
H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution
As the computed p-value is lower than the significance
level alpha=0.05, one should reject the null hypothesis H0, and
accept the alternative hypothesis Ha.
The risk to reject the null hypothesis H0 while it is true is lower than 2.60%.



Descriptive statistics for the intervals (BDE 99):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	210	6	0.316	0.002	0.119
210	420	7	0.368	0.002	0.147
420	630	2	0.105	0.001	0.156
630	840	0	0.000	0.000	0.142
840	1050	2	0.105	0.001	0.111
1050	1260	0	0.000	0.000	0.075
1260	1470	0	0.000	0.000	0.043
1470	1680	0	0.000	0.000	0.021
1680	1890	1	0.053	0.000	0.009
1890	2100	1	0.053	0.000	0.003

Distribution fitting (BDE 100):

Estimated parameters (BDE 100):

Parameter	Value
μ	185.016
sigma	278.722

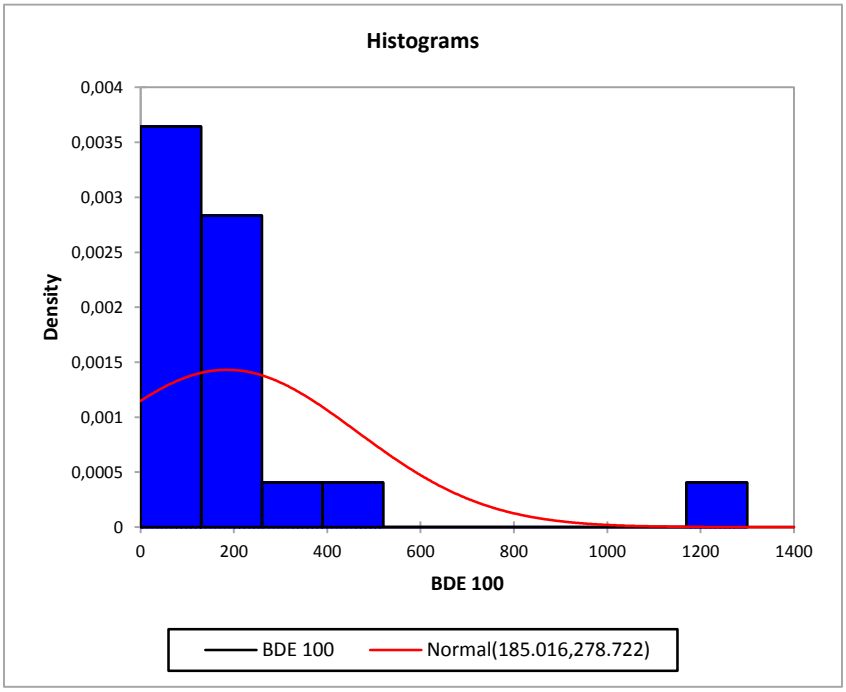
Statistics estimated on the input data and computed using the estimated parameters of the Normal distribution (BDE 100):

Statistic	Data	Parameters
Mean	185.016	185.016
Variance	77686.147	77686.147
Skewness (Pearson)	2.635	0.000
Kurtosis (Pearson)	7.056	0.000

Kolmogorov-Smirnov test (BDE 100):

D	0.279
p-value	0.086
alpha	0.05

Test interpretation:
H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution
As the computed p-value is greater than the significance level alpha=0.05, one cannot reject the null hypothesis H0.
The risk to reject the null hypothesis H0 while it is true is 8.57%.



Descriptive statistics for the intervals (BDE 100):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	130	9	0.474	0.004	0.168
130	260	7	0.368	0.003	0.184
260	390	1	0.053	0.000	0.163
390	520	1	0.053	0.000	0.116
520	650	0	0.000	0.000	0.067
650	780	0	0.000	0.000	0.031
780	910	0	0.000	0.000	0.012
910	1040	0	0.000	0.000	0.004
1040	1170	0	0.000	0.000	0.001
1170	1300	1	0.053	0.000	0.000

Distribution fitting (BDE 153):

Estimated parameters (BDE 153):

Parameter	Value
μ	374.512
sigma	514.663

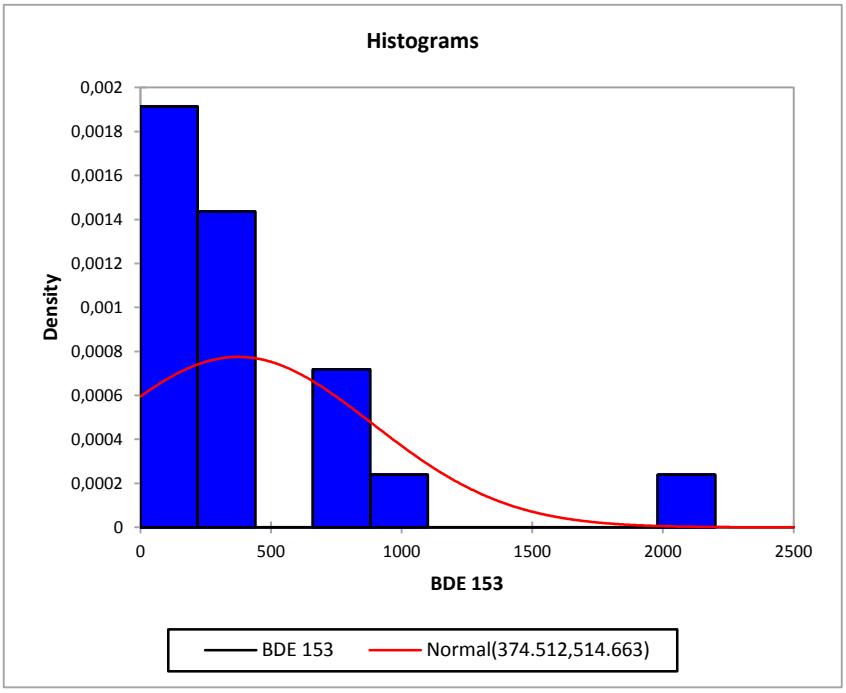
Statistics estimated on the input data and computed using the estimated parameters of the Normal distribution (BDE 153):

Statistic	Data	Parameters
Mean	374.512	374.512
Variance	264878.137	264878.137
Skewness (Pearson)	1.958	0.000
Kurtosis (Pearson)	3.890	0.000

Kolmogorov-Smirnov test (BDE 153):

D	0.233
p-value	0.219
alpha	0.05

Test interpretation:
H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution
As the computed p-value is greater than the significance level alpha=0.05, one cannot reject the null hypothesis H0.
The risk to reject the null hypothesis H0 while it is true is 21.90%.



Descriptive statistics for the intervals (BDE 153):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	220	8	0.421	0.002	0.149
220	440	6	0.316	0.001	0.169
440	660	0	0.000	0.000	0.160
660	880	3	0.158	0.001	0.127
880	1100	1	0.053	0.000	0.084
1100	1320	0	0.000	0.000	0.046
1320	1540	0	0.000	0.000	0.021
1540	1760	0	0.000	0.000	0.008
1760	1980	0	0.000	0.000	0.003
1980	2200	1	0.053	0.000	0.001

Distribution fitting (BDE 154):

Estimated parameters (BDE 154):

Parameter	Value
μ	167.913
sigma	256.721

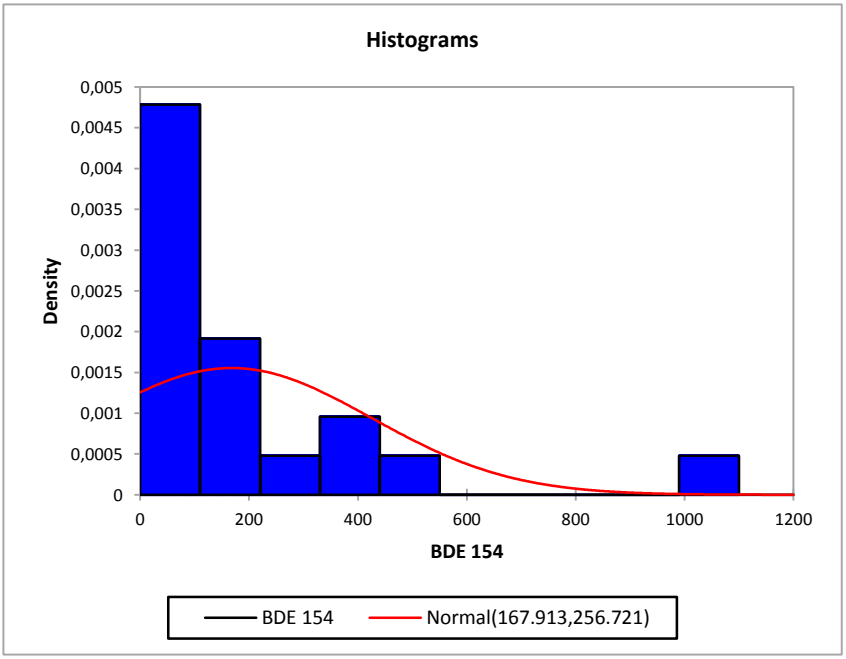
Statistics estimated on the input data and computed using
the estimated parameters of the Normal distribution (BDE 154):

Statistic	Data	Parameters
Mean	167.913	167.913
Variance	65905.574	65905.574
Skewness (Pearson)	1.935	0.000
Kurtosis (Pearson)	3.578	0.000

Kolmogorov-Smirnov test (BDE 154):

D	0.257
p-value	0.138
alpha	0.05

Test interpretation:
H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution
As the computed p-value is greater than the significance
level alpha=0.05, one cannot reject the null hypothesis H0.
The risk to reject the null hypothesis H0 while it is true is 13.84%.



Descriptive statistics for the intervals (BDE 154):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	110	10	0.526	0.005	0.154
110	220	4	0.211	0.002	0.170
220	330	1	0.053	0.000	0.156
330	440	2	0.105	0.001	0.119
440	550	1	0.053	0.000	0.076
550	660	0	0.000	0.000	0.041
660	770	0	0.000	0.000	0.018
770	880	0	0.000	0.000	0.007
880	990	0	0.000	0.000	0.002
990	1100	1	0.053	0.000	0.001

Distribution fitting (BDE 183):

Estimated parameters (BDE 183):

Parameter	Value
μ	391.877
sigma	502.218

Statistics estimated on the input data and computed
using the estimated parameters of the Normal distribution (BDE 183):

Statistic	Data	Parameters
Mean	391.877	391.877
Variance	252223.045	252223.045
Skewness (Pearson)	2.678	0.000
Kurtosis (Pearson)	7.352	0.000

Kolmogorov-Smirnov test (BDE 183):

D	0.250
p-value	0.159
alpha	0.05

Test interpretation:
H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution

As the computed p-value is greater than the significance level alpha=0.05, one cannot reject the null hypothesis H0.

Descriptive statistics for the intervals (BDE 183):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	230	9	0.474	0.002	0.156
230	460	4	0.211	0.001	0.180
460	690	4	0.211	0.001	0.170
690	920	1	0.053	0.000	0.130
920	1150	0	0.000	0.000	0.081
1150	1380	0	0.000	0.000	0.041
1380	1610	0	0.000	0.000	0.017
1610	1840	0	0.000	0.000	0.006
1840	2070	0	0.000	0.000	0.002
2070	2300	1	0.053	0.000	0.000

Distribution fitting (BDE 209):

Estimated parameters (BDE 209):

Parameter	Value
μ	1378.857
sigma	1541.066

Statistics estimated on the input data and computed using the estimated parameters of the Normal distribution (BDE 209):

Statistic	Data	Parameters
Mean	1378.857	1378.857
Variance	2374884.609	2374884.609
Skewness (Pearson)	1.410	0.000
Kurtosis (Pearson)	0.610	0.000

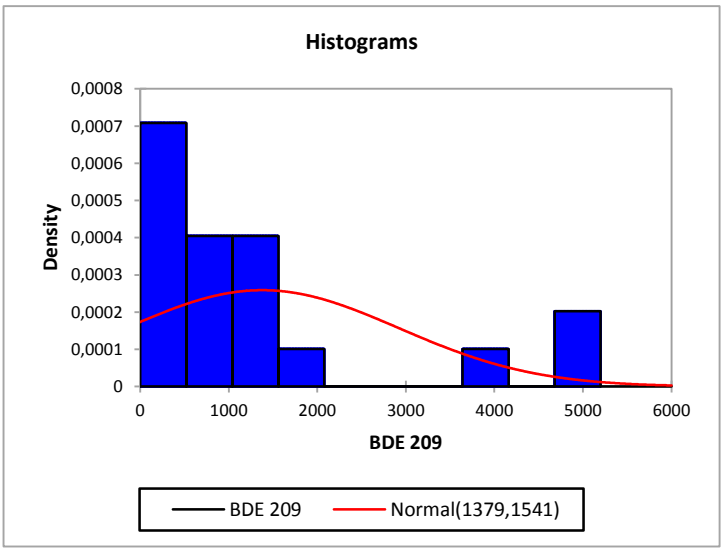
Kolmogorov-Smirnov test (BDE 209):

D	0.266
p-value	0.113
alpha	0.05

Test interpretation:
H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution

As the computed p-value is greater than the significance level alpha=0.05, one cannot reject the null hypothesis H0.

The risk to reject the null hypothesis H0 while it is true is 11.30%.



Descriptive statistics for the intervals (BDE 209):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	520	7	0.368	0.001	0.103
520	1040	4	0.211	0.000	0.124
1040	1560	4	0.211	0.000	0.134
1560	2080	1	0.053	0.000	0.129
2080	2600	0	0.000	0.000	0.111
2600	3120	0	0.000	0.000	0.085
3120	3640	0	0.000	0.000	0.058
3640	4160	1	0.053	0.000	0.036
4160	4680	0	0.000	0.000	0.019
4680	5200	2	0.105	0.000	0.010

3.6: Data comparison for the results obtained from UAE and SE

XLSTAT 2014.5.03 - Comparison of two samples (Wilcoxon, Mann-Whitney, ...) – on 11/23/2014 at 8:22:19 AM
Sample 1: Workbook = STATISTICAL ANALYSIS OF SRM 2585 EXTRACTED VIA ULTRASONIC AND SOXHLET.xlsx / Sheet = Sheet2 / Range = Sheet2!$K$7:$K$15 / 8 rows and 1 column
Sample 2: Workbook = STATISTICAL ANALYSIS OF SRM 2585 EXTRACTED VIA ULTRASONIC AND SOXHLET.xlsx / Sheet = Sheet2 / Range = Sheet2!$L$7:$L$15 / 8 rows and 1 column
Hypothesized difference (D): 0
Significance level (%): 5
p-value: Asymptotic p-value
Continuity correction: Yes

Summary statistics:

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
UAE	8	0	8	44.000	2806.100	574.800	944.267
SE	8	0	8	42.000	2650.000	553.188	895.227

Mann-Whitney test / Two-tailed test:

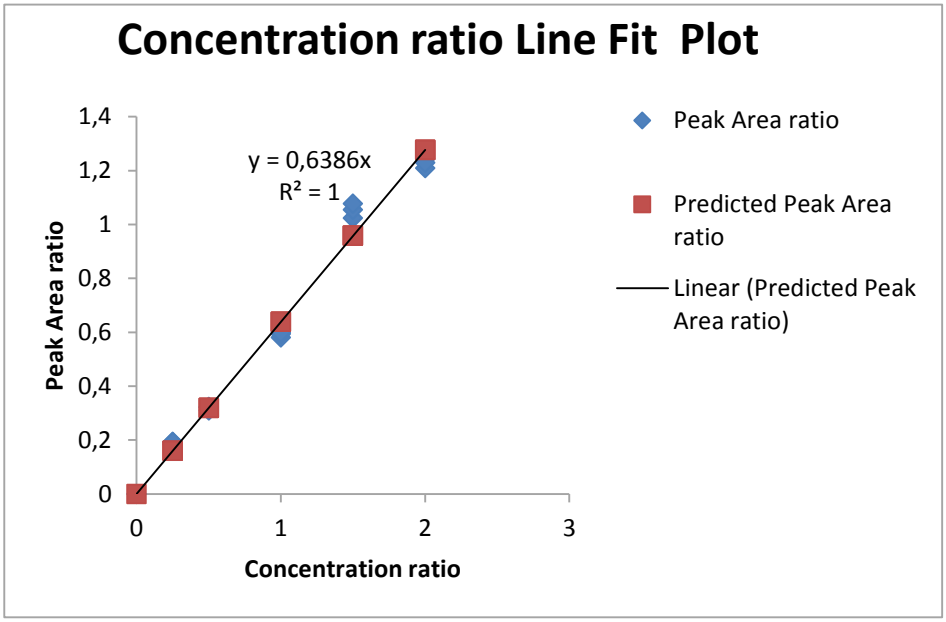
U	35.000
Expected value	32.000
Variance (U)	90.667
p-value (Two-tailed)	0.793
alpha	0.05

An approximation has been used to compute the p-value.

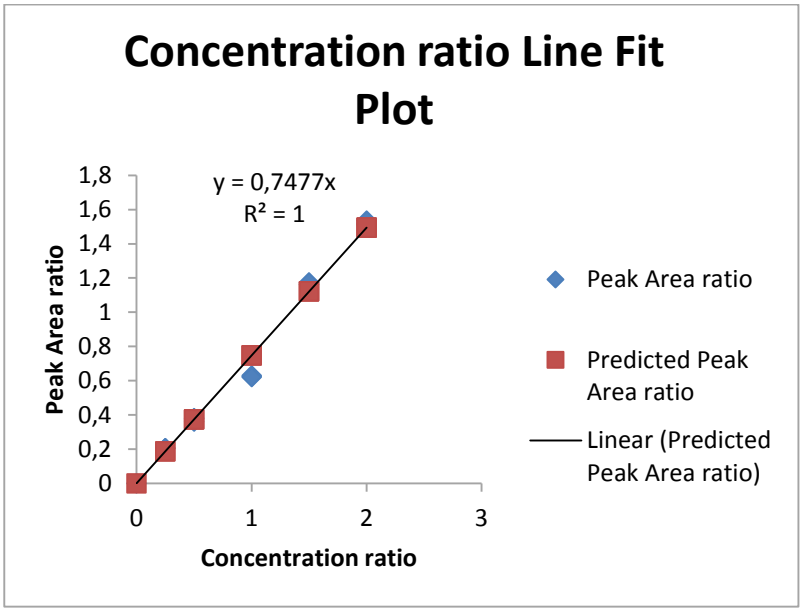
Test interpretation:
H0: The difference of location between the samples is equal to 0.
Ha: The difference of location between the samples is different from 0.
As the computed p-value is greater than the significance level
alpha=0.05, one cannot reject the null hypothesis H0.
The risk to reject the null hypothesis H0 while it is true is 79.29%.

Supplementary Material Figure S3. Calibration plots for representative BDE congeners.

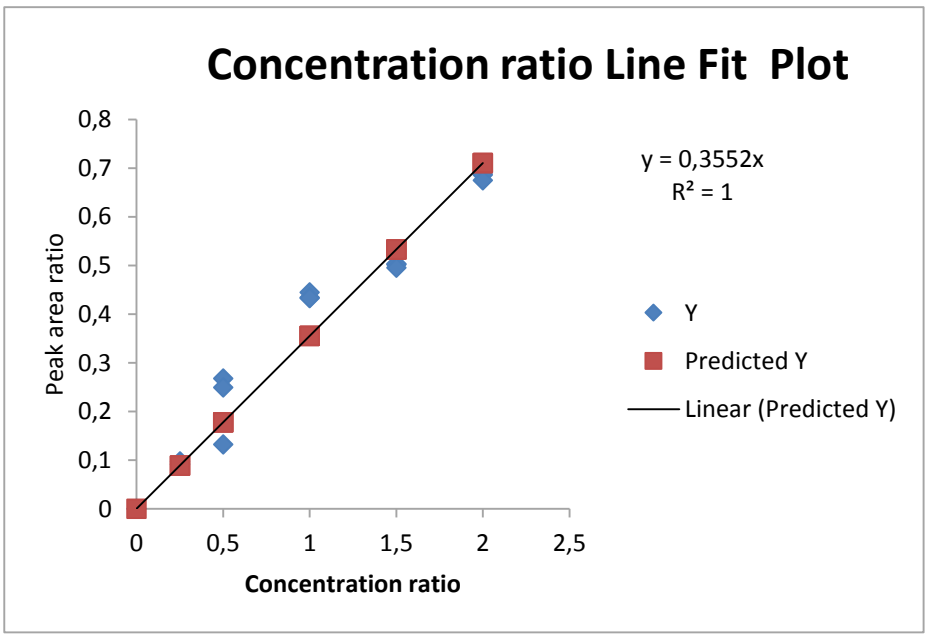
(a) BDE 28



(b) BDE 99



(c) BDE 153



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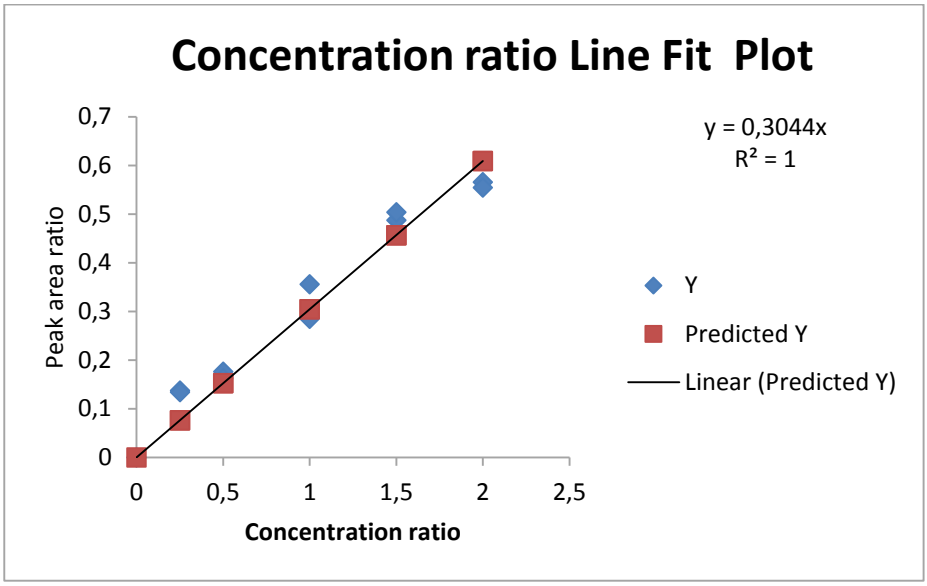
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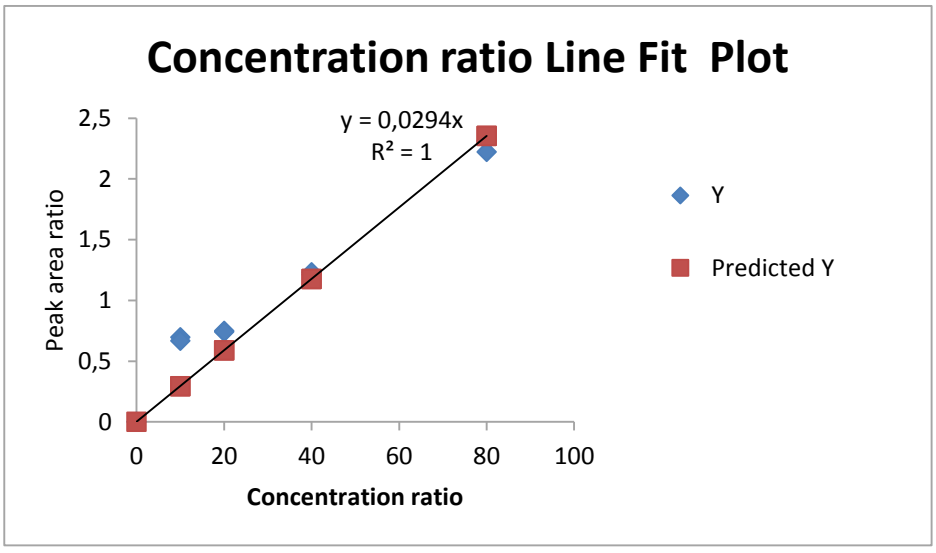
0,5
0,4
0,3
0,2
0,1
0
0
0,5
1
1,5
2
2,5
Concentration ratio
Y
Predicted Y
Linear (Predicted Y)

(e) BDE 209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2
1,5
1
0,5
0
0
20
40
60
80
100
Concentration ratio
Y
Predicted Y

Chapter 4

Determination of tetrabromobisphenol A in dust samples from electronic waste recycling sites (e-waste) by gas chromatography mass-spectrometry and liquid chromatography-tandem mass spectrometry

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Abstract

Two methods based on gas chromatography-electron impact ionization mass spectrometry (GC-EI-MS) and liquid chromatography–electrospray (negative) ionization tandem mass spectrometry (LC-ESI (-)–MS/MS) were optimized for the determination of tetrabromobisphenol A (TBBPA) in dust from e-waste recycling sites. Dust samples were extracted via ultrasonic-assisted extraction; and both silica gel and Florisil column chromatography were tested for the clean–up of the extracts. The cleaned extracts were derivatized with N,O-bis (trimethylsilyl)trifluoroacetamide (BSTFA) prior to analysis by GC-EI-MS. However, no derivatization was required for the LC-ESI (-)-MS/MS analysis. The LOD and LOQ were 0.35 ng g^{-1} and 1.15 ng g^{-1} for GC-EI-MS and 0.05 ng g^{-1} and 0.15 ng g^{-1} for LC-ESI-(-)-MS/MS respectively. Average recoveries from a spiked standard reference material (SRM 2585) ranged from 94 – 96 and 88 – 111 % for the GC-EI-MS and LC-ESI(-)-MS/MS methods respectively. The relative standard deviations (measured as intra and inter-day) were 0.86 % and 10.11 % for GC-EI-MS, and 0.48 % and 10.43 % for LC-ESI-(-)-MS/MS respectively. The mean concentration of TBBPA in dust samples from e-waste recycling sites 1 and 2 were 59150 ng g^{-1} and 14850 ng g^{-1}, respectively.

Keywords: *Method developmentGC-EI/MS, LC-ESI-MS/MS, Derivitization, BSTFA,Silical, Florisil*

4.1 Introduction

Tetrabromobisphenol A (TBBPA) is a widely used flame retardant with a worldwide market demand estimated at 170000 tonnes per year in 2004 (1, 2). Over 90 % of TBBPA is employed as a reactive flame retardant in printed circuit boards, with minor usage as an intermediate in the production of other brominated flame retardants, TBBPA derivatives and brominated epoxyl oligomers (1).

Toxicity studies have shown that human exposure to TBBPA can be hazardous. Studies has linked TBBPA with developmental defects, interference with thyroid hormone systems, and symptoms similar to those produced by dioxin (2). TBBPA has been shown to induce reactive oxygen species, affect natural human killer cells and be a potential endocrine-modulating chemical (2).

TBBPA has been detected in various environmental media including sediments, sewage sludge, surface water and biota (1); and air (1) and house dust (2, 3). However, more data are essential to better understand the levels and environmental fate of TBBPA in the indoor environment such as dust from electronic waste recycling sites (e-waste). Human exposure to high levels of TBBPA is a substantial risk for e-waste recyclers, owing to the fact that the largest additive use of TBBPA is found in television casings, PC monitor casings, and components in printers, fax machines and photocopiers (4), which characterize e-waste recycling facilities; since indoor dust is a recognized repository for organic pollutants such as TBBPA (3). Several studies have focussed on quantitative method development for the analysis of TBBPA in various media within the past decade (2). Most of these methods have been developed based on gas chromatography–mass spectrometry (GC-MS) following derivatization of the phenolic group with either diazomethane, methyl chloroformate, or N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) + 1% trimethylsilyl chloride (TMCS) (1), or liquid chromatography (LC) with various detection techniques such mass spectrometry (MS) (1, 3) and ultraviolet (UV) detection (5).

The present study aimed to develop, validate, and compare two highly sensitive, versatile and reliable analytical methods for the determination of TBBPA in indoor dust. These methods were based on LC-electrospray ionization (ESI) (negative)-MS/MS and GC-electron impact ionization (EI)/MS. The GC-MS method was developed following derivatization of TBBPA with BSTFA - a silylation reagent. The methods were applied for the determination of TBBPA in dust from e-waste recycling sites. To the best of our knowledge this is the first report of TBBPA concentrations in indoor dust from e-waste recycling facilities.

4.2 Experimental

4.2.1 Materials

A 100 mg of 3,3',5,5'–tetrabromobisphenol A analytical standard was obtained from Sigma-Aldrich. Similarly, silica gel 90 was from Sigma-Aldrich and Florisil PR 60-100 mesh was from Floridin Co., USA. The standard reference material (SRM 2585: Organic contaminants in house dust) was purchased from the National Institute of Standards and Technology (NIST). Anhydrous sodium sulfate was from Associated Chemical Enterprises (ACE), Johannesburg, South Africa. A Restek Rtx®–1614 fused silica (5% diphenyl 95% dimethyl polysiloxane) capillary column was obtained as a generous gift from Restek Corporation, Bellefonte, PA, USA. All solvents were high performance liquid chromatography grade obtained from Sigma Aldrich, South Africa. Both (N,O-bis(trimethylsilyl)acetamide) BSA and BSTFA were products of Supelco obtained from Sigma Aldrich, South Africa.

4.2.2 Sampling

Dust samples were collected from two e-waste dismantling/recycling facilities in KwaZulu-Natal province, South Africa. In the e-waste facilities, samples were collected from two locations. Point one in each recycling facility, comprised mainly stockpiles of televisions, computers, fridges and other electronic equipment including dismantled computer and television casings. Point two was characterized by electronic mother boards and other internal electronic components (see Supplementary Material Table S4.1). Sampling was carried out with a LG 1600W vacuum cleaner. The vacuum cleaner contained a dust unit which could easily be removed and emptied after each collection. Between each collection, it was cleaned with a disposable cloth wetted with iso-propanol. Samples were stored in amber glass bottles and stored at -10 °C until analysis.

4.2.3 Extraction and Clean-up

Non-dust particles, hair and debris were hand-picked from all samples. The two samples from each of the recycling sites were composited to one sample each per site. Samples were homogenized by sieving through a 212 µm stainless steel sieve. For dust extraction, approximately 1.0 g of dust sample was quantitatively weighed into a glass test tube. A volume of 10 mL *n*-hexane:methanol (1:3 v/v) was added. Samples were mixed in an orbital shaker for 10 mins and then extracted in an ultrasonic water bath at 40 °C for 30 mins. The mixing and extraction was repeated for a second time without addition of fresh solvent. The samples were then centrifuged at 3500 rpm for 10 mins and the supernatants were stored at <4 °C prior to clean-up. Silica gel 90 and Florisil (PR 60 to 100 mesh) were activated at 130 °C for 16 hours and anhydrous sodium sulfate was baked at 450 °C for 5 hours before use. Silica gel, Florisil and anhydrous sodium sulfate were subsequently cooled in a desiccator. A 30 cm × 1 cm glass column was packed with either 3 g of silica gel or 3 g of Florisil. Each column was topped with 0.8 g of anhydrous

sodium sulfate and then wetted with 30 mL of the extraction solvent. Extracts were loaded onto the columns just before the exposure of the sodium sulfate layer. TBBPA was eluted from the silica columns with 25 mL *n*-hexane and subsequently with 30 mL of diethyl ether/*n*-hexane (50:50 v/v). The column flow rates were maintained at 0.5 mL min^{-1}. All fractions were reduced to approximately 250 µL in a rotary evaporator at 55 °C. Similarly, TBBPA was fractionated and cleaned-up with Florisil column chromatography. Columns were eluted with 30 mL diethyl ether/*n*-hexane (6:94 v/v). Eluates were reduced and concentrated in a rotary evaporator to approximately 250 µL and stored in 1.5 mL amber glass GC/MS vials. All extracts were stored at <4 °C prior to further chemical analysis.

4.2.4 Derivatization

Different derivatization reagents such as bis(trimethylsilyl)acetamide (BSA) and BSTFA were tested under various reaction conditions. Optimum derivatization was obtained with 1:2 (v/v) sample extracts:BSTFA, with a one hour reaction time. The optimized derivatization procedure is: 50 µL BSTFA was added to 25 µL of the sample extract in a glass vial and mixture was reacted for 60 mins at 70 °C (Fig. 4.1). The volume of the reaction mixture was adjusted to 75 µL with n-hexane containing 2 µg PCB 209 as internal standard. Extracts were then ready for GC-MS analysis without further treatment.

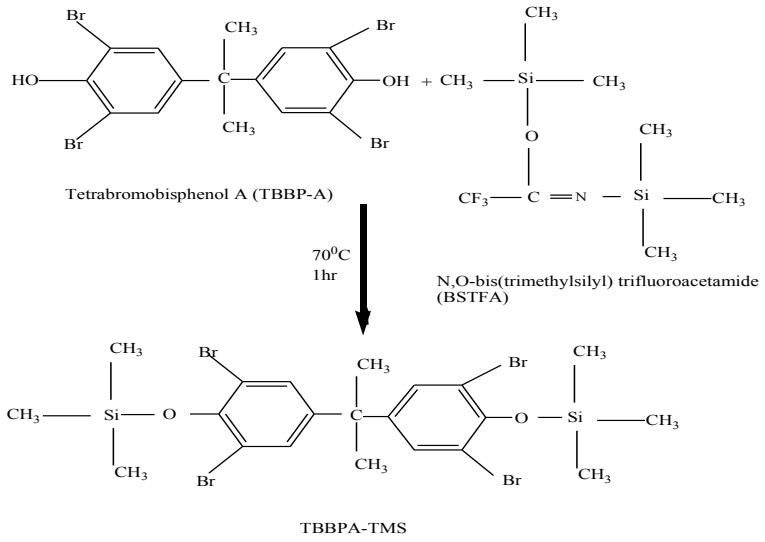


Figure 4.1 Derivitization of tetrabromobisphenol A with N,O-bis (trimethylsilyl)trifluoroacetamide.

4.2.5 Recovery Experiment

The recovery of TBBPA in dust was determined from a spiked standard reference material (SRM 2585 - Organic contaminants in house dust) as well as anhydrous sodium sulfate that had been spiked with TBBPA. A mass of 1.0 g of SRM 2585 or anhydrous sodium sulfate were spiked separately with 50, 200, 250, 500, 700, 1500, 2000 and 10000

ng of TBBPA respectively in triplicate. Samples were left to stand for at least 21 days at -10 °C. The spiked samples were extracted and cleaned-up following the procedure for real samples. For GC–MS, the extracts were derivatized prior to analysis. Extracts for LC-MS/MS analysis were reduced to incipient dryness and reconstituted in 100 µL of the mobile phase.

4.2.6 GC-EI/MS Analysis

An Agilent 6890 GC fitted with a Restek Rtx®–1614 fused silica (5% diphenyl, 95% dimethyl polysiloxane) capillary column (15 m × 250 µm × 0.1 µm) coupled to an Agilent 5973N series mass spectrometer was used for the separation, detection and quantitation of TBBPA. Injections were made in the pulsed splitless mode with the injector temperature set at 250 ºC. The injection volume was 1 µL. The GC oven temperature programme started at 50 ºC (held for 1 min), then was increased at 30 ºC min^{-1} to 280 ºC and held there for 2 min. Helium was used as the carrier gas at a flow rate of 1.2 mL min^{-1} and a constant linear velocity of 56 cm s^{-1}. For the MS, the ion source and transfer line temperatures were 230 ºC and 350 ºC, respectively; and the ionization energy was 70 eV. TBBPA mass spectra were obtained in full scan mode.

Quantitation was carried out by means of a multiple point internal standard method. TBBPA was quantified by using PCB-209 as the internal standard. The response factor was determined from the slope of a plot of the ratio of peak areas against the ratio of the concentrations. The values for the plots were obtained from a 5 point triplicate analysis of the TBBPA standard solution diluted to fall within a concentration range of 10 – 10000 ng mL^{-1} TBBPA.

4.2.7 LC-ESI (Negative)-MS-MS Analysis

An Agilent ion trap mass spectrometer LCQ Finnigan (Thermoquest) coupled to an Agilent 1100/1200 series liquid chromatography system was employed for all LC-ESI (negative)-MS/MS analysis. Data processing was with ChemStation software.

A 150 mm × 4.6 mm i.d, 5 µm particle size Zorbax Eclipse XDB C18 column was used to effect separation on the LC system. Separation was performed via isocratic elution with a mobile phase composition of 95% acetonitrile in 0.1% formic acid and 5% water in 0.1% formic acid, at a flow rate of 1.0 mL min^{-1}. A thermostatted autosampler kept at 20 °C was used to inject 20 µL of sample. The column temperature and pressure was 30 ºC and 36-38 bar, respectively. The MS was operated in electrospray ionization (ESI) negative mode. ESI parameters were: a dry temperature of 350 ºC, measured nebulizer of 9.96 l/min, HV capillary of 3500 V, 9.766 nA capillary current, 121.460 nA current end plate and HV end plate of -500 V. The optimized Smart Parameter Setting (SPS) was a target mass of 544 m/z, 50 % compound stability and 100 % trap drive level. Both selected ion monitoring (SIM) and MS/MS full scan were employed as acquisition methods. SIM at m/z 544 and 542.7 was monitored for TBBPA. The most abundant molecular ion m/z 542.7 was employed in the MS/MS experiment and mass spectra

between m/z 50 and m/z 550 were obtained. The fragmentation pattern was further confirmed by a MS^3 experiment. TBBPA was quantified by an external calibration method over a linear range of 5 – 7000 ng mL^{-1}.

Ions employed for the identification of TBBPA in both GC-MS and LC-MS/MS methods are presented in Table 4.1, together with ions reported by other workers.

4.2.8 Quality Control

Method blanks were analysed with samples. For the method blank, dust samples were replaced with anhydrous sodium sulfate and passed through all the analytical procedure carried out for real samples. The TBBPA concentration in the blanks was less than 3 % of the TBBPA concentrations in samples from the two e-waste recycling sites. Hence, samples were not blank corrected for TBBPA concentrations. Field blank samples (n = 3) were obtained by spreading anhydrous sodium sulfate on a pre-cleaned tiled floor. The floor was vacuumed following the same sampling protocol as for real samples and the samples subjected to the analytical procedure. Solvent blanks were injected after each sample injection and analysis.

Table 4.1 Ions (M/Z) of TBBPA derivative used for GC-MS and LC-MS/MS quantification with EI mode.

Instrument	Derivatization reagent	Monitored Ion	LOD/ ng g^{-1} ng mL^{-1} ng m^{-3}	Media	Reference
GC-EI/MS	BSTFA	673, 688	0.35	Dust	This work
LC-ESI-MS/MS		542.7→78.8	0.05	Dust	This work
LC-MS/MS		542.8→78.9, 79.9		Dust	(6)
LC-MS/MS		542.7→ 78.9, 80.9		Dust	(7)
LC-MS/MS			3.0	Sewage sludge	(8)
LC-MS/MS		542.6→78.6 548→80.6	11.0	Dust	(3)
HPLC-UV			1275	Dust	(5)
LC-ESI-MS/MS		543→ 444	0.02	Wastewater	(9)
LC-APCI-MS/MS		542.7→445.8	0.0002	Landfill leachate, sediment,	(10)

				soil and marine sediment	
LC-ESI-MS		542	0.01	River water	(11)
LC-ESI-MS/MS		542.7→417.8	-	Air	(12)
LC-ESI-MS		542	0.1	Air	(13)
LC-ESI-MS		540.9	0.5	Sediment and sewage sludge	(14)
LC-ESI-MS/MS		543→81	0.05	Sediment and sewage sludge	(15)
LC-ITD/MS		145-543	0.5	Sediment and sewage sludge	(16)
LC-TOF-MS		230-550	20	Egg	(17)
GC-LR/MS GC-HR/MS	Methyl chloroformate	556.76, 554.76	10 1		
GC- HR/MS	MSTFA	682.85, 684.85	0.2 – 4	Blood and serum	(18)
LC-UV-APCI-MS/MS		150 – 1000	10 -100	E-Waste polymer	(19)
LC-UV		NA	90,000	E- Waste polymer	(20)

4.3 Results and discussion

Both the GC-EI/MS and LC-EI-MS/MS methods were developed, validated and successfully applied for the determination of TBBPA in dust from two e-waste recycling sites. The sensitivity of the methods is compared with the limits of detection of other methods reported in the literature in Table 4.1.

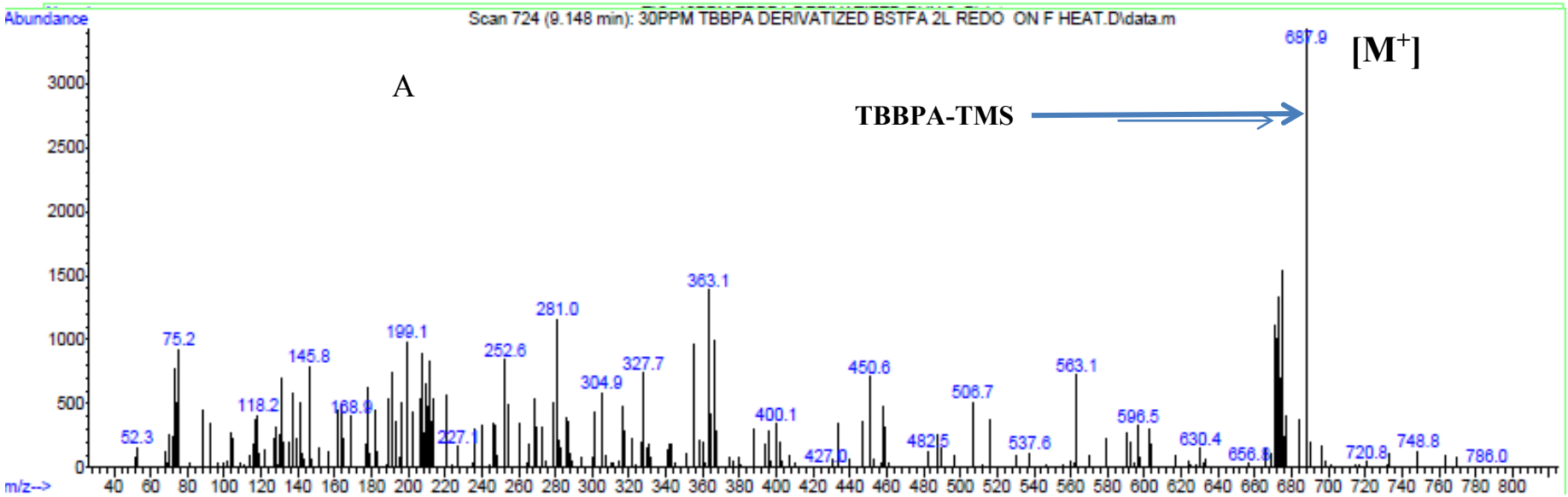
4.3.1 Derivatization

Derivatization of TBBPA and other phenolic compounds has been carried out by different research groups in other to enhance the selectivity and sensitivity of detection by GC-MS analysis. Methyl chloroformate, diazomethane (1); pentafluoropropionic acid anhydride (PFPA) (21); BSTFA+1% TMCS and BSTFA (1) have been applied for the derivatization of TBBPA in various environmental matrices. In this study, two silylation reagents (BSA and BSTFA) were employed to determine the optimum conditions for derivatization of TBBPA in house dust. The derivatization of TBBPA

with BSA was characterized by peak broadening and irreproducability of peak areas with %RSDs greater than 30 %. This is possibly the result of incomplete reaction of BSA with TBBPA after 3 hours of reaction time. However, good chromatographic peaks were obtained with BSTFA. The GC-MS chromatogram in the electron impact ionization mode showed a repeatedly sharp peak shape and excellent relative peak abundance (Fig 4.2). Several ratios of derivatization reagent to sample volume (1:1, 1:2, 2:1, and 1:3) were tested to obtain the optimum condition for derivatization of TBBPA in dust samples. Similarly, the reaction time (5, 15, 25, 35, 45, 60, 75, 90, 120 and 180 min) was tested for TBBPA derivatization. Excellent peak shape with good repeatability (n = 6) of 0.86 % and 10.11 % for both intra-day and inter-day %RSDs were obtained for a 2:1 (i.e. 50:25 µL) BSTFA:sample extract at 70 °C for 60 min. The stability of the product was tested by keeping the derivatized samples (n = 5) at 4 °C and analysed over a 60-day period. The results were reproducible with a satisfactory precision of 10.11 % measured as % RSD.

4.3.2 GC-EI/MS Analysis of TBBPA

The reaction of BSTFA with TBBPA results in the replacement of the active hydrogen of the phenolic groups in the TBBPA structure with a trimethylsilyl [-Si $(CH_3)_3$] group. The derivative obtained (Fig. 4.1A & B) is highly electrophilic, hence enabling the use of dissociative electron capture and electron capture as the principal ionization processes (1). The abundance of the molecular ion $[M]^+$ present in the mass spectrum at m/z of 687.9 was lower than that of the major fragment ion at m/z of 672.9. This corresponds to the loss of a $–CH_3$ group ($M-CH_3$) (Figs. 4.1B & C). The isotopic peaks are determined by the ratios of ^{79}Br, ^{81}Br, ^{28}Si, ^{29}Si and ^{30}Si in the molecular or fragment ion (1). The loss of [-Si $(CH_3)_3$] from the TBBPA derivative results in the peak at m/z of 73.2 (Fig 4.1C). The intensity of the m/z = 73.2 ion was low compared to other major ions; hence, it was used alongside the molecular peak as qualifier ions. However, the $[M-CH_3]$ ion was adopted for quantification.


Abundance
Scan 724 (9.148 min): 30PPM TBBPA DERIVATIZED BSTFA 2L REDO ON F HEAT.D\data.m
687.9
[M+]
A
TBBPA-TMS
3000
2500
2000
1500
1000
500
0
363.1
281.0
199.1
75.2
145.8
252.6
327.7
304.9
450.6
506.7
563.1
400.1
118.2
596.5
52.3
227.1
482.5
537.6
630.4
720.8
748.8
786.0
m/z-->
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540 560 580 600 620 640 660 680 700 720 740 760 780 800

B

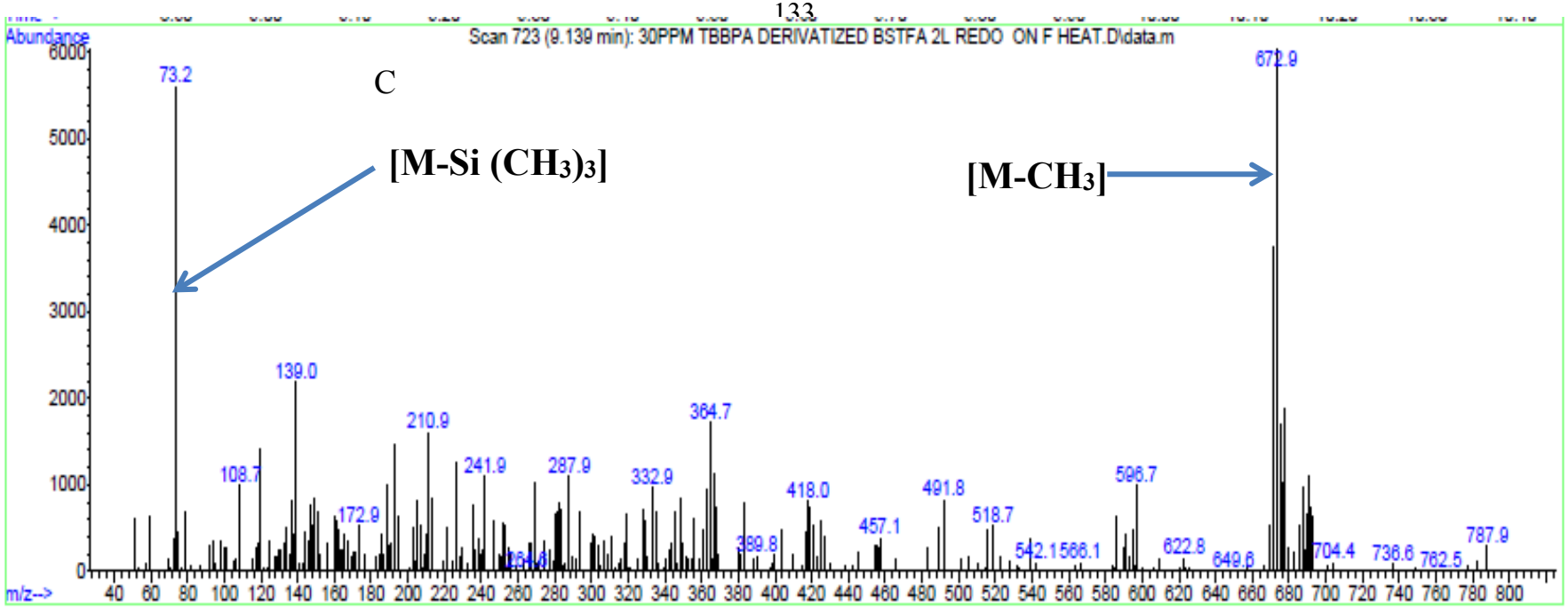


Figure 4.2 **(A)** GC-MS chromatogram and (B & C) full scan mass spectra of TBBPA derivatives (TBBPA-TMS). $[M]^+$ is the molecular ion and $[M\text{-}CH_3]^+$ and $[M\text{-}Si(CH_3)_3]$ are the major fragment ions.

4.3.3 LC-ESI-MS/MS

The mass spectrum of TBBPA in the negative ion mode was obtained during method development by directly infusing an aliquot of TBBPA standard solution into the mass spectrometer. The mass spectrum was acquired in full scan (mass range of 40 – 2200 m/z) (Fig. 4.3). The pseudo-molecular ion $[M-H]^-$, m/z = 542.7 was foremost in the mass spectrum of TBBPA.

The distribution of the isotopes was in accordance with the presence of the four bromine atoms on the ion (16). Two daughter ions at m/z = 527.8 and 447.8 correspond to the loss of a methyl group $[M-CH_3]$, preceding the loss of a bromine atom $[M-CH_3-Br]^-$, respectively. In the mass spectrum obtained in MS-MS mode with m/z = 542.7 as parent ion and a collision energy of 50 %, the ions at m/z = 447.8 and m/z = 527.8 were more prominent and were confirmed to have originated from the parent ion, i.e. $[M-H]^-$. Similarly, a third ion at m/z = 292.8 (Fig 4.3) is associated with the loss of a dibromophenol moiety with a rearrangement of the propyl group to give 4-iso-propylene-2,6-dibromophenol. The isotopic pattern is in agreement with the presence of two bromine atoms.

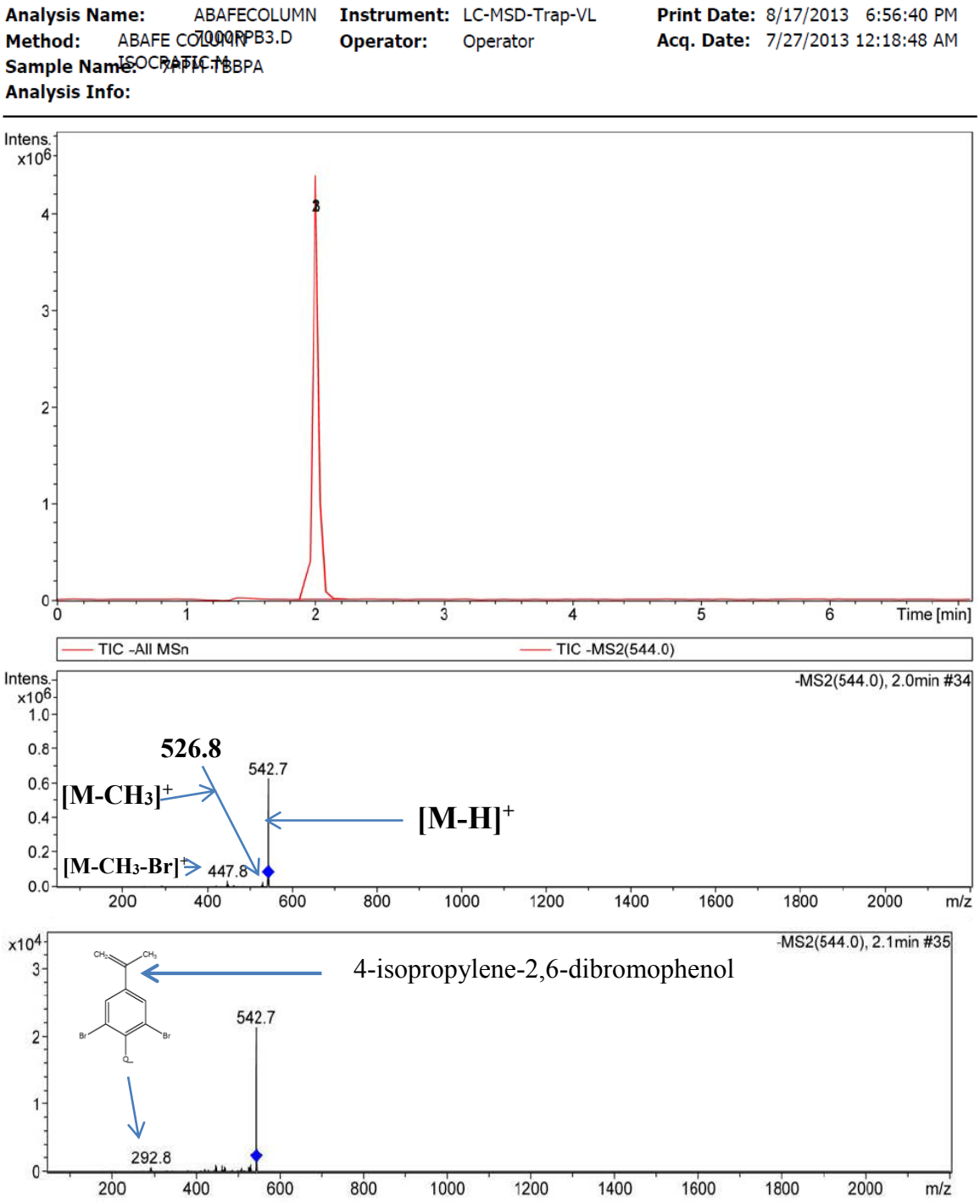


Figure 4.3 LC chromatogram and mass spectrum of TBBPA with its pseudomolecular ion $[M-H]^+$, two daughter ions corresponding to $[M-CH_3]^+$ snd $[M-CH_3-Br]^+$, and a third daughter ion–dibromophenol moiety.

4.3.4 Chromatographic Behaviour, Linearity, LOD and LOQ for GC-EI/MS and LC-ESI(-)MS/MS

Although, the pK_{a1} and pK_{a2} of the hydroxyl groups in the TBBPA structure are 7.5 and 8.5 (16), the loss of the first proton occurred probably due to the addition of the formic

acid to the acetonitrile:water mobile phase or as a result of electrospray processes. However, such chemical processes in the ESI mode have been reported previously without the addition of a buffer to the mobile phase (16). The retention time of TBBPA on the C18 column was approximately 2.10 min (Fig. 4.3**)** and the mass spectrum is similar to that obtained from direct infusion of TBBPA to the MS.

Good linearity of the absolute response of the mass spectrometer toward TBBPA was obtained for both GC-EI/MS and LC-ESI-MS/MS (Table 4.2). The concentration ranges employed for the preparation of calibration curves were 10 – 10000 ng mL^{-1} and 5.0 – 7000 ng mL^{-1} for GC-MS and LC-MS/MS, respectively.

Table 4.2 GC–MS versus LC-MS/MS method validation for TBBPA

Parameter	**GC-MS**	**LC-MS/MS**
Linear range ng mL^{-1}	10 – 10000	5.0 – 7000
R^2	0.975	0.997
LOD (ng g^{-1})	0.35	0.05
LOQ (ng g^{-1})	1.15	0.15
Accuracy (% Recovery)	93.79 – 95.63[a]	88.05 – 110.73[b]
Precision [Repeatability of peak area (n = 3)]		
Intra–day (% RSD)	0.86	0.48
Inter–day (% RSD)	10.11	10.43

[a]Spiked concentrations were 250, 700 and 10000 ng g^{-1}

[b]Spiked concentrations were 50, 200, 500, 700, 1500 and 2000 ng g^{-1}

Both Florisil and silica gel columns were employed for the clean-up of TBBPA. A number of solvent systems were tested for the elution of these columns. Higher recovery of TBBPA was obtained with a solvent mixture of n-hexane–diethyl ether as eluting solvent for the Florisil column as compared with the silica gel column (see Fig 4.4). These could be a result of the basic properties of magnesium silicate, since the presence of the –OH groups in TBBPA may result in a strong interaction with silica gel due to its polarity. Hence, the florisil column was used for clean-up of all sample extracts. The recovery of spiked TBBPA on the Florisil column is presented in Table 4.3.

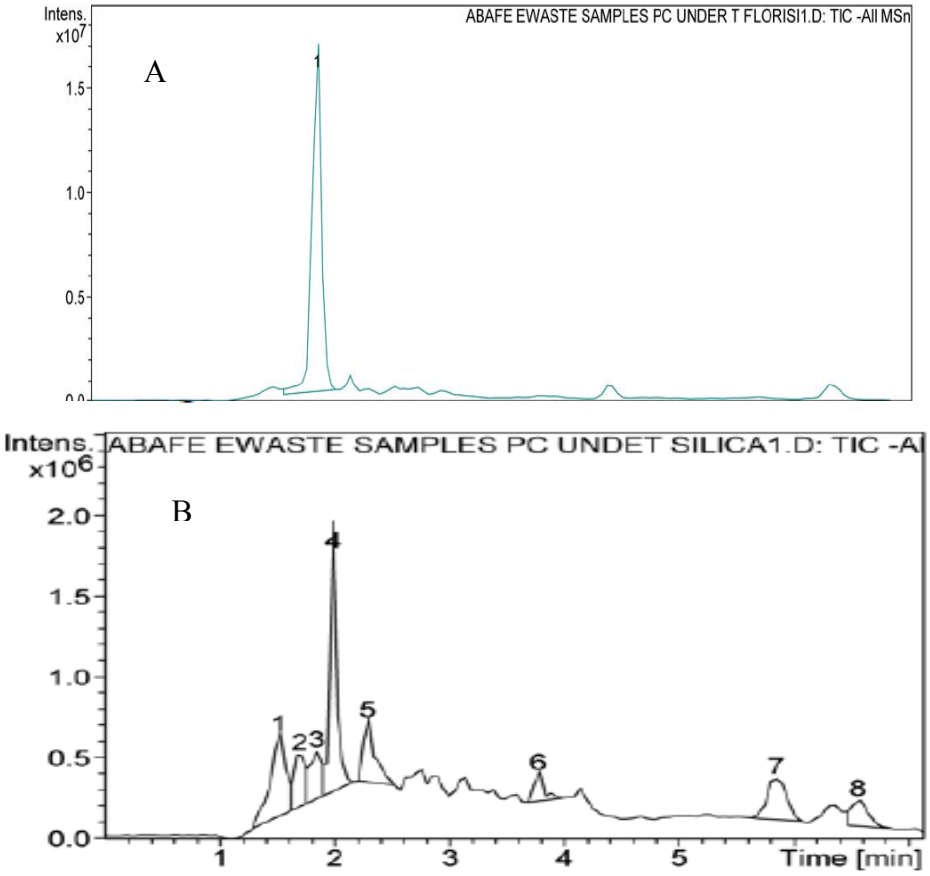


Figure 4.3 LC chromatogram of TBBPA in a typical e-waste dust sample: (A) Florisil clean-up fraction (B) Silica gel clean-up fraction.

Table 4.3 LC-MS/MS recovery of TBBPA on Florisil column

Spiked Concentration/ ng g^{-1}	50 (n = 3)	700 (n = 3)	1500 (n = 3)	2000 (n = 3)
Determined Concentration/ ng g^{-1}	49.3	701	1642.3	2100
Determined concentration/ ng g^{-1}	45.5	676	1351	1761
Determined concentration/ ng g^{-1}	55.4	701	1492.5	1967
Mean	50.0	693	1495.1	1943

STD	5.0	14.4	146	171
RSD	10.0	2.1	10	9.0
% Recovery	100.1	99	100	97.1

Limits of detection (LOD) and limits of quantitation (LOQ) were determined following Thomsen et al. (22). The LODs and LOQs calculated for the determination of TBBPA with both GC-EI/MS and LC-ESI (negative) MS/MS are shown alongside available literature reports in Table 4.1. The LODs obtained in this study compared favourably with available literature reports.

4.3.5 Application to real samples

Dusts from the floors of two major e-waste recycling sites in Durban, South Africa, were collected by extensively vacuum-cleaning each site. Site characteristics are presented in Supplementary Material Table S4.1. TBBPA was detected in high levels at both sites (Table 4.4). No data exist worldwide on the level of TBBPA in e-waste recycling sites, however, the concentrations found in these recycling sites resembles the levels reported by Yu and Hu (23) in dust from the inside of computers. Although TBBPA is used primarily as a reactive flame retardant in the manufacture of epoxy and polycarbonate resins (4), 18% of the global TBBPA usage has been as an additive flame retardant in the manufacture of acrylonitrile-butadiene-styrene (ABS) resins or high impact polystyrene (HIPS) (4). As an additive flame retardant, no chemical reactions with other components of the polymer exist, thus TBBPA may leach out of the product after incorporation (4), particularly during usage and recycling of the product. ABS resins are applied in automotive, pipes and fittings, refrigerators, copiers, printers and telephones; similarly, HIPS resins are used in electrical and electronic equipment, packaging, consumer products, furniture, building and construction materials (4). The largest additive use of TBBPA is in television casings, as well as PC monitor casings, components of printers, fax machines and photocopiers (4). These materials typify the products recycled in the e-waste recycling sites sampled, and hence the high levels of TBBPA were not unexpected.

Table 4.4 Concentrations of TBBPA in dust samples from e-Waste recycling plant.

Statistical parameter	Recycling site 1 LC-MS/MS	Recycling site 2 LC-MS/MS	Recycling site 1 GC-MS
Mean TBBPA concentrations/ng g^{-1}S1	55464	17313	42891
Mean TBBPA concentrations/ng g^{-1}S2	59548	12633	49435
Mean TBBPA concentrations/ng g^{-1}S3	62427	14599	Missing data
Mean/ng g^{-1}	59146	14848	46113

S1, S2 and S3 refers to replicated samples analysed.

4.4 Conclusions

Two chromatographic methods for the determination of TBBPA in dust samples have been optimized and applied to real samples. Both methods are cost-effective with the GC-MS method involving a simple derivatization of the phenolic groups with a derivatization reagent common in chemistry laboratories. Sample preparation for the LC-MS/MS method is fast and simple requiring no derivatization and ion suppression steps. LODs and LOQs for both methods compared favourably with reported methods. Concentrations of TBBPA were determined for the first time in dust samples from e-waste recycling sites. The concentrations of TBBPA in these facilities may present hazardous health effects to e-waste recyclers and the environment. Further studies detailing levels of TBBPA in dust and subsequent human exposure magnitude from multiple indoor microenvironments such as homes, offices, automobiles and computer laboratories are urgently required in South Africa and indeed the African continent; since this continent is a known receiver of e-waste from developed countries.

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Supplementary Material

Table S4.1: Sampling site description

Sampling site/ Code	Sampling point description
E-waste recycling facility SC point 1	Comprised mainly of stockpile of waste television and computers, also with plastic casing of dismantled computers and TVs
E-waste recycling facility SC point 2	Sampling point 2, characterized with printed circuit board and other internal e-waste components
E-waste recycling facility PCU point 1	Comprised mainly of stockpile of waste televisons and computers, also with plastic casing of dismantled computers and TVs
E-waste recycling facility PCU point 2	Sampling point 2, characterized with printed circuit board and other internal e-waste components

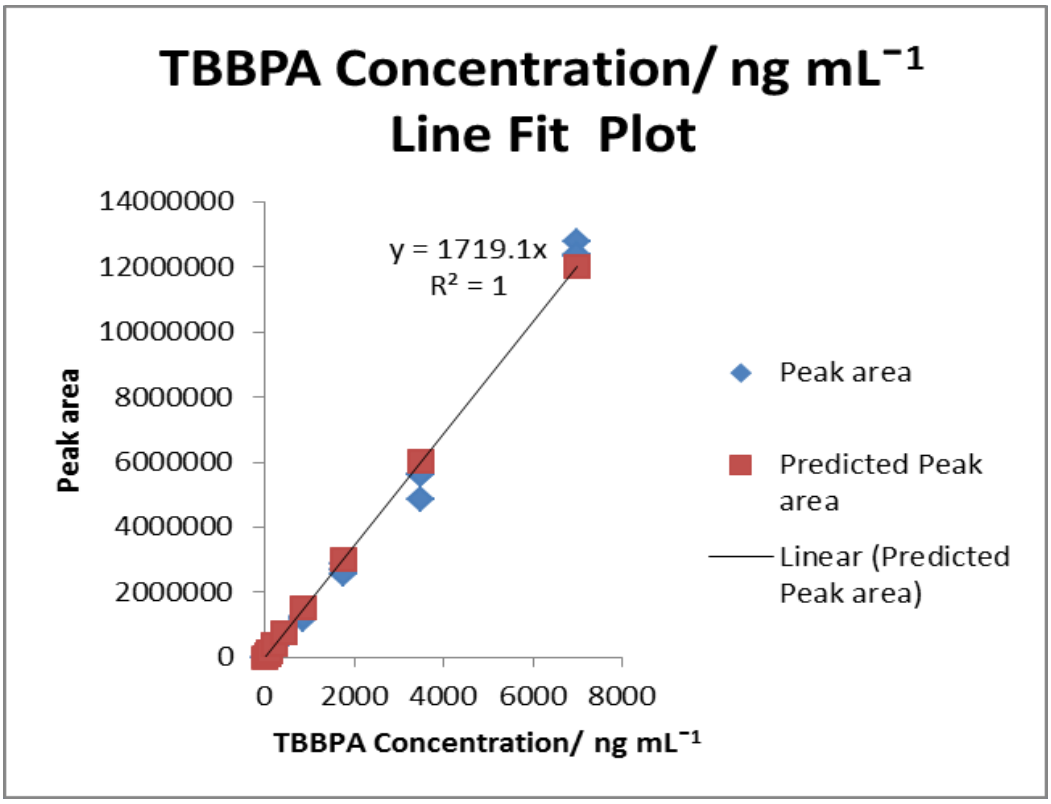

Figure S4.1 LC-MS/MS calibration curve for TBBPA.

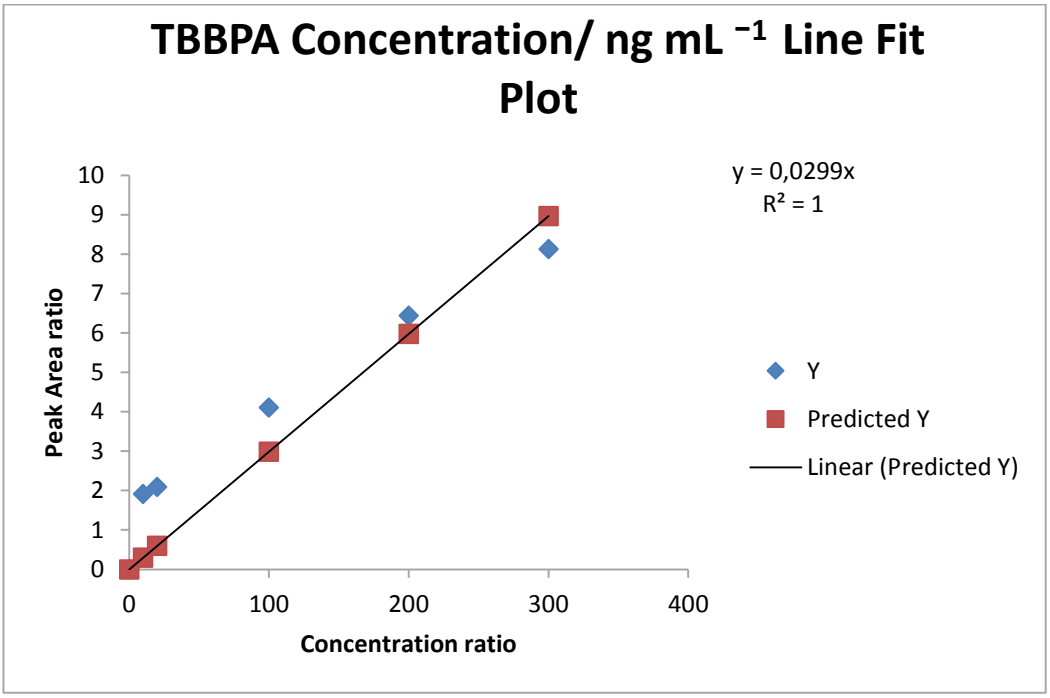

Figure S4.2 GC-MS calibration curve for TBBPA derivative.

Table S4.2 Analysis of Variance for LC-MS/MS and GC-MS Results for Recycling site 1

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
LC-MS/MS	2	115012	57506	8339528
GC-MS	2	92226	46113	22071368

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	129800449	1	129800449	8.53644358	0.09989854	18.51282051
Within Groups	30410896	2	15205448			
Total	160211345	3				

Chapter 5

Assessment of concentrations and human exposure magnitude of tetrabromobisphenol A in dust from microenvironments in Durban, South Africa

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Abstract

Tetrabromobisphenol A, the most widely used brominated flame retardant, is a phenolic organic contaminant used in a variety of consumer applications. Through manufacture, usage and recycling, TBBPA may leach out of products, thereby contaminating the environment. Indoor dust is a recognized receptor of TBBPA in the indoor environment. Therefore, in this study, we determined the concentrations of TBBPA in dust samples from automobile (n = 14); computer laboratories (n = 6); homes (n = 7); and offices (n = 9), in Durban, South Africa. Chromatographic and mass spectrometric techniques were applied for the separation, identification and quantitation of TBBPA after simple sample preparation steps. Median concentrations of TBBPA were 1157 ng g^{-1}, 268 ng g^{-1}, 120 ng g^{-1} and 492 ng g^{-1} in automobiles, computer laboratories, homes and offices, respectively. Analysis of variance showed no statistically significant differences in the concentrations of TBBPA in the various microenvironments. However, TBBPA was highest in automobiles, followed by homes, offices and computer laboratories. Household characteristics such as ventilation system, type of floor and frequency of cleaning, influenced the distribution of TBBPA in the various microenvironments. Building age had no influence on the profile of TBBPA in the indoor dust. Assessment of human daily exposure doses of TBBPA reveals dust ingestion as a major pathway of exposure for three human population groups – adults, teenagers and toddlers. Assuming an average dust ingestion rate, and a median TBBPA concentration, the $\sum$DED/ng kg^{-1} bw day^{-1} of TBBPA is 0.08, 0.08 and 0.60, respectively for an adult, teenager and toddler. These doses are higher than the average dietary intake of 0.04 ng kg^{-1} bw day^{-1} of TBBPA by the Dutch population.

Keywords: *TBBPA, Concentrations, Indoor, Environment, Sources, Household characteristics, Human Exposure, South Africa.*

5.1 Introduction

Tetrabromobisphenol A (TBBPA) is the most widely used brominated flame retardant (1), with a production volume covering almost 60% of the total brominated flame retardant (BFR) market (2). TBBPA is majorly used as a reactive flame retardant, in which it is covalently bonded to the host material, for example, in epoxyl and polycarbonate resins used in printed circuit boards and electronic equipment (3). A smaller amount of 18% of TBBPA is used as additive flame retardant in which TBBPA is mixed with the host material, for example in the manufacture of high impact polystyrene (HIPs) and acrylonitrile-butadiene-styrene (ABS) resins (2). Aside the additively incorporated TBBPA, excessive non-polymerized TBBPA is always present and can be emitted from a product in which TBBPA has been used as a reactive flame retardant (1); hence contaminating the environment. When released, TBBPA is probably associated with particulate matter due to its low vapour pressure (6.24×10^{-6} Pa) and low water solubility (63 μg L^{-1}) (4). TBBPA has been reported in several environmental compartments including air (3-7); dust (4, 8-13); sewage sludge and sediment (14-17); human serum (18, 19); egg (20); fish (Granby and Cederberg 2007); e-waste polymers (21, 22); water (23, 24); and wastewater (25). Toxicity studies have shown TBPPA to be associated with developmental defects and interference with thyroid hormones (1). TBBPA has also been identified as an endocrine disruptor, and has the potential to bind to human transthyretin and cause immunotoxicity (4).

Several studies have shown the significance of dust ingestion as an exposure pathway for humans to TBBPA, particularly for toddlers (38, 4, 12). Studies have revealed dust to contribute 34% and 90% of the mean overall exposure for adults and toddlers respectively (38). This is in harmony with the 100 – 1000 fold increases in dust concentrations of TBBPA in an office dust of a new building sampled over a year (Batterman et al., 2008). Despite the increases in TBBPA concentrations and the high levels of TBBPA found recently in dust from internet cafés (9), UK classrooms (38); UK cars, homes, public microenvironments, offices and classrooms (4), the fate of this brominated flame retardant is not well understood (Batterman et al., 2008). Data on the presence of TBBPA in indoor dust is scarce. There are currently no data on TBBPA indoor concentrations in Africa; and only a few studies have reported TBBPA in dust from indoor microenvironments worldwide. From the foregoing, the aim of the present study was to determine the concentrations of TBBPA in dust from automobiles, homes, offices and University students' computer laboratories, to compare TBBPA concentrations in various countries and also, to estimate human daily exposure doses of TBBPA among three typical population groups (toddlers, teenagers and adults) through dust ingestion; to further understand the human exposure magnitude of TBBPA, in line with past evidence of continual increases in TBBPA dust concentrations.

5.2 Experimental

5.2.1 Materials and chemicals

A 100 mg of 3,3',5,5'–tetrabromobisphenol A analytical standard was purchased from Sigma-Aldrich, South Africa. Florisil PR 60 -100 mesh was from Floridin Co., USA. The standard reference material (SRM 2585: Organic contaminants in house dust) was purchased from the National Institute of Standards and Technology (NIST), Gaithsburg, USA. Anhydrous sodium sulfate was from Associated Chemical Enterprises (ACE), Johannesburg, South Africa, BSTFA was obtained from Sigma Aldrich, South Africa. A Restek Rtx®–1614 fused silica (5% diphenyl 95% dimethyl polysiloxane) capillary column was obtained as a generous gift from Restek Corporation, Bellefonte, PA, USA. All solvents were high performance liquid chromatography grade obtained from Sigma Aldrich, South Africa.

5.2.2 Sampling

A total of 36 dust samples were collected from homes, n = 7, university students' computer laboratories, n = 6, and university staff offices, n = 9, between August and October 2012 in Durban, South Africa. Similarly, dust samples, n = 14, were collected between January and March, 2013 from personal and previously owned automobiles available for resale. The previously owned automobiles were sampled at a dealership in Durban, South Africa. All automobiles from the dealership had been through a thorough cleaning process on arrival at the dealership prior to resale. Similarly, personal automobiles sampled had not undergone any form of cleaning at least three days before sampling. Computer laboratory and office samples were collected with a LG 1600 W vacuum cleaner following the description of Harrad et al. (39) whilst a MoTo Quip super wet and dry auto vacuum cleaner was used for sampling automobiles. The vacuum cleaners contained a dust unit which could easily be removed and emptied after each collection. Between each collection it was cleaned with a disposable cloth wetted with *iso*-propanol. Samples from homes were obtained from the vacuum cleaner bags of each home collected under normal home use conditions as they reflect recently collected dusts, and thereby provide an estimate of residential exposure to TBBPA contamination. Samples were stored in amber glass bottles at -10 °C until analysis. Detailed questionnaires were used to obtain pertinent information on homes, offices and computer laboratories. This information included location, time since floor was last vacuumed, type of ventilation and flooring, and the number and types of electronic/electrical devices and furniture. Interviews were also conducted to obtain further information on building ages and to determine if, and when, any renovations were carried out. Details of automobiles such as manufacturer, model year and interior characteristics were obtained via questionnaires (Supplementary Material Table 5.1).

5.2.3 Extraction and Clean-up

Non-dust particles, hair and debris were hand-picked from all samples. Samples were homogenized by sieving through a 212 μm stainless steel sieve. For dust extraction,

approximately 0.8 g of dust sample was quantitatively weighed into a glass test tube. A volume of 10 mL *n*-hexane:methanol (1:3 v/v) was added. Samples were mixed in an orbital shaker for 10 mins and then extracted in an ultrasonic water bath at 40 °C for 30 mins. The mixing and extraction was repeated for a second time without addition of fresh solvent. The samples were then centrifuged at 3500 rpm for 10 mins and the supernatants were stored at <4 °C prior to clean-up. Florisil (PR 60 to 100 mesh) was activated at 130 °C for 16 hours and anhydrous sodium sulfate was baked at 450 °C for 5 hours before use. Florisil and anhydrous sodium sulfate were subsequently cooled in a desiccator. A 30 cm × 1 cm glass column was packed with 3 g of Florisil. Each column was topped with 0.8 g of anhydrous sodium sulfate and then wetted with 30 mL of the extraction solvent. Extracts were loaded onto columns just before the exposure of the sodium sulfate layer. Columns were eluted with 30 mL diethyl ether/*n*-hexane (6:94 v/v). Eluates were reduced and concentrated in a rotary evaporator to approximately 250 µL and stored in 1.5 mL amber glass GC/MS vials. All extracts were stored at <4 °C prior to further chemical analysis.

5.2.4 Derivatization

The sample extracts were derivatised as follows: 50 µL BSTFA was added to 25 µL sample extract in a glass vial and mixture was reacted for 60 mins at 70 °C. The volume of the reaction mixture was adjusted to 75 µL with n-hexane containing 140 ng PCB 209 as internal standard. Extracts were then ready for GC-MS analysis without further treatment. Results from GC-MS analysis were further confirmed with LC-ESI (negative) MS/MS for representative samples. Details of LC-ESI (-ve) MS/ MS can be found elsewhere (see Chapter 4, Section 4.2.7).

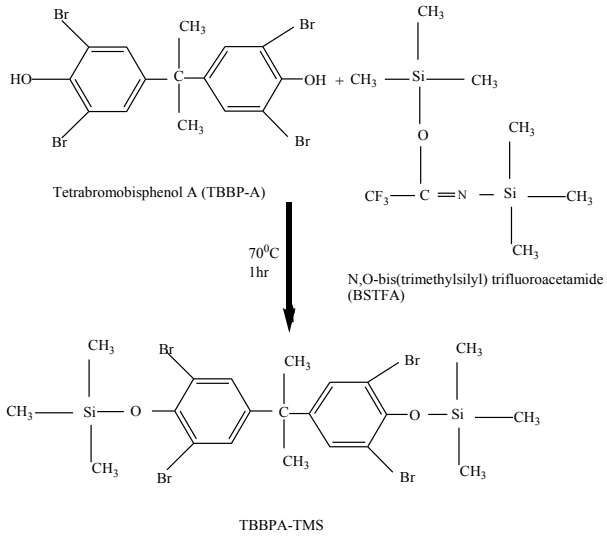

Figure 5.1 Derivitization of TBBPA with BSTFA.

5.2.5 GC-EI/MS Analysis

An Agilent 6890 GC fitted with a Restek Rtx®–1614 fused silica (5% diphenyl, 95% dimethyl polysiloxane) capillary column (15 m × 250 µm × 0.1 µm) coupled to an

Agilent 5973N series mass spectrometer was used for the separation, detection and quantitation of TBBPA. Injections were made in the pulsed splitless mode with the injector temperature set at 250 ºC. The Injection volume was 2 µL. The GC oven temperature programme started at 50 ºC (held for 1 min), then increased at 30 ºC min^{-1} to 280 ºC and was held there for 2 mins. Helium was used as carrier gas at a flow rate of 1.2 mL min^{-1} and a constant linear velocity of 56 cm s^{-1}. For the MS, the ion source and transfer line temperatures were 230 ºC and 350 ºC, respectively; and the ionization energy was 70 eV. TBBPA mass spectra were obtained in full scan mode.

Quantitation was carried out by means of a multiple point internal standard method. TBBPA was quantified by using PCB-209 as an internal standard. The response factor was determined from the slope of a plot of the ratio of peak areas against the ratio of the concentrations. The values for the plots were obtained from a 5 point triplicate analysis of the TBBPA standard solution diluted to fall within a concentration range of 10 – 10000 ng mL^{-1} TBBPA.

5.2.6 Recovery Experiment

Recoveries for TBBPA in dust were determined from a spiked standard reference material (SRM 2585 - Organic contaminants in house dust) as well as anhydrous sodium sulfate that had been spiked with TBBPA. A mass of 0.8 g SRM 2585 or anhydrous sodium sulfate was spiked separately with 250, 700, 10000 ng of TBBPA in triplicate. Samples were left to stand for at least 21 days at -10 °C. Spiked samples were extracted, cleaned-up and derivatised following the procedure for real samples.

5.2.7 Quality control

Method blanks were analysed with every batch of five samples. For the method blank, dust samples were replaced with anhydrous sodium sulfate and passed through all the analytical procedure carried out for real samples. The TBBPA concentration in the blanks was less than 3% of TBBPA in all samples with concentrations above LOD. Hence, samples were not blank corrected for TBBPA concentrations. Field blank samples (n = 3) were obtained by spreading anhydrous sodium sulfate on a pre-cleaned tiled floor. The floor was vacuumed following the same sampling protocol as for real samples and the sample subjected to the analytical procedure. Solvent blanks were injected after the analysis of at most three samples. For quality assurance, a spiked standard reference material (NIST SRM 2585 - Organic contaminants in house dust) was analysed. Table 5.1 shows the analytical method validation criteria for TBBPA.
All glassware was cleaned with laboratory wash solutions, rinsed with distilled water and then with organic solvents. Non-volumetric glassware was oven-dried prior to use. Direct ultraviolet light and plasticware was avoided throughout the analysis.

5.2.8 Statistics

Data were log normally transformed with SIMCA version 13.0 software. Descriptive statistics such as sum, mean, median, minimum, maximum, T-test and analysis of

variance (ANOVA) were calculated by using Microsoft Excel® 2010. Limits of detection (LOD) and quantitation (LOQ) were estimated following Thomsen et al. (26). Samples below the detection limit were treated as zero throughout the statistical analysis.

Table 5.1 GC–MS method validation for TBBPA.

Parameter	GC- MS
Linear range/ ng mL^{-1}	100 – 10000
R^2	0.975
LOD/ ng g^{-1}	0.35
LOQ/ ng g^{-1}	1.15
Accuracy /% Recovery	93.79 – 95.63[a]
Precision [Repeatability of peak area (n=3)]	
Intra –day (% RSD)	0.86
Inter – day (% RSD)	10.11

[a]Spiked concentrations were 250, 700 and 10000 ng g^{-1}

5.3 Results and discussion

Levels of TBBPA and subsequent human exposure, and the relationship between TBBPA concentrations in the indoor environment and household items, are reported in subsequent sections.

5.3.1 TBBPA contamination in various microenvironments

TBBPA was detected above the LOQ in 71% of homes, 86% of offices, 86% of automobiles and 100% of the computer laboratory samples. The statistical characteristics of TBBPA in the various microenvironments are summarized in Table 5.2. Median concentrations of TBBPA were 120 ng g^{-1}, 492 ng g^{-1}, 1157 ng g^{-1} and 268 ng g^{-1} in dust samples collected from homes, offices, automobiles and the students' computer laboratories, respectively. A single factor ANOVA showed no statistical difference among the concentrations of TBBPA in the various microenvironments. However, TBBPA concentrations in automobiles were highest compared to other studied microenvironments. TBBPA concentrations in automobiles correlated with the concentrations in the other microenvironments; as did concentrations in offices and computer laboratories. This could suggest similar sources of TBBPA in the studied microenvironments. Although, limited literature are available on the levels of TBBPA in indoor dust; the concentrations of TBBPA in automobiles in this study were higher than levels reported in United Kingdom cars (4). In terms of automobile make, TBBPA was generally more prevalent in automobiles made by Toyota, although the highest

concentration of TBBPA was found in a car manufactured by Honda (Supplementary Material Table S5.2.)

Table 5.2 Statistical description of TBBPA concentrations in indoor dust of the studied microenvironments.

Location	Statistical Parameter	TBBPA Concentration/ng g^{-1}
Cars (n = 14)	Average	1365
	Median	1157
	Minimum	ND
	Maximum	4578
	5th percentile	ND
	95th percentile	3368
Homes (n = 7)	Average	815
	Median	120
	Minimum	ND
	Maximum	3767
	5th percentile	ND
	95th percentile	3074
Computer laboratories (n = 6)	Average	321
	Median	268
	Minimum	71
	Maximum	709
	5th percentile	85
	95th percentile	661
Offices (n = 9)	Average	729
	Median	492
	Minimum	ND
	Maximum	2063
	5th percentile	30
	95th percentile	1901

ND – not detected

However, because of the small sample size, these observations are inconclusive. Higher TBBPA concentrations were found in automobiles available for resale in an auto dealership and in a personal car that was not cleaned over a 14-day period prior to sampling. With respect to automobile ages, no particular order was observed for TBBPA concentrations; this could imply that external contamination sources may contribute largely to the high levels of TBBPA found in these automobiles. The use of TBBPA as an additive flame retardant in the manufacture of ABS resins and HIPs (2), may account for the high levels of TBBPA in these automobiles since ABS resins are used in

automotive trim components and automotive bumper bars, as well as in automobile stereos, speakers and radios. As an additive flame retardant, TBBPA does not react chemically with the other components of the polymer, and hence may leach out of the polymer matrix after incorporation.

Contrary to the levels of TBBPA in automobiles, the median concentrations of TBBPA in homes (n = 7) fell within the range of internationally reported levels (4, 8, 10, 27) (see Table 5.3). Compared to the concentrations of octaBDE in an earlier study (Abafe and Martincigh, 2014, see Chapter 6); the median concentrations of TBBPA were three times higher than those of BDE-183. This trend was not unexpected as TBBPA has replaced octaBDE commercial formulations (2), and 18% of the total TBBPA produced is used as an additive flame retardant in HIPs and ABS resins used in plastic casings of electronic equipment. However, no statistical correlation existed between the TBBPA and BDE-183 concentrations in homes; this could imply different sources of contamination for this class of indoor microenvironment. The median concentrations of TBBPA in office dusts were seven and 14 times higher than levels reported in Flanders, Belgium (13) and Birmingham, United Kingdom (4), respectively.

Table 5.3 Comparison of TBBPA indoor dust concentrations in the present study and literature data.

Indoor Microenvironment	Number of samples	Median concentrations or range (ng g^{-1})	Location	Reference
Computer laboratories	6	268.44	Durban, South Africa	This study
Personal homes	7	120.02	Durban, South Africa	This study
Personal homes	20	ND – 470	Germany	(8)
Personal homes	45	62	Birmingham, U.K.	(4)
Personal homes	4	ND – 1728	Philippines	(9)
Personal homes	45	11.7	Flanders, Belgium	(13)
Personal homes	18	10 (1-1480)	Belgium	(10)
Personal homes	2	490 – 520	Japan	(27)
Offices	9	492.22	Durban, South Africa	This study
Offices	2	45 – 100	Belgium	(10)
Offices	10	70.4	Flanders, Belgium	(13)
Offices	28	36	Birmingham, U.K.	(4)
Automobiles	14	1156.88	Durban, South Africa	This study
Cars	20	2	Birmingham, U.K.	(4)
Child daycare centers and primary schools	43	17 – 1400	West Midlands, U.K.	(12)
Public microenvironment	4	230	Birmingham, U.K.	(4)

However, care must be taken in comparing these data; as the samples here were collected only recently, between August 2012 and February 2013, i.e. over 9 years after the replacement of commercial octaBDE with TBBPA in consumer products. Hence, higher levels of TBBPA are not unlikely in these samples compared with samples collected only a few years after the ban of commercial octaBDE formulations, as in the case of the work of Abdallah et al. (4) and D'Hollander et al. (13). The TBBPA concentrations in these offices were similar to levels reported in PCs, TVs and printers by Ali et al. (28), but lower than levels reported in dust from surface covers of electronic equipment (29). The high TBBPA concentrations in dust from two of the sampled homes and offices might be associated with indoor characteristics such as ventilation patterns, frequency of cleaning, house use pattern, floor type as well as number and ages of electronic equipment present in these microenvironments (Supplementary Table S5.2). The median concentration of TBBPA in students' computer laboratories was 268 ng g^{-1} (range 71 –

709). The detection frequency of TBBPA was highest in this microenvironment, but with the lowest abundance of TBBPA compared with the other microenvironments. The concentrations of TBBPA in this microenvironment typifies those reported in two Japanese homes (27), and those reported in Belgian homes and offices (13). These TBBPA concentrations were much lower than levels reported by Espino and Leon (9) in internet cafes from the Philippines. This was contrary to our expectations of higher TBBPA concentrations in the computer laboratories, because of the additive usage of TBBPA in the production of HIPs and ABS resins with applications in computer and television casings which characterize computer laboratories; and the fact that very high levels of TBBPA in indoor dust were reported for electronic waste recycling sites in a previous study (Abafe and Martincigh, 2014, see Chapter 4), and in dust from inside computers by (30). The low levels of TBBPA in this microenvironment were linked to the frequency of cleaning of the indoor environment; as these computer laboratories undergo biweekly vacuum cleaning, bearing in mind that dust does not only act as a receptor but also a concentrator of organic contaminants such as TBBPA (31-34). Hence, frequent household cleaning influences indoor TBBPA concentrations.

5.3.2 Estimated Daily Exposure to TBBPA via Dust Ingestion

In evaluating human exposure via dust ingestion of contaminants, we assumed 100% absorption of TBBPA from ingested dust in accordance with other studies (31, 34, 35). Average dust intake rates of 20 and 50 mg d^{-1} and high dust intakes of 50 and 200 mg d^{-1} for adults, teenagers and toddlers were used as reported by Ali et al. (34). Body weights of 70 kg and 12 kg were used for adults and toddlers respectively (34), and 52 kg for teenagers (36). Questionnaires were used to obtain the average number of hours spent per day by adults in offices, cars and homes. The average numbers of hour students (teenagers in this work) spend in computer laboratories (for lectures, studies and assignments) and in their residences per day were obtained by interview. The amount of the time spent per day in homes by toddlers (79.9 %) was the same as that of Ali et al. (35). Thus, the average time an adult spends in the office, car and home were taken as 33.3%, 4.2%, and 62.5%, respectively. For full-time undergraduate students (teenagers) it was estimated that they spend 54.2% and 45.83% of their time indoors in classrooms and in their residences respectively. Different exposure scenarios were calculated by using the 5th percentile, median, mean and 95th percentile concentrations from home, car, office and computer laboratory dusts. Thus, the daily exposure dose of contaminants (ΣDED TBBPA/ng kg^{-1} bw day^{-1}) via dust ingestion were calculated from the following equations reported by Ali et al. (34) with modifications:

For adult exposure estimation,

$$\Sigma DED/ng\ kg^{-1}\ bw\ day^{-1} = [(C_{HD}F_H) + (C_{ofD}F_{of}) + (C_{ATD}F_{ATD})]\ DIR/BW,$$

For teenager exposure assessment

$$\Sigma DED/ng\ kg^{-1}\ bw\ day^{-1} = [(C_{HD}F_H) + (C_{LD}F_L) + (C_{ATD}F_{ATD})]\ DIR/BW, \text{ and}$$

For toddlers exposure assessment,

$\Sigma DED/ng\ kg^{-1}\ bw\ day^{-1} = [(C_{HD}F_H) + (C_{ATD}F_{ATD})]\ DIR/BW$

where $C_{ATD,}$ $C_{HD,}$ C_{ofD} and C_{LD} are dust concentrations in automobiles, homes, offices and computer laboratories (5^{th} percentile, median, mean and 95^{th} percentile) and F_{ATD}, $F_{H,}$ F_{of} and F_L are the fraction of time spent in automobiles, homes, offices and computer laboratories, respectively. DIR is the dust intake rate and BW is the body weight.

Fig 5.2 shows the estimated daily exposure doses of TBBPA for adults, teenagers and toddlers in South Africa. The daily exposure doses of TBBPA via dust ingestion are of particular concern for the South African population. Considering an average dust intake rate for adults, teenagers and toddlers; and by using the median TBBPA concentrations, the $\Sigma DED/ng\ kg^{-1}\ bw\ day^{-1}$ of TBBPA are 0.08, 0.08 and 0.60, respectively for adults, teenagers and toddlers. These concentrations are well above the average dietary intake of 0.04 $ng\ kg^{-1}\ bw\ day^{-1}$ of TBBPA by the Dutch population (37). These results are in line with the observations of Abdallah et al. (4); implicating dust ingestion as the main pathway of exposure to TBBPA for UK toddlers. In the same light, our data show the relevance of dust ingestion as a major pathway of exposure to TBBPA by all population groups – adults, teenagers and toddlers. Despite the concern expressed for the South African population, it must be noted that TBBPA was detected in only few Dutch food samples, hence the dietary intake for the Dutch population is an estimate. Although, the daily exposure doses of TBBPA are far lower than the tolerable daily intake recommended (10^6 $ng\ kg^{-1}\ bw\ day^{-1}$) by the UK committee for toxicity (12); the importance of dust as a pathway of human exposure to TBBPA cannot be overlooked. Table 5.4 compares the daily exposure doses of TBBPA within the South African population with those from other parts of the world.

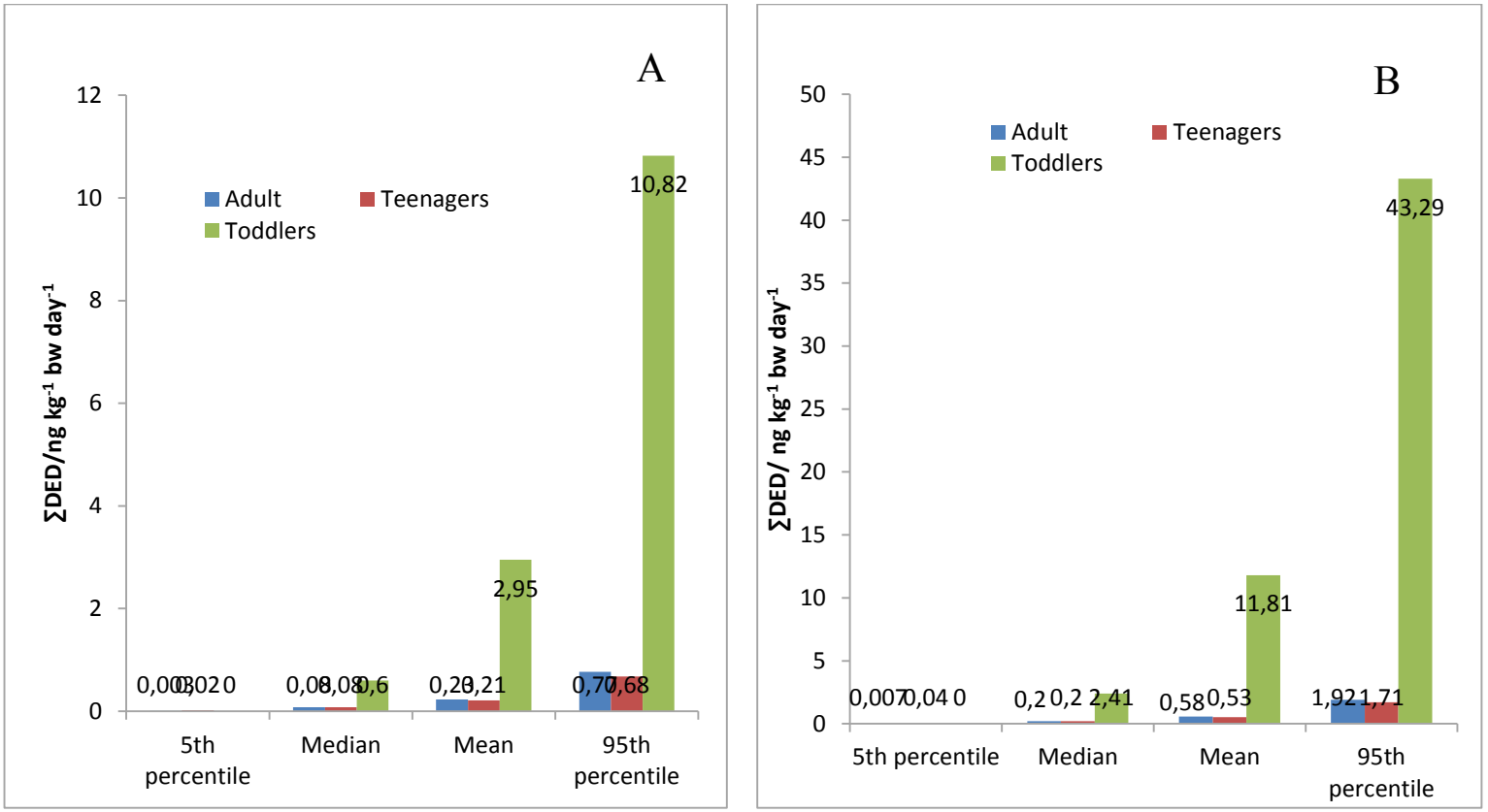

Figure 5.2 Estimated daily exposure doses (in ng kg^{-1} bw day^{-1}) of TBBPA for adults, teenagers and toddlers in South Africa: (A) assuming mean dust intake rates and (B) assuming high dust intake rate

Table 5.4 Comparison of human exposure magnitudes to TBBPA (in ng kg^{-1} bw day^{-1}) for various Countries.

Population group	Country	Microenvironment	Intake rates	5th percentile exposure	Average exposure	Median exposure	95th percentile exposure	References
Adult	South Africa	Automobile, homes and offices	Mean	0.003	0.23	0.08	0.77	This study
			High	0.007	0.58	0.20	1.92	This study
Teenager	South Africa	Homes and computer laboratories/ classrooms	Mean	0.02	0.21	0.08	0.68	This study
			High	0.04	0.53	0.20	1.71	This study
Toddler	South Africa	Automobile and homes	Mean	0.00	2.95	0.60	10.82	This study
			High	0.00	11.81	2.41	43.29	This study
Adult	United Kingdom	Car, home, office, outdoor and public microenvironment	Mean	0.006	0.02	0.02	0.05	(4)
			High	0.02	0.06	0.04	0.12	(4)

Toddler	United Kingdom	Car, home, outdoors and public microenvironment	Mean	0.13	0.44	0.33	0.85	(4)
			High	0.52	1.80	1.36	3.49	(4)
Child	United Kingdom	Car, classroom and house	Mean	0.05		0.17		(12)
			High				2.3	(12)
Child	United Kingdom	Classroom	Mean			0.06		(12)
Adult	United Kingdom	Office	Mean			0.002		(12)
Toddler	Germany	Home				0.2		(8)

Despite the apparent ease of release of TBBPA from treated products, human daily exposure doses of TBBPA are comparable to those of PBDEs, particularly for toddlers (Abafe and Martincigh, 2014, see Chapter 6). It is likely that TBBPA levels in dust will continue to increase all through the useful life of TBBPA-treated products due to incomplete polymerization, and unbound TBBPA in polymer matrices.

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Supplementary Materials

Table S5.1 Indoor characteristics of the various microenvironments

Location	TBBPA Concn. /ng g^{-1}	Manufacture/ construction year	Floor type	Automobile model and manufacturer	Automobile condition
automobile 01	0	2009	Carpet	Honda jazz	Personal
automobile 02	0	2012	Carpet	Toyota corolla	Personal
automobile 03	264.86	2012	Carpet	Audi A4	Personal
automobile 04	154.24	2009	Carpet	Honda jazz	Personal
automobile 05	2576.66	2011	Carpet	Kia rio	Personal
automobile 06	1557.57	2010	Carpet	Peugeots 406	Resale
automobile 07	670.92	2010	Carpet	Audi A6	Resale
automobile 08	4578.25	2010	Carpet	Turbo corsa	Resale
automobile 09	1904.23	1999	Carpet	Honda prelude	Resale
automobile 10	647.41	2008	Carpet	Chevrolet	Personal
automobile 11	1723.81	INB	Carpet	Toyota starlet	Personal
automobile 12	1194.57	1998	Carpet	Mazda 63	Personal
automobile 13	1117.18	1989	Carpet	Toyota corolla	Personal
automobile 14	2717.04	2007	Carpet	Toyota corolla	Personal
Home 1	0	1978	Carpet		
Home 4	1456.71	QNR	QNR		
Home 6	0	QNR	QNR		
Home 4	120.02	1991	tiled		
Home 8	3766.87	1973	Carpet and wooden floor		
Home 9	291.38	QNR	QNR		
Home 10	68.69	1981	tiled		
Computer lab 1	110.84	INB	tiled		
Computer lab 2	185.41	INB	tiled		
Computer lab 3	194.36	INB	tiled		
Computer lab 4	70.58	INB	tiled		

Computer lab 5	379.86	INB	tiled		
Computer lab 6	572.55	INB	tiled		
Office 1	120.03	Early 1970s	Carpet		
Office 2	1522.39	Early 1970s	Carpet		
Office 3	492.22	Early 1970s	Carpet		
Office 4	0	Early 1970s	Carpet		
Office 5	275.65	Early 1970s	Carpet		
Office 6	2062.92	Early 1970s	Carpet		
Office 7	632.57	Early 1970s	Carpet		
Office 8	708.6	Early 1970s	Carpet		
Office 9	342.51	Early 1970s	Carpet		

Table S5.2: Parametric statistics of TBBPA in indoor microenvironments

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	11	14077.95	1279.8136	1956925
Column 2	7	5703.67	814.81	1961322
Column 3	8	2564.71	320.58875	51248.1
Column 4	7	5105.78	729.39714	596997.4

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4373999	3	1457999.6	1.19854	0.32775	2.93402989
Within Groups	35277901	29	1216479.4			
Total	39651900	32				

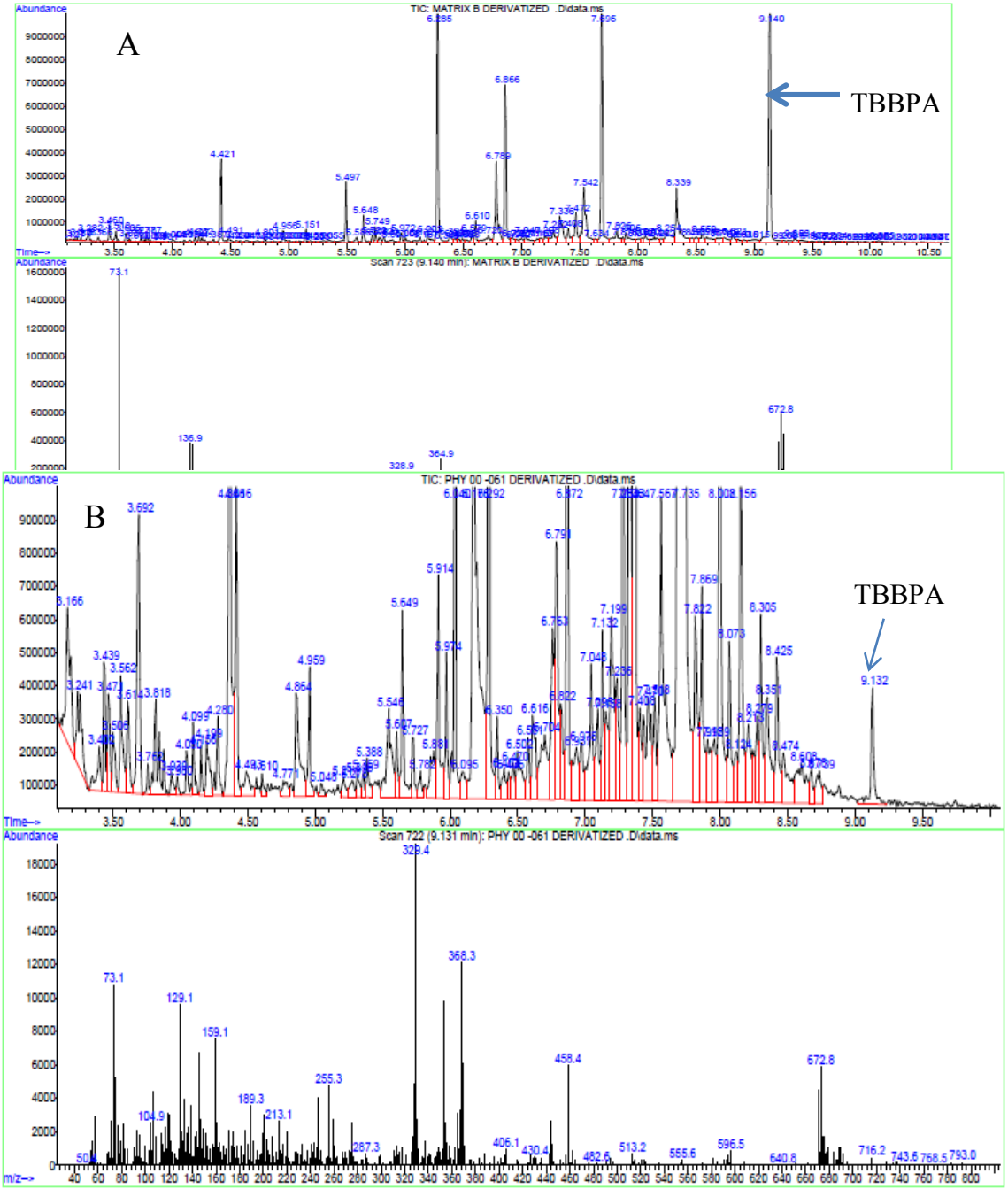

Figure S5.1. GC chromatogram and mass spectrum of TBBPA in (A) Spiked SRM 2585 and (B) an office dust sample.

Chapter 6

Polybrominated diphenyl ethers and polychlorinated biphenyls in indoor dust in Durban, South Africa

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ABSTRACT

Polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) were measured in indoor dust of three microenvironments in Durban, South Africa. The sum of eight PBDEs and three PCBs were quantified by gas chromatography with mass spectral detection. The mean concentrations of $\sum_{n=8}$ PBDEs and $\sum_{n=3}$ PCBs in 10 homes, 11 offices and 13 university students' computer laboratories were 1714, 1523, 818 ng g^{-1} and 891, 923, 1877 ng g^{-1} for PBDEs and PCBs, respectively. The concentration of PCBs found in homes were independent (p = 0.0625) of building construction year. Similarly, no relationship was observed between PCB concentrations and floor type. The concentrations of PBDEs correlated (r = 0.60) with PCB concentrations in homes, thus assuming similar sources. The elevated concentrations of PBDEs and PCBs may have significant implications for human exposure.

Keywords: PBDEs, PCBs, Indoor dust, Sources, Human exposure, Correlation

Practical Implications

This study provides vital data on the concentrations of the environmentally most abundant PCBs and PBDEs in three indoor microenvironments, namely, homes, offices and university students' computer laboratories, in Durban, South Africa. Potential sources of these contaminants were established in the various indoor environments. These data can be used to establish suitable legislation, and also for risk assessment and management of these ubiquitous persistent organic pollutants.

6.1 Introduction

The considerable length of time we spend indoors daily in homes, offices, schools, day-care centres and computer rooms, amongst other possible indoor locales, avails us ample opportunity for exposure to chemical contaminants such as polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs). For instance, PBDE-treated products, and abrasion, alongside evaporation of PCBs in building materials in homes and public buildings constructed prior to 1977, are a reservoir of PBDEs and PCBs in the indoor environment (Takigami et al., 2009). Recent studies have reported elevated concentrations of PBDEs in indoor dust from several countries: Japan (Takigami et al., 2009), United Kingdom (Harrad et al., 2008a), China (Yu et al., 2012), Sweden (de Wit et al., 2012), Belgium (D'Hollander et al., 2010), Philippines (Fulong and Espino, 2013), Germany (Sjödin et al., 2008; Fromme et al., 2014), Pakistan and Kuwait (Ali et al., 2013). Although few reports are available on indoor exposure to PCBs, some authors have reported possible PCB contamination in the indoor environment in several locations, including, Wisconsin, USA, (Knobeloch et al., 2012), California, USA (Whitehead et al., 2012), Boston, USA (Herrick et al., 2004), Switzerland (Kohler et al., 2005), and China (Xing et al., 2011).

Much attention has been focussed recently on the significance of indoor dust as a pathway of human exposure to PBDEs and other brominated flame retardants (BFRs) (Harrad et al., 2008a; Abdallah et al., 2009; Harrad and Abdallah, 2011). The relationship between dust and human body burdens is strongly implied by the correlation of PBDEs in household dusts and human milk (Wu et al., 2007), and dusts and human blood (Fischer et al., 2006).

Not much is known on the production, use, distribution and fate of PBDEs and PCBs in South Africa. However, recent studies have reported PBDEs in sediments (Olukunle et al., 2012; La Guardia et al., 2013), indoor dust from Pretoria (Kefeni et al., 2011; Kefeni and Okonkwo, 2012; Kefeni and Okonkwo, 2014; Kefeni et al., 2014), landfills (Odusanya et al., 2009), sewage sludge and wastewater effluent (Daso et al., 2012), bird eggs (Polder et al., 2008) and human breast milk (Darnerud et al., 2011). While PCBs were never produced in South Africa, PCB oils and equipment containing PCB oils were imported mainly for electricity generation (South Africa's Plan for the Implementation of the Stockholm Convention on Persistent Organic Pollutants, 2011). PCBs have been reported in water and fish tissues from the Isipingo Estuary (Grobler et al., 1996), outdoor air, soil and milk in KwaZulu-Natal, South Africa (Batterman et al., 2009a), soil and sediment samples from the industrialised Vaal Triangle region (Quinn et al., 2009) and human breast milk from Limpopo Province, South Africa (Darnerud et al., 2011). No published work is available on indoor PCB contamination in South Africa, and indeed the African continent. Furthermore, despite the increasing proof of the significant implications of indoor dusts for human exposure to PBDEs and PCBs, attempts to link indoor contaminants with probable source items has had limited success. A dearth of

information also exists for human exposure pathways to PBDEs and PCBs in Africa and other developing countries of the world.

To breach these research gaps, we seek to provide:
(1) A first report of PCB levels in indoor dust and to extend the range of microenvironments examined for PBDE contamination to include homes, offices and university students' computer laboratories in South Africa, and
(2) To study the relationship between PBDE and PCB levels and their probable sources in these indoor microenvironments.

6.2 Materials and methods

6.2.1 Chemicals

Method 1614 Native PAR PBDE stock solution [(1 μg mL^{-1} 2,4,4′-tribromodiphenyl ether, BDE-28; 2,2′,4,4′-tetrabromodiphenyl ether, BDE-47; 2,2,4,4,5′-pentabromodiphenyl ether, BDE-99; 2,2,4,4,6′ pentabromodiphenyl ether, BDE-100; 2,2,4,4,5,5-hexabromodiphenyl ether, BDE-153; 2,2,4,4,5,6-hexabromodiphenyl ether, BDE-154; 2,2′,3,4,4′,5,6′-heptabromodiphenyl ether, BDE-183) and (10 μg mL^{-1} 2,2′,3,3′,4,4′,5,5′,6,6′-decabromodiphenyl ether, BDE-209)] was received as a kind donation from Cambridge Isotope Laboratories, Andover, MA, USA. 2,4,4′-Trichlorobiphenyl (PCB-28); 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB-153); 2,2′,3,4,4′,5,5′-heptachlorobiphenyl (PCB-180) and decachlorobiphenyl (PCB-209) were purchased from Sigma-Aldrich, South Africa. $^{13}C_{12}$-labelled decachlorobiphenyl ($^{13}C_{12}$ PCB-209) was obtained from Wellington Laboratories, Guelph, Ontario, Canada. Silica gel 90 was from Sigma-Aldrich and Florisil PR 60-100 mesh was from Floridin Co., USA. The standard reference material (SRM 2585: Organic contaminants in house dust) was purchased from the National Institute of Standards and Technology (NIST). Anhydrous sodium sulfate was from Associated Chemical Enterprises (ACE), Johannesburg, South Africa. A Rtx® – 1614 fused silica (5% diphenyl, 95% dimethyl polysiloxane) capillary column was obtained as a generous gift from Restek Corporation, Bellefonte, PA, USA. All solvents were high performance liquid chromatography or pesticide grade obtained from Sigma Aldrich, South Africa.

6.2.2 Sampling

A total of 34 dust samples were collected from homes, n = 10, university students' computer laboratories, n = 13, and university staff offices, n = 11, between August and October 2012 in Durban, South Africa. Computer laboratory and office samples were collected with a LG 1600 W vacuum cleaner following the description of Harrad et al. (2008a). The vacuum cleaner contained a dust unit which could easily be removed and emptied after each collection. Between each collection it was cleaned with a disposable cloth wetted with *iso*-propanol. Samples from homes were obtained from the vacuum cleaner bags of each home collected under normal home use conditions as they reflect

recently collected dusts, and thereby provide an estimate of residential exposure to PBDE and PCB contamination. Samples were stored in amber glass bottles at -10 °C until analysis. Detailed questionnaires were used to obtain pertinent information on homes, offices and computer laboratories. This information included location, time since floor was last vacuumed, type of ventilation and flooring, and the number and types of electronic/electrical devices and furniture. Interviews were also conducted to obtain further information on building ages and to determine if, and when, any renovations were carried out. These data were used to relate PBDE and PCB concentrations to potential sources.

6.2.3 Extraction and clean-up

Non-dust particles, hair and debris were hand-picked from all samples. Samples were homogenized by sieving through a 212 μm stainless steel sieve. Dusts were analysed following the United States Environmental Protection Agency (US EPA) methods 3550c, 3620c, 1614 and 1668a with modifications. Briefly, approximately 0.8 g of sample was quantitatively weighed into a glass test tube and spiked with 50 ng PCB-209 as the internal standard. A volume of 10 mL *n*-hexane:methanol (1:3 v/v) was added. Samples were mixed in an orbital shaker for 10 mins and then extracted in an ultrasonic water bath at 40 °C for 30 mins. The mixing and extraction was repeated for a second time without addition of fresh solvent. The samples were then centrifuged at 3500 rpm for 10 mins and the supernatants were stored at <4 °C prior to clean-up. Silica gel 90 and Florisil (PR 60 to 100 mesh) were activated at 130 °C for 16 hours and anhydrous sodium sulfate was baked at 450 °C for 5 hours before use. Silica gel, Florisil and anhydrous sodium sulfate were subsequently cooled in a desiccator. A 30 cm x 1 cm glass column was packed with either 3 g of silica gel or 3 g of Florisil. Each column was topped with 0.8 g of anhydrous sodium sulfate and then wetted with 30 mL of the extraction solvent. Extracts were loaded onto columns just before the exposure of the sodium sulfate layer. PBDEs were eluted on silica columns with 25 mL *n*-hexane. This was kept as Fraction 1 and contained essentially BDE-209. The columns were further eluted with 30 mL of diethyl ether/*n*-hexane (50:50 v/v), and kept as Fraction 2. The column flow rates were maintained at 0.5 mL min^{-1}. All fractions were reduced to approximately 250 μL in a rotary evaporator at 55 °C. PCBs were fractionated and cleaned-up with Florisil column chromatography. Columns were eluted with 30 mL diethyl ether/*n*-hexane (6:94 v/v). Eluates were reduced and concentrated in a rotary evaporator to approximately 250 μL and stored in 1.5 mL amber glass GC/MS vials. All extracts were stored at <4 °C until instrumental analysis.

6.2.4 Instrumental Analysis

PBDE analysis was performed on an Agilent 6890 (Palo Alto, CA, USA) gas chromatograph (GC), coupled to a 5973N series mass spectrometer (MS) operated in electron impact (EI) ionization mode. A Restek Rtx® – 1614 fused silica (5% diphenyl, 95% dimethyl polysiloxane) capillary column (15 m x 250 μm x 0.1 μm) was used to effect separation and the MS was operated in the selected ion monitoring (SIM) mode.

The injections were made in the pulsed splitless mode with the injector temperature set at 285 °C. The injection volume was 1 µL. The GC oven temperature programme started at 90 °C (held for 2 mins), then increased at 20 °C min^{-1} to 270 ºC, followed by 10 °C min^{-1} to 325 °C and held for 5 mins. Helium was employed as the carrier gas at a flow rate of 1.2 mL min^{-1} and a constant linear velocity of 58 cm s^{-1}. For the MS the ion source and transfer line temperatures were 230 °C and 350 °C, respectively, and the ionization energy was 70 eV. The molecular ions $[M]^+$ or $[M+2]^+$ and fragment ions $[M-Br_2]^+$ or $[M-Br_2+2]^+$ were monitored for tri- through hepta-BDEs. M/Z 400 and 800 were monitored for BDE-209. Data were acquired with ChemStation software. PCBs were analysed with the same GC-MS but this time fitted with a Restek 5MS (30.0 m x 250 µm x 0.25 µm) capillary column. A 1 µL volume of sample was injected in the pulsed splitless mode with helium as the carrier gas at a flow rate of 0.7 mL min^{-1} and a pulse pressure of 150 kPa for 1 min. The injector temperature was 250 °C. The GC oven conditions for the PCBs were: an initial temperature of 90 °C, held for 1 min, then increased at 30 °C min^{-1} to 280 °C and held at 280 °C for 10 mins. The MSD and interphase temperatures were 350 °C and 280 °C respectively. The GC column pressure was set at 36.6 kPa and the total column flow was 43.6 mL min^{-1}. The MS was operated in the SIM mode. The molecular ion $[M]^+$ was monitored for all PCB congeners.

Quantitation was carried out by means of a multiple point internal standard method. Unlabelled PCB-209 and $^{13}C_{12}$ labelled PCB-209 were employed as internal standards for PCBs and PBDEs respectively. The response factors were determined from the slope of a plot of the ratio of peak areas against the ratio of the concentrations. The values for the plots were obtained from a 5 – 6 point triplicate analysis of the PBDE standard solution diluted to fall within a concentration range of 5 – 500 ng mL^{-1} and 0.1 to 4 µg mL^{-1} for tri- to octa-BDEs and BDE-209, respectively; similarly PCB calibrations were made with pure PCB standards in a similar concentration range as the tri- to octa-BDEs.

6.2.5 Quality control

Method blanks were analysed with every batch of five samples. For the method blank, dust samples were replaced with anhydrous sodium sulfate and passed through all the analytical procedure carried out for real samples. Traces of PCB-180 were found in the method blank (n = 6), thus PCB-180 concentrations reported were corrected for the blank. Field blank samples were obtained (n = 3) by spreading anhydrous sodium sulfate on a pre-cleaned tiled floor. The floor was vacuumed following the same sampling protocol as for real samples and the sample subjected to the analytical procedure. Solvent blanks were injected after the analysis of at most three samples. For quality assurance, indoor dust reference material (NIST SRM 2585 - Organic contaminants in house dust) was analysed. As can be seen from the results presented in Table 1, the measured concentrations generally fell within the range of the certified values and the relative standard deviations were low indicating good precision of the method. The LOD and LOQ values ranged from 0.03 – 0.16 ng g^{-1} and 0.31 – 0.54 ng g^{-1} for the PBDEs and

from 0.03 – 0.08 ng g^{-1} and 0.10 – 0.26 ng g^{-1} for the PCBs respectively. All glassware was cleaned with laboratory wash solutions, rinsed with distilled water and then with organic solvents. Non-volumetric glassware was oven-dried prior to use. Direct ultraviolet light and plasticware was avoided throughout the analysis.

6.2.6 Statistics

The distribution of PBDE and PCB concentrations in the different microenvironments was tested with the Shapiro-Wilk test of normality. Non-parametric statistics such as Wilcoxon Signed-Ranks test, Kendall tau test and Spearman rank correlation were performed with Analyse-it® software in Microsoft Excel 2010. The Kruskal-Wallis test was employed to test for differences in location by using XLSTAT 2014 software. Limits of detection (LOD) and quantitation (LOQ) were estimated following Thomsen et al. (2003). Samples below the detection limit (<dl) were treated as zero throughout the statistical analysis.

Table 6.1 Results of the analysis of certified reference material (NIST SRM 2585 - Organic Contaminants in House Dust).

PBDE/PCB congener	Measured Concentrations (ng g^{-1})	Certified Concentrations (µg kg^{-1})	Percent Recovery (%)	Method Accuracy (%)	Method Precision (% RSD) (n = 4)
BDE-28	48.12 ± 0.1	46.90 ± 4.4	102.6	-2.6	0.2
BDE-47	480.0 ± 2.4	497.0 ± 46	96.6	3.4	0.5
BDE-99	854.0 ± 1.0	892.0 ± 53	95.7	4.3	0.1
BDE-100	152.3 ± 0.2	145.0 ± 11	105.0	-5.0	0.1
BDE-153	121.2 ± 0.1	119.0 ± 1	101.9	-1.9	0.1
BDE-154	92.74 ± 0.2	83.50 ± 2	111.1	-11.	0.2
BDE-183	43.99 ± 0.04	43.00 ± 3.5	102.3	-2.3	0.1
BDE-209	2806.1 ± 0.6	2510 ± 190	111.8	-12.	0.02
PCB-28	10.11 ± 0.02	13.4 ± 0.5	75.4	24.6	0.2
PCB-153	33.52 ± 0.1	40.2 ± 1.8	83.4	16.6	0.3
PCB-180	18.83 ± 0.1	18.4 ± 3.2	102.3	-2.3	0.5

6.3 Results and discussion

6.3.1 Levels of polychlorinated biphenyls in South African indoor dust

PCBs were detected in 9 of the 10 samples from homes, 12 of the 13 computer laboratory and 8 of the 11 office samples. Table 2 summarises the concentrations (in ng g^{-1}) of the three PCB congeners, namely PCB-28, PCB-153 and PCB-180, that were quantified in the dust samples collected from these three microenvironments. The full list of the concentrations measured in each of the samples is presented in the Supplementary Material Table S1. The sum of the three PCBs monitored (ΣPCB) showed the widest range for the computer laboratories (<dl to 19050 ng g^{-1} with a median of 360 ng g^{-1}) whilst the homes and offices showed similar ranges (<dl to 2196 ng g^{-1} with a median of

724 ng g^{-1} for homes, and <dl to 2053 ng g^{-1} with a median of 1036 ng g^{-1} for offices). One of the samples from the computer laboratories had an exceedingly large value. If that outlier value is not considered the range becomes <dl to 1559 ng g^{-1} with a mean of 446 ng g^{-1} and a median of 353 ng g^{-1}. These values are now comparable to those found for homes and offices. Analysis of variance ($p < 0.05$) showed no statistically significant difference in the ΣPCB from the three indoor microenvironments. All congeners were normally distributed ($p < 0.05$) in the computer laboratory but not in the home and office samples.

Table 6.2 Concentrations (in ng g^{-1}) of PCBs in dust samples from indoor microenvironments in Durban, South Africa.

Location	Statistical Parameter	PCB-28	PCB-153	PCB-180	ΣPCB
Homes	Mean	16.1	173	702	891
$n = 10$	Median	10.9	150	585	724
	Maximum	55.5	450	2050	2200
Computer	Mean	8.31	358	1510	1880
laboratories	Median	<dl	62.2	307	360
$n = 13$	Maximum	72.7	3560	15500	19100
Offices	Mean	64.4	170	689	923
$n = 11$	Median	28.3	136	812	1040
	Maximum	389	647	1530	2050

<dl denotes below detection limit

The most abundant congener of the three measured PCBs in all samples was PCB-180. It accounted for 79, 81 and 75 % of the total PCBs measured in homes, computer laboratories and university offices, respectively. The global production of PCB-180 was smaller than for a number of other congeners and production was phased out earlier than for lighter congeners as a result of increased awareness of its more persistent nature (Breivik et al., 2002). The fact that it was found in greater amounts reflects this more persistent nature and the fact that the heavier congeners are more likely to be found adsorbed to settled particulate matter rather than in the vapour phase. The finding that the more chlorinated congener is more prevalent is similar to the findings observed in Eastern Romania (Dirtu et al., 2012) but unlike the observations in the UK, Canada and the USA. The greater abundance of PCB-180 is of concern because this congener has been linked to an increased risk of non-Hodgkin lymphoma (Colt et al., 2005). It is interesting to note that despite the prevalence of PCB-180 in settled dust samples it appeared to be depleted in Durban air (Batterman et al., 2009a). The generally high prevalence of PCB-180 and PCB-153 in this study is akin to high levels of these

congeners in wild bird eggs reported recently in South Africa (Quinn et al., 2013), and in fish tissues quantified as Aroclor 1254 in the Isipingo Estuary (Grobler et al., 1996).

The levels of PCBs measured in this study are compared with those reported for other parts of the world in Table 3. No previous reports exist on PCB contamination of indoor dust for South Africa. However, the values found here for homes and offices are similar to those reported for the USA by Roberts and Dickey (1995) and those for Kuwait City by Ali et al. (2013). The levels observed in the computer laboratories are much higher and fall within the range reported by Vorhees et al. (1999) for homes near New Bedford harbour monitored during dredging of PCB-contaminated harbour sediments. The higher levels of PCBs reported for sites in the USA are in keeping with the fact that the USA was the largest producer and user of PCBs. It is therefore surprising that such high levels were measured in Durban, South Africa, particularly since PCB production was phased out in the 1970s. However, such variation in reported concentrations could be due to differences in methods used for calculations as well as differences in sampling methodology and dust particle size fraction employed for analysis. PCBs were never produced in South Africa but they did find use in various sectors of the economy, including the mining, transport, energy, cement manufacturing, chemical manufacturing, and petrochemical industries. Further imports of PCBs are prohibited. South Africa is a signatory to the Stockholm Convention and measures are in place to destroy PCBs but to date only a small fraction of the PCBs have been destroyed (South Africa's Plan for the Implementation of the Stockholm Convention on Persistent Organic Pollutants, 2011).

The high levels of PCBs obtained in the university computer laboratories could partly be attributed to both indoor characteristics and possible outdoor sources: visitors, students and other university officials visit the laboratories daily with their shoes on, that might have been contaminated from outdoor sources and thereby transfer such contamination to dust in the indoor environment. Also, these venues were carpeted and carpets are known to retain more dust than tiled or bare floors particularly if they are not vacuumed frequently. The study conducted by Whitehead et al. (2012) showed higher PCB loadings in homes where residents do not remove their shoes as opposed to those that do, and more PCBs in carpets that were not vacuumed frequently. The elevated concentrations of PCBs found in homes in this study were independent of home characteristics. Building construction year was independent of PCB concentrations ($p = 0.0625$) in the respective buildings. Similarly, no relationship was observed between PCB concentrations and floor type contrary to the observations of Whitehead et al. (2012), Knobeloch et al. (2012) and Vorhees et al. (1999). These differences could be due to the small sample size in the present study. Other sources of PCB-contamination in indoor environments include paints, plaster, sealants, wood floor finishes, caulking compounds, old fluorescent light fixtures and appliances. The main transport route of PCBs in building materials to the indoor environment is volatilization from these materials and adsorption onto dust

particles. Consequently, houses built/renovated with PCB-entrenched building materials are likely to have elevated amounts of PCBs in indoor dusts and air.

6.3.2 Levels of polybrominated diphenyl ethers in South African indoor dust

All the dust samples analysed (10 from homes, 11 from computer laboratories and 10 from offices) contained PBDEs. The concentrations (in ng g^{-1}) of the eight congeners quantified in the different microenvironments are given in Table 4. The full list of the concentrations measured in each of the samples is presented in the Supplementary Material Table S2.

The average of the sum of the eight PBDEs monitored (ΣPBDE) increased in the order computer laboratories < offices < homes. The widest range was observed for the office samples. BDE-28 was the least abundant congener for all the samples. On the other hand, BDE-209 was the most abundant congener in homes and offices: it accounted for 43 % and 45% of all congeners, respectively (see Fig. S1 in Supplementary Material). However, BDE-153 was the most abundant congener in the computer laboratories and comprised 38 % of all congeners in that microenvironment. The dominance of BDE-209 is not surprising since most other studies have found this to be the major congener present (see Table 5). It was also the major congener detected in other South African studies (Olukunle et al., 2012; La Guardia et al., 2013). This also reflects the fact that the deca-BDE mixture is still in use. BDE-209 is particularly prevalent in dust samples because of its low volatility (Thuresson et al., 2012). The prevalence of BDE-153 in the computer laboratories is surprising. It was a constituent of the pentaBDE mix whose use is now banned. However, both Daso et al. (2012) and Odusanya et al. (2009) found the prevalence of constituents of the pentaBDE formulation in samples from a wasterwater treatment plant and landfills in South Africa thereby indicating its usage in products in the country. The penta-formulation was used in particular to flame retard polyurethane foams in carpet underlay, vehicle interiors, furniture, bedding, printed circuit boards and microprocessor packaging in computers (Harrad et al., 2008b). The computer laboratories sampled in this study were all refurbished between 2006 and 2007. All carpeting, furniture, blinds, computers and printers were replaced. In addition, in some rooms the dividing walls were replaced with plasterboard. The prevalence of BDE-153 is a concern because these less brominated congeners have longer half-lives and are therefore likely to accumulate to higher concentrations in humans (Sjödin et al., 2008). The Kruskal-Wallis test showed statistical differences ($p = 0.011$) in the distributions of the PBDE congeners in the three microenvironments.

Table 6.3 Concentrations (in ng g^{-1}) of ΣPCBs in indoor dusts reported in other studies.

Location	n		ΣPCB	min	max	mean	median	Reference
Guangzhou, China	20	Homes	37	51.9	264	139	130	Wang et al., 2013
Hong Kong, China	20	Homes	37	17.4	137	81.8	80.8	Wang et al., 2013
Hanoi, Vietnam	7	homes (suburban)	62	3.6	20		5.4	Tue et al., 2013
Hanoi, Vietnam	6	homes (urban)	62	5.6	85		10	Tue et al., 2013
Singapore	31	Homes	28	<dl	44	9.2	5.6	Tan et al., 2007
Hokkaido, Japan	2	homes	10	15	22			Takigami et al., 2009
Wellington, New Zealand	20	Homes	9	11	260	67	46	Harrad et al., 2009
Gujrat, Pakistan	31	homes and mosques	7	0.3	6.10	0.75	0.67	Ali et al., 2012
Faisalabad, Pakistan	15	Homes	17	1	38		2.7	Ali et al., 2013
Kuwait City	15	Homes	17	1	3080		3.6	Ali et al., 2013
Iasi, Eastern Romania	47	Homes	21	10	900		35	Dirtu et al., 2012
Iasi, Eastern Romania	18	Homes	8				26.5	Dirtu and Covaci, 2010
Birmingham, UK	20	Homes	9	5.7	860	110	48	Harrad et al., 2009
West Midlands, UK	36	child daycare centres and primary schools	8	1.2	560	41	15	Harrad et al., 2010
Toronto, Canada	10	Homes	9	56	820	290	260	Harrad et al., 2009
Amarillo, Austin, TX, USA	20	Homes	9	47	620	220	200	Harrad et al., 2009
Wisconsin, USA	20	Homes	89	8.8	1186			Knobeloch et al., 2012
Davis, CA, USA	11	10 homes and 1 community hall	54	<10	570	75	38	Hwang et al., 2008
New Bedford, MA, USA	19	homes near harbour dredging	65	320	23000	1400*	880	Vorhees et al., 1999
New Bedford, MA, USA	15	Homes	65	260	3600	690*	710	Vorhees et al., 1999
North Carolina, USA	9	child day care centres	20	120	3150		525	Wilson et al., 2001

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Table 6.4 Concentrations (in ng g^{-1}) of PBDEs in dust samples from indoor microenvironments in Durban, South Africa.

Location	Statistical Parameter	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-209	ΣPBDE
Homes	Mean	14.8	183	541	46.9	77.6	76.2	44.0	731	1710
n = 10	Median	11.5	156	507	36.9	64.5	97.2	44.5	656	1550
	Minimum	1.12	56.1	150	<dl	33.6	<dl	5.65	59.2	689
	Maximum	30.0	466	1140	150	182	134	106	2190	3290
Computer	Mean	11.5	31.2	58.6	21.6	310	40.1	175	170	818
Laboratories	Median	10.6	31.8	55.5	20.4	84.8	41.6	145	145	628
n = 11	Minimum	<dl	<dl	<dl	<dl	<dl	<dl	73.9	24.8	319
	Maximum	33.2	51.9	138	68.8	1580	102	421	465	2720
Offices	Mean	17.3	135	461	25.2	83.5	52.8	70.2	678	1520
n = 10	Median	14.2	119	148	<dl	87.9	50.9	75.0	324	871
	Minimum	<dl	15.4	45.5	<dl	<dl	<dl	37.2	78.4	266
	Maximum	54.9	388	1930	95.1	145	122	95.1	2750	5020

*geometric mean, <dl denotes below the detection limit

The levels of PBDEs measured in this study are compared with those reported for other parts of the world in Table 5. No previous reports exist for values of PBDEs in computer laboratories in South Africa. The levels observed in this work for homes and computer laboratories fall within the ranges measured in Toronto, Canada by Harrad et al. (2008b) and in Shanghai, China by Yu et al. (2012). The levels are not as high as those observed in the USA and United Kingdom where the usage of PBDEs is much greater and there is a bigger emphasis on the use of flame-retarded products. However, they are not as low as values reported for Germany, New Zealand and Vietnam, for example. The levels of PBDEs measured in homes and offices are much higher than those reported by Kefeni and Okonkwo (2012) for offices and Kefeni et al. (2014) for homes in Pretoria, South Africa. However, it must be kept in mind that Durban is a larger city than Pretoria and more industrialised. In addition, it is the busiest port in Africa. The university offices sampled in this work are within a five-kilometer radius of the Umgeni Business Park downstream of which La Guardia and co-workers (2013) measured the highest brominated flame retardant levels in their study.

BDE-47 and -99, which are major congeners in the pentaBDE mix, correlated ($r = 0.59$ and 0.36) in homes and offices indicative of similar sources for both. The high levels of BDE-100, -153 and -154 can possibly be linked to debromination of BDE-209 (Yang et al., 2013). This is supported by a positive correlation ($r = 0.66$, 0.76 and 0.22 for homes, computer laboratories and offices, respectively) of BDE-209 with BDE-153 concentrations; as well as the influence of penta- and octa-BDE commercial formulations with known application in printed circuit-board components (Labunska et al., 2013). Similarly, the results obtained for tetra- through hexa-BDEs were in line with levels reported in indoor dust consistent with concentrations in human serum and milk of the US general population (Sjödin et al., 2008). However, the levels of BDE-183 can be attributed to the commercial octaBDE formulation, which contains about 80 % of total octaBDE; since this formulation was known to be used as a flame retardant in plastic computer monitors and television housings (Yang et al., 2013). Likewise, it has been reported that technical decaBDE is used primarily in combination with antimony trioxide in high impact polystyrenes for use in electronic enclosures (e.g., television cabinets, computers, electrical boxes, wire and cable, connectors, etc.); these household appliances were present in the microenvironments studied, and thus could be linked as possible sources of the high levels of BDE-209 found in this study. This is supported further by the correlation of BDE-209 with electronic appliances in the indoor microenvironments. PBDE concentrations correlated positively ($r = 0.60$) with the concentrations of PCBs in homes but not with those in offices and computer laboratories, hence indicating similar sources but compound-specific differences in the indoor fate and transport of these ubiquitous contaminants (Ali et al., 2011).

Table 6.5 Concentrations (in ng g^{-1}) of ΣPBDEs in indoor dusts reported in other studies.

City	major congener	n		min	max	mean	median	Reference
Pretoria, South Africa	BDE 209	31	homes		234	16.2	15.2	Kefeni et al., 2014
Hanoi, Vietnam	BDE209	7	homes (suburban)	38	610		120	Tue et al., 2013
Munich, Germany[a]	BDE183	20	homes	6	1546		42	Fromme et al., 2014
Munich, Germany[b]	BDE209	20	homes	10	3748		950	Fromme et al., 2014
Hanoi, Vietnam	BDE209	6	homes (urban)	40	270		230	Tue et al., 2013
Faisalabad, Pakistan	BDE209	15	homes	30	2150		145	Ali et al., 2013
Gujrat, Pakistan	BDE209	31	homes	3	1595		27.7	Ali et al., 2012
Gujrat, Pakistan		12	mosques	6.4	337		50.4	Ali et al., 2012
Shanghai, China	BDE209	11	homes	132	3887	948		Yu et al., 2012
Hokkaido, Japan		2	homes	240	730			Takigami et al., 2009
Japan	BDE209	19	homes	140	3000		700	Suzuki et al., 2006
Kuwait City	BDE209	15	homes	90	19 200		356	Ali et al., 2013
South East Queensland, Australia		5	homes	87	733	376	294	Toms et al., 2009
Brisbane, Australia	BDE209	10	homes	500	13 000		1200	Sjodin et al., 2008
Wellington, New Zealand[c]		20	homes	13	680	160	96	Harrad et al., 2008b
Iasi, Eastern Romania	BDE209	18	homes				495	Dirtu and Covaci, 2010
Stockholm, Sweden	BDE209	10	homes	53	4000		510	Thuresson et al., 2012
Germany	BDE209	10	homes	17	550		74	Sjodin et al., 2008
Birmingham, UK		16	homes	360	520000	45 000	2900	Harrad et al., 2008b
Newcastle upon Tyne, UK	BDE209	10	homes	950	54 000		10 000	Sjodin et al., 2008
Toronto, Canada		7	homes	750	3500	1400	950	Harrad et al., 2008b

Atlanta, GA, USA	BDE209	10	homes	520	29 000		4200	Sjodin et al., 2008
Michigan,USA		12	homes	1	290 000	49 000	21 000	Batterman et al., 2009b
Amarillo and Austin, TX, USA		17	homes	920	17 000	4800	3500	Harrad et al., 2008b
Davis, CA, USA		11	10 homes and 1 community hall	1780	25200		9020	Hwang et al., 2008
Beijing, China	BDE209	28	offices	nd	5455.4			Cao et al., 2013
Pretoria	BDE99	16	offices	21.4	578.6	169	162	Kefeni and Okonkwo, 2012
Birmingham, UK		15	offices	790	280 000	31 000	7400	Harrad et al., 2008a
Stockholm, Sweden	BDE209	10	offices	800	13 000		1200	Thuresson et al., 2012
Japan	BDE209	14	offices				1800	Suzuki et al., 2006
South East Queensland, Australia		4	offices	583	3070	1547	1268	Toms et al., 2009
Stockholm, Sweden	BDE209	10	daycare centers	420	3900		1200	Thuresson et al., 2012
West Midlands, UK	BDE209	43	primary school and daycare centre classrooms	72	89000	8600	5100	Harrad et al., 2010

[a]data for tri- to – hepta BDEs, [b]data for BDE209 only, [c]hepta-, octa- and deca-BDEs not analysed.

6.3.3 Correlation of PBDE levels with potential sources

BDE-47 correlated (r = 0.77) with mattresses and foam-containing furniture in homes as did BDE-209 with electronic appliances (r = 0.54). Similarly, BDE-183 correlated (r = 0.54) with electronics in offices. These results thus implicate mattresses and furniture as probable sources of pentaBDE congeners in homes; and electronic appliances as sources for octa- and deca-BDEs in offices and homes. These observations are in agreement with those of de Wit et al. (2012), indicating the possible use of foam in furniture and beds containing the penta-mix which has previously found wide application in polyurethane foams. Approximately 95 % of technical pentaBDE was used in the production of supple polyurethane foam applied as cushioning in upholstery, foam-based laminated automotive applications, domestic furniture, and in foam-based packaging. Technical octaBDE has been majorly applied in the preparation of acrylonitrile-butadiene-styrene terpolymer (ABS), which is used in the production of computer and business equipment housings, adhesives and coatings.

6.4 Conclusions

This study provides data on PCB indoor dust contamination in South Africa and adds to reports on PBDE indoor contamination in multiple microenvironments. Concentrations of PCBs in indoor dust were generally lower than those of PBDEs in this study; but significantly high in university students' computer laboratories, thus suggesting an exposure of residents to high levels of organohalogens. Whilst PBDE concentrations in this study were generally lower than levels reported in most developed countries, the concentrations of PCBs were similar and in some cases higher than levels reported in some parts of the world. Building construction materials and household characteristics such as electronics and furniture in homes have been implicated as probable sources of these ubiquitous contaminants in the indoor environment; also contributions from outdoor sources in the case of PCBs cannot be precluded. Further studies are required to illustrate human exposure to PBDEs and PCBs via multiple routes including dust ingestion and dermal contact, and inhalation and dietary exposure.

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Supplementary Information

Table S1 Concentrations (in ng g^{-1}) of PCBs in dust from three microenvironments in Durban, South Africa.

Sample ID	PCB-28	PCB-153	PCB-180	∑PCB
Home 1	56	235	781	1071
Home 2	11	143	283	437
Home 3	<dl	<dl	648	648
Home 4	<dl	150	2046	2196
Home 5	<dl	151	1161	1312
Home 6	48	152	521	721
Home 7	11	338	377	726
Home 8	14	450	820	1285
Home 9	<dl	<dl	<dl	<dl
Home 10	21	106	384	512
Lab 1	5.2	146	445	597
Lab 2	<dl	<dl	<dl	<dl
Lab 3	1.5	<dl	117	119
Lab 4	7.2	23	156	186
Lab 5	<dl	39	307	346
Lab 6	<dl	3557	15492	19050
Lab 7	16	90	254	360
Lab 8	4.9	62	166	233
Lab 9	<dl	125	404	529
Lab 10	<dl	46	95	140
Lab 11	<dl	38	474	512
Lab 12	<dl	87	686	773
Lab 13	73	444	1042	1559
Office 1	146	184	812	1142
Office 2	<dl	<dl	<dl	<dl
Office 3	42	<dl	<dl	42
Office 4	28	136	872	1036
Office 5	<dl	<dl	<dl	<dl
Office 6	<dl	647	1405	2053
Office 7	<dl	<dl	<dl	<dl
Office 8	<dl	271	1534	1805
Office 9	64	209	535	807
Office 10	40	3.2	1368	1411
Office 11	389	421	1049	1859

Table S2 Concentrations (in ng g^{-1}) of PBDEs in dust from three microenvironments in Durban, South Africa.

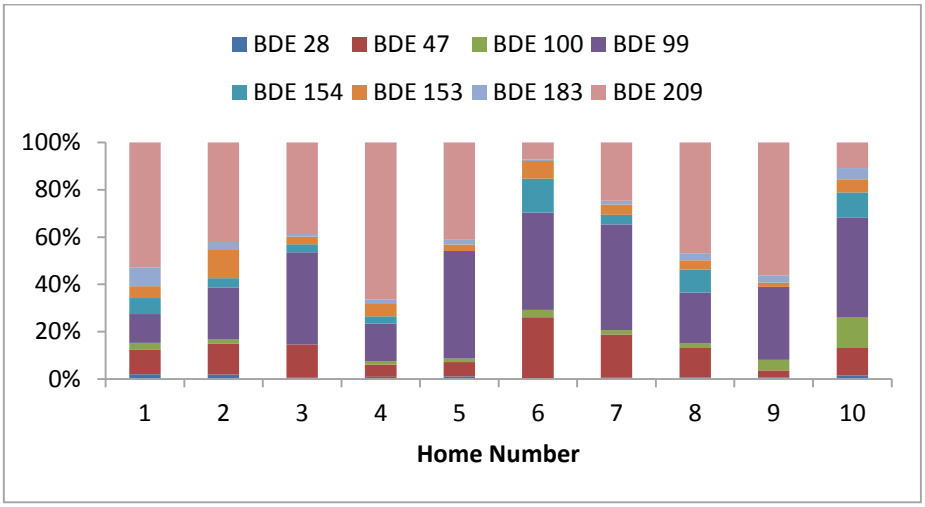
Sample ID	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-209	∑PBDE
Home 1	27	139	163	40	68	92	106	712	1348
Home 2	12	90	150	14	82	27	23	290	689
Home 3	8.0	243	674	<dl	57	59	17	668	1726
Home 4	30	170	519	49	182	105	47	2186	3287
Home 5	23	143	1055	34	62	<dl	48	952	2316
Home 6	1.1	217	345	26	63	120	5.6	59	837
Home 7	11	466	1139	48	109	103	42	625	2541
Home 8	8.2	172	296	25	55	134	40	643	1372
Home 9	10	56	575	83	34	<dl	57	1047	1863
Home 10	17	136	495	150	66	123	54	126	1166
Lab 1	15	36	54	57	82	102	312	27	686
Lab 3	28	46	84	<dl	1580	94	421	465	2718
Lab 4	11	<dl	35	20	61	44	122	25	319
Lab 5	<dl	52	<dl	<dl	685	<dl	156	219	1112
Lab 7	8.3	32	68	26	141	57	174	145	652
Lab 8	<dl	32	80	<dl	436	<dl	145	150	842
Lab 9	33	50	44	69	<dl	41	95	294	626
Lab 10	13	21	29	30	82	<dl	166	68	408
Lab 11	11	26	56	<dl	181	<dl	74	120	468
Lab 12	7.6	32	57	35	85	61	125	137	539
Lab 13	<dl	18	138	<dl	76	42	138	216	628
Office 1	<dl	140	1925	<dl	79	41	82	2752	5019
Office 2	12	104	176	<dl	97	58	48	472	967
Office 3	55	119	120	55	60	18	94	116	636
Office 4	<dl	388	728	<dl	142	73	73	232	1636
Office 5	17	163	1026	<dl	145	<dl	77	620	2048
Office 6	30	143	79	69	<dl	88	95	217	721
Office 7	27	120	366	<dl	124	47	83	1643	2410
Office 8	11	98	45	95	71	55	37	362	775
Office 9	17	15	75	<dl	<dl	25	55	78	266
Office 10	4.3	58	71	33	116	122	56	287	748

Table S3 Description of indoor microenvironments.

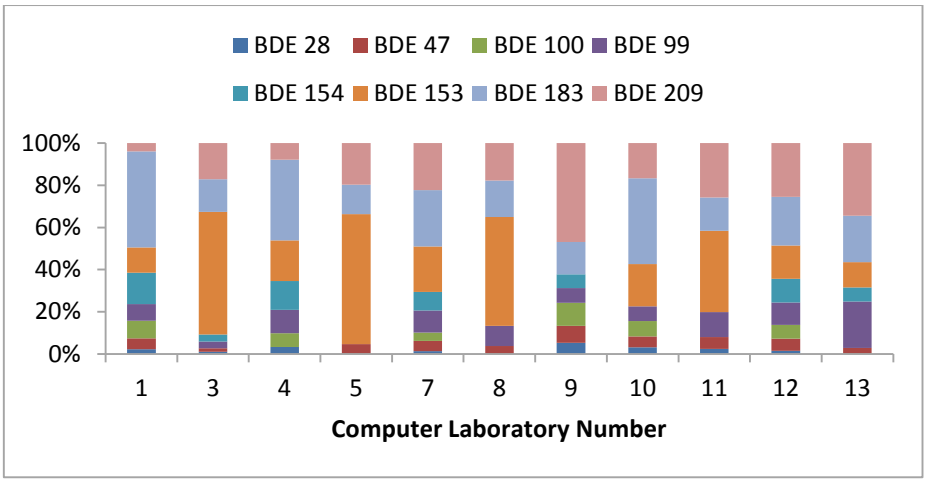
Indoor Micro-environment	Construction Year	Floor type	Number of electronics	Number of Foam-containing furniture	ΣPBDEs /ng g^{-1}	ΣPCBs/ ng g^{-1}
Home 1	1978	Carpet	5	8	1348	1071
Home 2	1993	tiled	INB	INB	689	437
Home 3	1979	tiled	6	12	1726	648
Home 4	QNR	QNR	QNR	QNR	3287	2196
Home 5	1977	tiled	6	3	2316	1312
Home 6	QNR	QNR	QNR	QNR	837	721
Home 7	1991	tiled	6	INB	2541	726
Home 8	1973	Carpet and wooden floor	5	10	1372	1285
Home 9	QNR	QNR	QNR	QNR	1863	ND
Home 10	1981	Tiled	8	22	1166	512
Office 1	Early 1970s	Carpet	1	4	5019	1142
Office 2	Early 1970s	Carpet	1	3	967	ND
Office 3	Early 1970s	Carpet	2	3	636	42
Office 4	Early 1970s	Carpet	4	3	1636	1036
Office 5	Early 1970s	Carpet	3	3	2048	ND
Office 6	Early 1970s	Carpet	1	1	721	2053
Office 7	Early 1970s	Carpet	1	3	2410	ND
Office 8	Early 1970s	Carpet	INB	INB	775	1805
Office 9	Early 1970s	Carpet	3	4	532	807
Office 10	Early 1970s	Carpet	5	5	750	1411
Office 11	Early 1970s	Carpet	2	1	Missing data	1859

INB: Information not obtained, ND: Not detected, QNR: Questionnaire not returned

(a)



(b)



(c)

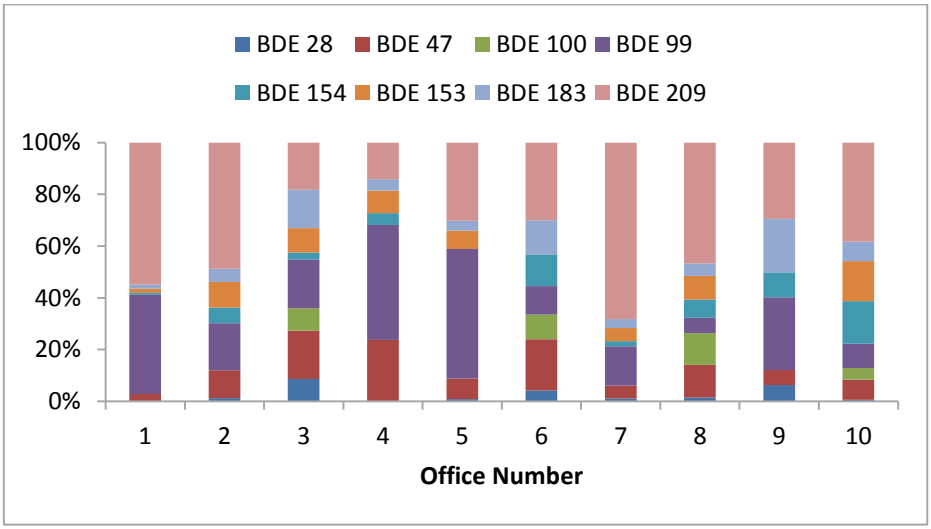


Fig. S1. Percentage abundance of PBDE congeners in (a) homes, (b) computer laboratories and (c) offices.

Supplementary Material S6.1

Estimated Daily Exposure to PBDEs and PCBs

In evaluating human exposure via dust ingestion of contaminants, we assumed 100 % absorption of contaminants from ingested dust in accordance with other studies (Jones-Otazo et al., 2005; Ali et al., 2012; Ali et al., 2013). Average dust intake rates of 20 and 50 mg d^{-1} and high dust intakes of 50 and 200 mg d^{-1} for adults, teenagers and toddlers were used as reported by Ali et al. (2013). Body weights of 70 kg and 12 kg were used for adults and toddlers respectively (Ali et al., 2013), and 52 kg for teenagers (Johnson-Restrepo and Kannan, 2009). Questionnaires were used to obtain the average number of hours spent per day by adults in offices and homes. The average number of hours students spend in computer laboratories (for lectures, studies and assignments) and in their residences per day were obtained by interview. The amount of the time spent per day in homes by toddlers (79.9 %) was the same as that of Ali et al. (2012). Thus, the average time an adult spends in the office, car and home were taken as 33.3 %, 4.2 %, and 62.5 %, respectively. For full-time undergraduate students (teenagers) it was estimated that they spend 54.2 % and 45.83 % of their time indoors in classrooms and in their residences respectively. Different exposure scenarios were calculated by using the 5th percentile, median, mean and 95th percentile concentrations from home, office and computer laboratory dusts. Thus, the daily exposure dose of contaminants (ΣDED PBDEs or PCBs/ng kg^{-1} bw day^{-1}) via dust ingestion were calculated from the following equations reported by Ali et al. (2013) with modifications.

$\Sigma DED/ng\ kg^{-1}\ bw\ day^{-1} = [(C_{HD}F_H) + (C_{ofD}F_{of})]DIR/BW$, for adult exposure estimation, and

$\Sigma DED/ng\ kg^{-1}\ bw\ day^{-1} = [(C_{HD}F_H) + (C_{LD}F_L)]DIR/BW$, for teenager exposure assessment,

where $C_{HD,}$ C_{ofD} and C_{LD} are dust concentrations in homes, offices and computer laboratories (5th percentile, median, mean and 95th percentile) and $F_{H,}$ F_{of} and F_L are the fraction of time spent in homes, offices and computer laboratories, respectively. DIR is the dust intake rate and BW is the body weight.

In the case of toddlers, the ΣDED of contaminants was assessed as the sum of exposure via dust ingestion and dermal absorption. The latter contribution was calculated in the same manner as Johnson-Restrepo and Kannan (2009), with slight modifications.

Exposure via dust ingestion = $\Sigma DED/ng\ kg^{-1}\ bw\ day^{-1} = [C_{HD}F_H]DIR/BW$, and

Exposure via dermal absorption = $\Sigma DED/ng\ kg^{-1}\ bw\ day^{-1}$ = [C.BSA.DAS.AF.IEF]/[BW.1000]

where C is the concentration of dust from homes (5th percentile, median, mean and 95th percentile), BSA is the body surface area, DAS is the dust adhered to skin, AF is the fraction of contaminant absorbed in the skin and IEF is the indoor exposure fraction.

The estimated daily exposure doses for PBDEs and PCBs with different exposure scenarios for adults, teenagers and toddlers are presented in Tables 6-9. The daily exposure dose of PBDEs at the 5th and 95th percentile concentrations were much lower

than the EPA reference doses (RfD) of 0.1, 0.1 and 0.2 µg kg^{-1} day^{-1} for BDE-47, -99 and -153 respectively (Liberda et al., 2011) and 7.0 µg kg^{-1} day^{-1} for BDE-209 (de Wit et al. 2012). However, the daily exposure doses of PCBs were close to the reference dose for the best-case scenario but higher than the EPA RfD of 20 ng kg^{-1} day^{-1} for Aroclor 1254 (main congeners: PCB105, 118, 138 and 153) (Knobeloch et al., 2012) for the worst-case scenario. The high daily exposure doses of PBDEs and PCBs should be a concern, particularly for toddlers and teenagers.

Table S6

Estimated daily exposure doses (in ng kg^{-1} bw day^{-1}) of PBDEs for adults and teenagers in South Africa.

Dust Intake Rate	Exposure of Adults				Exposure of Teenagers			
	5^{th} percentile	Median	Mean	95^{th} percentile	5^{th} percentile	Median	Mean	95^{th} percentile
Mean	0.19	0.36	0.45	0.89	0.21	0.40	0.47	0.92
High	0.47	0.90	1.1	2.23	0.52	1.0	1.2	2.30

Table S7

Estimated daily exposure doses (in ng kg^{-1} bw day^{-1}) of PBDEs for toddlers in South Africa.

Exposure Pathway	Mean dust intake rate				High dust intake rate			
	5^{th} percentile	Median	Mean	95^{th} percentile	5^{th} percentile	Median	Mean	95^{th} percentile
Dust ingestion	2.52	5.16	5.71	9.83	10.06	20.62	22.82	39.30
Dermal Absorption	0.36	0.76	0.84	1.45	0.36	0.76	0.84	1.45
ΣDED	2.88	5.92	6.55	11.28	10.42	21.38	23.66	40.75

Table S8
Estimated daily exposure doses (in ng kg^{-1} bw day^{-1}) of PCBs for adults and teenagers in South Africa.

Dust Intake Rate	Exposure of Adults				Exposure of Teenagers			
	5^{th} percentile	Median	Mean	95^{th} percentile	5^{th} percentile	Median	Mean	95^{th} percentile
Mean	0.04	0.23	0.25	0.51	0.05	0.20	0.55	2.1
High	0.09	0.57	0.62	1.27	0.12	0.51	1.37	5.30

Table 9
Estimated daily exposure doses (in ng kg^{-1} bw day^{-1}) of PCBs for toddlers in South Africa.

Exposure Pathway	Mean dust intake rate				High dust intake rate			
	5^{th} percentile	Median	Mean	95^{th} percentile	5^{th} percentile	Median	Mean	95^{th} percentile
Dust ingestion	0.65	2.41	2.97	5.99	2.62	9.63	11.86	23.94
Dermal Absorption	0.10	0.36	0.44	0.88	0.10	0.36	0.44	0.88
ΣDED	0.75	2.77	3.41	7.87	2.72	9.99	12.30	24.92

These observations are in agreement with reports of studies that affirm dust ingestion as a primary route of exposure to PBDEs, especially for toddlers and infants (Stapleton et al., 2004; Wilford et al., 2005; Hwang et al., 2008; Stapleton et al., 2011). Studies have shown that children contained two to five orders of magnitude higher levels of PBDEs in blood relative to their parents, and the PBDE congener profiles in children's serum are more closely related to the profiles in house dust (Fischer et al., 2006). The implication is that toddlers and infants are exposed to PBDEs and PCBs via unintentional dust ingestion, and as a result are more susceptible to adverse health effects. Contrary to young children's exposure, the low exposure doses of PCBs and PBDEs for adults may be likened to reports that dust ingestion is not a primary exposure route for adults to toxic organohalogens, since PBDE congener profiles reported in human blood and milk differ from those in house dust (Hites, 2004; Fischer et al., 2006; Hwang et al., 2008). Various adverse effects of PBDEs and PCBs to children have been reported. Studies have associated PBDEs with congenital cryptorchidism (Main et al., 2007). Also, associations of prenatal PBDE exposures with decreased total and free thyroxin (T4) levels in infants

born by spontaneous delivery have been reported (Kodavanti and Curras-Collazo, 2010). Kodavanti and Curras-Collazo (2010) showed a relationship of PBDEs with longer time to pregnancy in women and reduced development of children at school age that includes psychomotor development index and full scale intelligent quotient (IQ) performance. Several other adverse effects of PBDEs in man has been reported (Darnerud, 2003; Rice et al., 2007; Viberg et al., 2007; Van der Ven et al., 2008; Fujimoto et al., 2011).

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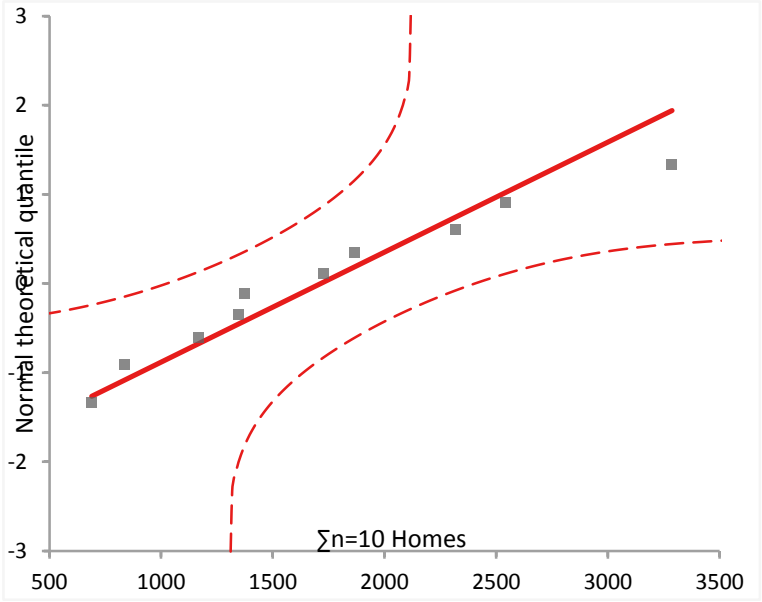
Supplementary Material S6.2: Statistical Analysis of PBDEs and PCBs

Analyse-it

Descriptives

N	10					
	Mean	SE	SD	Variance	Skewness	Kurtosis
∑n=10 Homes	1714.483	256.3473	810.6 1	657139.331	0.7	-0.03

Normality



0.95

H0: F(Y) = N(μ, σ)

The distribution of the population is normal ith unspecified mean and s andard deviation.

H1: F(Y) ≠ N(μ, σ)

The distribution of the pop lat on is not normal.

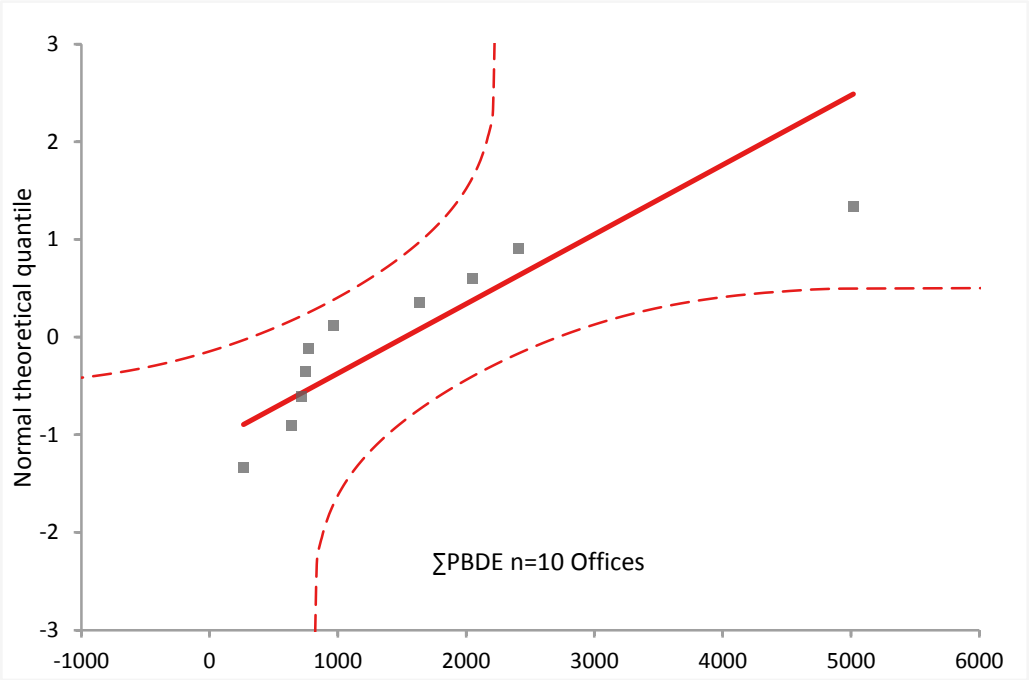
1 Do not reject the null hypothesis at the 5% significance level.

Distribution: ∑PBDE n=10 Offices

Analyse-it v3.76.1

Sheet1 A1: 12

Mean	SE	SD	Variance	Skewness	Kurtosis
1522.468	444.3913	1405.289	1974836.479	2.0	4.30


Normal theoretical quantile
3
2
1
0
-1
-2
-3
-1000
0
1000
2000
3000
4000
5000
6000
∑PBDE n=10 Offices

Shapiro-Wilk test

W statistic	0.78
p-value	1
	0.0072

H0: F(Y) = N(μ, σ)

The distribution of the population is normal with unspecified mean and standard deviation.

H1: F(Y) ≠ N(μ, σ)

The distribution of the popula io is not norm l.

1 Reject the null hypothesis in favour of the alternative hypothesis at th 1% significance level.

XLSTAT 2014.3.01 Comparison of k samples (Kruskal-Wallis, Friedman, ...) - on 5/28/2014 at 2:05:34 PM
Samples: Workbook = Book1 / Sheet = Sheet1 / Range = Sheet1!$A$2:$C$13 / 11 rows and 3 columns
Significance level (%): 5
p-value: Asymptotic p-value

Summary statistics:

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
∑n=10 Homes	11	1	10	689.380	3287.080	1714.483	810.641
∑PBDE n=11 LANs	11	0	11	318.530	2718.370	817.999	665.933
∑PBDE n=10 Offices	11	1	10	265.000	5018.520	1522.468	1405.289

Kruskal-Wallis test:

K (Observed value)	8.988
K (Critical value)	5.991
DF	2
p-value (Two-tailed)	0.011
alpha	0.05

An approximation has been used to compute the p-value.

Test interpretation:
H0: The samples come from the same population.
Ha: The samples do not come from the same population.
As the computed p-value is lower than the significance level alpha=0.05, one should reject the null hypothesis H0, and accept the alternative hypothesis Ha.
As the computed p-value is lower than the significance level alpha=0.05, one should reject the null hypothesis H0,

and accept the alternative hypothesis Ha.
The risk to reject the null hypothesis H0 while it is true is lower than 1.12%.

Correlation: ∑PCBs/, Construction Year

Sheet1 A10:C16

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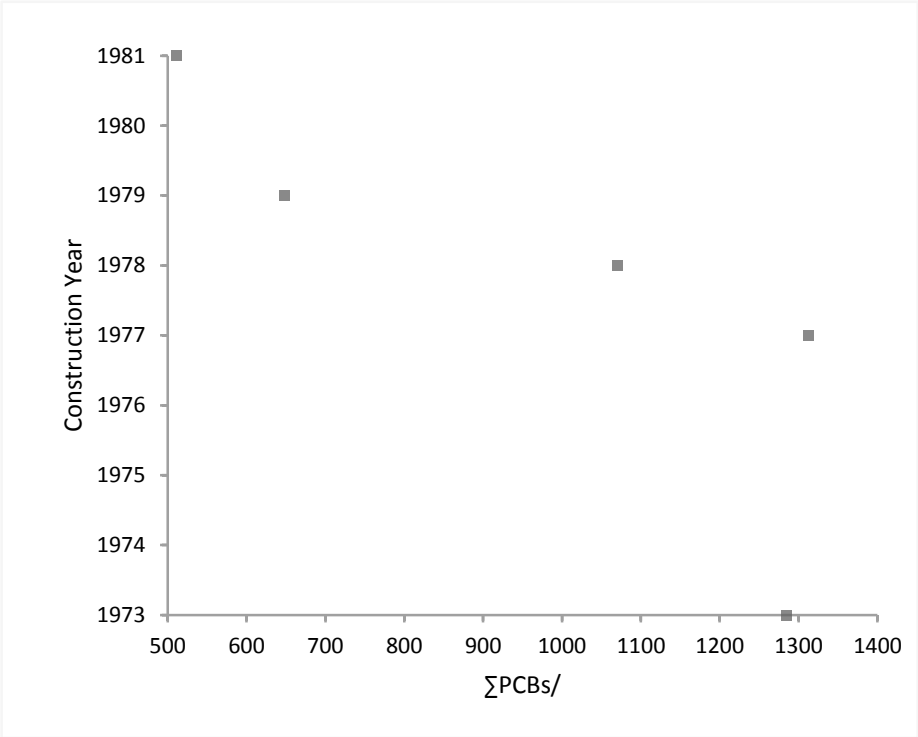
Descriptives



Analyse-it v3.76.1

Sheet25 E3:G6

Last updated 28 May 2014 at 15:00 by user

Descriptives

N	2

	Minimum	1st Quartile	Median	3rd Quartile	Maximum	Inter-quartile range
Carpet	1071.03	1071.030	1177.950	1284.870	1284.87	213.840
Tiled	648.45	648.450	980.245	1312.040	1312.04	663.590

Location

Hodges-Lehmann shift	-197.705	
50% CI	-422.580	to 27.170

$F(_{Tiled})=F(_{Carpet}+\Delta)$

Wilcoxon te t

Hypothesized difference	1

Sign	n	Rank sum	Mean rank
Positive	1	1.0	1.00
Negative	1	2.0	2.00
Zero	0		

T statistic	1.00	
Exact p-value		[1]
	1.0000	

H0: Δ = 1The shift in location between the distributions of the populations is equal to 1. H1: Δ ≠ 1The shift in location between the distributions of the populations is not equal to 1.

[1] Do not reject the null hypothesis at the 5% significance level.

CHAPTER 7

An assessment of polybrominated diphenyl ethers and polychlorinated biphenyls in the indoor dust of e-waste recycling facilities in South Africa: Implications for occupational exposure

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Abstract

Workplace exposure to persistent organic pollutants is a concern for human health. This study examined the presence of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in the indoor dust from two major e-waste recycling sites and a university electronic equipment repair workshop in Durban, South Africa, in order to evaluate the implication of dust for occupational exposure. The mean $\sum_{(n=8)}$ PBDEs and $\sum_{(n=3)}$ PCBs were 20094 ng g^{-1} and 235 ng g^{-1} respectively. The levels of PBDEs and PCBs obtained in one of the recycling sites (123 – 27530 ng g^{-1} and 162 – 593 ng g^{-1}) were significantly higher than the levels obtained (91 – 7686 ng g^{-1} and <DL – 42 ng g^{-1} respectively) in the same site after site clean-up/maintenance. Occupational exposure was assessed via different exposure scenarios by using the 5th and 95th percentile, and median and mean concentrations measured at the sites. By assuming a mean and a high dust intake rate, the average and 95th percentile daily exposure doses ($\sum$DED/ng kg^{-1} bw d^{-1}) of PBDEs were 3.98, 8.52 and 7.58, 16.19 respectively, and of PCBs were 0.047, 0.094 and 0.089, 0.179 respectively. The $\sum$DED of PBDEs and PCBs were lower than the reference (RfD) values for BDE 47, BDE 99 and BDE 153 and BDE 209

Keywords*: PBDEs, PCBs, Occupational exposure, Facility maintenance*

7.1 Introduction

Waste electronic and electrical equipment (WEEE) or electronic waste or e-waste for short has received increasing attention in recent years occasioned by the rapid increase of obsolete or end-of-life electronic goods ranging from computers, printers, televisions, mobile phones and digital cameras to smart appliances (1, 2). Recently, e-waste poses one of the major tasks facing the solid waste management community owing to constant generation of huge amounts through legal or illegal imports and domestic use (2). Although e-waste has been identified as a unique waste stream there is a growing acknowledgement of the potential human and environmental health challenges resulting from the mishandling of e-waste. Though any new and expanding waste stream encounters issues regarding storage, collection, recycling, disposal and the environment, e-waste is a particular problem because of the enormous array of chemicals and components used to manufacture electrical and electronic equipment (1). However, the demands for recycled materials and potential economic benefit have resulted in the increase of the e-waste recycling industry. Hence, various private firms have embraced e-waste recycling business in various locations of South Africa, but nevertheless, at the expense of having thousands of personnel involved in primitive recycling operations without adequate occupational safety awareness. These primitive operations include removal of electronic components from circuit boards via heating in a grill; metal stripping in open pit acid baths; mechanical and physical dismantling of e-waste polymer casings; and combustion of cables to recover valuable metals (2). Through these operations and evaporation, leakage, volatilization, etc., many toxic chemicals, such as polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs), are released into the environment and partly enter the human body through several exposure routes, such as inhalation, dermal contact and dust ingestion (2-6).

Both PBDEs and PCBs are persistent organic pollutants (POPs) of great concern due to their environmental persistence, bioaccumulation in the food web, long-range atmospheric transport and potential for toxic effects in human (4, 7). Due to their ubiquity and adverse health effects such as endocrine system disruption, and reproductive and neurodevelopmental toxic effects (4, 8-10), these chemicals have been listed in the Stockholm Convention on persistent organic pollutants (4, 7) and penta- and octa-BDEs, and PCBs have been banned or withdrawn from commerce (4, 11). However, as a result of mass production and widespread use in the past, for example, PBDEs added into electronic products as flame retardants (4) and PCBs as coolants in capacitors and as insulating fluids in transformers (12), during uncontrolled recycling of e-waste, these pollutants find their way into the environment. Substantial inventories have been created for these contaminants in developing countries of Asia and Africa, as well as developed countries (7, 9, 13-15).

House dust is a significant medium and exposure route for PBDEs and PCBs, and it is a subject of increasing interest in recent years (9). Indoor dust has been implicated as a

receptacle and a concentrator of many organic contaminants, hence levels of contaminants in indoor dust can be used as a proxy to assess the exposure potential to contaminants in the indoor environment (5, 6, 16-18).

Hence, an assessment of occupational exposure to PBDEs and PCBs in e-waste recycling sites is needed in order to evaluate subsequent health effects (4). The present study aimed to (i) Assess the levels of PBDEs and PCBs in e-waste recycling sites, (ii) present for the first time, data on PBDEs and PCBs in the workplace in Africa (iii) assess the magnitude of contamination by comparing results obtained with international data (iv) evaluate the influence of recycling site maintenance on the profiles of PBDEs and PCBs, and (v) estimate occupational exposure to PBDEs and PCBs via dust ingestion and dermal absorption.

7.2 Materials and methods

7.2.1 Materials and chemicals

Method 1614 Native PAR PBDE stock solution [(1 μg mL^{-1} 2,4,4′-tribromodiphenyl ether, BDE-28; 2,2′,4,4′-tetrabromodiphenyl ether, BDE-47; 2,2,4,4,5′-pentabromodiphenyl ether, BDE-99; 2,2,4,4,6′ pentabromodiphenyl ether, BDE-100; 2,2,4,4,5,5-hexabromodiphenyl ether, BDE-153; 2,2,4,4,5,6-hexabromodiphenyl ether, BDE-154; 2,2′,3,4,4′,5,6′-heptabromodiphenyl ether, BDE-183) and (10 μg mL^{-1} 2,2′,3,3′,4,4′,5,5′,6,6′-decabromodiphenyl ether, BDE-209)] was received as a kind donation from Cambridge Isotope Laboratories, Andover, MA, USA. 2,4,4′-Trichlorobiphenyl (PCB-28); 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB-153); 2,2′,3,4,4′,5,5′-heptachlorobiphenyl (PCB-180) and decachlorobiphenyl (PCB-209) were purchased from Sigma-Aldrich, South Africa. $^{13}C_{12}$-labelled decachlorobiphenyl ($^{13}C_{12}$ PCB-209) was obtained from Wellington Laboratories, Guelph, Ontario, Canada. Silica gel 90 was from Sigma-Aldrich and Florisil PR 60-100 mesh was from Floridin Co., USA. The standard reference material (SRM 2585: Organic contaminants in house dust) was purchased from the National Institute of Standards and Technology (NIST). Anhydrous sodium sulfate was from Associated Chemical Enterprises (ACE), Johannesburg, South Africa. A Rtx® – 1614 fused silica (5% diphenyl, 95% dimethyl polysiloxane) capillary column was obtained as a generous gift from Restek Corporation, Bellefonte, PA, USA. All solvents were high performance liquid chromatography or pesticide grade obtained from Sigma Aldrich, South Africa.

7.2.2 Sampling

Dust samples were collected from two e-waste dismantling/recycling facilities and one university Information and Communication Technology (ICT) electronic equipment repair workshop in Durban, South Africa. In the e-waste facilities, samples were collected from two locations. Point one in each recycling facility, comprised mainly stockpiles of televisions, computers, fridges and other electronic equipment including dismantled computer and television casings. Point two was characterized by electronic

mother boards and other internal electronic components (see Supplementary Material Table 7.1). At the ICT workshop, samples were collected from the entire facility as there was no special sorting being carried out. In one e-waste facility, sampling was conducted in two seasons prior to (winter 2012) and after (summer 2013) e-waste facility maintenance. In this facility, the indoor environment was vacuumed cleaned and industrially washed a few days before the summer sampling. Sampling was carried out with a LG 1600 W vacuum cleaner. The vacuum cleaner contained a dust unit which could easily be removed and emptied after each collection. Between each collection it was cleaned with a disposable cloth wetted with *iso*-propanol. Samples were stored in amber glass bottles at -10 °C . In this work, the sampling sites are coded as SC and PCU for the two e-waste recycling sites and ICT for the university ICT workshop.

7.2.3 Extraction

Non-dust particles, hair and debris were hand-picked from all samples. Samples were homogenized by sieving through a 212 μm stainless steel sieve. Dusts were analysed following the United States Environmental Protection Agency (US EPA) methods 3550c, 3620c, 1614 and 1668a with modifications. Briefly, approximately 0.8 g of sample was quantitatively weighed into a glass test tube and spiked with 50 ng PCB-209 as the internal standard. A volume of 10 mL *n*-hexane:methanol (1:3 v/v) was added. Samples were mixed in an orbital shaker for 10 mins and then extracted in an ultrasonic water bath at 40 °C for 30 mins. The mixing and extraction was repeated for a second time without addition of fresh solvent. The samples were then centrifuged at 3500 rpm for 10 mins and the supernatants were stored at <4 °C prior to clean-up.

For Soxhlet extraction, 1.0 g dust was Soxhlet extracted with 100 mL 1:3 n-hexane: methanol (v/v) at 70 °C for 8 hours. Extracts were reduced to approximately 10 mL prior to clean up.

7.2.4 Clean-up

Silica gel 90 and Florisil (PR 60 to 100 mesh) were activated at 130 °C for 16 hours and anhydrous sodium sulfate was baked at 450 °C for 5 hours before use. Silica gel, Florisil and anhydrous sodium sulfate were subsequently cooled in a desiccator. A 30 cm × 1 cm glass column was packed with either 3 g of silica gel or 3 g of Florisil. Each column was topped with 0.8 g of anhydrous sodium sulfate and then wetted with 30 mL of the extraction solvent. Extracts were loaded onto columns just before the exposure of the sodium sulfate layer. PBDEs were eluted on silica columns with 25 mL *n*-hexane. This was kept as Fraction 1 and contained essentially BDE-209. The columns were further eluted with 30 mL of diethyl ether/*n*-hexane (50:50 v/v), and kept as Fraction 2. The column flow rates were maintained at 0.5 mL min^{-1}. All fractions were reduced to approximately 250 μL in a rotary evaporator at 55 °C. PCBs were fractionated and cleaned-up with Florisil column chromatography. Columns were eluted with 30 mL diethyl ether/*n*-hexane (6:94 v/v). Eluates were reduced and concentrated in a rotary

evaporator to approximately 250 µL and stored in 1.5 mL amber glass GC/MS vials. All extracts were stored at <4 °C until instrumental analysis.

7.2.6 Chromatographic Analysis

PBDE analysis was performed on an Agilent 6890 (Palo Alto, CA, USA) gas chromatograph (GC), coupled to a 5973N series mass spectrometer (MS) operated in electron impact (EI) ionization mode. A Restek Rtx® – 1614 fused silica (5% diphenyl, 95% dimethyl polysiloxane) capillary column (15 m × 250 µm × 0.1 µm) was used to effect separation and the MS was operated in the selected ion monitoring (SIM) mode. The injections were made in the pulsed splitless mode with the injector temperature set at 285 °C. The injection volume was 1 µL. The GC oven temperature programme started at 90 °C (held for 2 mins), then increased at 20 °C min^{-1} to 270 ºC, followed by 10 °C min^{-1} to 325 °C and held for 5 mins. Helium was employed as the carrier gas at a flow rate of 1.2 mL min^{-1} and a constant linear velocity of 58 cm s^{-1}. For the MS the ion source and transfer line temperatures were 230 °C and 350 °C, respectively, and the ionization energy was 70 eV. The molecular ions $[M]^+$ or $[M+2]^+$ and fragment ions $[M-Br_2]^+$ or $[M-Br_2+2]^+$ were monitored for tri- through hepta-BDEs. M/Z 400 and 800 were monitored for BDE-209. Data were acquired with ChemStation software. PCBs were analysed with the same GC-MS but this time fitted with a Restek Rxi®-5MS fused silica (diphenyl dimethyl polysiloxane) capillary column (30.0 m × 250 µm × 0.25 µm). A 1 µL volume of sample was injected in the pulsed splitless mode with helium as the carrier gas at a flow rate of 0.7 mL min^{-1} and a pulse pressure of 150 kPa for 1 min. The injector temperature was 250 °C. The GC oven conditions for the PCBs were: an initial temperature of 90 °C, held for 1 min, then increased at 30 °C min^{-1} to 280 °C and held at 280 °C for 10 mins. The MSD and interphase temperatures were 350 °C and 280 °C respectively. The GC column pressure was set at 36.6 kPa and the total column flow was 43.6 mL min^{-1}. The MS was operated in the SIM mode. The molecular ion $[M]^+$ was monitored for all PCB congeners.

Quantitation was carried out by means of a multiple point internal standard method. Unlabelled PCB-209 and $^{13}C_{12}$ labelled PCB-209 were employed as internal standards for PCBs and PBDEs respectively. The response factors were determined from the slope of a plot of the ratio of peak areas against the ratio of the concentrations. The values for the plots were obtained from a 5 – 6 point triplicate analysis of the PBDE standard solution diluted to fall within a concentration range of 5 – 500 ng mL^{-1} and 0.1 to 4 µg mL^{-1} for tri- to octa-BDEs and BDE-209, respectively; similarly PCB calibrations were made with pure PCB standards in a similar concentration range as the tri- to octa-BDEs

7.2.7 Quality Control and Quality Assurance

Analysis of solvent, method and field blank samples was carried out simultaneously with all sample batches. Simulated laboratory dusts were used for field blanks to check the effects of sampling techniques on analyte concentrations in samples. In this case,

anhydrous sodium sulfate was spread on a bare floor with no electrical or other possible sources of PBDEs and PCBs. The samples (n = 3) were subjected to sampling, employing the same sampling protocol as for real the dust samples. Samples were then passed through all the analytical procedure as for real samples. Method blanks (n = 10) were prepared by taking 1.0 g of anhydrous sodium sulfate and passing it through the extraction, clean-up and chromatographic analysis procedure to check for possible laboratory contamination sources. Standard reference material (SRM 2585: Organic Contaminants in House Dusts) was analysed employing both extraction techniques for quality assurance.

7.2.8 Statistics

Descriptive statistics such as sum, mean, median, minimum, maximum, t-test and analysis of variance (ANOVA) were calculated by using Microsoft Excel® 2010. Distributions of PBDEs and PCBs were tested with Kolmogorov-Smirnov test by using XLSTATS 2014. Other non-parametric statistical tests were performed with XLSTATS 2014. Limits of detection (LOD) and quantitation (LOQ) were estimated following Thomsen et al. (35). Samples below the detection limit were treated as zero throughout the statistical analysis. The confidence intervals were calculated as described by Bunhu (36)

7.3 Results and discussion

The results of the concentrations of PBDEs and PCBs and the subsequent human exposure doses are presented in subsequent sections. Details of the method validation criteria can be found in Chapter 3 Section 3.3.2.

7.3.2 Levels of PBDEs

PBDEs were detected at high levels in all samples (Fig.7.1). Information on the concentrations profiles of PBDEs is presented in Supplementary Materials Table S7.3. The PBDE concentrations varied widely between the two sampling points at each e-waste facility.

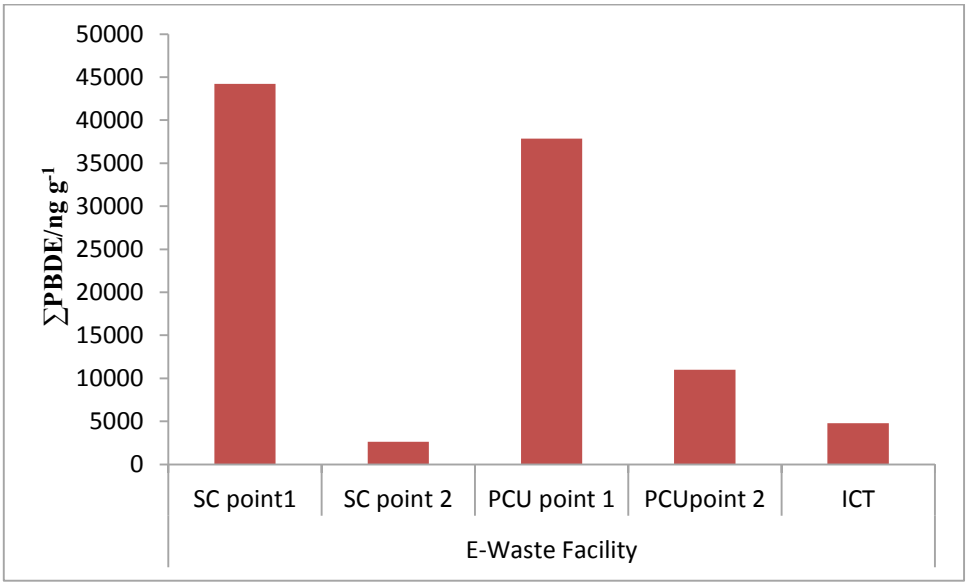

Figure 7.1 Levels of PBDEs in e-waste samples

The levels of PBDEs ranged from 123 to 27526 ng g^{-1} ($\sum_{n=8}$PBDEs = 44200 ng g^{-1}) and 205 to 34013 ng g^{-1} ($\sum_{n=8}$PBDEs =37859 ng g^{-1}) for SC point 1 and PCU point 1 samples, respectively (Table 7.1). On the other hand, the levels of PBDES in SC point 2 and PCU point 2 ranged from 11 ng g^{-1} to 1862 ng g^{-1} ($\sum_{n=8}$PBDEs = 2633 ng g^{-1}) and <DL to 9897 ng g^{-1} ($\sum_{n=8}$PBDEs = 10994 ng g^{-1}) respectively. This variability in concentrations could be attributed to the sampling point characteristics. The SC point 1 and PCU point 1 sampling points were characterized by e-waste polymers, unlike SC point 2 and PCU point 2 that comprised mainly of printed circuit boards and other internal components of PCs, mobile phones and fridges. The levels of PBDEs in ICT ranged from <DL to 2862 ng g^{-1} ($\sum_{n=8}$PBDEs = 4784 ng g^{-1}). Overall, the levels of PBDEs were generally higher in samples collected around e-waste polymers. Generally, BDE congeners 209 and 99 were the most prevalent among the studied congeners (Fig 7.2). Both congeners accounted for 76% and 7%, respectively of the total PBDE concentration in all sites. The distribution pattern of PBDEs was BDE 28 < BDE 100 < BDE 183 < BDE 153 < BDE 154 < BDE 47 < BDE 99 < BDE 209.

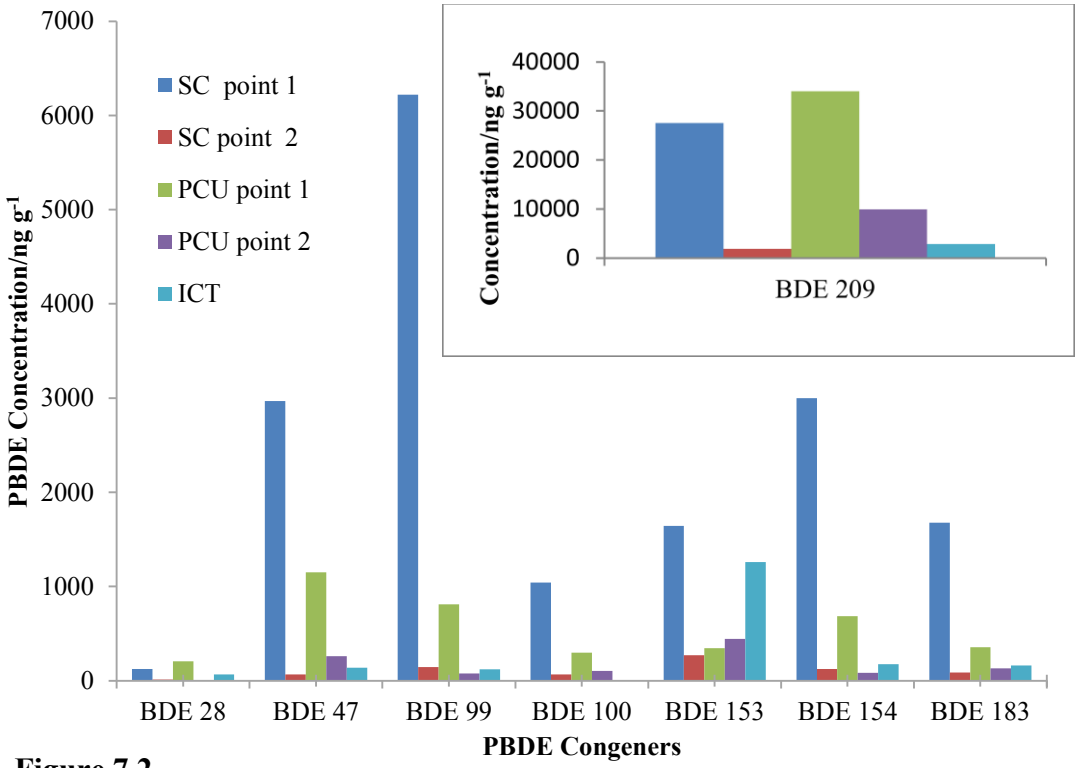

Figure 7.2
Congener profiles of PBDEs in e-waste recycling facilities.

The concentrations of PBDEs in this study were higher than levels reported in Guiyu, China (13) in indoor dust from e-waste workshops, and also higher than levels reported by Tue et al. (8) in settled house dust around Vietnamese e-waste recycling sites, but several orders of magnitude lower than levels recently reported in indoor dust from e-waste recycling sites in Thailand and Southern China (Muenhor et al., 2010; Labunska et al., 2013). The levels of pentaBDEs in this study could be related to those reported by Yang et al. (4), in which the profiles of $PBDE_{4-6}$ were found to be similar to the commercial pentaBDE products (with trade name, DE-71 and Bromkal 70 – 5DE), thus implicating the use of these two products in e-wastes. Similarly, the high levels of BDE 99, 100, 153 and 154 could be linked to debromination of BDE-209 (4); as well as the influence of penta- and octa-BDE commercial formulations with known applications in printed circuit board components (19). Similarly, our results for tetra- through hexaBDEs were in line with levels reported in indoor dust which were consistent with concentrations in human serum and milk of the US general population (21). However, the levels of BDE-183 can be attributed to the commercial octa-BDE formulation, which contains about 80 % of total octa-BDE; since this formulation was used as a flame retardant in plastic computer monitors and television housings (4). The high concentrations of BDE 209 in this study resemble those reported in Poland (22), Denmark (23) and USA (21). This high levels of BDE 209 could be aligned with the fact the decaBDE mix, which is the commercial mixture added to polymers employed in the production of the housings of electronic equipment, contains about 97-99% BDE 209

(22). Congeners with longer half-lives such as the tetra- through hexaBDEs may be of greater concern from a toxicological point of view (24); even though these congeners were found at levels lower than BDE 209, but higher than BDE 183 in this study. The fact that they are more likely to accumulate to higher concentrations in occupationally exposed workers over time, has been observed in studies measuring human body burdens of PBDEs in the USA (21, 25-28).

Table 7.1 Descriptive statistics for PBDE concentrations/ng g^{-1} in e-waste facilities.

	BDE 28	BDE 47	BDE 99	BDE 100	BDE 153	BDE 154	BDE 183	BDE 209	∑ PBDE
SC point 1	123	2968	6220	1042	1643	3000	1677	27530	44203
SC point 2	11	67	144	66	270	124	88	1862	2632
PCU point 1	205	1151	809	297	345	683	355	34010	37855
PCU point 2	0	260	77	103	445	83	130	9897	10995
ICT	67	136	121	0	1261	175	163	2862	4785
Mean	81.2	916.4	1474.2	301.6	792.8	813	482.6	15232.2	20094
Median	67	260	144	103	445	175	163	9897	10995
Minimum	0	67	77	0	270	83	88	1862	2632
Maximum	205	2968	6220	1042	1643	3000	1677	34010	44203
5th percentile	2.2	80.8	85.8	13.2	285	91.2	96.4	2062	3062.6
95th percentile	188.6	2604.6	5137.8	893	1566.6	2536.6	1412.6	32714	42933.4

7.3.3 Levels of PCBs Contamination in Samples

The concentrations of PCBs in this study varied among sampling points (Table 7.2). PCB levels ranged from 10 – 342 ng g^{-1} ($\sum_{(n=3)}$PCBs = 490 ng g^{-1}) and 7.2 – 109 ng g^{-1} ($\sum_{(n=3)}$PCBs = 163 ng g^{-1}) for SC point 2 and PCU point 2, respectively.

Similarly, levels of PCBs in SC point 1, PCU point 1 and ICT ranged from <DL to 42 ($\sum_{(n=3)}$PCBs =55 ng g^{-1}), 7.1 – 38 ng g^{-1}($\sum_{(n=3)}$PCBs =55) and <DL to 342 ng g^{-1} ($\sum_{(n=3)}$PCBs =412 ng g^{-1}, respectively (Fig. 7.3).

Table 7.2 Descriptive statistics for PCB concentrations/ng g^{-1} in e-waste facilities.

	CB 28	CB 153	CB 180	∑PCB
SC point 1	13	0	42	55
SC point 2	10	342	138	490
PCU point 1	7	47	109	163
PCU point 2	9	7	38	54
ICT	0	69	342	411
Mean	7.8	93	133.8	234.6
Median	9	47	109	163
Minimum	0	0	38	54
Maximum	13	342	342	490
5th percentile	1.4	1.4	38.8	54.2
95th percentile	12.4	287.4	301.2	474.2

< DL below the detection limit

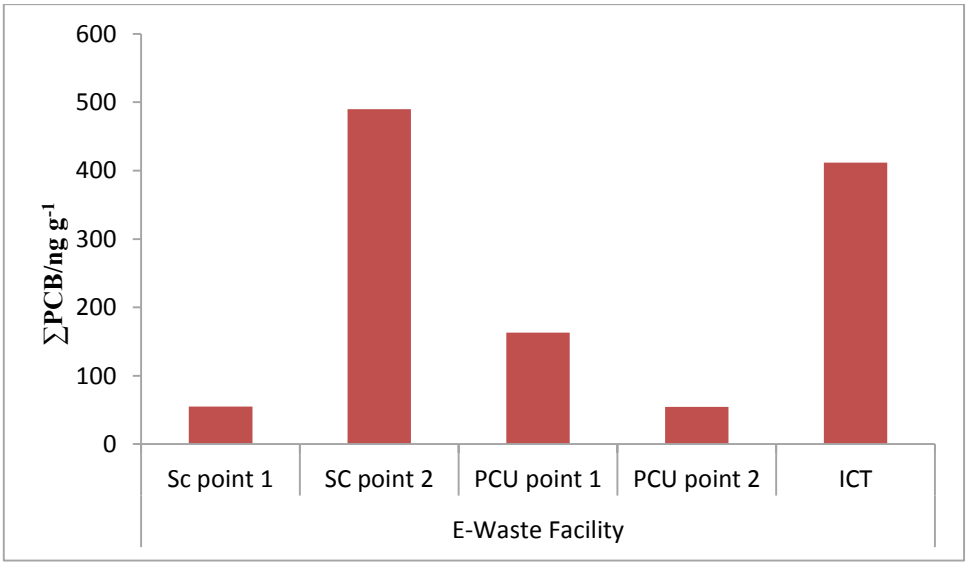

Figure 7.3 Levels of PCBs in e-waste samples.

PCB 180 was the most abundant of all studied congeners (Fig 7.4) in samples with the exception of SC point 2 samples in which PCB-153 dominated. PCB-180 accounted for 57% of total PCBs in all samples. The distribution pattern of PCBs was in the order PCB-28 < PCB-153 < PCB-180. These PCB concentrations could be linked to the application of PCBs as coolants and dielectric fluids in transformers, capacitors and electric motors, which might have been released into the environment during dismantling of e-waste (4). PCB concentrations in this study were a few order of magnitude lower than levels measured in hair of e-waste dismantling site workers (9) and several orders of magnitude lower than levels reported in dust from e-waste dismantling sites (9).

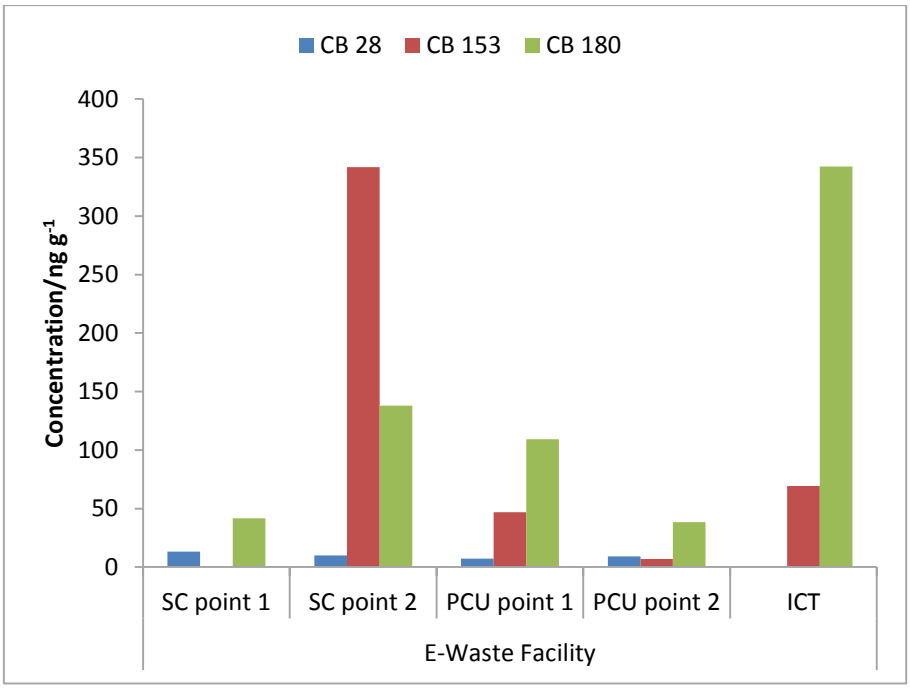

Figure 7.4 Congener profiles of PCBs in e-waste recycling facilities.

7.3.4 Variation in PBDE and PCB Profiles after Site Clean-up/Maintenance

The levels of PBDEs and PCBs varied widely between two sampling periods at the same facility, occasioned by e-waste facility maintenance. The concentrations of PBDEs and PCBs in the winter of 2012, prior to facility maintenance ranged from 123 – 27525 ng $g^{-1}$and 161 – 593 ng g^{-1} respectively (Supplementary Material Table S7.4 and S7.5). These values were one order of magnitude (for PBDEs and for PCBs) higher than levels (91 - 7686 ng g^{-1} and <DL – 42 ng g^{-1} for PBDEs and PCBs, respectively) obtained in the summer of 2013 after facility maintenance (Fig 7.5 and 7.6). The results obtained were significantly different ($p = 0.010$) for PBDEs and ($p = 0.033$) for PCBs. This significant difference could be associated with the maintenance carried out at the e-waste

facility in the summer of 2013 prior to sampling. This result points to the fact that regular indoor maintenance of e-waste facilities could significantly reduce the occupational exposure to persistent organic pollutants.

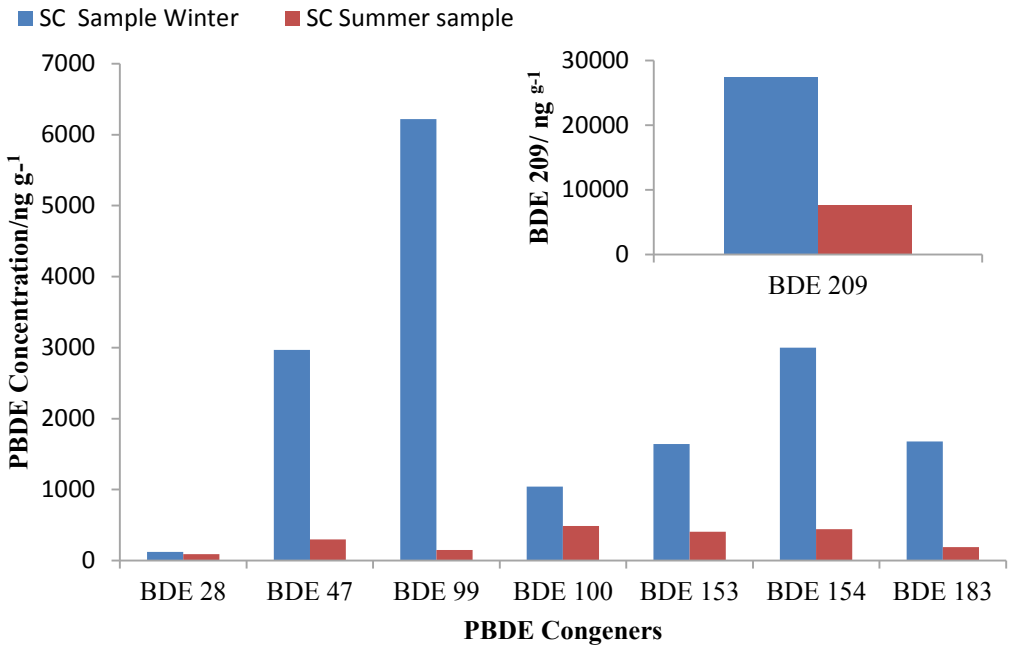

Figure 7.5 Variation in PBDE congener profiles before (blue bar) and after (red bar) site maintenance, BDE-209 (inset).

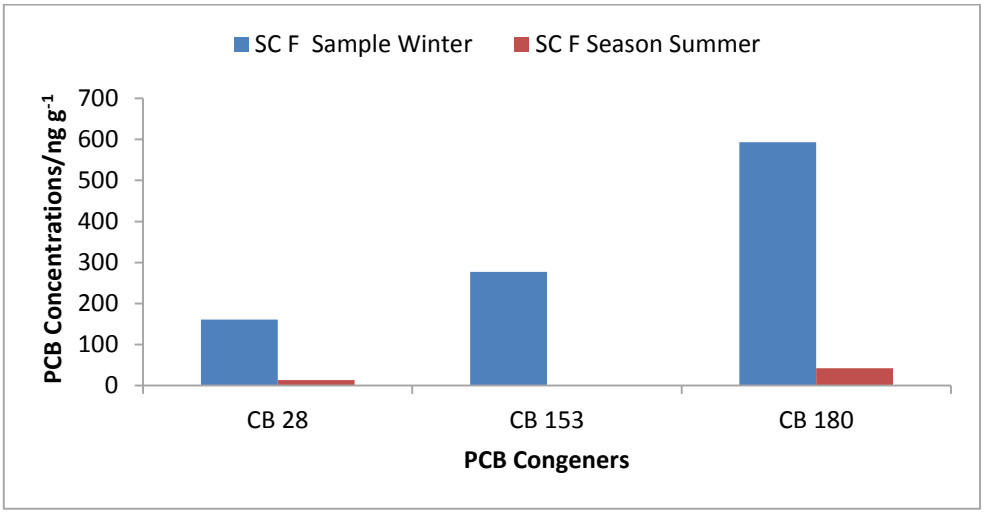

Figure 7.6 Variation in PCB congener profiles before (blue bar) and after (red bar) site maintenance.

7.3.4 Occupational Exposure Assessment

Dietary intake is commonly considered the main exposure route for many lipophilic and persistent organohalogen compounds, such as PCBs and persistent pesticides (21). However, in the case of PBDEs, evidence suggests that food consumption is not the major pathway, but indoor exposure through ingestion, inhalation and skin contact may be of higher importance (21). Available data on dust ingestion rates are limited and subject to uncertainties. However, reports shows that the magnitude of exposure is highly dependent on daily activities (21). The importance of dust as an exposure route of PBDEs has been shown at electronic waste recycling sites in the USA (18), Sweden (21) and other locations (5, 12, 17, 29-31). In this study, the daily exposure dose of PBDEs and PCBs ($\sum$DED/ng kg^{-1} bw day^{-1}) were estimated by using the dust exposure factors of Jones-Otazo et al.; Ali et al. and Johnson-Restrepo and Kannan (5, 6, 32). The daily occupational exposure time, i.e., the time spent daily at recycling sites, were obtained by interviews at the various recycling sites. Thus, an average of 10 hours per day was employed in the present study. A 100 % absorption of contaminants from ingested dust was assumed in this study, in accordance to other reports (6). Diverse exposure scenarios were examined by exploiting the 5^{th} percentile, median, mean and 95^{th} percentile concentrations obtained from samples. The estimated daily intake of PBDEs and PCBs in the work place through different exposure scenarios is presented in Table 7.3. The $\sum$DED of PBDEs obtained at (mean 95^{th} percentile) for both mean and high dust intake rates were relatively lower than the EPA reference dose (RfD) of 0.1, 0.1, 0.2 and 0.7 µg kg^{-1} bw day^{-1} for BDE-47 BDE-99, BDE-153 and BDE 209 (6, 33). Similarly, the $\sum$DED of PCBs obtained in this study were lower than the reference doses of 0.02 µg kg^{-1} bw day^{-1} and 0.07 µg kg^{-1} bw day^{-1} for Aroclor 1254 and 1016 (34). This high $\sum$DED of PBDEs in this study is in agreement with the evidence that occupational dust exposure via ingestion may be the primary exposure mechanism for workers (9, 20). Overall, the levels of daily exposure doses to PBDEs and PCBs in this study suggest that both dust ingestion and dust dermal absorption are major pathways of exposure for workers to these contaminants in the workplace. Thus, South African e-waste recyclers employing primitive recycling technologies are exposed to high doses of the more bioaccumulative tetra- to hexa–BDEs, and to some extent BDE-209 and PCBs.

Table 7.3 Assessment of occupational exposure to PBDEs and PCBs via dust ingestion and dust dermal absorption (ng kg^{-1}bw day^{-1}) by using mean and high dust intake rates. 100% dust absorption and intake was assumed in this study (5, 7, 31).

	Occupational Exposure							
	Mean dust ingestion[a]				High dust ingestion[b]			
	5th Percentile	Median	Mean	95th Percentile	5th Percentile	Median	Mean	95th Percentile
PBDEs								
Dust Ingestion	0.36	1.31	2.39	5.12	0.91	3.27	5.99	12.79
Dust Dermal Absorption	0.24	0.87	1.59	3.40	0.24	0.87	1.59	3.40
∑DED PBDEs	**0.60**	**2.18**	**3.98**	**8.52**	**1.15**	**4.14**	**7.58**	**16.19**
PCBs								
Dust Ingestion	0.006	0.019	0.028	0.056	0.016	0.049	0.070	0.141
Dust Dermal Absorption	0.004	0.013	0.019	0.038	0.004	0.013	0.019	0.038
∑DED PCBs	**0.010**	**0.032**	**0.047**	**0.094**	**0.020**	**0.062**	**0.089**	**0.179**

[a]Mean dust ingestion rate for adults = 20 mg day^{-1}

[b] High dust ingestion rate for adults = 50 mg day^{-1}

7.4 Conclusions

The levels and congener profiles of PBDEs and PCBs have been documented in indoor dust from e-waste recycling/repair sites in South Africa. To our knowledge, this is the first study emanating from the African region on the presence of these pollutants in the workplace. The daily exposure doses of these contaminants were generally lower than their corresponding reference doses and levels obtained were comparable to levels obtained in China and Vietnam. Hence, the study suggests a high exposure of South African recyclers to these pollutants and therefore calls for urgent recycling guidelines and contaminated site mitigation to reduce overall occupational exposure and subsequent adverse effects to human health. It was shown that regular cleaning / site maintenance can reduce these contaminants significantly.

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Supplementary Material

Supplementary Material S7.1

Determination of Concentrations of individual PBDEs and PCBs in samples

$$\text{Concentration} = \frac{A_{TP}}{A_{IS}} \times \frac{1}{RR_F} \times \frac{M_{IS}}{SS}$$

A_{IS} = Peak area of internal standard in sample

A_{TP} = Peak area of target pollutant

RR_F = Relative response factor for the target pollutant

M_{IS} = Mass of internal standard added to sample (ng)

SS = Sample size (g)

Supplementary Material S7.2

Estimation of daily exposure dose of polybrominated diphenyl ethers and polychlorinated biphenyls from house dust (ng kg^{-1}bw day^{-1}) for five age groups in the South African environment

The Restrepo and Kannan equation as adapted from USEPA was applied for the estimation of the daily exposure doses.

$$\text{Dust ingestion DED} = \frac{C.\,SIR.\,IEF}{BW}$$

$$\text{Dust dermal absorption DED} = \frac{C.\,BSA.\,SAS.\,AF.\,IEF}{BW.\,1000}$$

C = ∑ PBDEs or PCBs concentration (ng / g dry weight)

BSA = Body surface area (cm^2 day^{-1}) = 4615

SIR = dust ingestion rate (mg day^{-1}) = 20 & 50

BW = Body weight (kg) = 70

SAS = dust adhered to skin (mg cm^{-2}) = 0.096

AF = Fraction of PBDEs or PCBs adsorbed in the skin = 0.03

IEF = Indoor exposure fraction (hours spent over a day in an indoor environment) = 0.417

The values assumed for SAS and AF were 0.096 mg/cm^2 and 0.03 respectively, as reported by Johnson-restrepo and Kannan (32).

Table S7.1: Variation in PBDE congeners profile (in ng g^{-1}) in an e-waste facility before (winter season) and after (summer season) facility maintenance.

BDE/ CB Congener	SC F Sample Winter	SC F Season Summer
BDE 28	123.37	90.80
BDE 47	2968.41	301.00
BDE 99	6219.55	150.67
BDE 100	1041.69	487.69
BDE 153	1643.2	408.67
BDE 154	3000.36	441.51
BDE 183	1677.07	189.33
BDE 209	27525.98	7685.69
∑PBDE in Facility	44199.63	9755.36
Mean	5524.95	1219.42
Median	2322.74	354.84
Minimum	123.37	90.80
Maximum	27525.98	7685.69
5th Percentile	444.78	111.75
95th Percentile	20068.73	5166.39

Table S7.2 Variation in PCB congeners profile (in ng g^{-1}) in an e-waste facility before (winter season) and after (summer season) facility maintenance.

BDE/ CB Congener	SC F Sample Winter	SC F Season Summer
CB 28	160.61	13.22
CB 153	276.75	>DL
CB 180	593.22	41.6
∑PBDE in Facility	1030.58	54.82
Mean	343.53	18.27
Median	276.75	13.22
Minimum	160.61	>DL
Maximum	593.22	41.6
5th Percentile	172.22	1.32
95th Percentile	561.57	38.76

Table S7.3 PBDE concentrations (in ng g^{-1}) at e-waste recycling facilities.

PBDE Congener	E-Waste Facility					
	SC point 1	SC point 2	PCU point 1	PCU point 2	ICT	∑ PBDE
BDE 28	123	11	205	0	67	406
BDE 47	2968	67	1151	260	136	4582
BDE 99	6220	144	809	77	121	7371
BDE 100	1042	66	297	103	0	1507
BDE 153	1643	270	345	445	1261	3964
BDE 154	3000	124	683	83	175	4067
BDE 183	1677	88	355	130	163	2412
BDE 209	27526	1862	34013	9897	2862	76159
∑PBDE in Facility	44200	2633	37859	10994	4784	100470
Mean	5525	329	4732	1374	598	12559
Median	2323	106	519	116	149	4015
Minimum	123	11	205	0	0	406
Maximum	27526	1862	34013	9897	2862	76159
5th Percentile	445	30	237	27	23	792
95th Percentile	20069	1305	22511	6589	2301	52084

Table S7.4 PCB concentrations (in ng g^{-1}) in e-waste recycling facilities.

PCB Congener	E-Waste Facility					
	SC point 1	SC point 2	PCU point 1	PCU point 2	ICT	∑ PCB
CB 28	13	10	7	9	0	39
CB 153	0	342	47	7	69	465
CB 180	42	138	109	38	342	670
∑PCB in Facility	55	490	163	55	412	1174
Mean	18	163	54	18	137	391
Median	13	138	47	9	69	465
Minimum	0	10	7	7	0	39
Maximum	42	342	109	38	342	670
5th Percentile	1	23	11	7	7	82
95th Percentile	39	321	103	35	315	649

Table S7.5 Distribution of PCBs in the sampling sites

XLSTAT 2014.6.01 - Distribution fitting - on 2014/12/02 at 04:08:13 PM
Data: Workbook = Book1 / Sheet = Sheet1 / Range =
Sheet1!$I$3:$M$6 / 3 rows and 5 columns
Significance level (%): 5
Distribution: Normal
Estimation method: Moments

Summary statistics:

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
SC point 1	3	0	3	0	42	18	21
SC point 2	3	0	3	10	342	163	167
PCU point 1	3	0	3	7	109	54	51
PCU point 2	3	0	3	7	38	18	17
ICT	3	0	3	0	342	137	181

Distribution fitting (SC point1):

Estimated parameters (SC point 1):

Parameter	Value
μ	18.290
sigma	21.283

Statistics estimated on the input data and computed using the estimated parameters of the Normal distribution
(SC point 1):

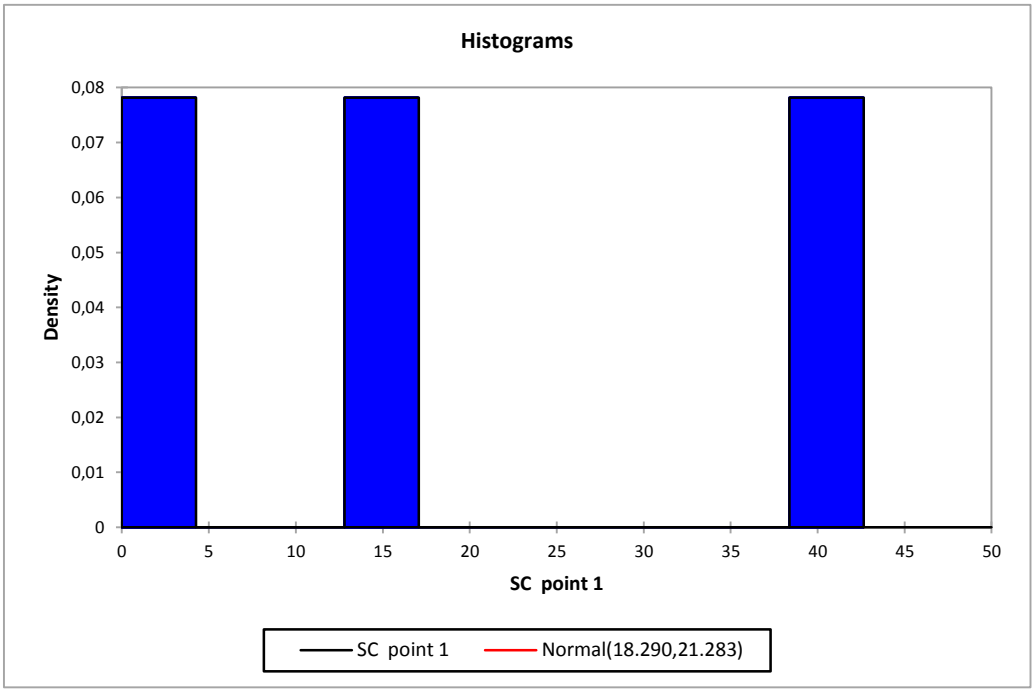
Statistic	Data	Parameters
Mean	18.290	18.290

Variance	452.959	452.959
Skewness (Pearson)	0.225	0.000
Kurtosis (Pearson)	-2.333	0.000

Kolmogorov-Smirnov test (SC point 1):

D	0.261
p-value	0.964
alpha	0.05

Test interpretation:
H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution
As the computed p-value is greater than the significance level alpha=0.05, one cannot reject the null hypothesis H0.
The risk to reject the null hypothesis H0 while it is true is 96.42%.



Descriptive statistics for the intervals (SC point 1):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	4.265	1	0.333	0.078	0.060
4.265	8.53	0	0.000	0.000	0.068
8.53	12.795	0	0.000	0.000	0.075
12.795	17.06	1	0.333	0.078	0.079
17.06	21.325	0	0.000	0.000	0.080
21.325	25.59	0	0.000	0.000	0.078
25.59	29.855	0	0.000	0.000	0.072
29.855	34.12	0	0.000	0.000	0.065
34.12	38.385	0	0.000	0.000	0.056
38.385	42.65	1	0.333	0.078	0.046

Distribution fitting (SC point 2):

Estimated parameters (SC point 2):

Parameter	Value
μ	163.243
sigma	167.314

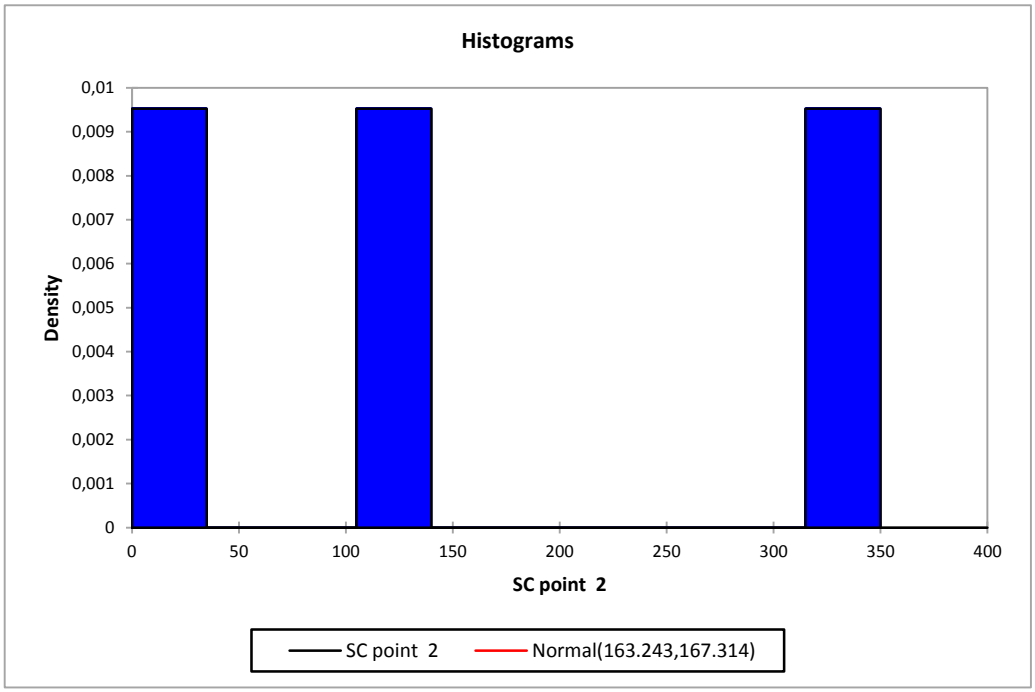
Statistics estimated on the input data and computed using the estimated parameters of the Normal distribution (SC point 2):

Statistic	Data	Parameters
Mean	163.243	163.243
Variance	27994.010	27994.010
Skewness (Pearson)	0.147	0.000
Kurtosis (Pearson)	-2.333	0.000

Kolmogorov-Smirnov test (SC point 2):	
D	0.226
p-value	0.992
alpha	0.05

Test interpretation:

H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution
As the computed p-value is greater than the significance level alpha=0.05, one cannot reject the null hypothesis H0.
The risk to reject the null hypothesis H0 while it is true is 99.18%.



Descriptive statistics for the intervals (SC point 2):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	35	1	0.333	0.010	0.057
35	70	0	0.000	0.000	0.067
70	105	0	0.000	0.000	0.075
105	140	1	0.333	0.010	0.081
140	175	0	0.000	0.000	0.083
175	210	0	0.000	0.000	0.082
210	245	0	0.000	0.000	0.077
245	280	0	0.000	0.000	0.070
280	315	0	0.000	0.000	0.060
315	350	1	0.333	0.010	0.050

Distribution fitting (PCU point 1):

Estimated parameters (PCU point 1):

Parameter	Value
μ	54.383
sigma	51.448

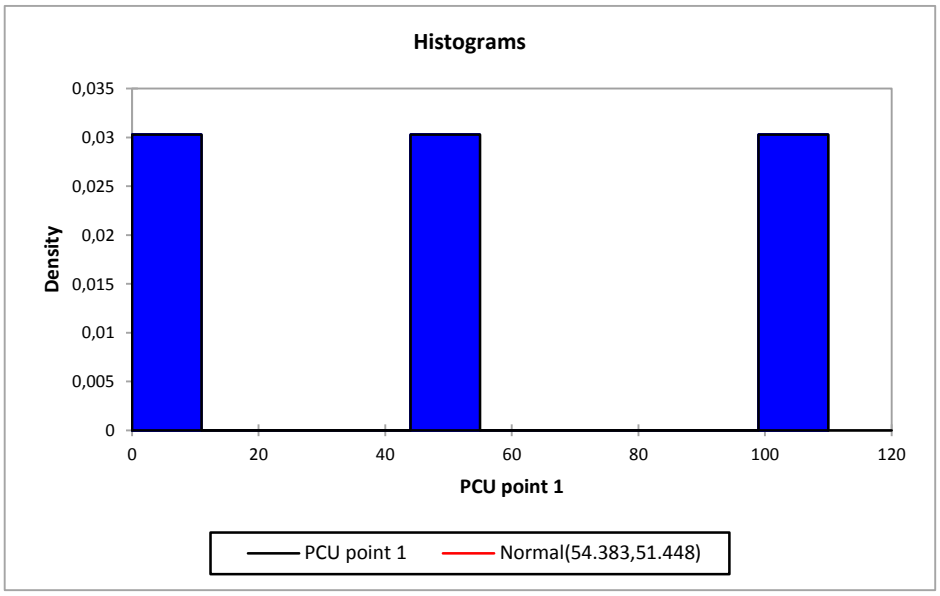
Statistics estimated on the input data and computed using the estimated parameters of the Normal distribution (PCU point 1):

Statistic	Data	Parameters
Mean	54.383	54.383
Variance	2646.909	2646.909
Skewness (Pearson)	0.145	0.000
Kurtosis (Pearson)	-2.333	0.000

Kolmogorov-Smirnov test (PCU point 1):

D	0.225
p-value	0.992
alpha	0.05

Test interpretation:
H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution
As the computed p-value is greater than the significance level alpha=0.05,
one cannot reject the null hypothesis H0.
The risk to reject the null hypothesis H0 while it is true is 99.22%.



Descriptive statistics for the intervals (PCU point 1):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	11	1	0.333	0.030	0.054
11	22	0	0.000	0.000	0.065
22	33	0	0.000	0.000	0.074
33	44	0	0.000	0.000	0.081
44	55	1	0.333	0.030	0.085
55	66	0	0.000	0.000	0.085
66	77	0	0.000	0.000	0.081
77	88	0	0.000	0.000	0.073
88	99	0	0.000	0.000	0.064
99	110	1	0.333	0.030	0.053

Distribution fitting (PCU point 2):

Estimated parameters (PCU point 2):

Parameter	Value
μ	18.197
sigma	17.477

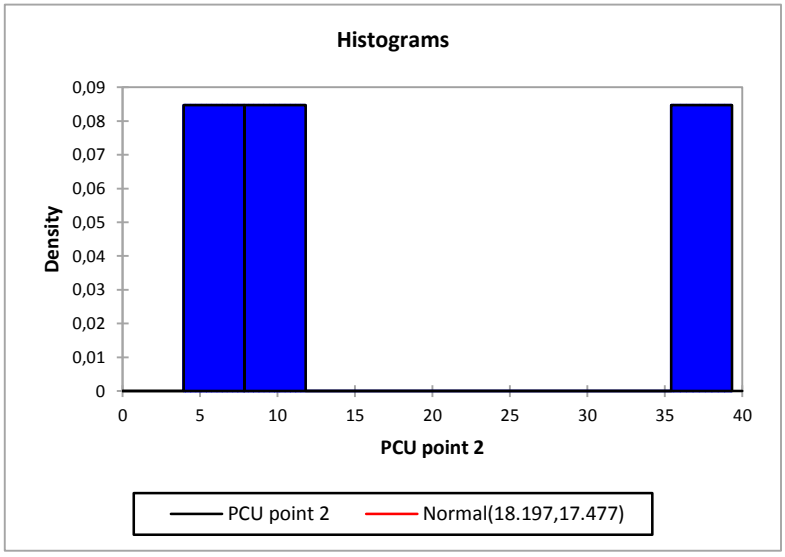
Statistics estimated on the input data and computed using the estimated parameters of the Normal distribution (PCU point 2):

Statistic	Data	Parameters
Mean	18.197	18.197
Variance	305.428	305.428
Skewness (Pearson)	0.379	0.000
Kurtosis (Pearson)	-2.333	0.000

Kolmogorov-Smirnov test (PCU point 2):

D	0.364
p-value	0.717
alpha	0.05

Test interpretation:
H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution
As the computed p-value is greater than the significance level alpha=0.05, one cannot reject the null hypothesis H0.
The risk to reject the null hypothesis H0 while it is true is 71.67%.



Descriptive statistics for the intervals (PCU point 2):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	3.934	0	0.000	0.000	0.058
3.934	7.868	1	0.333	0.085	0.070
7.868	11.802	1	0.333	0.085	0.080
11.802	15.736	0	0.000	0.000	0.087
15.736	19.67	0	0.000	0.000	0.090
19.67	23.604	0	0.000	0.000	0.088
23.604	27.538	0	0.000	0.000	0.082
27.538	31.472	0	0.000	0.000	0.073
31.472	35.406	0	0.000	0.000	0.061
35.406	39.34	1	0.333	0.085	0.049

Distribution fitting (ICT):

Estimated parameters (ICT):

Parameter	Value
μ	137.273
sigma	181.034

Statistics estimated on the input data and computed using the estimated parameters of the Normal distribution (ICT):

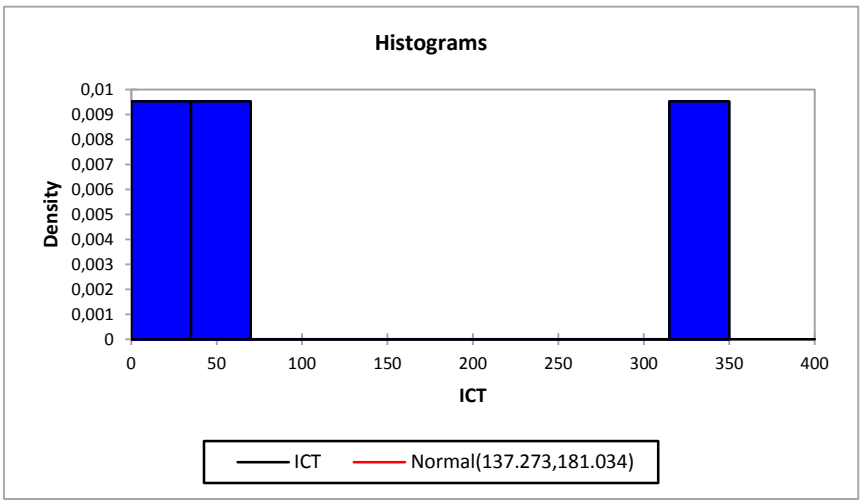
Statistic	Data	Parameters
Mean	137.273	137.273
Variance	32773.417	32773.417
Skewness (Pearson)	0.322	0.000
Kurtosis (Pearson)	-2.333	0.000

Kolmogorov-Smirnov test (ICT):

D	0.313
p-value	0.865
alpha	0.05

Test interpretation:
H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution
As the computed p-value is greater than the significance level alpha=0.05,
one cannot reject the null hypothesis H0.

The risk to reject the null hypothesis H0 while it is true is 86.52%.



Descriptive statistics for the intervals (ICT):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	35	1	0.333	0.010	0.062
35	70	1	0.333	0.010	0.069
70	105	0	0.000	0.000	0.074
105	140	0	0.000	0.000	0.077
140	175	0	0.000	0.000	0.077
175	210	0	0.000	0.000	0.074
210	245	0	0.000	0.000	0.068
245	280	0	0.000	0.000	0.061
280	315	0	0.000	0.000	0.052
315	350	1	0.333	0.010	0.043

Supplementary Material Table S7.6 Distribution of PBDEs in the sampling sites

XLSTAT 2014.6.01 - Distribution fitting - on 2014/12/02 at 03:51:39 PM
Data: Workbook = Book1 / Sheet = Sheet1 / Range = Sheet1!$B$2:$F$10 / 8 rows and 5 columns
Significance level (%): 5
Distribution: Normal
Estimation method: Moments

Summary statistics:

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
SC point 1	8	0	8	123	27526	5525	9076
SC point 2	8	0	8	11	1862	329	624
PCU point 1	8	0	8	205	34013	4732	11835
PCU point 2	8	0	8	0	9897	1374	3446
ICT	8	0	8	0	2862	598	1001

Distribution fitting (SC point 1):

Estimated parameters (SC point 1):

Parameter	Value
μ	5524.954
sigma	9075.777

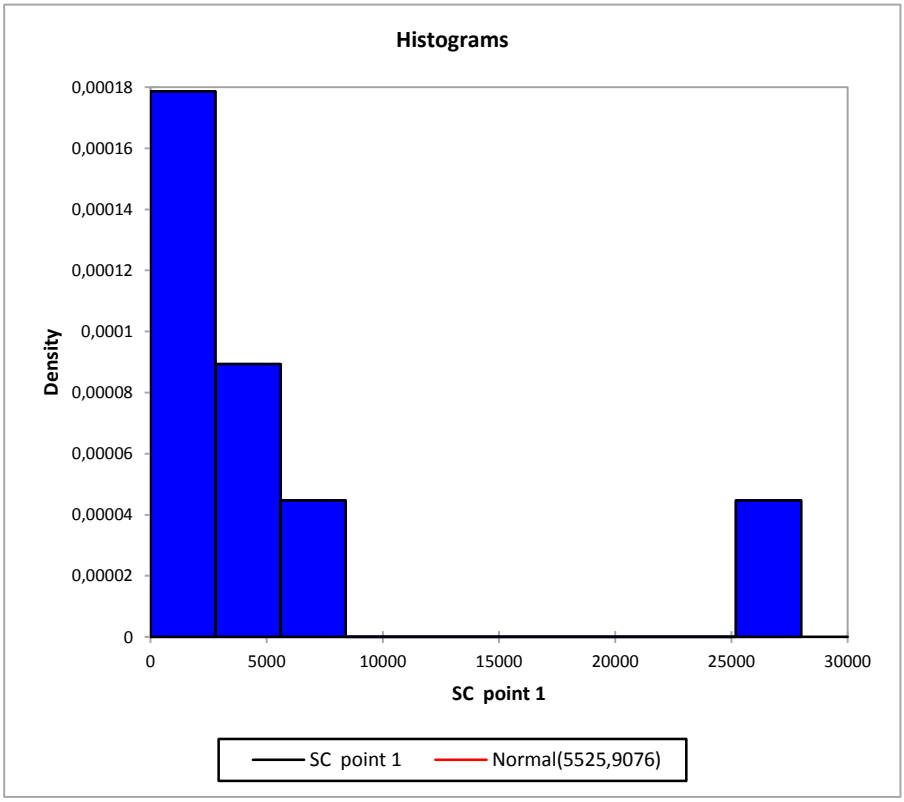
Statistics estimated on the input data and computed using the estimated parameters of the Normal distribution (SC point 1):

Statistic	Data	Parameters
Mean	5524.954	5524.954
Variance	##########	82369727.420
Skewness (Pearson)	1.715	0.000
Kurtosis (Pearson)	1.350	0.000

Kolmogorov-Smirnov test (SC point 1):

D	0.360
p-value	0.199
alpha	0.05

Test interpretation:
H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution
As the computed p-value is greater than the significance level alpha=0.05,
one cannot reject the null hypothesis H0.
The risk to reject the null hypothesis H0 while it is true is 19.88%.


Histograms
0,00018
0,00016
0,00014
0,00012
0,0001
0,00008
0,00006
0,00004
0,00002
0
Density
0
5000
10000
15000
20000
25000
30000
SC point 1
SC point 1
Normal(5525,9076)

Descriptive statistics for the intervals (SC point 1):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	2800	4	0.500	0.000	0.111
2800	5600	2	0.250	0.000	0.121
5600	8400	1	0.125	0.000	0.121
8400	11200	0	0.000	0.000	0.110
11200	14000	0	0.000	0.000	0.091
14000	16800	0	0.000	0.000	0.068
16800	19600	0	0.000	0.000	0.047
19600	22400	0	0.000	0.000	0.029
22400	25200	0	0.000	0.000	0.016
25200	28000	1	0.125	0.000	0.008

Distribution fitting (SC point 2):

Estimated parameters (SC point 2):

Parameter	Value
μ	329.130
sigma	624.228

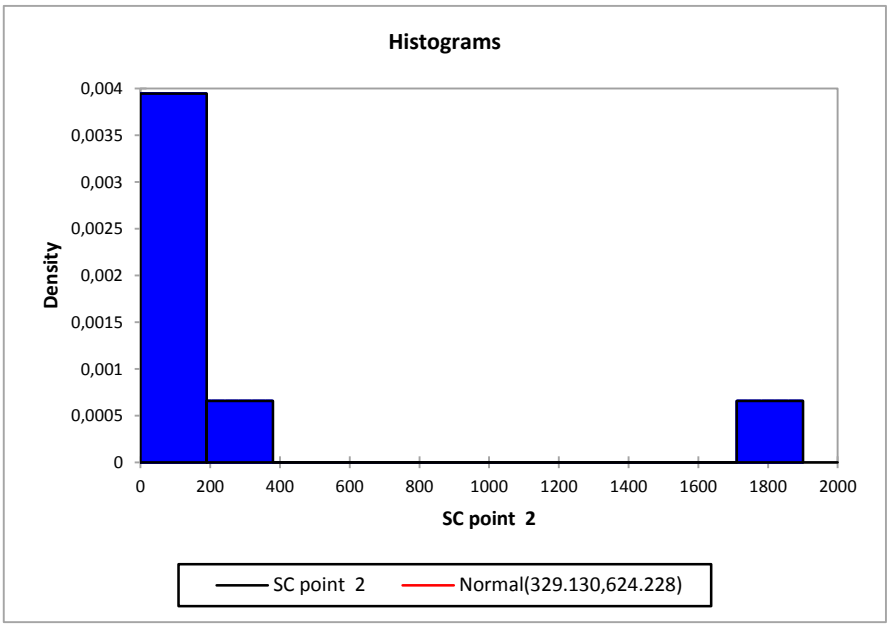
Statistics estimated on the input data and computed using the estimated parameters of the Normal distribution (SC point 2):

Statistic	Data	Parameters
Mean	329.130	329.130
Variance	389660.613	389660.613
Skewness (Pearson)	1.802	0.000
Kurtosis (Pearson)	1.570	0.000

Kolmogorov-Smirnov test (SC point 2):

D	0.413
p-value	0.096
alpha	0.05

Test interpretation:
H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution
As the computed p-value is greater than the significance level alpha=0.05, one cannot reject the null hypothesis H0.
The risk to reject the null hypothesis H0 while it is true is 9.59%.



Descriptive statistics for the intervals (SC point 2):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	190	6	0.750	0.004	0.113
190	380	1	0.125	0.001	0.121
380	570	0	0.000	0.000	0.118
570	760	0	0.000	0.000	0.105
760	950	0	0.000	0.000	0.085
950	1140	0	0.000	0.000	0.063
1140	1330	0	0.000	0.000	0.043
1330	1520	0	0.000	0.000	0.026
1520	1710	0	0.000	0.000	0.015
1710	1900	1	0.125	0.001	0.008

Distribution fitting (PCU point 1):

Estimated parameters (PCU point 1):

Parameter	Value
μ	4732.350
sigma	11835.338

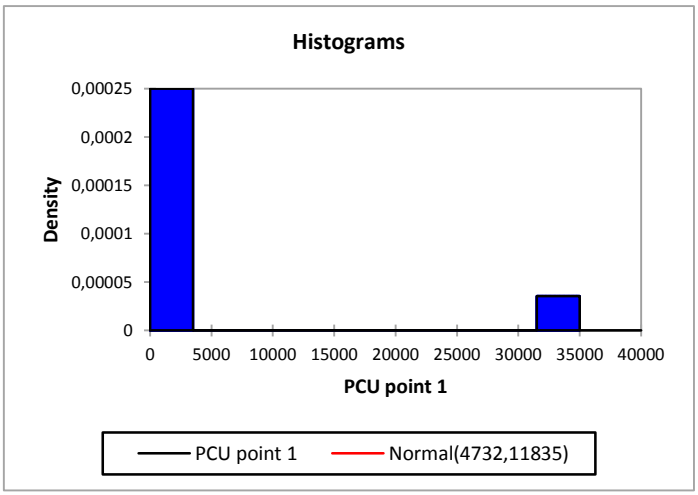
Statistics estimated on the input data and computed using the estimated parameters of the Normal distribution (PCU point 1):

Statistic	Data	Parameters
Mean	4732.350	4732.350
Variance	##########	###########
Skewness (Pearson)	1.853	0.000
Kurtosis (Pearson)	1.697	0.000

Kolmogorov-Smirnov test (PCU point 1):

D	0.494
p-value	0.026
alpha	0.05

Test interpretation:
H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution
As the computed p-value is lower than the significance level alpha=0.05,
one should reject the null hypothesis H0, and accept the alternative hypothesis Ha.
The risk to reject the null hypothesis H0 while it is true is lower than 2.57%.



Descriptive statistics for the intervals (PCU point 1):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	3500	7	0.875	0.000	0.114
3500	7000	0	0.000	0.000	0.117
7000	10500	0	0.000	0.000	0.111
10500	14000	0	0.000	0.000	0.096
14000	17500	0	0.000	0.000	0.076
17500	21000	0	0.000	0.000	0.056
21000	24500	0	0.000	0.000	0.037
24500	28000	0	0.000	0.000	0.023
28000	31500	0	0.000	0.000	0.013
31500	35000	1	0.125	0.000	0.007

Distribution fitting (PCU point 2):

Estimated parameters (PCU point 2):

Parameter	Value
μ	1374.308
sigma	3446.421

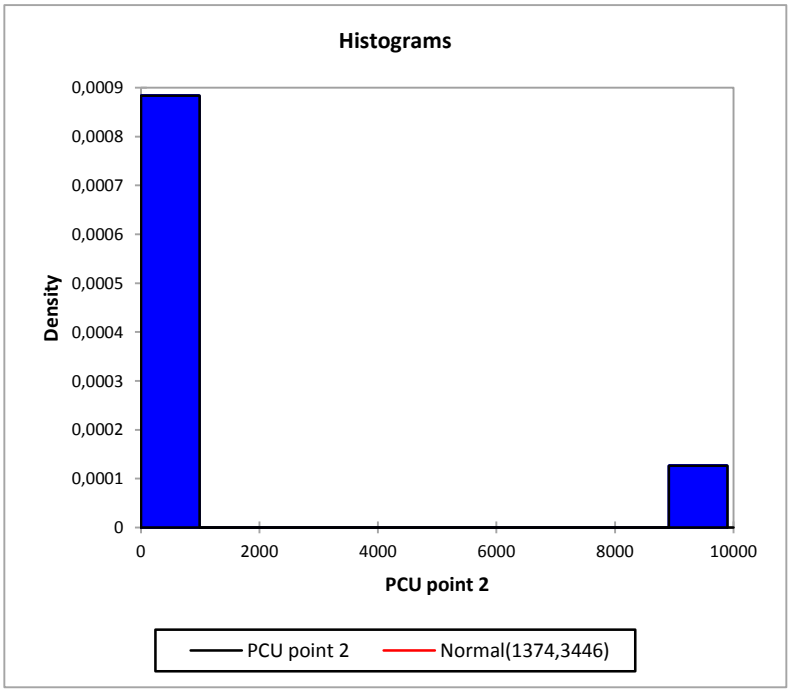
Statistics estimated on the input data and computed using the estimated parameters of the Normal distribution (PCU point 2):

Statistic	Data	Parameters
Mean	1374.308	1374.308
Variance	##########	11877817.074
Skewness (Pearson)	1.850	0.000
Kurtosis (Pearson)	1.689	0.000

Kolmogorov-Smirnov test (PCU point 2):

D	0.481
p-value	0.032
alpha	0.05

Test interpretation:
H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution
As the computed p-value is lower than the significance level alpha=0.05,
one should reject the null hypothesis H0, and accept the alternative hypothesis Ha.
The risk to reject the null hypothesis H0 while it is true is lower than 3.20%.



Descriptive statistics for the intervals (PCU point 2):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	990	7	0.875	0.001	0.111
990	1980	0	0.000	0.000	0.114
1980	2970	0	0.000	0.000	0.109
2970	3960	0	0.000	0.000	0.095

3960	4950	0	0.000	0.000	0.077
4950	5940	0	0.000	0.000	0.057
5940	6930	0	0.000	0.000	0.039
6930	7920	0	0.000	0.000	0.025
7920	8910	0	0.000	0.000	0.014
8910	9900	1	0.125	0.000	0.008

Distribution fitting (ICT):

Estimated parameters (ICT):

Parameter	Value
μ	598.015
sigma	1000.839

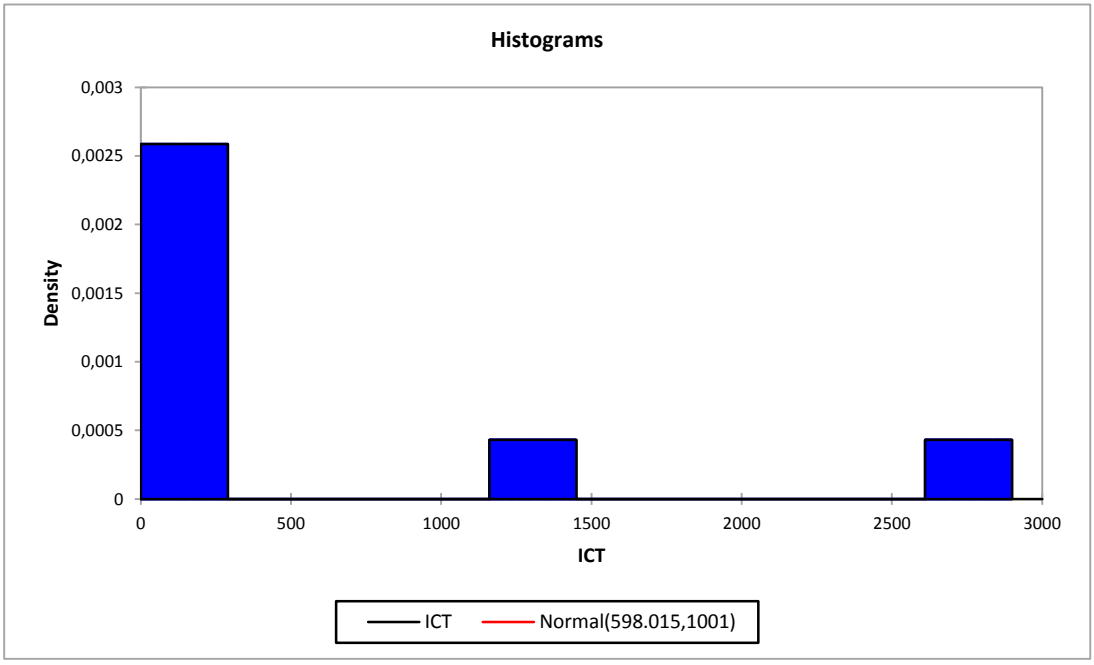
Statistics estimated on the input data and computed using the estimated parameters of the Normal distribution (ICT):

Statistic	Data	Parameters
Mean	598.015	598.015
Variance	1001679.644	1001679.644
Skewness (Pearson)	1.391	0.000
Kurtosis (Pearson)	0.341	0.000

Kolmogorov-Smirnov test (ICT):

D	0.414
p-value	0.094
alpha	0.05

Test interpretation:
H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution
As the computed p-value is greater than the significance level alpha=0.05,
one cannot reject the null hypothesis H0.
The risk to reject the null hypothesis H0 while it is true is 9.44%.



Descriptive statistics for the intervals (ICT):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	290	6	0.750	0.003	0.104
290	580	0	0.000	0.000	0.114
580	870	0	0.000	0.000	0.114
870	1160	0	0.000	0.000	0.106
1160	1450	1	0.125	0.000	0.090
1450	1740	0	0.000	0.000	0.070
1740	2030	0	0.000	0.000	0.051
2030	2320	0	0.000	0.000	0.034
2320	2610	0	0.000	0.000	0.020
2610	2900	1	0.125	0.000	0.011

Table S7.7 Kruskal-Wallis, Friedman Comparison of PBDEs in the three sample sites.

XLSTAT 2014.6.01 - Comparison of k samples (Kruskal-Wallis, Friedman, ...) - on 2014/12/02 at 04:17:58 PM
Samples: Workbook = XLSTAT for FRs in e-waste.xlsx / Sheet = Sheet1 / Range = Sheet1!$B$26:$D$34 / 8 rows and 3 columns
Significance level (%): 5
p-value: Asymptotic p-value

Summary statistics:

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
TOTAL SC	8	0	8	134.380	29388.280	5854.084	9688.041
Total PCU	8	0	8	205.200	43909.630	6106.658	15279.074
ICT	8	0	8	0.000	2861.570	598.015	1000.839

Friedman's test:

Q (Observed value)	10.750
Q (Critical value)	5.991
DF	2
p-value (Two-tailed)	0.005
alpha	0.05

Test interpretation:
H0: The samples come from the same population.
Ha: The samples do not come from the same population.
As the computed p-value is lower than the significance level alpha=0.05, one should reject the null hypothesis H0, and accept the alternative hypothesis Ha.

The risk to reject the null hypothesis H0 while it is true is lower than 0.46%.

Supplementary Material Table S7.8 Kolmogorov-Smirnov Test for Comparison of PCB profiles in the Winter and Summer Seasons

XLSTAT 2014.6.01 - Comparison of two distributions (Kolmogorov-Smirnov,) - on 2014/12/02 at 04:11:39 PM
Sample 1: Workbook = Book1 / Sheet = Sheet1 / Range = Sheet1!$F$15:$F$18 / 3 rows and 1 column
Sample 2: Workbook = Book1 / Sheet = Sheet1 / Range = Sheet1!$G$15:$G$18 / 3 rows and 1 column
Hypothesized difference (D): 0
Significance level (%): 5
p-value: Asymptotic p-value
Summary statistics:

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
SC Sample Winter	3	0	3	161	593	344	224
SC Summer sample	3	0	3	0	42	18	21

Two-sample Kolmogorov-Smirnov test / Two-tailed test:

D	1.000
p-value	0.033
alpha	0.05

An approximation has been used to compute the p-value.

Test interpretation:
H0: The two samples follow the same distribution.
Ha: The distributions of the two samples are different.

As the computed p-value is lower than the significance level alpha=0.05, one should reject the null hypothesis H0, and accept the alternative hypothesis Ha.

The risk to reject the null hypothesis H0 while it is true is lower than 3.26%.

Table S7.9 Kolmogorov-Smirnov Test for Comparison of PBDE profiles in the Winter and Summer Seasons

XLSTAT 2014.6.01 - Comparison of two distributions (Kolmogorov-Smirnov, ...) - on 2014/12/02 at 04:04:11 PM
Sample 1: Workbook = Book1 / Sheet = Sheet1 / Range = Sheet1!$A$15:$A$23 / 8 rows and 1 column
Sample 2: Workbook = Book1 / Sheet = Sheet1 / Range = Sheet1!$B$15:$B$23 / 8 rows and 1 column
Hypothesized difference (D): 0
Significance level (%): 5
p-value: Asymptotic p value

Summary statistics:

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
SC Sample Winter	8	0	8	123.370	27525.980	5524.954	9075.777
SC Summer sample	8	0	8	90.800	7685.690	1219.420	2616.735

Two-sample Kolmogorov-Smirnov test / Two-tailed test:

D	0.750
p-value	0.010
alpha	0.05

An approximation has been used to compute the p-value.

Test interpretation:
H0: The two samples follow the same distribution.
Ha: The distributions of the two samples are different.
As the computed p-value is lower than the significance level alpha=0.05, one should reject the null hypothesis H0, and accept the alternative hypothesis Ha.

The risk to reject the null hypothesis H0 while it is true is lower than 0.98%.

Chapter 8

Organophosphate flame retardants and plasticizer in indoor dust from South Africa: implications for personal exposure via inadvertent dust ingestion

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Abstract

Organophosphate esters (OPEs) have been widely reported as alternative flame retardants due to the restrictions on the use of polybrominated diphenyl ethers. To investigate the presence of these flame retardants and plasticizers in South Africa, we analysed 50 dust samples from homes (n = 10); offices (n = 9); university computer laboratories (n = 12) and automobiles (n = 19) for tris(2-chloroethyl) phosphate (TCEP), tris(1-chloro-2-propyl) phosphate (TCPP); tris(1,3-dichloro-2-propyl) phosphate (TDCPP) and triphenylphosphate (TPP). OPEs were detected in all samples with the exception of one automobile and one computer laboratory sample in which TDCPP was not detected. The median concentrations $\sum_{(n=4)}$ OPEs were 22940 ng g^{-1}, 26930 ng g^{-1}, 19565 ng g^{-1} and 49010 ng g^{-1} respectively in homes, offices, university computer laboratories and automobiles. Significant association of indoor characteristics with OPE concentrations was observed. OPEs positively correlated (r = 0.22, p value = 0.4862) with electronics and correlated (r = 0.522, p value = 0.0675) with foams and furniture in homes. Employing the median concentrations and an average dust intake rate, the exposure doses (ng day^{-1}) were 169.4 (TCEP), 73.89 (TCPP), 162.3 (TDCPP) and 55.34 (TPP) for adults; 159.0 (TCEP), 70.3 (TCPP), 107.6 (TDCPP) and 57.4 (TPP) for teenagers; 316.6 (TCEP), 152.1 (TCPP), 334.2 (TDCPP) and 93.9 (TPP) for toddlers. The predominance and exposure magnitude of OPEs in the South African environment requires further investigations to determine cumulative human health effects arising from the mixtures of these compounds through multiple exposure routes.

Keywords*: Organophosphate ester flame retardants and plasticizers, Indoor dust, Correlation, Indoor characteristics, Human exposure, Microenvironments, South Africa.*

8.1 Introduction

Organophophate esters (OPEs) are used in a variety of applications. The chlorinated alkylphosphates such as tris(1-chloro-2-propyl) phosphate (TCPP), tris(1,3-dichloro-2-propyl) phosphate (TDCPP) and tris(2-chloroethyl) phosphate (TCEP) are used mainly as phosphorus flame retardants (PFRs) in polyurethane foams (1), electronic equipment, textiles, plastic and building materials (2), whilst the non-derivatized organophosphates, such as triphenyl phosphate (TPP), are majorly used as plasticizers, lubricants, varnishes, glues, airplane hydraulic fluids and to regulate pore sizes such as in concrete (3). They are sometimes used as substitute flame retardants for the halogenated compounds, e.g., TPP in electronic devices (1). There has been a great increase in the use of PFRs to meet fire safety regulations in various consumer products since the restrictions of penta, octa and decaBDE technical mixtures (3). Because PFRs are not covalently bound to the materials but used as additive flame retardants, they may off-gas and leach from products through abrasion and/or volatilization into the environment (3, 4). Little is known of the toxicity of OPEs, however, studies have reported harmful effects of OPEs to include altered hormone levels and decreased sperm concentrations (5); neurotoxic, mutagenic and carcinogenic effects in rats and mice; hemolytic and reproductive effects in humans (6) and potential human carcinogens (4). PFRs have been detected in various environmental media including air (7-12); surface and drinking waters, and sediments (13-18); biota (19, 20) and indoor dust of various microenvironments in several locations worldwide (3, 4, 7, 21-24). Much attention has been given recently to the significance of indoor dust as a pathway of human exposure to OPEs. The relationship between dust and human body burdens is strongly implied by association of PFRs in household dusts and human semen quality and hormone levels (5). Nothing is known on the production, use, distribution and fate of PFRs in South Africa. Moreover, despite the increasing proof of the significant implications of indoor dusts for human exposure to PFRs, attempts to link indoor contaminants with probable source items has had limited success. A dearth of information also exists for human exposure and pathways to PFRs. To breach these gaps, the aim of the present study was to investigate indoor dust contamination of four OPEs (TCEP, TCPP, TDCPP and TPP) in multiple indoor environments in South Africa as no data is currently available, as well as to compare the profiles of PFRs in the different microenvironments (automobiles, homes, offices and university computer laboratories). We also aimed to establish the relationships of various household products and the concentrations of PFRs in dust in order to identify possible sources of OPEs in the indoor environment. Finally, we estimated the exposure magnitude of OPEs among different population groups, utilizing various exposure scenarios.

8.2 Materials and methods

8.2.1 Chemicals

Pure standards of TCEP, TCPP, TDCPP, and TPP were purchased from Sigma-Aldrich, South Africa. The internal standard, $^{13}C_{12}$ –labelled decachlorobiphenyl ($^{13}C_{12}$ PCB-20

9), was obtained from Wellington Laboratories, Guelph, Ontario, Canada. Anhydrous sodium sulfate was from Associated Chemical Enterprises (ACE), Johannesburg, South Africa. Silica gel 90 was from Sigma-Aldrich. A Restek Rtx®-1614 fused silica (5% diphenyl 95% dimethyl polysiloxane) capillary column was obtained as a generous gift from Restek Corporation, Bellefonte, PA, USA. All solvents were high performance liquid chromatography grade purchased from Sigma-Aldrich, South Africa.

8.2.2 Sampling

A total of 50 dust samples were collected from homes, n = 10, university students' computer laboratories, n = 12, and university staff offices, n = 9, between August and October 2012 in Durban, South Africa. Similarly, dust samples, n = 19, were collected between January and March, 2013 from personal and previously owned automobiles available for resale. The previously owned automobiles were sampled at a dealership in Durban, South Africa. All automobiles from the dealership had been through a thorough cleaning process on arrival at the dealership prior to resale. Similarly, personal automobiles sampled had not undergone any form of cleaning at least three days before sampling. Computer laboratory and office samples were collected with a LG 1600 W vacuum cleaner following the description of Harrad, et al. (25). The vacuum cleaner contained a dust unit which could easily be removed and emptied after each collection. Between each collection it was cleaned with a disposable cloth wetted with *iso*-propanol. Samples from homes were obtained from the vacuum cleaner bags of each home collected under normal home use conditions as they reflect recently collected dusts, and thereby provide an estimate of residential exposure to OPE contamination. Samples were stored in amber glass bottles at -10 °C until analysis. Detailed questionnaires were used to obtain pertinent information on automobiles, homes, offices and computer laboratories. This information included location, time since floor was last vacuumed, type of ventilation and flooring, and the number and types of electronic/electrical devices and furniture, model and manufacturer of automobile, year of manufacture, etc. Interviews were also conducted to obtain further information on building ages and to determine if, and when, any renovations were carried out. Since only 50% and 89% of the questionnaires were returned for homes and offices, respectively, correlation analyses were based on those samples for which questionnaires were returned.

8.2.3 Extraction and Clean-up

Non-dust particles, hair and debris were hand-picked from all samples. Samples were homogenized by sieving through a 212 µm stainless steel sieve. Dusts were analysed as follows. Approximately 1.0 g of sample was quantitatively weighed into a glass test tube and spiked with 2 µg $^{13}C_{12}$ CB-209. A volume of 12 mL *n*-hexane:methanol (1:3 v/v) was added. Samples were mixed in an orbital shaker for 15 mins and then extracted in an ultrasonic water bath at 40 °C for 30 mins. The mixing and extraction was repeated for a second time without addition of fresh solvent. The samples were then centrifuged at 3500 rpm for 10 mins and the supernatants were stored at <4 °C prior to clean-up. Representative samples (n = 5) from each microenvironment were analyzed in triplicate.

Silica gel was activated at 130 °C for 16 hours and anhydrous sodium sulfate was baked at 450 °C for 5 hours before use. Silica gel and anhydrous sodium sulfate were subsequently cooled in a desiccator. A 30 cm × 1 cm glass column was packed with 4 g deactivated silica gel. Each column was topped with 2.0 g of anhydrous sodium sulfate and then 30 mL of the extraction solvent was passed through column. Extracts were loaded onto columns just before the exposure of the sodium sulfate layer. OPEs were eluted with 30 mL *n*-hexane. This was kept as fraction 1, columns were further eluted with 30 mL of diethyl ether:*n*-hexane (50:50 v/v), and kept as fraction 2, both fractions were mixed together. Finally, columns were eluted with 30 mL acetone:dichloromethane (1:1 v/v) as fraction 3. Eluates were reduced to approximately 250 µL in a vacuum rotary evaporator at 55 °C and stored in 1.5 mL amber glass GC/MS vials. The column flow rates were maintained at 0.5 mL min^{-1}. All extracts were stored at <4 °C until instrumental analysis.

8.2.4 GC-EI/MS Analysis

An Agilent 6890 GC fitted with a Restek Rtx® – 1614 fused silica (5% diphenyl, 95% dimethyl polysiloxane) capillary column (15 m × 250 µm × 0.1 µm) coupled to an Agilent 5973N series mass spectrometer was used for the separation, detection and quantitation of all OPEs. Injections were made in the pulsed splitless mode with the injector temperature set at 250 ºC. The injection volume was 2 µL. The GC oven temperature programme started at 90 ºC (held for 2 mins), then increased at 20 ºC min^{-1} to 270 ºC and held for 1 min and finally increased at 10 ºC min^{-1} to 290 ºC and held for a minute. Helium was used as the carrier gas at a flow rate of 1.2 mL min^{-1} and a constant linear velocity of 37 cm s^{-1}. For the MS, the ion source and transfer line temperatures were 230 ºC and 350 ºC, respectively; and the ionization energy was 70 eV. OPE mass spectra were obtained in full scan mode to select prominent ions that were utilized in the selected ion monitoring (SIM) mode (see Supplementary Material Figure S8.1).

Quantitation was carried out by means of a multiple point internal standard method. A $^{13}C_{12}$ labelled PCB-209 was employed as an internal standard for all studied OPEs. The response factors were determined from the slope of a plot of the ratio of peak areas against the ratio of the concentrations. The values for the plots were obtained from a 5 – 6 point triplicate analysis of the OPE standard solution diluted to fall within a concentration range of 2 – 10 µg mL^{-1}.

8.2.5 Quality Control

Recovery for PFRs in dust was determined from spiked anhydrous sodium sulfate at different spike concentrations (Table 8.2). Samples were left to stand for at least 21 days at -10 °C. Spiked samples were extracted and cleaned-up following the procedure for real samples. Method blanks were analysed with every batch of ten samples. For the method blank, dust samples were replaced with anhydrous sodium sulfate and passed through all the analytical procedure carried out for real samples. TPP concentrations in samples were blank corrected as concentrations found in method blank (n = 5) were as

high as 1.6 % of the concentrations in samples. Solvent blanks were injected after the analysis of at most three samples. All glassware was cleaned with laboratory wash solutions, rinsed with distilled water and then with organic solvents. Non-volumetric glassware was oven-dried prior to use. Direct ultraviolet light and plasticware was avoided throughout the analysis.

Table 8.1 shows the selected ions employed for identification and quantitation of OPEs in the dust samples as revealed by full scan mass spectrum of individual pure analytical standards of the OPEs. The instrument responses to pure OPE analytical standards employed for calibration were linear with $r^2 > 0.99$ for all OPEs studied. Limits of detection (LODs) ranged from 0.56 ng g^{-1} for TCEP to 100.2 ng g^{-1} for TPP; limits of quantitation (LOQ) were in the range 1.68 ng g^{-1} (TCEP) and 300.60 ng g^{-1} (TPP). Matrix spike recovery samples were prepared by adding 200 ng g^{-1} – 10000 ng g^{-1} of each OPE to glass test tubes filled with 1.0 g anhydrous sodium sulfate for each concentration listed in Table 8.2. Matrix spikes were subjected to all analytical protocols employed for real samples. The average recovery and the percentage relative standard deviation (%RSD) for each of the OPEs at the respective spiked concentrations are shown in Table 8.2.

8.2.6 Statistics

The distribution of OPE concentrations in the different microenvironments was tested with the Shapiro-Wilk test of normality. Descriptive statistics such as sum, mean, median, minimum, maximum and parametric statistics such as t-test and analysis of variance (ANOVA) were calculated by using Microsoft Excel® 2010. Non-parametric statistics such as Wilcoxon Signed-Ranks test, Kendall tau test and Spearman rank correlation were performed with Analyse-it® software in Microsoft Excel 2010. The Kruskal-Wallis test was employed to test for differences in location by using XLSTAT 2014 software. Principal component analysis (PCA) was performed with SIMCA version 13 statistical software. Limits of detection (LOD) and quantitation (LOQ) were estimated following Thomsen et al. (26). Samples below the detection limit were treated as zero throughout the statistical analysis.

Table 8.1 Qualifying and quantitation ions, r^2, limits of detection and quantitation for TCEP, TCPP, TDCCP and TPP.

Analyte	Qualifier and quantifier ion	R^2	LOD[a]/ ng g^{-1}	LOQ[a] /ng g^{-1}
TCEP	205, 249	0.999	0.56	1.68
TCPP	201, 277	0.998	50.65	151.95
TDCPP	321, 381	0.995	91.4	274.2
TPP	326, 325	0.992	100.2	300.6
$^{13}C_{12}$-PCB 209[b]	510			
PCB 209[b]	498			

[a] Thomsen, et al. (26)

[b] Internal standard.

Table 8.2 Recovery of individual OPEs.

Analytes	Spiked concentration (n = 4)/µg g^{-1}	Average determined concentration (n = 4)/µg g^{-1}	Standard deviation	% Recovery	% relative standard deviation (%RSD)
TCEP	0.20	0.19	0.05	95	25
	1.00	1.22	0.002	122	0.2
	2.00	1.74	0.03	87	1.7
	6.00	5.16	0.44	86	8.5
TCPP	0.20	0.19	0.09	95	47.4
	1.00	0.74	0.10	74	13.5
	2.00	1.63	0.15	82	9.2
	6.00	5.92	0.22	99	3.7
TDCPP	0.60	0.57	0.04	95	7.0
	2.00	2.71	0.76	136	28
	10.0	12.87	0.82	129	6.4
TPP	0.60	0.61	0.10	102	16.4
	2.00	2.03	0.05	102	2.5
	10.0	10.79	0.16	108	1.5

8.3 Results and discussion

Levels of OPEs measured in the indoor environment and the potential human exposure magnitude arising from the ingestion of indoor dust are presented in subsequent sections.

8.3.1 House Dust Concentrations of OPEs

The four organophosphate esters were detected in 100 % of dust samples collected from homes in South Africa (Fig 8.1). A full list of concentrations of OPEs in home samples is presented in Supplementary Material Table S8.1. Descriptive statistics for the distribution of these OPEs are summarized in Table 8.3. The strong positive correlation of OPEs in this microenvironment (Supplementary Material Tables S8.5-S8.11) is indicative of similar sources of the PFRs in the South African homes. Nothing is known on the production and use of organophosphate flame retardants in South Africa. However, due to industrialization in South Africa comparable to most countries in the European Union, and because of the very high levels of these OPEs found in indoor dust of two industrial sites – a textile and polyurethane industry (see Chapter 10); we hypothesize the possible indoor contamination of OPEs from the products of these industries and others which eventually end up indoors. The OPE profiles in the house dust were TDCPP > TCEP > TCPP > TPP. The distribution pattern of OPEs in this study is similar to those reported in Swedish homes (23), German homes (21) and Egyptian homes (4); in which TDCPP dominated in the house dust.

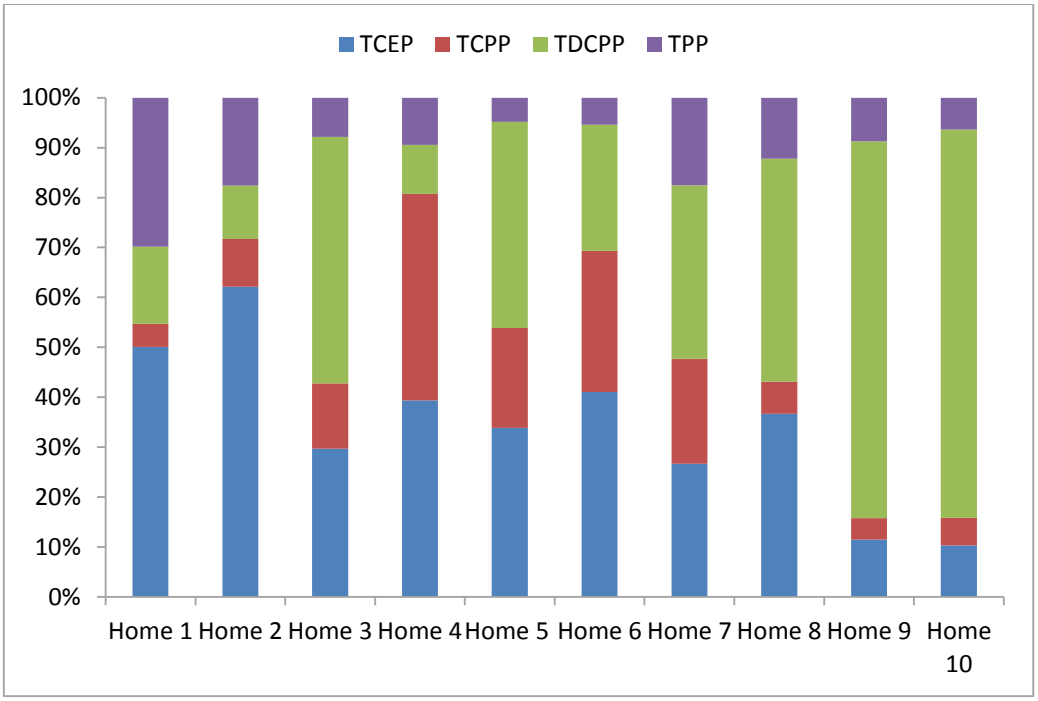

Figure 8.1 Percentage contribution of individual OPEs in dust collected from homes in South Africa.

Despite, the replacement of TCEP by TCPP, the house dust concentrations of TCEP in this study overwhelmed those of TCPP; and TCEP was found to be second most abundant OPE in South African homes similar to the observations of Bergh et al. (23) in Swedish homes. However, the concentrations of TCEP strongly correlated (r = 0.82) with TCPP in this microenvironment. A statistically significant difference (p = 0.022) was observed among the concentrations of the individual OPEs in South African homes. The high levels of TCEP in these house dust samples suggests a high volume use of TCEP and possibly the widely-used Chinese flame retardant V6 [2,2–bis (chloromethyl)propane-1,3-diyltetrakis(2-chloroethyl)biphosphate] which is applied to polyurethane foam commonly present in furniture and automobile foam (27). The high vapour pressure (1.1×10^{-4} mmHg) of TCEP is likely responsible for its easy migration from treated products during the product's useful life. The elevated TCEP concentrations in South African homes should be of particular interest due to the reported carcinogenicity of TCEP (24, 27). The OPE concentrations did not correlate well (r = 0.04) with corresponding PBDE concentrations reported earlier in these samples (28). This suggests differences in source and/or release mechanism of these flame retardants in the house dust samples. The correlations of ∑OPE concentrations in dust and the number of electronics (r = 0.22, p value of 0.4862) and the number of foams and furniture (r = 0.522, p value of 0.0675) in these homes, strongly implicate these household items as sources of OPEs.

Table 8.3 Summary of descriptive statistics for OPEs in indoor dust from several microenvironments in Durban, South Africa.

	Concentrations of OPEs/ng g^{-1}				
Statistical descriptor	TCEP	TCPP	TDCPP	TPP	∑OPEs
$\sum_{n=10}$ Home	97650	47860	327510	47510	520530
Mean	9765	4786	32751	4751	52053
Median	7390	3545	7695	2025	22490
Minimum	2400	220	740	760	4790
Maximum	34250	12870	226200	26120	299440
$\sum_{n=9}$ Office	88690	38880	60690	65420	253680
Mean	9854	4320	6743	7269	28187
Median	10820	3810	8320	3730	26930
Minimum	6900	1240	1450	650	14920
Maximum	11720	10320	11680	18370	49210

$\sum_{n=12}$ University Computer Laboratory	109750	48420	58520	49250	265940
Mean	9145	4035	4877	4104	22162
Median	8420	3485	3415	3580	19565
Minimum	5040	830	<MDL	1420	10070
Maximum	18350	11350	11450	9990	43390
$\sum_{n=19}$ Automobile	667410	191310	1694670	177210	2730600
Mean	35126	10069	89193	9327	143716
Median	10200	5000	12770	6170	49010
Minimum	2000	770	<MDL	670	14020
Maximum	245230	56250	697100	34100	773590
5th percentile	4664	2417	1953	823	21625
95th percentile	131173	35424	396725	31562	431176

8.3.2 Office Dust Concentrations of OPEs

All four OPEs were detected in 100% of the dust samples from offices in South Africa (Fig 8.2). A full list of the concentrations of the individual OPEs in this microenvironment can be found in Supplementary Material Table S8.2 and the descriptive statistics are given in Table 8.3. Unlike the distribution of OPEs in home samples where TDCPP was predominant, TCEP was most abundant in the office dust samples. The relative abundance of OPEs were TCEP > TPP > TDCPP >TCPP.

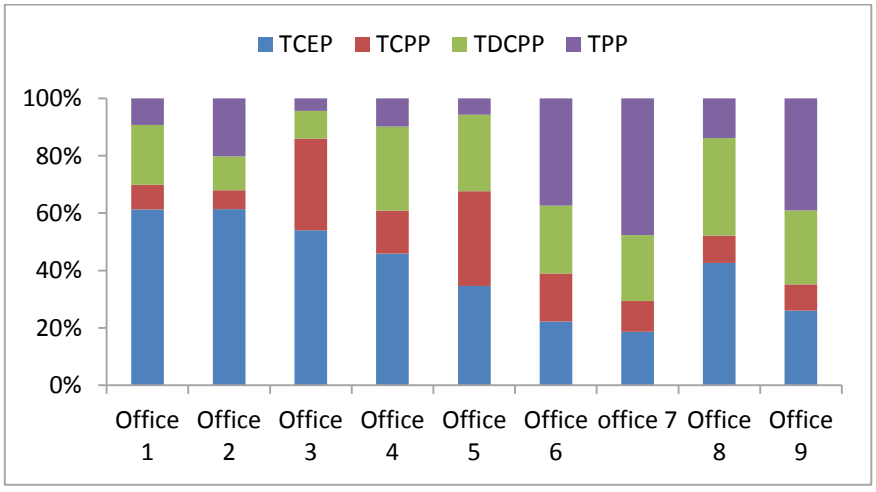

Figure 8.2 Percentage contribution of individual OPEs in dust collected from offices in South Africa.

Limited data are available on indoor dust TCEP concentrations worldwide. The concentrations of TCEP were higher than reported concentrations in two offices in

Sweden (i.e. the minimum concentrations of TCEP in the South African offices were much higher than the maximum concentrations of TCEP reported in the Swedish offices) (7). The high levels of TCEP in the office dust could be associated with indoor characteristics; however, there were no correlations between the concentrations of OPEs and office characteristics such as electronics or furniture in these offices. It should be noted that the Swedish study by Marklund et al. (7) was conducted prior to the ban of commercial penta- and octa-PBDE mixtures which had wider application in foam and furniture compared with organophosphate flame retardants. The abundance of TCEP and TPP may be indicative of the possible use of V6 and Firemaster 550 (FM550) in flame retarding various office products in South Africa. TCEP is a component of V6 and TPP is a major component of Firemaster 550 (FM550) (24). Fang et al. (27) showed a significant relationship between V6 and TCEP in dust samples and concluded that V6 is an important source of TCEP in the environment. This observation is supported by the poor correlation of TCEP with the other studied OPEs, whilst TCPP and TDCPP concentrations are significantly correlated ($r = 0.49$); similar to the strong correlation ($r = 0.73$) of TDCPP and TPP concentrations in the offices (Supplementary Material Table S8.7). These relationships suggest similar sources for TCPP, TDCPP and TPP in the office dust samples. We cannot exclusively associate the presence of TPP in this microenvironment to the flame retardant FM550 since TPP has other applications as a plasticizer in various indoor products which may also account for a source of TPP in the office dust (3).

8.3.3 University Computer Laboratory (classrooms) Concentrations of OPEs

The percentage contribution of individual OPE in this microenvironment is presented in Fig. 8.3. The descriptive statistics of OPEs in this microenvironment are presented in Table 8.3.

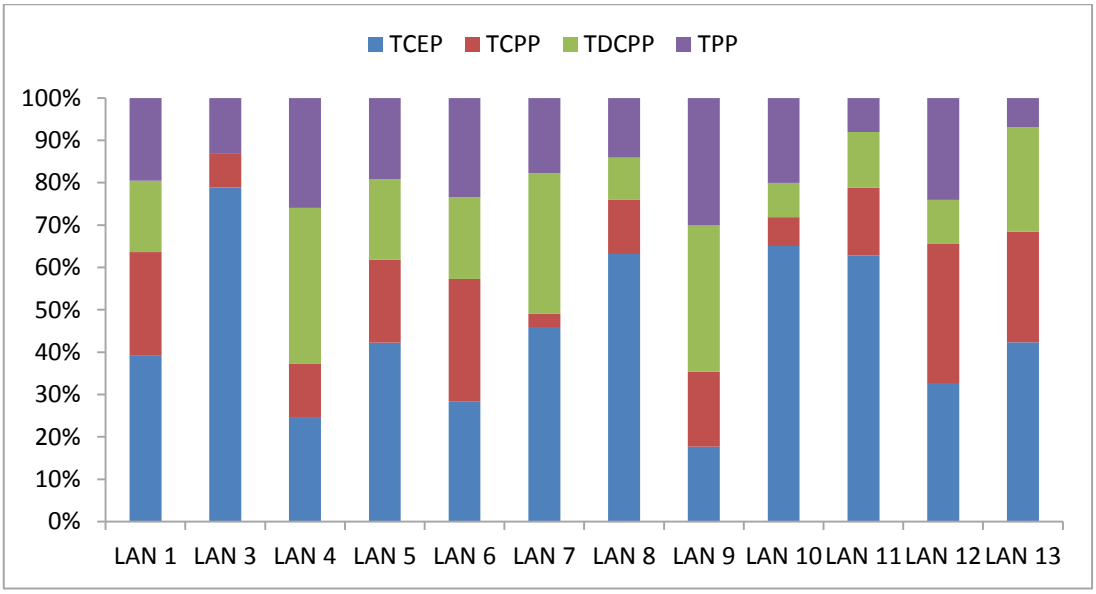

Figure 8.3 Percentage contribution of individual OPEs in dust collected from University computer laboratories (classrooms) in South Africa.

As observed for OPEs in offices, TCEP was the major OPE found in these laboratories. The concentration profiles of the OPEs were in the order TCEP > TDCPP > TPP > TCPP. The dominance of TCEP in this microenvironment is not surprising as a previous study had reported high levels of TCEP in the indoor air of a lecture hall with computers as opposed to a hall without computers in which TCEP was not detected in the same study (29). The authors also reported TCEP as high as 10 ng m^{-3} in air of an electronic dismantling facility (29). The concentration of TCEP in these computer laboratories calls for concern owing to the fact that these microenvironments were refurbished between 2006 and 2007. All carpeting, furniture, blinds, computers and printers were replaced and the dividing walls in some of the rooms were also replaced with plasterboard. Though no report on OPEs in a similar microenvironment is available in the literature, the levels of TCEP in this study exceeded the levels reported in dust from homes in New Zealand (30) and dust from Belgian homes (31). All OPEs were normally distributed in this microenvironment. A single factor ANOVA ($p < 0.05$) showed statistical differences in the concentrations of all four OPEs determined in dust from the computer laboratories. However, the exclusion of TCEP from the statistics, showed that no significant difference ($p < 0.05$) in the concentrations of TCPP, TDCPP and TPP. The correlation matrix (Supplementary Material Table S8.8) indicates a good relationship between the concentrations of the studied OPEs. However, the concentrations of TCEP negatively correlated with those of TPP. The correlation of TDCPP and TPP concentrations in the computer laboratories resembles that of the offices; hence providing further evidence for source similarities of both OPEs in these microenvironments. The concentrations of TPP in these microenvironments is not surprising as TPP concentrations in excess of 500000 ng g^{-1} have been reported in liquid crystal display (LCD) televisions and laptop computers in Japan (22). In the same light, Brandsma et al. (3) showed high contamination levels of TPP in dust samples collected from electronic equipment consistent with the observations of Kajiwara et al. (22) in which TPP was the major PFR detected in electronic equipment from the Japanese market. TPP is used as flame retardant and a plasticizer in variety of products, which may also account for some of the sources of TPP in this microenvironment. The high volatilities of TDCPP and TPP are most likely responsible for their concentrations in the indoor dust since they can be easily released from products into the environment (6).

8.3.4 Automobile Dust Concentrations of OPEs

All OPEs were detected in the automobiles sampled for this study with the exception of a car in which TDCPP was not detected. Fig. 8.4 shows the percentage contribution of each OPE in the automobile dust samples in South Africa. A full list of the concentrations of OPEs in this microenvironment can be found in Supplementary Material Table S8.4. The concentration profile of OPEs in this microenvironment is typical of the profile in house dust. The abundance of OPEs in these automobiles is in the order TDCPP > TCEP > TCPP > TPP. A summary of the descriptive statistics for OPEs in automobiles is given in Table 8.3.

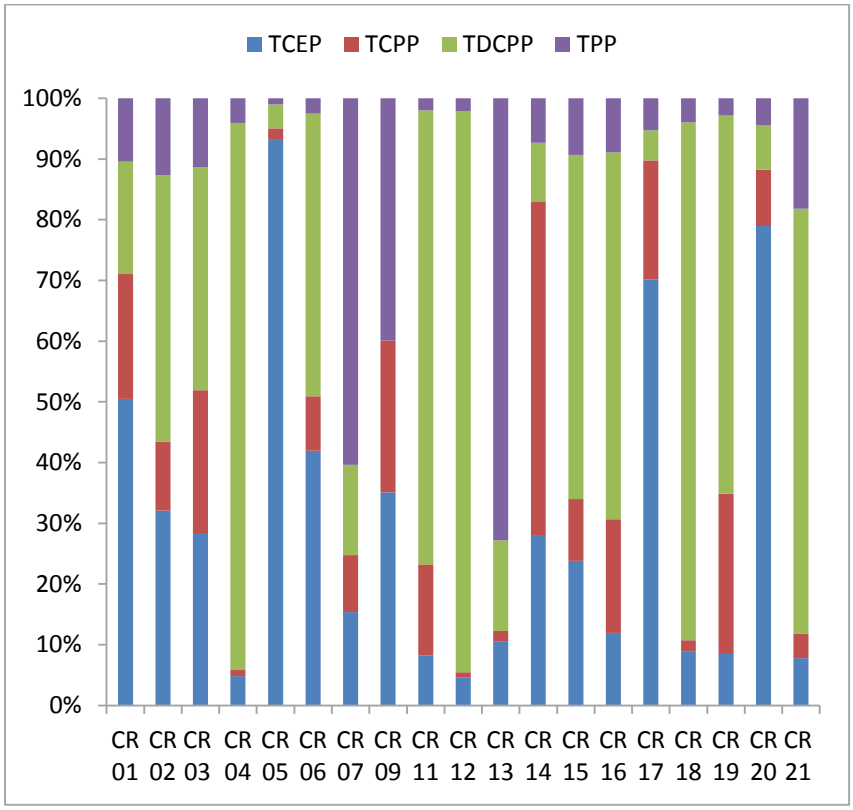

Figure 8.4 Percentage contribution of individual OPEs in dust collected from automobiles in South Africa.

The concentration profiles of TDCPP in these automobiles are similar to concentrations reported in car dust from the Netherlands (32) and Germany (21). The high TDCPP concentrations in automobile dust are in tandem with its usage as a flame retardant in flexible and rigid polyurethane foams (PUF) (7). Unlike the observations of Brommer et al. (21), in which high TDCPP concentrations were associated with older cars, the concentrations of TDCPP did not show a particular trend with the year of manufacture of the vehicle in South Africa. The highest TDCPP concentrations reported in this study were found to be 697100 ng g^{-1} in a car manufactured in 2009. However, a similar car from the same manufacturer, of the same model and year, had a TDCPP concentration of only 2600 ng g^{-1}. The cause of this discrepancy could not be ascertained, since information obtained showed that both cars were cleaned at approximately the same time (i.e. 90 days) prior to the date of sampling. However, Brommer et al. (21) postulated that intensive car usage leads to greater abrasion of vehicle upholstery with an attendant increase in the release of flame retarded upholstery fabric fibres which may be accountable together with external sources for the variations of TDCPP levels in the two cars. No direct relationship was found between OPE concentrations and automobile manufacture year. Whilst TDCPP and TPP showed weak positive correlations of $r = 0.14$ and $r = 0.15$ with automobile manufacture year; TCEP and TCPP only showed weak negative correlations of $r = -0.12$ and $r = -0.04$ with automobile manufacture year

(Supplementary Material Table S8.15 – S8.18). The correlation matrix (Supplementary Material Table S8.9) shows the relationship among OPE concentrations in the automobile dust. TCEP, TCPP and TDCPP are weakly correlated while TDCPP and TPP showed significant positive correlation (r = 0.43) in the automobile samples similar to observations in the three other microenvironments; further suggesting peculiarity in the source of TDCPP and TPP in the South African indoor environment. A boxplot (Fig 8.5) of the concentrations of OPEs and automobiles grouped by manufacturer depicts a wide range in the concentrations of PFRs in automobiles manufactured by HONDA and AUDI.

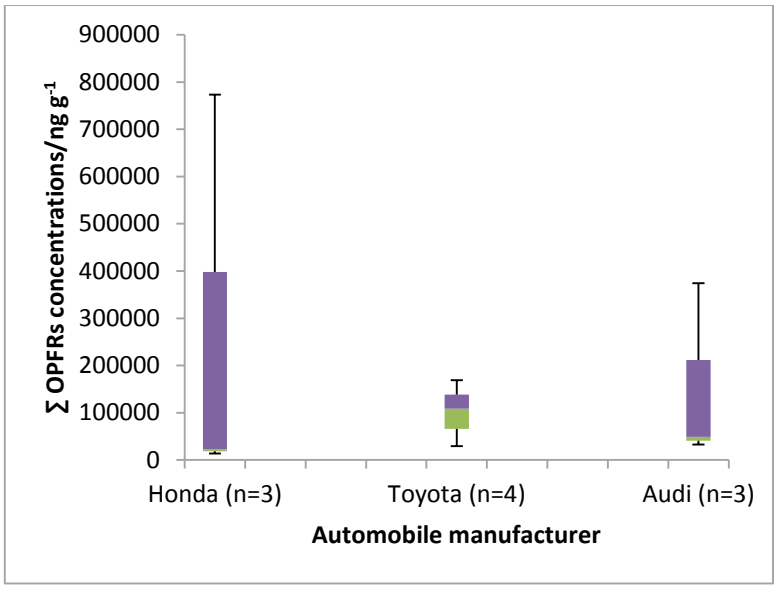

Figure 8.5 Box and Whisker plot for OPE concentrations and automobile manufacturer.

This observation may indicate the large volume use of OPEs (majorly TDCPP) in some applications, such as flame retardants for flexible and rigid PUF in vehicles; the observation could also be reflective of a small sample size (i.e. n = 3) for each of the two automobile manufacturers. Contrary to the observation above, OPE concentrations showed a similar distribution pattern in automobiles (n = 4) manufactured by Toyota. TCEP and TCPP seemed to have a similar use pattern in cars made by Toyota, as a strong positive correlation (r = 0.995) (Supplementary Material Table S8.10) was observed between the concentrations of TCEP and TCPP in cars made by Toyota.

The automobiles in this study were grouped in terms of year of manufacture: the first group (n = 4) reflects automobiles manufactured prior to 2004, i.e. before the ban of penta-BDE commercial formulations in PUF; the second group are automobiles manufactured between 2005 – 2012 (n=15) reflecting automobiles manufactured after the replacement of penta-BDE with alternative flame retardants such as PFRs in PUFs. The boxplot (Fig. 8.6) indicates wide a variation in the concentrations of OPEs in the

latter group. This could mean an increased use of OPEs in automobiles manufactured after 2004.

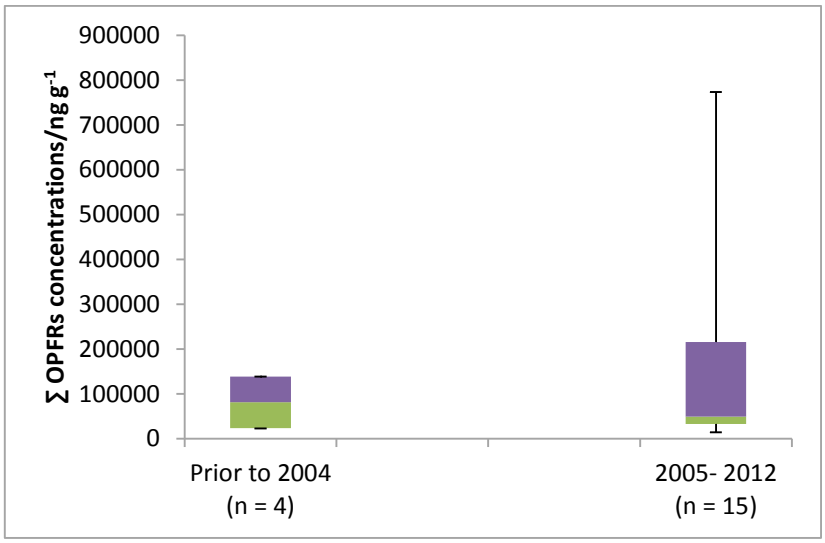

Figure 8.6 Box and Whisker plot for OPE concentrations and automobiles grouped into periods of manufacture.

8.3.5 Comparison of OPE Levels in Different Microenvironments in South Africa with Levels Reported in other Countries

The target OPEs (with the exception of TDCPP which was below the detection limit in one automobile and one university computer laboratory sample) were detected in all samples from the various microenvironments. The Kruskal-Wallis test showed statistical differences ($p = 0.003$) in the concentrations of the OPEs in the various microenvironments.

To investigate the distinctions and similarities between the concentration profiles of the OPEs in the four microenvironments, we used principal component analysis (PCA) to analyze the data obtained for all the microenvironments. No obvious differences were identified between the concentration profiles of the OPEs in the dust of the different microenvironments. The loadings, scores and biplots of the PCA are presented in Figure 8.8. The PCA hotelling T2 plot showed the distance from the origin in the score space (model plane) for each observation; the distance to the model DModX/ DModY which corresponds to the residual standard deviations; and the normal probability plot for the observations are presented in Supplementary Material Figs. S8.1 - S8.3. The PCA loadings (OPEs) plot indicates that all OPEs have significant influence on clustering in the observations (Score) plot. The implication is that all target OPEs might have originated from similar sources in the four microenvironments.

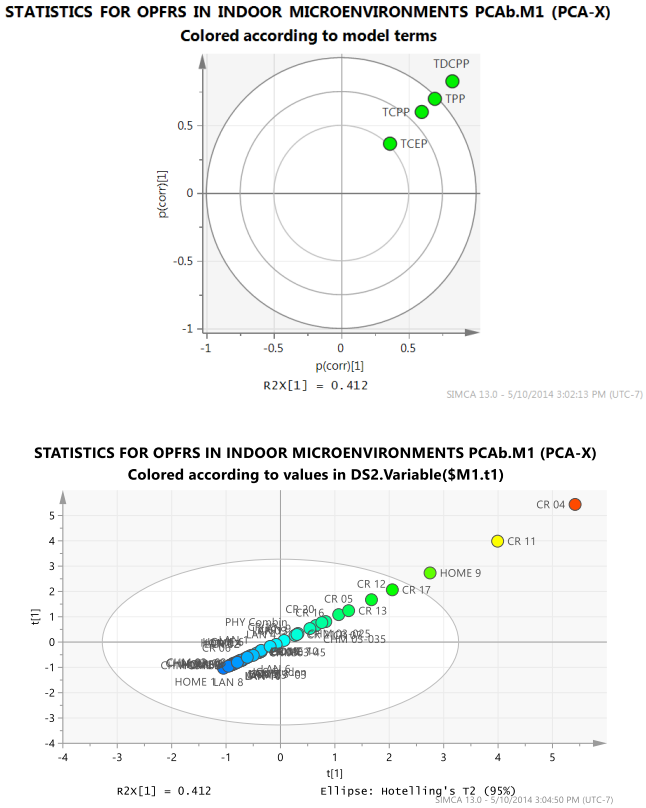

Figure 8.7 PCA scores, loadings and biplots of OPEs in dust samples from automobiles, homes, offices and university computer laboratories. Top panel – loadings (variables); Middle panel - Scores (observations) and Bottom panel – biplots

Table 8.4 compares the median concentrations and range in parenthesis of OPEs in the studied microenvironments to data reported in different countries. Similar to data from the USA (27), Germany (21), and the Netherlands (3), automobiles contain the bulk of the OPEs compared with the other indoor microenvironments. Tris(1,3-dichloro-2-propyl) phosphate (TDCPP) dominates the phosphorus flame retardants contaminating

South Africa's indoor environments in line with the profile in many countries worldwide (Table 8.4).

Table 8.4 Comparison of PFR concentrations in the present study with other studies worldwide

Country	Microenvironment	Median Concentrations/ng g^{-1} (range in parentheses)				Reference
		TCEP	TCPP	TDCPP	TPP	
South Africa	Homes (n=10)	7390 (2400-34250)	3545 (220-12870)	7695 (740 – 226200)	2025 (760 – 26120)	This study
South Africa	Offices (n=9)	10820 (6900-11720)	3810 (1240 – 10320)	8320 (1450 – 11680)	3730 (650 – 18730)	This study
South Africa	University computer laboratories (n=12)	8420 (5040 – 18350)	3485 (830 – 11350)	3415 (ND – 11450)	3580 (1420 – 9990)	This study
South Africa	Automobiles (n=19)	10200 (2000-245230)`	5000 (770 – 56250)	12770 (ND- 697100)	6170 (670 – 34100)	This study
Sweden	Homes (n=10)	2100 (ND-33000)	1600 (700-11000)	10000 (2200 – 27000)	1200 (100 – 4200)	(23)
Sweden	Daycare centres (n=10)	30000 (2500 – 150000)	3100 (800 – 12000)	9100 (3900 -150000)	1900 (300 – 17000)	(23)
Sweden	Workplaces (n=10)	6700 (1300 – 260000)	19000 (3400 – 120000)	17000 (3300 – 91000)	5300 (900 – 32000)	(23)
Netherlands	House dust around	1300 (220 – 6900)	1300 (480 – 3800)	280 (70 – 3200)	820 (680 – 11000)	(3)

	electronics (n=8)					
Netherlands	House dust on electronics (n=8)	880 (520 – 2200)	1300 (580 – 4500)	680 (100 – 7400)	6500 (1600 – 21000)	(3)
Netherlands	Car dashboards (n=8)	2800 (1100 – 5700)	5700 (1800 – 110000)	17000 (6000 – 150000)	1700 (360 – 14000)	(3)
Netherlands	Car seats (n=8)	600 (240 – 5600)	4300 (1400 – 110000)	110000 (3800 – 1100000)	2400 (670 – 43000)	(3)
Japan	Hotels (n=8)	(82 – 2300)	(1000 – 9800)	(69 – 18000)	(110 – 2600)	(33)
USA	House (n=50)	-	(<140 – 5490)	(<90 – 56090)	(<150 – 1780000)	(24)
USA	Houses (n=20)	50.2 (<20 – 1350)	-	-	-	(27)
USA	Cars (n=20)	1080 (<20 – 50120)	-	-	-	(27)
USA	Airplanes (n=40)	-	-	3850 (580 – 22000)	-	(34)
Sweden	Houses (n=2)	(190 – 270)	(470 – 930)	(390 – 1100)	(850 – 990)	(7)
Sweden	Offices (n=2)	(1000 – 48000)	(5300 – 73000)	(560 – 67000)	(2200 – 6800)	(7)
Sweden	Aircraft (n=1)	4200	2200	860	4400	(7)
Philippines	Houses (n=17)	34 (<0.44 – 1200)	-	-	89 (8.5 – 2100)	(35)

Germany	Homes (n=6)	(140 – 280)	(370 – 960)	(80 – 110)	(180 – 1300)	(21)
Germany	Offices (n=10)	(<80 – 170)	(<180 – 9400)	(80 – 290)	(470 – 4800)	(21)
Germany	Cars (n=12)	(<80 – 5800)	(1400 – 4300)	(<80 – 620000)	(500 – 11000)	(21)
Egypt	Houses (n=20)	22 (<LOQ – 132)	28 (<LOQ – 700)	72 (<LOQ – 557)	67 (8 – 289)	(4)
Egypt	Office (n=20)	31 (<LOQ- 125)	80 (<LOQ – 700)	49 (<LOQ – 490)	73 (11 – 337)	(4)
Egypt	Cars (n=20)	127 (<LOQ – 572)	291 (<LOQ – 1425)	61 (<LOQ – 283)	135 (26 – 1872)	(4)
Egypt	Public microenvironments (n= 11)	234 (<LOQ – 538)	232 (<LOQ – 465)	416 (<LOQ – 1616)	629 (116 – 2357)	(4)
Spain	Houses (n=8)	(250 – 9800)	(350 – 10300)	(NQ – 1100)	(290 – 9500)	(36)
Belgium	Houses (n=8)	(75 – 1350)	(85 – 711)	(95 – 544)	(236 – 2640)	. (37)
Romania	Houses (n=3)	(40 – 1450)	(8-1020)	(19 – 666)	(105 – 3750)	(37)
Kuwait	Houses (n=15)	710	1460	360	430	(38)
Kuwait	Car (n=15)	1765	30725	7630	1760	(38)
Pakistan	Houses (n=15)				175	(38)
Pakistan	Cars (n=15)				245	(38)

LOQ refers to limit of quantitation

8.3.6 Implications for Human Exposure via Accidental Dust Ingestion

In evaluating human exposure via dust ingestion of contaminants, we assumed 100 % absorption of OPEs from ingested dust in accordance with other studies (4, 24, 38). Average dust intake rates of 20 and 50 mg d^{-1} and high dust intakes of 50 and 200 mg d^{-1} for adults, teenagers and toddlers were used as reported by Ali et al. (38). Body weights of 70 kg and 12 kg were used for adults and toddlers respectively (38), and 52 kg for teenagers (39). Questionnaires were used to obtain the average number of hours spent per day by adults in offices, cars and homes. The average number of hour students spend in computer laboratories (for lectures, studies and assignments) and in their residences per day were obtained by interview. The amount of the time spent per day in homes by toddlers (79.9%) was the same as that of Ali et al. (30). Thus, the average time an adult spends in the office, car and home were obtained as 33.3%, 4.2%, and 62.5%, respectively. For full-time undergraduate students (teenagers) it was estimated that they spend 54.2% and 45.83% of their time indoors in classrooms and in their residences respectively. Different exposure scenarios were calculated by using the 5th percentile (low end), median, mean and 95th percentile (high end) concentrations from homes, cars, offices and computer laboratory dusts. Thus, the daily dose of OPEs (ΣDED/ng day^{-1}) via dust ingestion were calculated from the following equations reported by Ali et al. (38) with modifications.

$\Sigma DED/ng\ day^{-1} = [(C_{HD}F_H) + (C_{ofD}F_{of}) + (C_{ATD}F_{ATD})]\ DIR$, for adult exposure estimation,
$\Sigma DED/ng\ day^{-1} = [(C_{HD}F_H) + (C_{LD}F_L) +]\ DIR$, for teenager exposure assessment, and
$\Sigma DED/ng\ day^{-1} = [(C_{HD}F_H) + (C_{ATD}F_{ATD})]\ DIR$, for toddlers exposure assessment,
where $C_{ATD,}$ $C_{HD,}$ C_{ofD} and C_{LD} are dust concentrations in automobiles, homes, offices and computer laboratories (5th percentile, median, mean and 95th percentile) and F_{ATD}, $F_{H,}$ F_{of} and F_L are the fraction of time spent in automobiles, homes, offices and computer laboratories, respectively. DIR is the dust intake rate and BW is the body weight.

Table 8.6 shows the daily dose of individual OPEs and the $\sum$OPEs among different population groups in South Africa compared with the toxicological reference dose (RfD) values for each OPE.

The results indicate that the high end exposure (i.e using 95th percentile concentration) may cause significant health implications for toddlers. At this exposure scenario, young children ingest as high as 36859 ng of $\sum_{n=4}$PFRs per day following inadvertent dust ingestion. For the individual OPEs, the worse-case scenario results in daily ingestion of 4939 ng TCEP, 2002 ng TCPP, 26978 ng TDCPP and 2940 ng TPP by toddlers. The worst-case daily exposure dose of TDCPP is at par with the toxicological reference dose of 30000 ng d^{-1} (4). The dominance of two chlorinated phosphorus flame retardants – TDCPP and TCEP – in exposure doses of studied OPEs in the South African population may pose significant health challenges particularly among toddlers who are exposed to a higher magnitude of these flame retardant chemicals. Studies have reported that TDCPP

is mutagenic, carcinogenic in rats and humans, and a moderate hazard for reproductive and developmental effects (24). Similarly, TCEP is reportedly carcinogenic for animals, neurotoxic to rats and mice, induces adverse reproductive effects in rats, and exhibits hemolytic and reproductive effects such as reduced fertility, longer estrous cycle length, and reduced sperm motility and density, in humans. TCEP was associated with the acute death of dogs after ingestion of car seat cushions containing an enormous amount of TCEP (6). Several toxicological effects have also been reported for TCPP and TPP (6).

8.4 Conclusions

We have reported for the first time the presence of organophosphate esters in the South African indoor environment. The results of the study indicate the wide usage of organophosphate esters, particularly the chlorinated phosphorus flame retardants, at levels comparable to those in the European Union. The abundance of TCEP in these microenvironments suggests V6 as a possible replacement of pentaBDE in polyurethane foams in South Africa. Automobiles contained the highest levels of OPEs among the microenvironments and TDCPP was the most abundant OPE found among the studied OPEs in South African. The relationships between OPEs and some indoor characteristics implicate furniture and electronics as reservoirs of OPEs in the indoor environment, particularly in homes. The result of the present study is reflective of a shift to OPEs as flame retardants, probably in response to international regulations. Generally, the current exposure of OPEs should be of interest as TDCPP and TCEP, which are known mutagens and carcinogens, majorly contributed to the overall exposure of the South African population, particularly young children, to OPEs. The current levels, profiles and magnitude of exposure indicate further investigations are required on other exposure routes such as dietary and inhalations; as well as patterns and human health implications of exposure to mixtures of these organophosphate esters.

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Table 8. 5 Summary of adult, teenager and toddler exposure to target OPEs (ng day^{-1}) via accidental indoor dust ingestion using different exposure scenarios.

OPEs	Reference dose		Exposure Scenario	Adult				Teenager				Toddler			
	Adult	Toddler		Low end	Median	Average	High end	Low end	Median	Average	High end	Low end	Median	Average	High end
			Mean	100	169	217	488	92	159	189	383	159	317	464	1235
TCEP	154000	44000	High	249	423	543	1219	229	398	472	958	634	1267	1856	4939
			Mean	18	74	97	226	16	70	88	189	30	152	212	500
TCPP	560000	160000	High	46	185	243	566	40	176	219	473	119	608	849	2002
			Mean	26	162	529	2258	15	108	353	1476	43	334	1496	6745
TDCPP	105000	30000	High	64	406	1323	5646	37	269	883	3691	171	1337	5983	26978
			Mean	20	55	116	356	26	57	88	248	44	94	209	735
TPP	490000	140000	High	51	138	289	890	65	143	220	619	177	375	838	2940

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Supplementary Material

Table S8.1 Concentrations (in ng g^{-1}) of OPEs in house dust samples.

Microenvironment	TCEP	TCPP	TDCPP	TPP	∑OPEs
HOME 1	2400	220	740	1430	4790
HOME 2	7200	1110	1240	2040	11590
HOME 3	7630	3370	12710	2010	25720
HOME 4	7580	7970	1890	1820	19260
HOME 5	5340	3160	6530	760	15790
HOME 6	11500	7920	7080	1520	28020
HOME 7	8010	6330	10450	5270	30060
HOME 8	6810	1190	8310	2270	18580
HOME 9	34250	12870	226200	26120	299440
HOME 10	6930	3720	52360	4270	67280

Table S8.2. Concentrations of OPEs in office dust samples.

Sample code	Concentrations of analytes/ng g^{-1}				
Microenvironment	TCEP	TCPP	TDCPP	TPP	∑ OPEs
Office 1	9700	1360	3300	1460	15820
Office 2	11720	1240	2270	3850	19080
Office 3	8060	4760	1450	650	14920
Office 4	8210	2680	5250	1750	17890
Office 5	10820	10320	8320	1790	31250
Office 6	10930	8230	11680	18370	49210
office 7	6900	3930	8490	17550	36870
Office 8	11480	2550	9170	3730	26930
Office 9	10870	3810	10760	16270	41710

Table S8.3 Concentrations (in ng g^{-1}) of OPEs in university computer laboratories (classrooms).

	TCEP	TCPP	TDCPP	TPP	∑ OPEs
LAN 1	9660	6050	4160	4820	24690
LAN 3	10250	1050	0	1690	12990
LAN 4	7180	3780	10790	7610	29360
LAN 5	6880	3190	3090	3120	16280
LAN 6	5530	5630	3740	4570	19470
LAN 7	11090	830	8020	4310	24250
LAN 8	6360	1290	1000	1420	10070
LAN 9	5870	5850	11450	9990	33160
LAN 10	11190	1170	1390	3450	17200
LAN 11	12350	3140	2600	1570	19660
LAN 12	5040	5090	1580	3710	15420
LAN 13	18350	11350	10700	2990	43390

Table S8.4 Concentrations (in ng g^{-1}) of OPEs in automobile dust samples

	TCEP	TCPP	TDCPP	TPP	∑ OPFRs
CR 01	7080	2880	2600	1460	14020
CR 02	9320	3290	12770	3670	29050
CR 03	9220	7640	11950	3680	32490
CR 04	37270	7940	697100	31280	773590
CR 05	245230	4980	10480	2540	263230
CR 06	14110	3000	15670	840	33620
CR 07	6430	3930	6220	25250	41830
CR 09	8360	5950	0	9510	23820
CR 11	30700	56250	279870	7360	374180
CR 12	18120	3330	363350	8330	393130
CR 13	4960	770	6990	34100	46820
CR 14	6280	12380	2170	1640	22470
CR 15	11670	5000	27770	4570	49010
CR 16	10200	16020	51690	7620	85530
CR 17	118500	33110	8360	8870	168840
CR 18	12280	2600	118260	5460	138600
CR 19	2000	6240	14710	670	23620
CR 20	109630	12860	10140	6170	138800
CR 21	6050	3140	54570	14190	77950

Table S8.5 Correlation of PBDEs and OPEs.

	∑PBDE	*∑ OPEs*
∑PBDE	1	
∑ OPFRs	0.04331505	1

Table S8.6 Correlation matrix for OPEs in homes.

	TCEP	*TCPP*	*TDCPP*	*TPP*
TCEP	1			
TCPP	0.823179	1		
TDCPP	0.949279	0.706493	1	
TPP	0.960371	0.732696	0.982565	1

Table S8.7 Correlation matrix for OPEs in offices.

	TCEP	*TCPP*	*TDCPP*	*TPP*
TCEP	1			
TCPP	0.10262	1		
TDCPP	0.264909	0.492358	1	
TPP	-0.04578	0.22207	0.733693	1

Table S8.8 Correlation matrix for OPEs in University computer laboratories.

	TCEP	*TCPP*	*TDCPP*	*TPP*
TCEP	1			
TCPP	0.345128	1		
TDCPP	0.219374	0.535362	1	
TPP	-0.35845	0.265227	0.736748	1

Table S8.9 Correlation matrix for OPEs in automobiles.

	TCEP	*TCPP*	*TDCPP*	*TPP*
TCEP	1			
TCPP	0.172324	1		
TDCPP	-0.054	0.179259	1	
TPP	-0.1401	-0.10413	0.428412	1

Table S8.10 Correlation matrix of OPEs in automobiles manufactured by Toyota (n = 4).

	TCEP	*TCPP*	*TDCPP*	*TPP*
TCEP	1			
TCPP	0.994713	1		
TDCPP	-0.48644	-0.56182	1	
TPP	-0.21118	-0.16235	0.122225	1

Table S8.11 Correlation of TCEP and TCPP concentrations in homes.

	TCEP	*TCPP*
TCEP	1	
TCPP	0.823179	1

Table S8.12 Correlation matrix of OPEs and home characteristics.

Home charateristics	*Number of electronics*	*Number of foam containing furniture*	*∑PFRs/ng g-1*
Number of electronics	1		
Number of foam containing furniture	0.76	1	
∑PFRs/ng g-1	0.86	0.90	1

Table S8.13 Characteristics of indoor microenvironments.

Indoor Microenvironment	Construction Year	Floor type	Number of Electronics	Number of foam containing furniture	∑PFRs/ng g^{-1}
Home 1	1978	Carpet	5	8	4790
Home 3	1979	tiled	6	12	25720
Home 5	1977	tiled	6	3	15790
Home 7	1973	Carpet+ Wooden	5	10	30060
Home 10	1981	Tiled	8	22	67280
Office 1	INB	Carpet	1	4	15820
Office 2	INB	Carpet	1	3	19080
Office 3	INB	Carpet	2	3	14920
Office 4	INB	Carpet	4	3	17890
Office 5	INB	Carpet	3	3	31250
Office 6	INB	Carpet	1	1	49210
Office 7	INB	Carpet	1	3	36870
Office 9	INB	Carpet	5	5	26930

Table S8.14 Details of automobiles.

Microenvironment	Model and Manufacturer	Model year	Automobile condition
CR 01	Honda Jazz	2009	Personal
CR 02	Toyota Corolla	2012	Personal
CR 03	Audi A4	2012	Personal
CR 04	Honda Jazz	2009	Personal
CR 05	Kia Rio	2011	Personal
CR 06	Peugeot 406	2010	Resale
CR 07	Mercedes C180	2008	Resale
CR 09	BMW X5	2011	Resale
CR 11	Audi A6	2010	Resale
CR 12	Isuzu KB 250	2010	Resale
CR 13	Opel Turbo Corsa	2010	Resale
CR 14	Honda Prelude	1999	Resale
CR 15	Audi Q7	2012	Resale
CR 16	Opel Corsa Utility	2011	Resale
CR 17	Chevrolet	2008	Personal
CR 18	Toyota Starlet		Personal
CR 19	Mazda 63	1998	Personal
CR 20	Toyota Corolla	1989	Personal
CR 21	Toyota Corolla	2007	Personal

Table S8.15 – S8.18 Correlations of OPEs with Vehicle Manufacture Year.

	Year	*TCEP*
Year	1	
TCEP	-0.12114	1

	Year	*TCPP*
Year	1	
TCPP	-0.0391	1

	Year	*TPP*
Year	1	
TPP	0.154249	1

	Year	*TDCPP*
Year	1	
TDCPP	0.143508	1

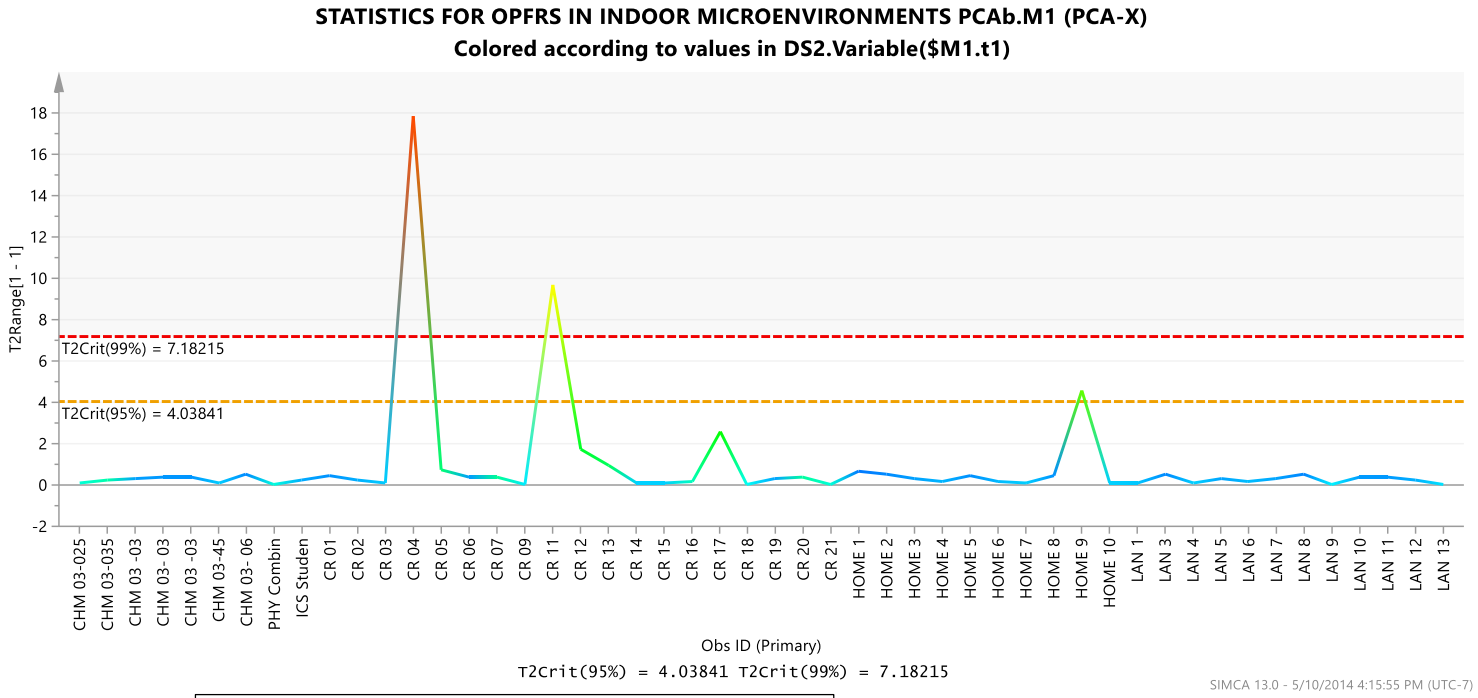

Figure 8.1 PCA hotelling T2 plot

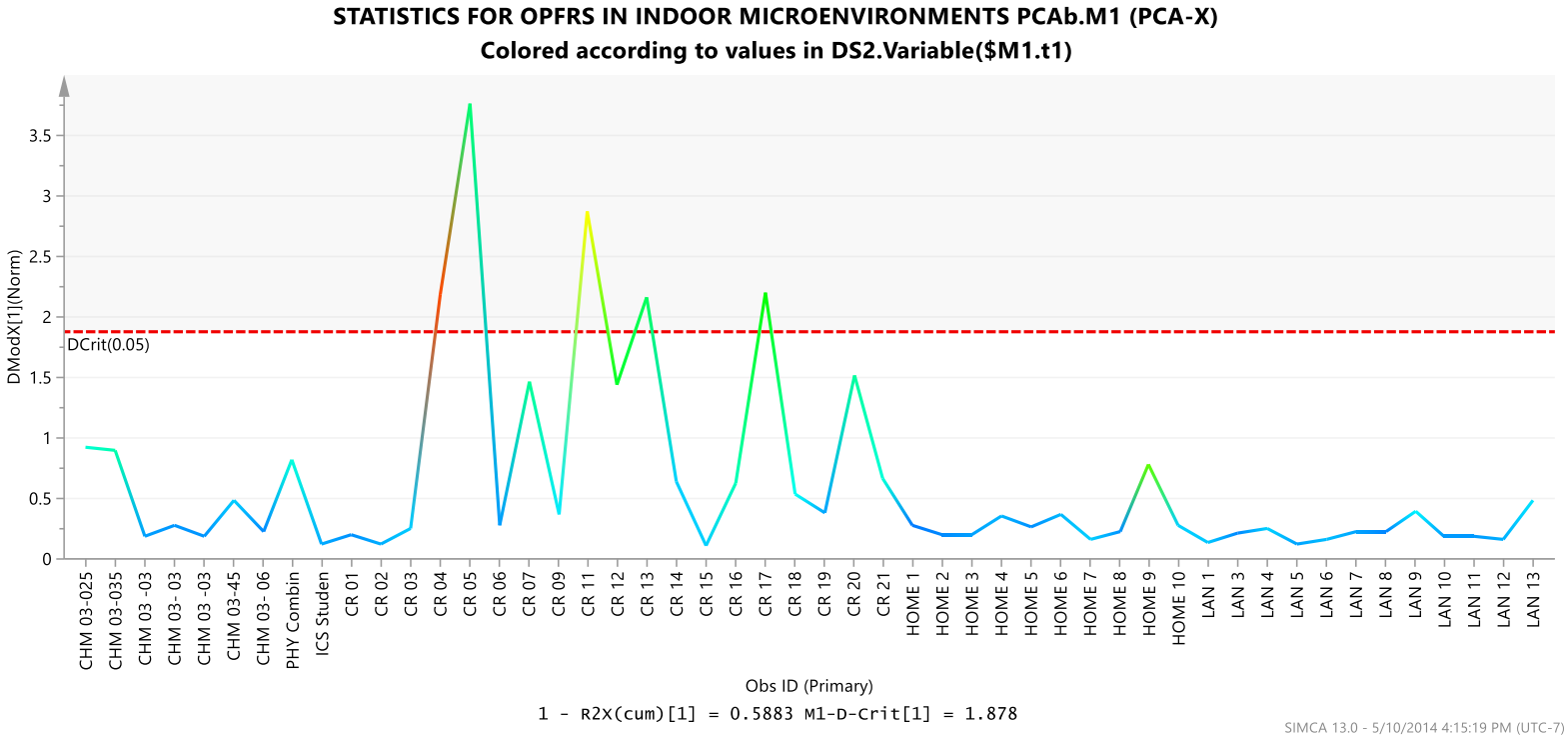

figure 8.2 PCA DModX/DModY

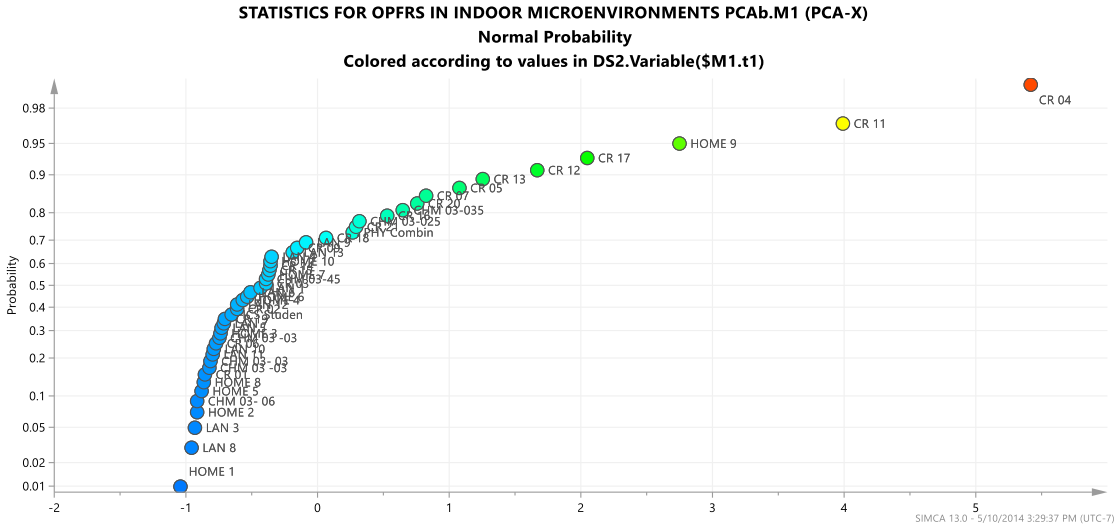

Figure S8.3 Normal probability plot for PCA observations.

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File       :C:\msdchem\1\data\ABAFE NBFR\Abafe NBFR\100PPM MULTI OPFR ST
...         D RUN 2.D
Operator   : abafe
Instrument :   5973n
Acquired   :  4 Jan 2014  16:10     using AcqMethod abafebde2013 NBFR DEC 2013 sim.M
Sample Name: 100ppm multi opfr std run 2
Misc Info  :
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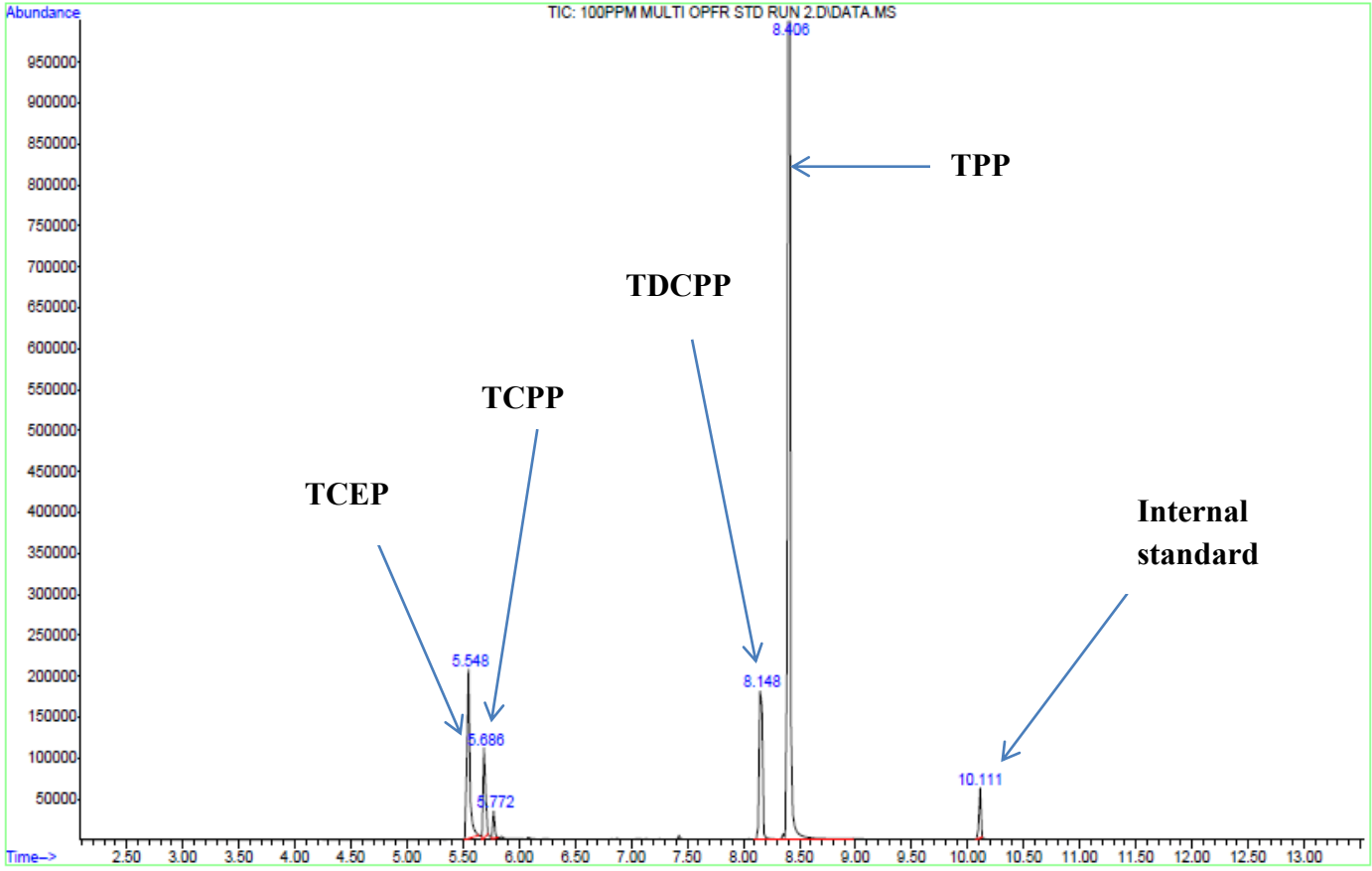

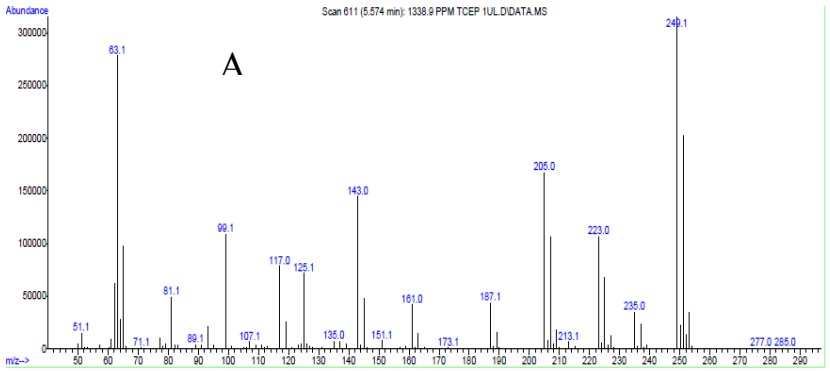
A
Abundance
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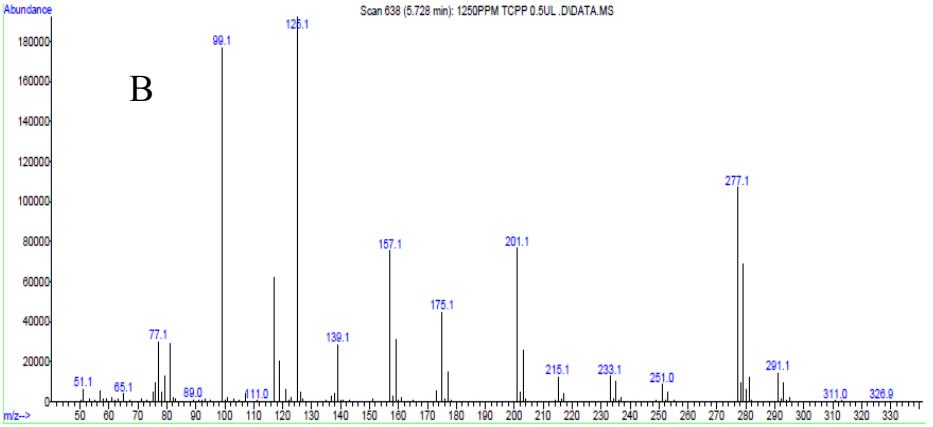
m/z-->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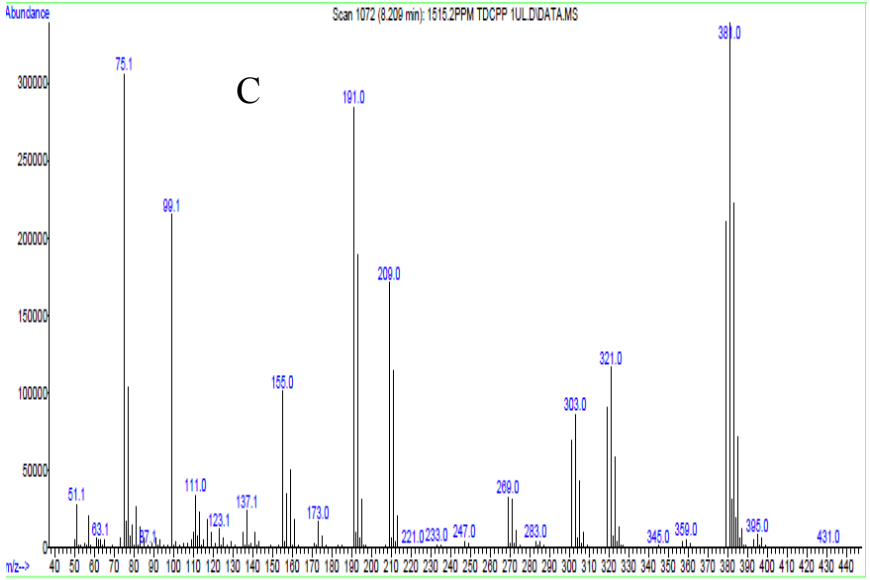
Abundance
Scan 638 (5.728 min): 1250PPM TCPP 0.5UL .D\DATA.MS
m/z-->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Abundance
Scan 1072 (8.209 min): 1515.2PPM TDCPP 1UL.D\DATA.MS
m/z-->

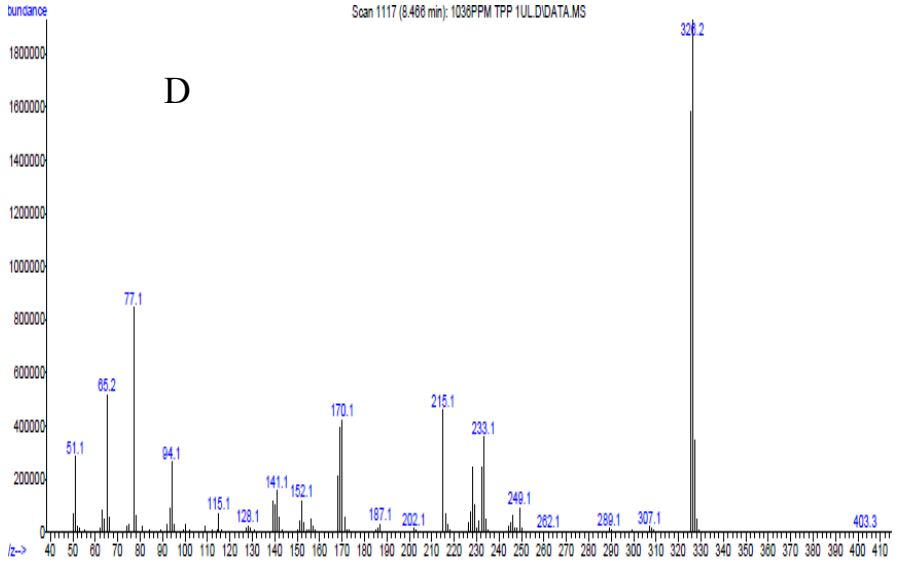
D
Scan 1117 (8.466 min): 1038PPM TPP 1UL.D\DATA.MS
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Figure S8.4 GC-MS chromatogram (top) and full scan mass spectral of pure analytical standard of (A) TCEP (B) TCPP (C) TDCPP and (D) TPP.

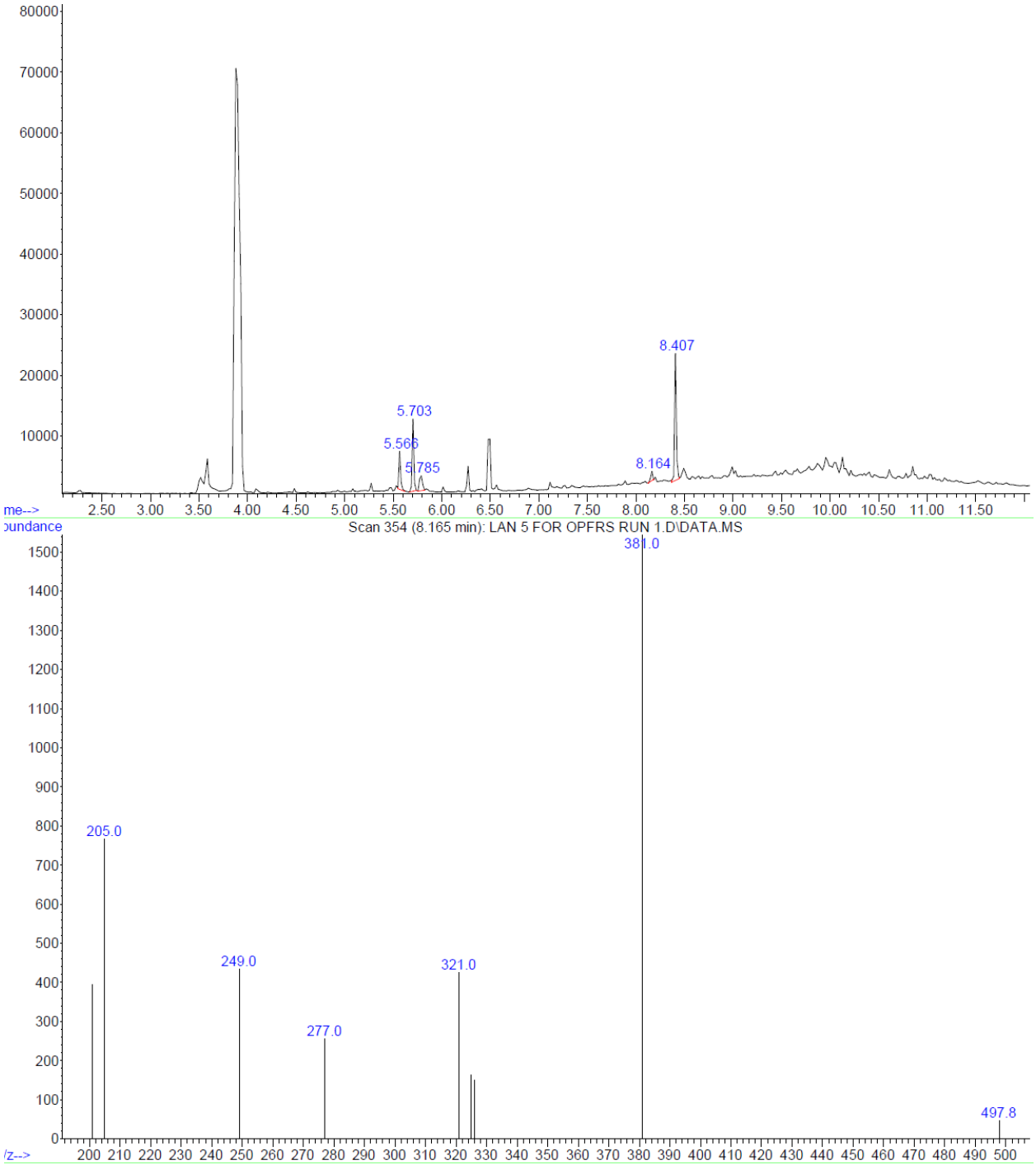


Figure S8.5 GC chromatogram and mass spectrum of OPEs in a typical computer laboratory sample.

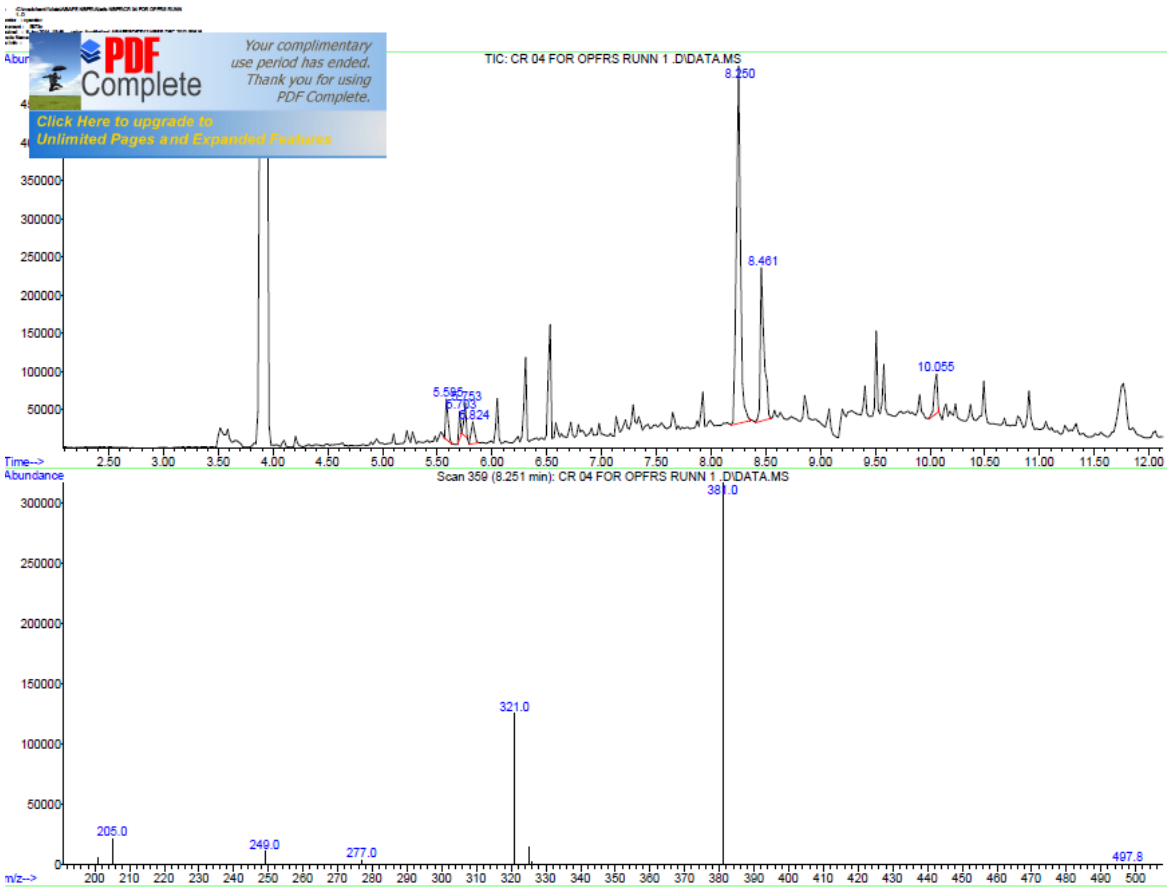


Figure S8.6 GC chromatogram and mass spectrum of OPEs in a typical automobile dust sample.

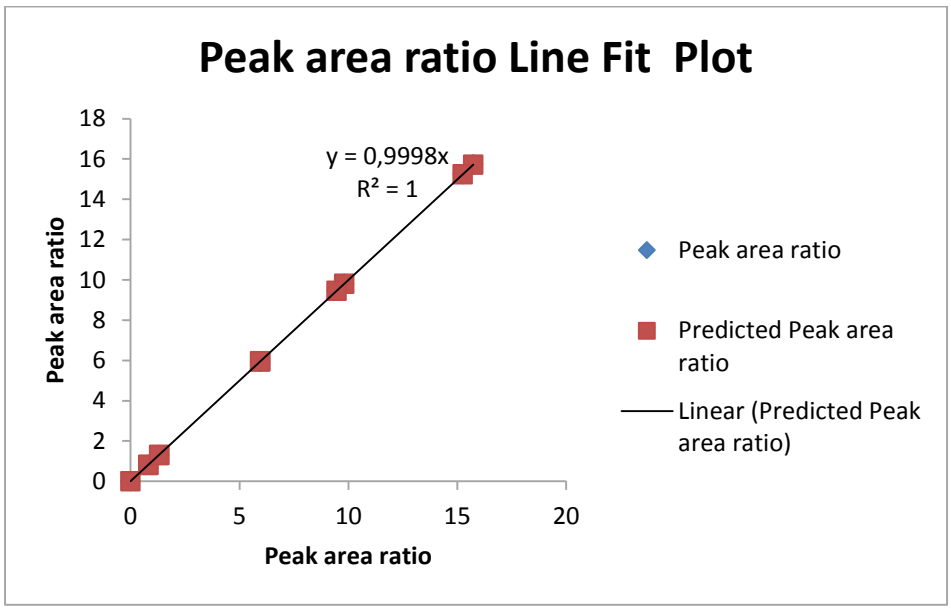


Figure S8.7 Calibration curve for TCEP.

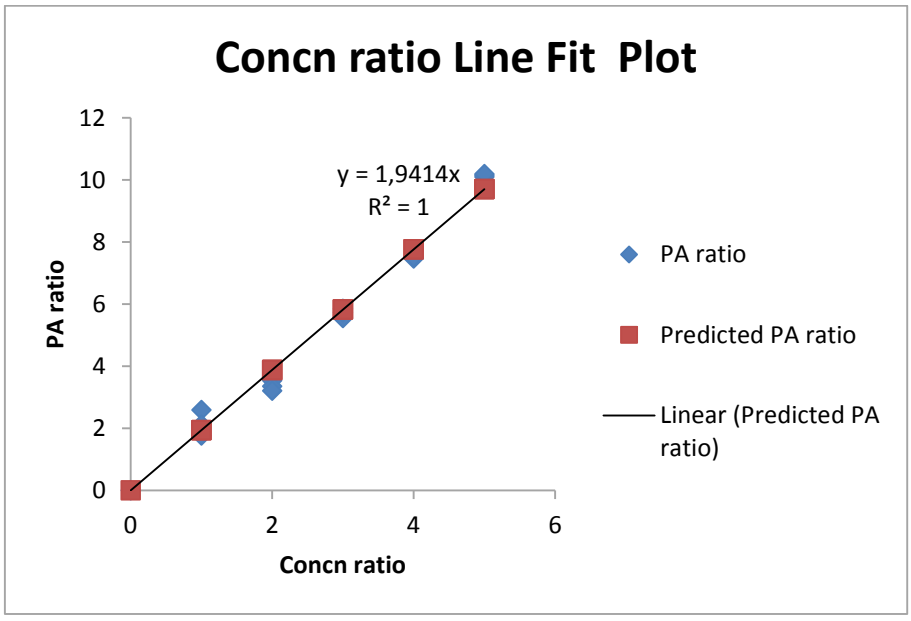


Figure S8.8 Calibration curve for TCPP.

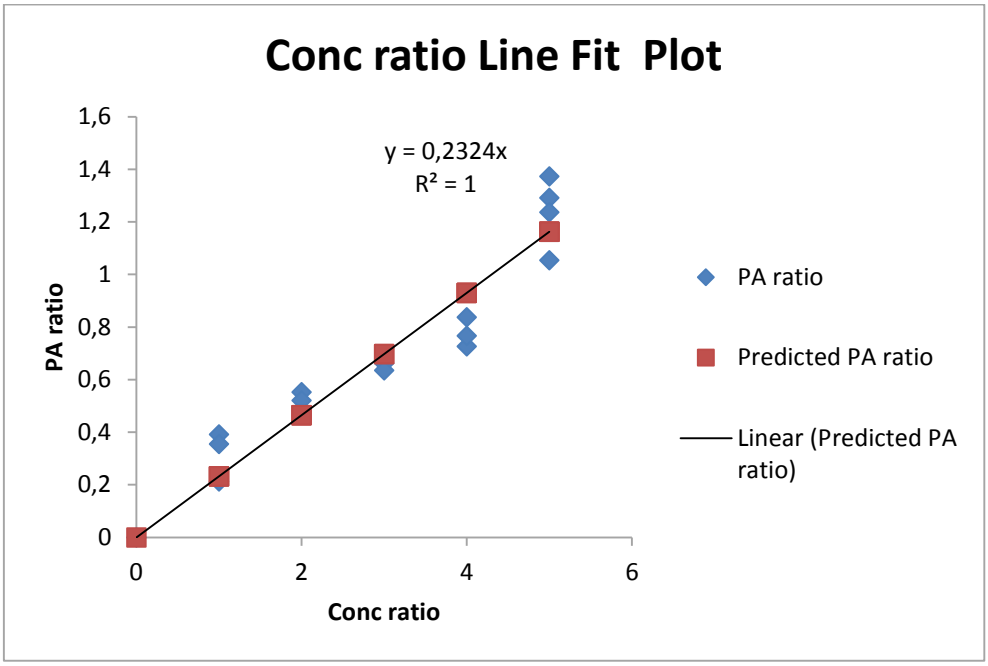


Figure S8.9 Calibration curve for TDCPP.

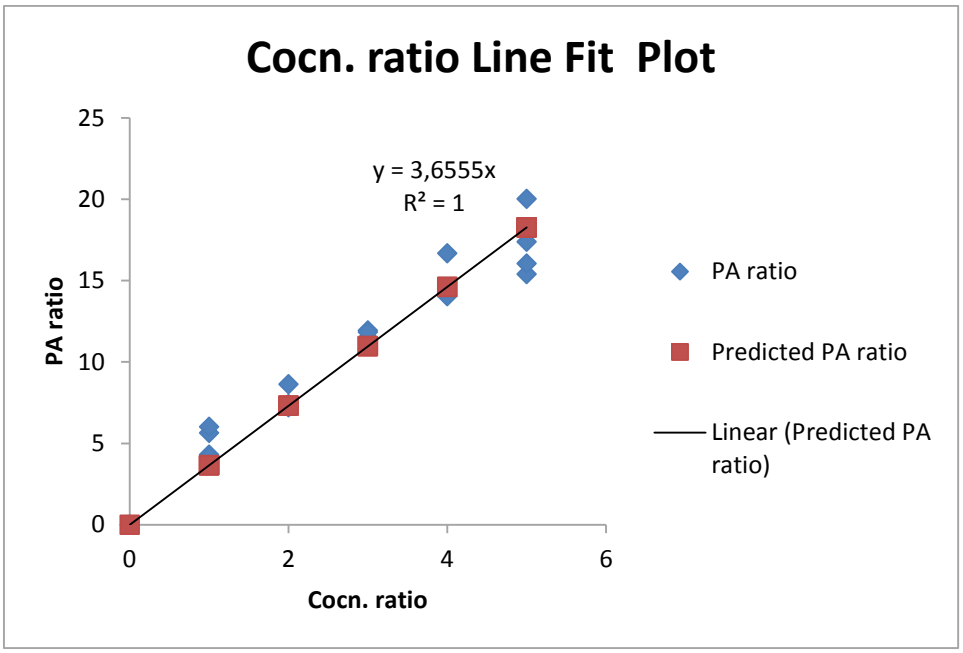


Figure S8.10 Calibration curve for TPP.

Supplementary Material Non parametric statistics for OPEs
(Distribution fitting of TCEP, TCPP, TDCPP and TPP)

XLSTAT 2014.3.01 - Distribution fitting - on 6/11/2014 at 3:09:41 AM
Data: Workbook = Book1 / Sheet = Sheet1 / Range = Sheet1!$B$2:$E$52 / 50 rows and 4 columns
Significance level (%): 5
Distribution: Normal
Estimation method: Moments

Summary statistics:

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
TCEP	50	0	50	2000	245230	19270	39136
TCPP	50	0	50	220	56250	6529	8957
TDCPP	50	0	50	0	697100	42828	117932
TPP	50	0	50	650	34100	6788	7986

Distribution fitting (TCEP):

Estimated parameters (TCEP):

Parameter	Value
μ	19270.000
Sigma	39135.855

Statistics estimated on the input data and computed using the estimated parameters of the Normal distribution (TCEP):

Statistic	Data	Parameters
Mean	19270.000	19270.000
Variance	1531615142.857	1531615142.857
Skewness (Pearson)	4.404	0.000
Kurtosis (Pearson)	20.629	0.000

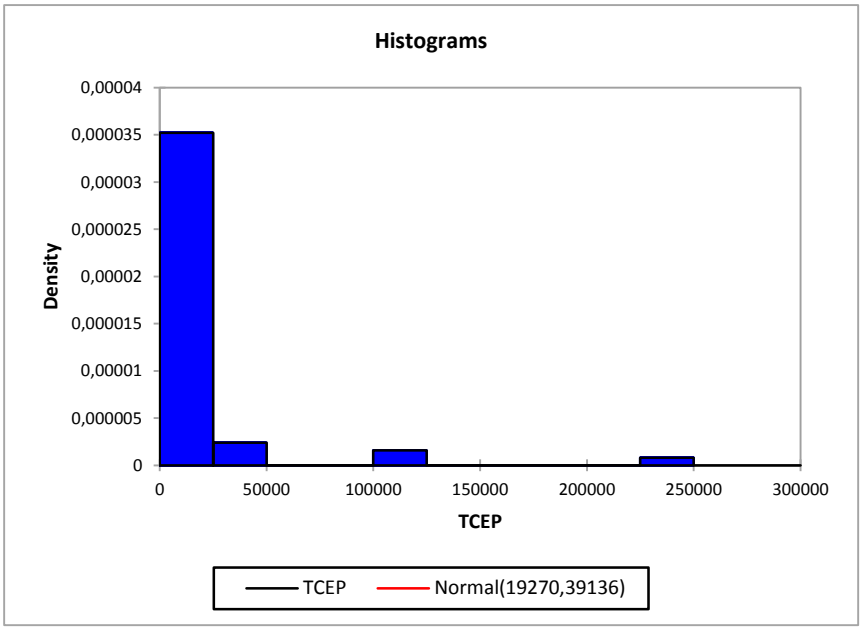
Kolmogorov-Smirnov test (TCEP):

D	0.392
p-value	< 0.0001
Alpha	0.05

Test interpretation:
H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution

As the computed p-value is lower than the significance level alpha=0.05,
one should reject the null hypothesis H0, and accept the alternative hypothesis Ha.

The risk to reject the null hypothesis H0 while it is true is lower than 0.01%.



Descriptive statistics for the intervals (TCEP):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	25000	44	0.880	0.000	0.247
25000	50000	3	0.060	0.000	0.226
50000	75000	0	0.000	0.000	0.139
75000	100000	0	0.000	0.000	0.058
100000	125000	2	0.040	0.000	0.016
125000	150000	0	0.000	0.000	0.003
150000	175000	0	0.000	0.000	0.000
175000	200000	0	0.000	0.000	0.000
200000	225000	0	0.000	0.000	0.000
225000	250000	1	0.020	0.000	0.000

Distribution fitting (TCPP):

Estimated parameters (TCPP):

Parameter	Value
μ	6529.400
Sigma	8957.430

Statistics estimated on the input data and computed using
the estimated parameters of the Normal distribution (TCPP):

Statistic	Data	Parameters
Mean	6529.400	6529.400
Variance	80235548.612	80235548.612
Skewness (Pearson)	3.927	0.000
Kurtosis (Pearson)	17.614	0.000

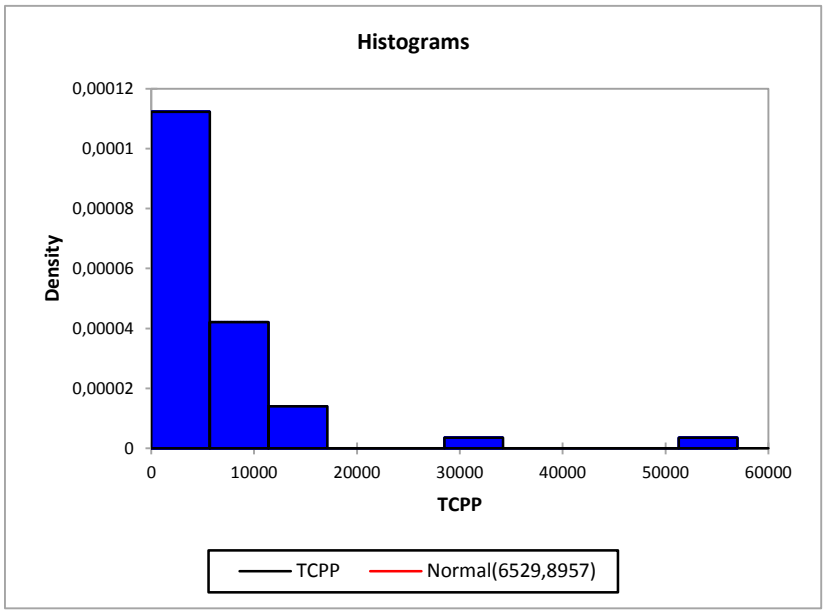
Kolmogorov-Smirnov test (TCPP):

D	0.265
p-value	0.001
Alpha	0.05

Test interpretation:
H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution

As the computed p-value is lower than the significance level alpha=0.05,
one should reject the null hypothesis H0, and accept the alternative hypothesis Ha.

The risk to reject the null hypothesis H0 while it is true is lower than 0.14%.



Descriptive statistics for the intervals (TCPP):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	5700	32	0.640	0.000	0.230
5700	11400	12	0.240	0.000	0.244
11400	17100	4	0.080	0.000	0.174
17100	22800	0	0.000	0.000	0.084
22800	28500	0	0.000	0.000	0.028
28500	34200	1	0.020	0.000	0.006
34200	39900	0	0.000	0.000	0.001
39900	45600	0	0.000	0.000	0.000
45600	51300	0	0.000	0.000	0.000
51300	57000	1	0.020	0.000	0.000

Distribution fitting (TDCPP):

Estimated parameters (TDCPP):

Parameter	Value
μ	42827.800
Sigma	117932.222

Statistics estimated on the input data and computed using the estimated parameters of the Normal distribution (TDCPP):

Statistic	Data	Parameters
Mean	42827.800	42827.800
Variance	13908008911.388	13908008911.388
Skewness (Pearson)	4.035	0.000
Kurtosis (Pearson)	17.493	0.000

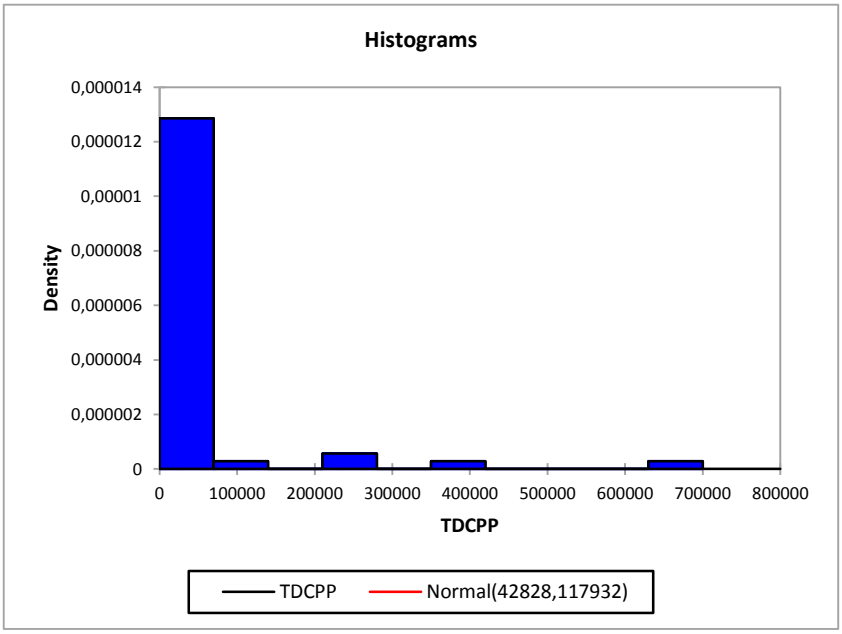
Kolmogorov-Smirnov test (TDCPP):

D	0.411
p-value	< 0.0001
Alpha	0.05

Test interpretation:
H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution

As the computed p-value is lower than the significance level alpha=0.05,
one should reject the null hypothesis H0, and accept the alternative hypothesis Ha.

The risk to reject the null hypothesis H0 while it is true is lower than 0.01%.



Descriptive statistics for the intervals (TDCPP):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	70000	45	0.900	0.000	0.233
70000	140000	1	0.020	0.000	0.204
140000	210000	0	0.000	0.000	0.127
210000	280000	2	0.040	0.000	0.056
280000	350000	0	0.000	0.000	0.018
350000	420000	1	0.020	0.000	0.004
420000	490000	0	0.000	0.000	0.001
490000	560000	0	0.000	0.000	0.000
560000	630000	0	0.000	0.000	0.000
630000	700000	1	0.020	0.000	0.000

Distribution fitting (TPP):

Estimated parameters (TPP):

Parameter	Value
μ	6787.800
sigma	7986.049

Statistics estimated on the input data and computed using the estimated parameters of the Normal distribution (TPP):

Statistic	Data	Parameters
Mean	6787.800	6787.800
Variance	63776980.776	63776980.776
Skewness (Pearson)	1.945	0.000
Kurtosis (Pearson)	3.053	0.000

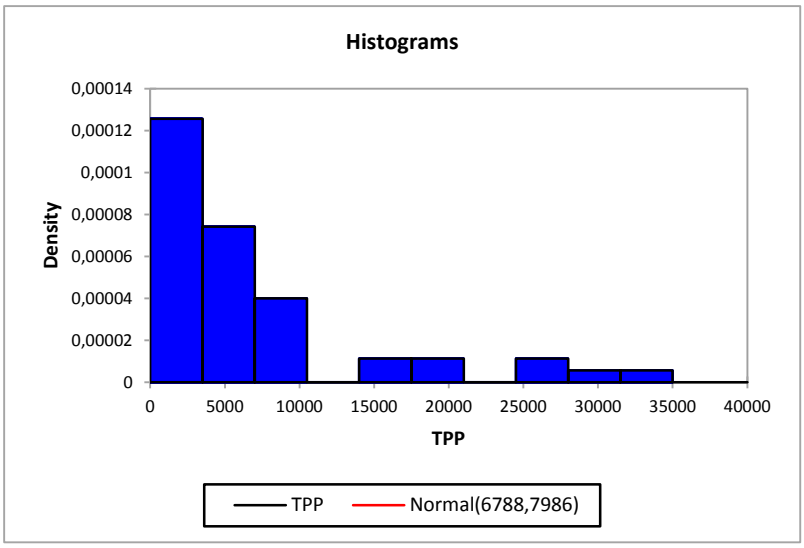
Kolmogorov-Smirnov test (TPP):

D	0.246
p-value	0.004
alpha	0.05

Test interpretation:
H0: The sample follows a Normal distribution
Ha: The sample does not follow a Normal distribution

As the computed p-value is lower than the significance level alpha=0.05, one should reject the null hypothesis H0, and accept the alternative hypothesis Ha.

The risk to reject the null hypothesis H0 while it is true is lower than 0.37%.



Descriptive statistics for the intervals (TPP):

Lower bound	Upper bound	Frequency	Relative frequency	Density (Data)	Density (Distribution)
0	3500	22	0.440	0.000	0.143
3500	7000	13	0.260	0.000	0.170
7000	10500	7	0.140	0.000	0.168
10500	14000	0	0.000	0.000	0.138
14000	17500	2	0.040	0.000	0.093
17500	21000	2	0.040	0.000	0.052
21000	24500	0	0.000	0.000	0.024
24500	28000	2	0.040	0.000	0.009
28000	31500	1	0.020	0.000	0.003
31500	35000	1	0.020	0.000	0.001

Non parametric statistics for OPEs (Kruskal-Wallis test for comparison of OPEs in the different microenvironment)

XLSTAT 2014.3.01 - Comparison of k samples (Kruskal-Wallis, Friedman, ...) - on 6/11/2014 at 3:27:53 AM
Samples: Workbook = Book1 / Sheet = Sheet2 / Range = Sheet2!$A$1:$D$20 / 19 rows and 4 columns
Significance level (%): 5
p-value: Asymptotic p-value

Summary statistics:

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
∑ OPFRs offices	19	10	9	1492	49210	28187	12395
∑ OPFRs automobile	19	0	19	1402	77359	14372	191693
∑ OPFRs in Homes	19	9	10	4790	29944	52053	88549
∑ OPFRs in LANs	19	7	12	1007	43390	22162	9452

Kruskal-Wallis test:

K (Observed value)	14.147
K (Critical value)	7.815
DF	3
p-value (Two-tailed)	0.003
alpha	0.05

An approximation has been used to compute the p-value.

Test interpretation:

H0: The samples come from the same population.
Ha: The samples do not come from the same population.

As the computed p-value is lower than the significance level alpha=0.05,
one should reject the null hypothesis H0, and accept the alternative hypothesis Ha.

The risk to reject the null hypothesis H0 while it is true is lower than 0.27%.

Chapter 9

Modifications to the Unified Bioaccessibility Method for the determination of oral bioaccessibility of polybrominated diphenyl ethers in contaminated dust of e-waste recycling sites and standard reference materials

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Abstract

The Unified Bioaccessibility Method (UBM) of Europe was modified to study the oral bioaccessibility of polybrominated diphenyl ethers (PBDEs) in indoor dust of contaminated e-waste recycling sites and in a standard reference material (SRM 2585). The gastric pH of the FED and UNFED states condition was optimized; and the optimum gastrointestinal tract (GIT) incubation time was established for PBDE congeners. Batch and sequential extraction formats were employed to determine the percentage bioaccessible fractions (% BAF) of PBDEs. Results revealed higher bioaccessibility of PBDEs in the FED–state sequential format of the GIT extraction. The % BAF of PBDEs under the FED condition ranged between 25.1 – 70.1 % for tri- to hepta-BDEs and 21.7 % for BDE-209 in SRM dust samples; and 11.5 – 104.5 % for tri- to hepta-BDEs, and 35.1 – 40.0 % for BDE–209 in e-waste samples. On the other hand, the % BAF of PBDEs in the UNFED condition ranged from 4.1 – 30.3 % for tri- to heptaBDEs and 12.0 % for BDE-209 in SRM 2585 samples; and 6.5 – 27.9 % for tri- to heptaBDEs and 9.5 % for BDE-209 in e-waste samples. A positive correlation ($r = 0.62$) was obtained for BDE congeners and their respective log K_{ow}. No statistically significant differences (p value of 0.496) were obtained for the % BAFs of PBDEs in SRM and e-waste samples for the FED and UNFED conditions. Significant statistical differences (p value of 0.0014) were obtained between the FED and UNFED state % BAFs of PBDEs in SRM and e-waste samples. However, no differences (p value of 0.480) were obtained for the % BAF of PBDEs following both the batch and sequential extraction formats in the UNFED condition.

Keywords*: Oral, Bioaccessibility, Unified Bioaccessibility Method, FoREhST, Dust, Ingestion.*

9.1 Introduction

Polybrominated diphenyl ethers (PBDEs) are legacy environmental contaminants used widely and largely as flame retardants in consumer products (1). Due to stricter fire regulations and increased demand for personal computers and other electronic equipment, there has been a rise in the production of PBDEs since they were first introduced in the early 1970s (2). PBDEs were commercially produced as three major formulations: penta-, octa- and deca-BDE. However, the potential human health risks, such as endocrine disruption, neurodevelopmental and behavioural disorders, hepatic abnormalities and possibly cancer, posed by PBDEs has consequently led to the ban of the penta- and octa-BDE formulations in 2004 and deca-BDE formulations in 2008 by the European Union (2). The commercial octa- and deca-BDE mixtures found application as flame retardants in electronic equipment and were physically incorporated into polymers and other substrates of electronic equipment (3). During manufacture, usage, repair, recycling and disposal of electronic products, PBDEs can be released thereby contaminating the environment.

Electronic waste (e-waste) has recently received much attention particularly for the African and Asian continents, which are known dumpsites for legally or illegally imported e-waste (3). Associated with the concern for e-waste, are the consequent potential human and environmental health challenges resulting from mishandling of e-waste. In Africa and Asia, various mechanized and primitive operations are employed in the recovery of useful products from e-waste. Such operations include metal stripping in open acid baths, removal of electronic components from circuit boards by heating in a grill, physical and mechanical dismantling of e-waste polymer casings, as well as combustion of cables to recover metals [see Chapter 7 and Zhao et al. (4)]. Through these operations, as well as evaporation, leakage, volatilization, etc., PBDEs are released into the environment and enter the human body via multiple exposure routes (5, 6).

Indoor dust is an established pathway of human exposure to PBDEs through inadvertent dust ingestion and hand-to-mouth activity, particularly for children, and is a subject of increasing concern (7-9). The relationship between dust and human body burdens is strongly suggested by the correlation of PBDEs in indoor dust and human milk (10), and dust and human blood (11).

Total contaminant concentrations are frequently used in risk assessment of contaminated sites to human health (12). Such assessment though advantageous for precautionary measures, may lead to overestimation of the amount of contaminant absorbed by humans (12). These overestimations have significant implications for cost and sustainability of brownfield remediation; hence, the use of bioaccessible and bioavailable fractions of contaminants for site specific risk assessment is a very important parameter. The idea of bioaccessibility and oral bioavailability are essential for quantifying the risks that are associated with oral exposure to environmental contaminants (13). Several *in vivo* and *in vitro* methodologies have been developed recently to study both accessible and

available fractions of PBDEs and other persistent organic pollutants (2, 14-16). Bioavailability is the fraction of a contaminant available for uptake across an organism's cellular membrane at any given time. The use of vertebrates and invertebrates for *in vivo* bioavailability studies of contaminants gives realistic measurements of the bioavailable fractions, however, ethical considerations of using mammals, high cost, low sample through-put, differing physiologies and ecologies compromises the extrapolation of a contaminant's bioavailability in animals to humans; thus making *in vivo* methods unsuitable for routine laboratory testing purposes (16). The use of *in vitro* bioaccessibility tests has recently gained much attention in studying human uptakes of various contaminants such as heavy metals, PCDD/Fs, PAHs, PCBs, and PBDEs in various matrices following various approaches utilizing both 'Fed' and 'Fasted' state conditions (2, 14-19). Bioacessibility is the fraction of the total target contaminant introduced that dissolves in the gastrointestinal tract (GIT), and is therefore available for systemic absorption (14, 16). The use of GIT extraction systems, such as the physiologically based extraction test (PBET), is a valuable tool in assessing the human health risk of persistent organic pollutants. These extraction processes tend to imitate the process of the human digestive system to determine the bioacessibility of PBDEs accidentally or intentionally ingested (14, 16). For the bioaccessibility of heavy metals, the universal bioaccessibility method (UBM) shows that the fasted state gives the most conservative estimate of the bioaccessible fraction as this will result in lower pH conditions compared with the fed state (13, 20). It has recently been shown that the presence of food components in the GIT during intestinal transit modulates the release of hydrophobic contaminants such as PBDEs. Such release has been ascribed to the more hydrophobic character that food components give to the aqueous solution; similarly, soluble soil organic matter forms microscale hydrophobic environments in the aqueous phase, thus acting as a mobile sorbent for hydrophobic compounds such as PBDEs, PCBs and PAHs (21). The types and concentrations of bile in PBET, GIT extraction time, gastric pH and transit time in each GIT medium play a significant role in the release of PBDEs and other persistent organic pollutants (2, 14, 15).

To better understand the implication of the ingestion of dust from e-waste recycling sites for human exposure, the objectives of the present study include: (1) to determine the optimum GIT conditions for determining the bioaccessibility of PBDEs in dust, (2) to apply the optimum conditions to determine the bioaccessibilities of eight environmentally relevant PBDE congeners, (3) to compare the effectiveness of two GIT procedures – the so called unified bioaccessibility method (fasted–state) of Europe and the FOREhST (fed–state) conditions reported by Lorenzi et al. (15) after parameter modifications, and (4) to determine the effectiveness of two extraction formats – batch and sequential – for studying the oral bioaccessibility of PBDEs. This *in vitro* model is based on four compartments: saliva, gastric, duodenum and bile phases, mimicking the enzymatic and physiochemistry preponderant in the human gastrointestinal transit for UNFED and FED state conditions. To our knowledge, this is the first study detailing the

parameters responsible for oral bioaccessibility of PBDEs from ingested dusts *vis-a-vis* the application of two GIT models to study the oral bioaccessibility of PBDEs in contaminated e-waste sites.

9.2 Materials and Methods

9.2.1 Chemicals for GIT media

Reagents used for the GIT extraction procedure were purchased from different reagent suppliers in South Africa or elsewhere as stated: $CaCl_2.2H_2O$ from NT Laboratory Supplies (Pty) Ltd, HCl and KCl from Promark Chemicals, KSCN from Merck Laboratory Supplies (Pty) Ltd, $MgCl_2.6H_2O$ from Saarchem Pty Ltd, NaCl from SMM Instruments, NaOH, Na_2SO_4 from Associated Chemical Enterprise, NH_4Cl from PAL Chemicals, $NaHCO_3$ from Merck Laboratory Supplies (Pty) Ltd, and $NaH_2PO_4.2H_2O$ from May and Baker Ltd, Dagenham, England. α-Amylase from porcine pancreas, uric acid, urea, pepsin from porcine gastric mucosa, D-glucuronic acid, glucosamine hydrochloride (Fluka), D(+) – glucose, pancreatin, bile salts (Fluka Analyticals), and lipase were obtained from Sigma Aldrich, South Africa; bovine serum albumin was purchased from Melford, United Kingdom and gastric mucin was purchased from Hangzhou Dayangchem Co, Ltd. HiPP organic creamy oats porridge was purchased from a local retail store. All organic solvents were high performance liquid chromatography grade from Sigma Aldrich; water was from a millipore Elix system with a resistance of 18 Ω.

9.2.2 Sampling and Analysis of Total PBDEs in Samples

Details of the sample collection and analysis for total PBDEs in the samples, SRM 2585 dust sample and residual PBDEs in solutes after GIT extraction can be found elsewhere (see Chapters 3 & 7).

9.2.3 Preparation of Gastrointestinal Fluids

The physiologically based extraction test in the present study was based on the Unified BARGE method (UBM) of Europe (15). All GIT fluids were prepared in advance and stored at < 4 ºC. The major modifications of our methods compared with those of Lorenzi et al. (15) are the prolonged extraction time, increased bile fluid for extraction (i.e. 9 mL) and optimum gastric pH for FORhEST.

9.2.3.1 Simulated saliva fluid

Simulated saliva fluid was prepared by adding 146 mg of α-amylase, 56 mg mucin and 18 mg uric acid to a 1 L Duran bottle. Separately, inorganic saliva components were prepared by adding 900.30 mg KCl, 900.90 mg $NaH_2PO_4.2H_2O$, 207.20 mg KSCN, 581.10 mg Na_2SO_4, 300.0 mg NaCl and 1.8 mL 1.0 M HCl into a 500 mL Duran bottle and made up to the mark with Millipore water. For the organic saliva component, 222 mg urea was added into a 500 mL Duran bottle and made to the mark with Millipore water. The entire contents of the inorganic and organic saliva components were

simultaneously poured into the 1 L Duran bottle. The entire content of the bottle was thoroughly mixed in a shaker. The pH of the simulated saliva fluid was measured and when necessary adjusted to be within 6.50 ± 0.5. The pH was adjusted with either 1.0 M NaOH or 10.32 M HCl.

9.2.3.2 Simulated gastric fluid

Gastric fluid was prepared by adding 1123 mg bovine serum albumin (BSA), 3018 mg mucin and 1017 mg pepsin to a 1 L Duran bottle. Then, 827 mg KCl, 276 mg $NaH_2PO_4.2H_2O$, 401 mg $CaCl_2.2H_2O$, 314 mg NH_4Cl, 2740 mg NaCl and 8.30 mL 10.32 M HCl were added to a 500 mL Duran bottle and made up to the mark with Millipore water to form the inorganic gastric components. To a second 500 mL Duran bottle, 664 mg D (+) glucose, 28 mg D-glucuronic acid, 88 mg urea and 328 mg glucosamine hydrochloride were added and made up to the mark with Millipore water to form the organic gastric components. The inorganic and organic gastric components were simultaneously poured into the 1 L Duran bottle. The entire content of the bottle was thoroughly mixed in a shaker. The pH of the gastric fluid component was measured and adjusted when necessary to be within 0.9 – 1.0. The pH was adjusted with either 1.0 M NaOH or 10.32 M HCl.

9.2.3.3 Simulated duodenal fluid

The duodenal fluid was prepared by adding 216 mg $CaCl_2$, 1061 mg BSA, 3013 mg pancreatin and 500 mg lipase into a 1 L Duran bottle. Then, the inorganic duodenal component was prepared by adding 578 mg KCl, 88 mg KH_2PO_4, 58 mg $MgCl_2.6H_2O$, 5629.7 mg $NaHCO_3$, 7092.0 mg NaCl and 180 µL of 10.32 M HCl into a 500 mL Duran bottle and made up to the mark with Millipore water. For the organic duodenal component, 115.7 mg urea was added into a 500 mL Duran bottle and made up to the mark with Millipore water. Simultaneously, the inorganic and organic duodenal components were added into the 1 L Duran bottle and the contents were mixed thoroughly in a shaker. The pH of the duodenal fluid component was measured and adjusted when necessary to be within 7.31 ± 0.2. The pH of the solution was adjusted with either 1.0 M NaOH or 10.32 M HCl.

9.2.3.4 Simulated bile fluids

The simulated bile fluid was prepared by adding 246.8 mg $CaCl_2$, 1807.9 mg BSA and 6007.6 mg bile into a 1 L Duran bottle. Separately, 385.4 mg KCl, 5771.6 mg $NaHCO_3$, 5299.1 mg NaCl and 180 µL of 10.32 M HCl were added into a 500 mL Duran bottle and made up to the mark with Millipore water. The organic bile component was prepared by adding 251.9 mg urea into a 500 mL Duran bottle and made up to the mark with Millipore water. Both inorganic and organic bile components were simultaneously added to the 1 L Duran bottle. The content of the bottle was mixed thoroughly in a shaker. After complete dissolution of the solid reagents, the pH of the solution was measured and where possible adjusted to be within 8.03 ±0.1. The pH was adjusted with either 1 M NaOH or 10.32 M HCl.

9.2.3.5 Selection of optimum gastric pH and GIT extraction time

Gastric pH plays a very important role in the bioaccessibility of organic contaminants such as PBDEs. To check the influence of gastric pH on the oral bioaccessibility of PBDEs, masses of 0.2 g SRM 2585 dust sample were placed in each of five separate 25 mL Sartedst bottle. Each sample was extracted with 18 mL gastric fluid at pH 0.99, 1.67, 2.33, 3.71 and 4.27. Extraction was carried out for 2 hours on a thermostatted end-over-end shaking water bath operated at 37 ± 2°C. Extracts were centrifuged at 7000 g for 10 mins. Aliquots of 3 mL of the supernatants were filtered through 0.45 µm hydrophilic PVDF syringe filters and then concentrated to approximately 1 mL in a rotary evaporator. Extracts were kept at <4 °C until analysis.

Similarly, GIT extraction time was optimized for PBDEs. An extraction time of 8.05 hrs was found sufficient for tri- to hexa-BDEs and 16.05 hrs was sufficient for BDE-183 and BDE-209.

9.2.4 Gastrointestinal (GIT) extraction

The Fed Organic Estimation human Simulation (FOREhST) procedure described by Lorenzi et al. (15) was modified for this study. Both batch and sequential extraction formats were employed in this study. The same reagents as used by Lorenzi et al. (15) were used in this study. Apart from the incubation time and the pH that were optimized for PBDEs in this study, every other parameter was the same as those of Lorenzi et al. (15) apart from slight modifications such as the volume of the fluid used for the extraction. Samples for GIT extraction were prepared by quantitatively weighing 0.301 – 0.304 g of the sample into a 50 mL screw-cap BIOLOGIX tube containing 0.814 g HIPP organic creamy porridge, 2.45 mL Millipore water, and 50 µL pure sunflower oil. Then 4.5 mL of simulated saliva fluid was added and the solution was rotated in an end-over-end shaking water bath for 5 minutes at approximately 35 rounds per minute and kept at 37 ± 2 ºC. Then, 9 mL of simulated gastric fluid was added to the solution by pipette, and samples were incubated in the shaking water bath under the same conditions for a further 2 hours. After incubation, the pH of the solutions was measured (ca. pH 2.67 ± 0.1 for SRM dust samples; 2.36 ± 0.1 for sample from e-waste recycling site 1; 2.45 ± 0.1 for sample from e-waste recycling site 2 and 1.44 ± 0.2 for extraction method blanks). Then, 9 mL of duodenal fluid and 9 mL of bile fluid were added. The solution pH was checked and adjusted when necessary to be within the tolerance of an intestinal pH of 6.0 ± 0.5. The solution was incubated in the shaking water bath under the same conditions for a further 6 hours for tri- to hexa-BDE and 14 hours for BDE-183 and BDE-209. Then, the solution pH was checked to be within 6.0 ± 0.5. The solutions were centrifuged at 3500 g for 10 minutes. Then, 3 mL of the supernatant was quantitatively transferred into a 50 mL amber bottle. The sample was then saponified with 5 mL of 5 M saturated potassium hydroxide in methanol at 60 ºC in the oven. The sample was then ready for liquid-liquid extraction.

For the modified Unified Bioaccessibility Method (so-called fasted state), the same reagents as used in the FOREhST method were used. The UBM GIT extraction was carried out by quantitatively weighing 0.3008 g – 0.3032 g of SRM 2585 dust sample and e-waste recycling site 1 and 2 dust samples into a 45 mL BIOLOGIX tube, followed by the addition of 2.45 mL Millipore water. Then 4.5 mL of simulated saliva fluid was added and the solution was rotated in an end-over-end shaking water bath for 5 minutes at approximately 35 rounds per minute and kept at 37 ± 2 ºC. Then, 9 mL of simulated gastric fluid was added to the solution by pipette, and samples were incubated in the shaking water bath under the same conditions for a further 2 hours. After the extraction, the pH of the solutions was measured (ca. pH 1.64 ± 0.1 for SRM dust samples; 1.37 ± 0.1 for sample site 1 dust samples; 1.44 ± 0.1 for sample site 2 and 1.44 ± 0.2 for extraction method blanks). Then, 9 mL of duodenal fluid and 9 mL of bile fluid were added. The solution pH was checked and adjusted when necessary to be within the tolerance of an intestinal pH of 6.0 ± 0.5. The solution was incubated in the shaking water bath under the same conditions for a further 6 hours for tri- to hexa-BDE and 14 hours for BDE-183 and BDE-209. Then, the solution pH was checked to be within 6.0 ± 0.5. The solutions were centrifuged at 3500 rpm for 10 minutes prior to liquid–liquid extraction.

Two formats of GIT extraction were used in this study: (a) batch, in which test substrates were exposed to each GIT compartment in isolation, and (b) sequential, in which test substrates were exposed to the four compartments in succession.

9.2.5 Liquid-Liquid Extraction of Supernatants after GIT Extraction

A 3 mL aliquot of the supernatants from the GIT solutions was extracted with 15 mL × 2 of 3:1 methanol:n-hexane mixture in a separating funnel. The extracts were filtered through a 0.45 μm hydrophilic PVDF (Millipore Millex-HV) syringe filter and then subjected to silica gel column chromatography prior to GC-MS analysis. Details of the clean-up and GC-MS analysis can be found elsewhere (see Chapter 7). Also, residual PBDEs in solutes after GIT extractions were extracted with a previously optimized ultrasonic-assisted extraction method (see Chapter 7)

9.2.6 Calculation of Bioaccessibility

We defined bioaccessibility as the fraction of PBDEs detected in the supernatant of the centrifuged GIT extracts. This was calculated as the percentage of the average amount of each PBDE (all experiments were performed in triplicate) in the supernatant of the respective FOREhST or UBM medium to the average amount of each target PBDE originally present in the dust sample extracted (Tables 9.1 & 9.2) as follows:

$$\text{Bioaccessibility}(\%) = \frac{Average\ amount\ of\ each\ PBDE\ in\ the\ supernatant\ of\ GIT\ medium}{Average\ amount\ of\ each\ PBDE\ \ originally\ present\ in\ extracted\ dust} \times 100 \ldots \text{Eqn. 1}$$

9.2.7 Quality Control

All analyses for total PBDE concentrations in SRM 2585 and contaminated dust samples were carried out in triplicate. Similarly, supernatants arising from both the modified FOREhST and UBM protocols in all treatments were analysed in triplicate (Supplementary Material Tables S9.1-S9.6). Duplicate measurements were reported for all residual PBDEs in samples after *in vitro* extractions. No analyte of interest was found in the method blanks (n = 6) above the detection limit; for this, anhydrous sodium sulfate replaced the dust samples, and was treated as for real samples. Methods were validated with SRM 2585. Equation 2 gives a mass balance exercise carried out with both SRM 2585 dust samples and e-waste contaminated samples to determine the recovery of each of the PBDE congeners following *in vitro GIT* extractions.

$$\%\ recovery = \frac{Average\ mass\ of\ PBDE\ congener\ in\ supernatants\ of\ modified\ FOREhST\ or\ UBM + average\ mass\ of\ PBDEs\ in\ solute\ after\ GIT\ \ extraction}{Average\ mass\ of\ PBDE\ congener\ in\ the\ extracted\ dust} \times 100 \quad \ldots\ldots \quad \text{Eqn. 2}$$

Good recoveries were obtained for most of the PBDE congeners with values ranging from 34% to 92% (modified FOREhST protocol); 42% - 94% (modified UBM protocol) in SRM 2585 dust samples (Tables 9.1, 9.2 and 9.3).

Table 9.1 Mean % recovery of PBDEs in SRM 2585 dust samples following the modified FOREhST protocol.

BDE congener	Mean Supernatant Concn./ ng g^{-1} (n = 3)	% RSD	Mean solute Concn./ ng g^{-1} (n = 2)	% RSD	% BAF	% Recovery
BDE 28	29	28	13	9	60	87
BDE 47	274	9	166	8	57	92
BDE 100	38	4	82	9	25	34
BDE 99	446	8	14	4	52	62
BDE 154	35	9	22	3	38	56
BDE 153	88	6	17	2	70	91
BDE 183	12	2	24	17	26	81
BDE 209	596	7	1373	15	22	70

Table 9.2 Mean % recovery of PBDEs in SRM 2585 dust samples following the modified UBM protocol.

BDE congener	Mean Supernatant	% RSD	Mean solute Concn. / ng	% RSD	% BAF	% Recovery

	Concn./ ng g^{-1} (n = 3)		g^{-1} (n = 2)			
BDE 28	2	48	43	9	4	94
BDE 47	87	3	297	4	18	80
BDE 100	19	3	281	17	12	42
BDE 99	96	2	46	7	11	44
BDE 154	6	0.8	74	3	7	70
BDE 153	37	21	58	0.5	30	91
BDE 183	11	9	22	3	25	74
BDE 209	338	12	2278	5	12	93

Similarly, % recovery ranged from 61% – 103% for the PBDE congeners in contaminated e-waste recycling sites (Table 9.3)

Table 9. 3 Mean % recovery of PBDEs in contaminated e-waste dust samples (site 1) following the modified FOREhST protocol.

BDE Congener	Mean total PBDE concentrations in original sample (n = 3)	Mean Concn. FOREhST Supernants in site 1 (n = 3)	%RSD Supernant (n = 3)	Mean Concn. FOREhST Solute in site 1 (n = 3)	% RSD Solute (n = 3)	Mean% BAF (n = 3)	Mean% Recovery (n = 3)
BDE 28	123	114	7	ND	ND	92	99
BDE 47	2968	656	5	2399	2	22	103
BDE 100	1042	532	6	101	0.1	51	61
BDE 99	6220	1384	4	3340	2	22	76
BDE 154	3000	346	15	1567	13	12	64
BDE 153	1643	506	3	1187	10	31	103
BDE 183	1677	468	4	1158	14	28	97
BDE 209	27526	11001	14	8721	3	40	72

9.3 Results and discussion

The results obtained from the application of the optimized GIT extraction for the oral bioaccessibilities of PBDEs in contaminated e-waste dust and in a standard reference material are presented in subsequent sections.

9.3.1 The Influence of pH on the Bioaccessibility of PBDEs

pH plays an important role in the bioaccessibility of organic contaminants such as PBDEs. Usually, the pH of the various gastrointestinal tract (GIT) fluids is adjusted to physiologic values as determined in man. Selecting an appropriate gastric pH is often

difficult because children's gastric pH varies between individuals, and depends largely on nutritional status. Typically, the gastric pH can be as low as 1 and as high as 6 for the UNFED and FED state conditions, respectively. Similarly, the secretion of the gastric, duodenal and bile fluids increases for the FED state, whilst the presence of food components delays the gastric emptying rate (2). Contrarily, the human duodenum has pH values ranging from 5.5 – 7.5 and are hardly affected by the presence of food components (2). Gastric pH for an UNFED condition is usually between 1 – 2; in which the presence of a meal results in a transient increase of gastric pH from 3 to 7 because of the dilution and buffering effects of ingested food components (2, 22). In this study we investigated the influence of pH (0.99, 1.67, 2.33, 3.71 and 4.27) on the oral bioaccessibility of tri- to deca-BDE congeners determined in the supernatant of the simulated gastric fluid compartment (Fig. 9.1).

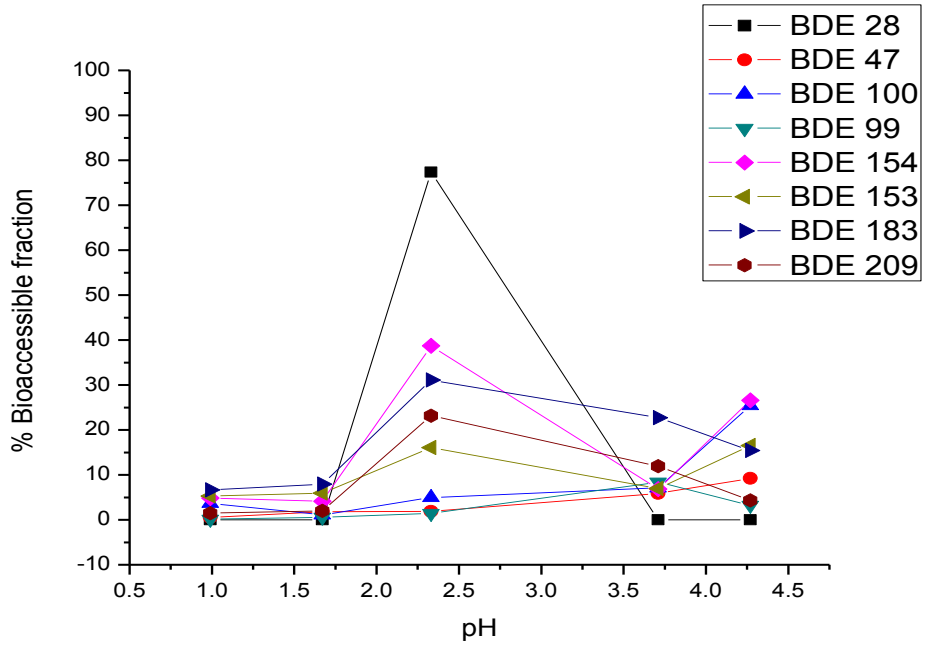

Figure 9.1 Influence of gastric pH on the oral bioaccessibility of PBDEs in dust.

The bioaccessibilities of tri- through to hexa-BDEs tend to increase with increasing pH. These observations are consistent with the gastric pH–dependence release of PBDEs in grass carp (2). At pH > 2.2, the bioaccessibility of tri- to hexa-BDE ranged between 1.4 - 27% in the supernatants of the gastric fluid compartments. However, a drop in bioaccessibility of BDE-28, BDE-183 and BDE-209 was observed at gastric pH > 2.33. Both the heptaBDE and decaBDE were bioaccessible to as high as 31 and 23% for BDE-183 and BDE-209 respectively in the supernatants of the simulated gastric fluid compartments (Fig. 9.1 & Supplementary Material Table S9.1). The formation of extra bicarbonate during hydrochloric acid secretion following ingestion of a meal could

account for the pH dependent bioaccessibility of PBDEs in the gastric fluid compartments. Similarly, the production of zymogen and pepsinogen in the gastric fluid results in the transformation of native protein into proteoses and peptones, hence providing an enriched environment for the uptake of PBDEs. This process occurs by maintaining a close equilibrium with the lipid content of the extracellular fluid of the body due to the relatively slow metabolic clearance of PBDEs (23). BDE-153 and BDE-154 showed two patterns of release in the gastric fluid compartment: first, there was an increase in the bioaccessible fractions up to 16% and 38% for BDE-153 and BDE-154 respectively at the starting of pH 0.99, and then reached equilibrium at pH 2.33, then there was a marked drop in the % BAF at pH 3.71 and finally, a slight increase up to 17% and 28% BAF for BDE-153 and BDE-154 respectively at pH 4.27. hence a gastric pH of 2.33 for the fed state and pH of 0.99 for the fasted state condition was found optimum for all PBDE congeners.

9.3.2 Optimum GIT Incubation Time

The bioaccessibility of the smaller BDE congeners (tri- to hexaBDE) increased markedly until equilibrium was attained after 8 hours of incubation time. A slight decline in the % BAF of these BDE congeners was observed on further incubation after 8 hours (Fig. 9.2). However, the larger BDE congeners (BDE-183 and BDE-209) showed a successive increase in the % BAF (Fig. 9.2) up to 16 hours of incubation. However, after 10 hours of incubation, the GIT media had noticeable active microbial growth evidenced by a peculiar smell and brownish–black colouration. Tilston et al. (12) attributed such microbial growth to sulfur-reducing bacteria which arise from introduced artifacts during chemical analysis. Thus, in this work an incubation time of 8:05 hrs and 16:05 hrs were used for tri- to hexa-BDEs and BDE-183 and BDE-209, respectively.

9.3.3 Comparison of the UBM and the FOREhST protocols for oral bioaaccessibility of PBDEs in SRM 2585

By employing the modified UBM protocol i.e. the “fasted state” for the assessment of PBDEs, the oral bioaccessibilities for SRM 2585 resulted in % BAF ranging from 4.1 – 30 following batch extraction (Supplementary Material Table. S9.2). The % BAF was lowest for BDE-28 (4.1%) and highest for BDE-153 (30%). Figure 9.3 shows the mean concentrations of the PBDE congeners present in the supernatants of the SRM sample after UBM incubation.

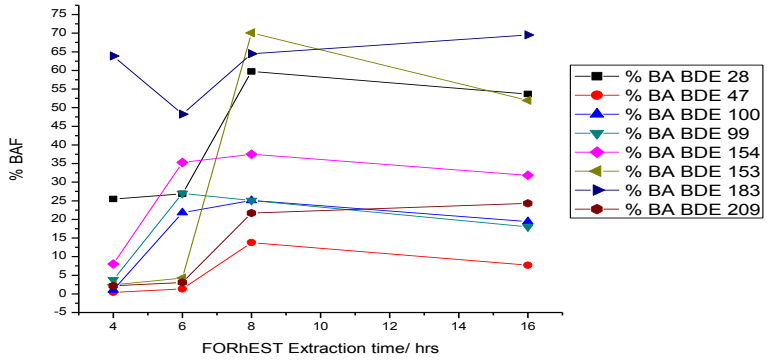

Figure 9.2 Influence of GIT incubation time on the oral bioaccessibility of PBDE in dust.

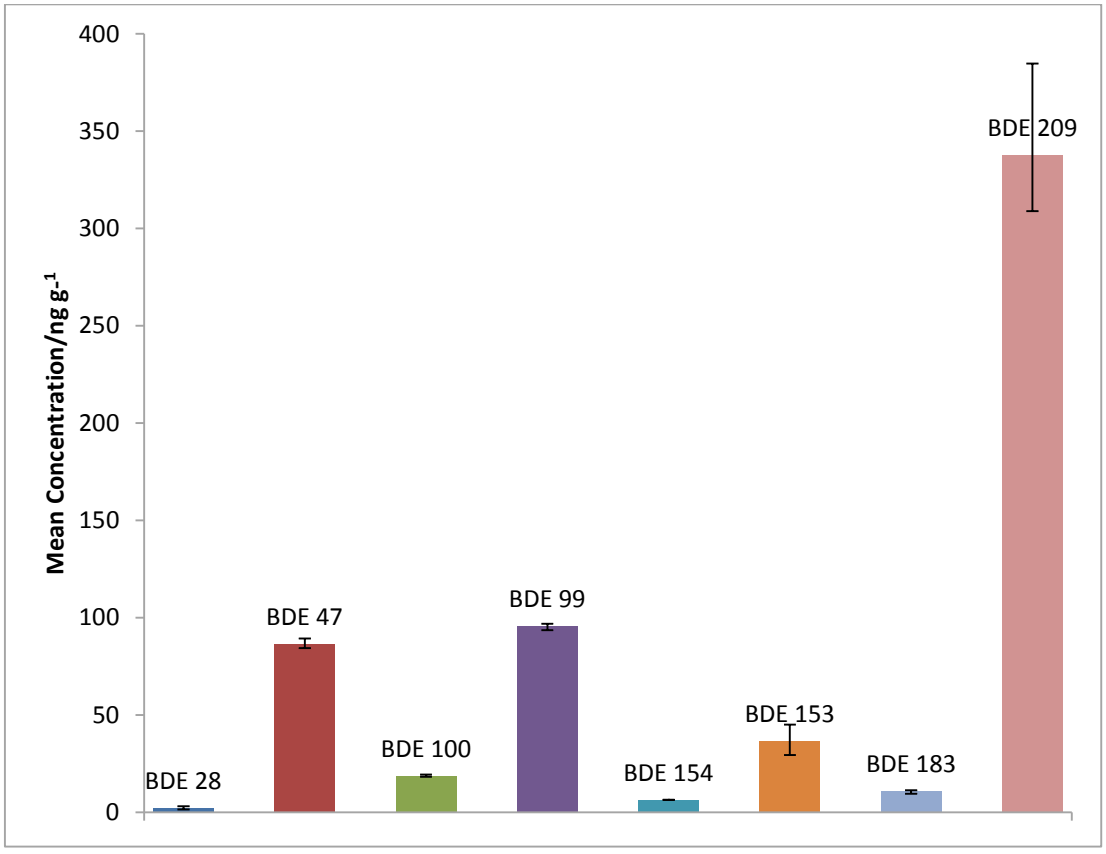

Figure 9.3 Mean ± standard deviation (n = 3) of individual PBDE congeners in the supernatants of UBM extracted SRM 2585 dust samples.

Unlike, the results observed with the UBM protocol, for the FOREhST protocol, the % BAF of PBDE congeners ranged from 22% to 70% (Table 9.1). BDE-209 and BDE-100 are the least bioaccessible with the FOREhST method. Though no study has ever shown

the % BAF of BDE-209 to be as high as 22% , it was recognized that the bioaccessibility of higher BDE congeners (BDE-183 and BDE-209) is highly dependent on incubation time as observed in Fig. 9.2. There was a constant decrease in the levels of BDE-183 and BDE-209 in the residual fractions over the incubation period. Figure 9.4 Shows mean ± standard deviations (n = 3) of PBDEs congeners present in the supernatants of the SRM sample following incubation with the modified FOREhST protocol.

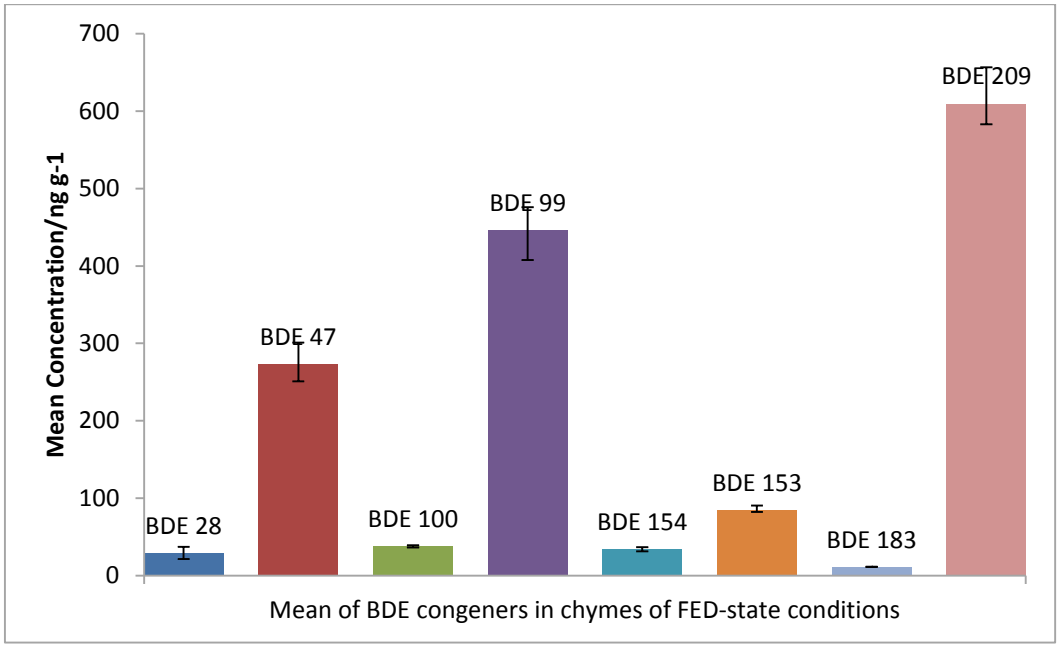

Figure 9.4 Mean ± standard deviation (n = 3) of individual PBDE congeners in the supernatant of FOREhST extracted SRM 2585 dust samples..

The sharp contrast in the % BAF (Fig 9.5) of PBDE congeners derived by using both the modified UBM and FOREhST protocols might be related to the pH-dependence of the BDE congeners (Fig 9.1).

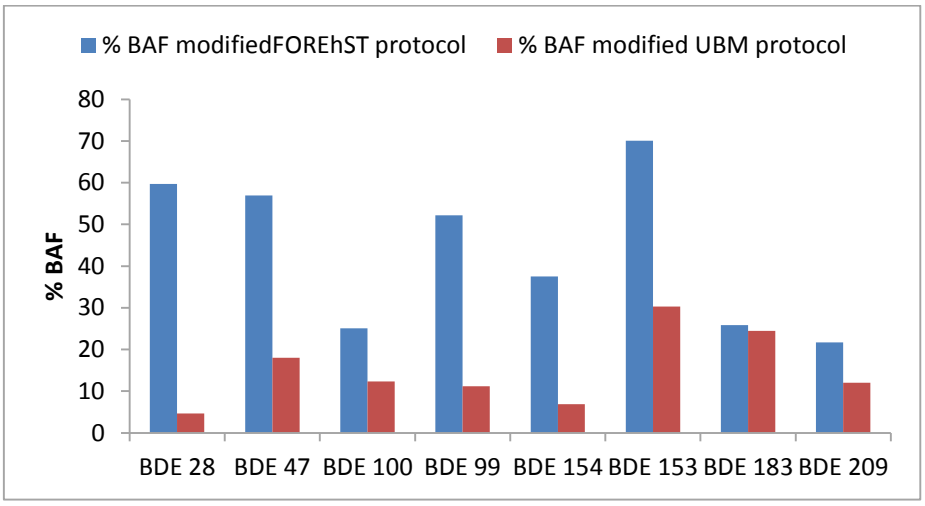

Figure 9.5 Comparison of % BAF for PBDE congeners following *in vitro* human GIT extraction by using both the modified FOREhST and UBM extraction protocols for SRM dust 2585 dust samples.

Yu et al. (2) have shown that low pH results in the precipitation of bile salts, which are otherwise responsible for solubilizing PBDEs and forming micelles. Bile plays an important role in fat digestion and absorption and hence provides an enriched environment for lipophilic organic contaminants such as PBDEs. Whilst the presence of proteinaceous and fatty foods stimulates the secretion of the hormone cholecystokinin which stimulates the secretion of bile by the liver, at pH < 4, bile salts precipitate up to 60% (2). Similarly, the presence of food in the FOREhST protocol might be an important factor for the higher % BAF of PBDEs. The presence of food has been shown to increase the transit time of the stomach (24); and hence, increases the period of mobilization, which is of particular importance for compounds in which dissolution is rate-limiting (24). Similarly, an increased solubilizing capacity of the digestive mixture, due to the increased flow of the digestive fluids or to the presence of food particles, may cause an increase in the mobilization of contaminants from soil (24). Specifically, bile is known to increase the solubilizing capacity for poorly water-soluble compounds, as bile salts form, micelles that have an apolar interior (24). Thus, these surfactant properties of bile salts, play a major role in increasing wetting hence and the degree of mobilization of PBDEs from dust (22).

9.3.4 Oral Bioaccessibility of PBDEs in Contaminated Sites (E-waste Recycling Sites) by Using the Two in vitro GIT Protocols

Both the modified UBM and FOREhST extraction protocols were applied to study the oral bioaccessibility of PBDEs in known contaminated sites. The % BAF of PBDEs ranged between 9.5 and 28 % following batch extraction by using the modified UBM protocol (Table 9.4). BDE-209 was the least bioaccessible congener among the eight

PBDE congeners studied. The mean value of 9.5 % BAF (n = 3) for BDE-209 following the UBM protocol falls within the range of % BAF (7 – 14 %) reported in previous *in vitro* GIT studies (16, 25) and the 4 - 26 % bioavailable fractions in previous animal studies (26). The low % BAF of BDE-209 is consistent with its low water solubility of <0.1 μg L^{-1} (27) at a neutral pH and a log K_{ow} of 9.97 (27). The oral bioaccessibility of tri- to hepta-BDEs ranged from 11 - 28% in the UBM protocol. A strong positive correlation (r = 0.62) was observed for the % BAF of the eight BDE congeners and their respective log K_{ow} (Supplementary Material Table S9.2) in the modified UBM protocol. The % BAF of PBDEs in the highly contaminated e-waste samples obtained by using the UNFED state extraction protocol are slightly higher than their corresponding % BAF in SRM 2585 samples. However, analysis of variance (Supplementary Material Table S9.11) showed no statistically significant ($p < 0.05$) difference in the mean % BAF of both the SRM and the highly contaminated e-waste samples.

Interestingly, the application of the modified Fed Organic Estimation human Simulation Test (FOREhST) method resulted in over a 50% increase in the % BAF of most of the BDE congeners studied. The % BAF following FOREhST protocols ranged from 17 for BDE-153 to 92% for BDE-28 (Table 9.4).

The average % BAF of tri- to hepta-BDE ranged from 17 to 92% and not detected (ND) to 105% for e-waste recycling sites 1 and 2 respectively. Whilst there were significant increases in the % BAF of the lower BDE congeners; no correlation exists between the % BAF and the log K_{ow} of the BDE congeners in the FOREhST protocol. This observation could indicate the effect of the partition mechanism of PBDEs in dust to the lipid enriched FOREhST GIT medium. Previous studies have shown that a dietary matrix in the presence of bile salts and pancreatin, mobilizes PBDEs from the matrix and then extracts the fat-loving compounds from the bulk mass, thus creating an apolar environment in the interior of bile salt micelles for hydrophobic compounds (such as PBDEs) thereby increasing their solubility. Except for BDE-47 and 183 (Fig. 9.6), the % BAF of the BDE congeners via the FOREhST protocol were systematically higher than those of the UBM protocol. A single factor ANOVA showed a statistically significant difference (at 95 % confidence level) in the average % BAF of PBDEs by using the FOREhST and UBM *in vitro* gastrointestinal test protocols.

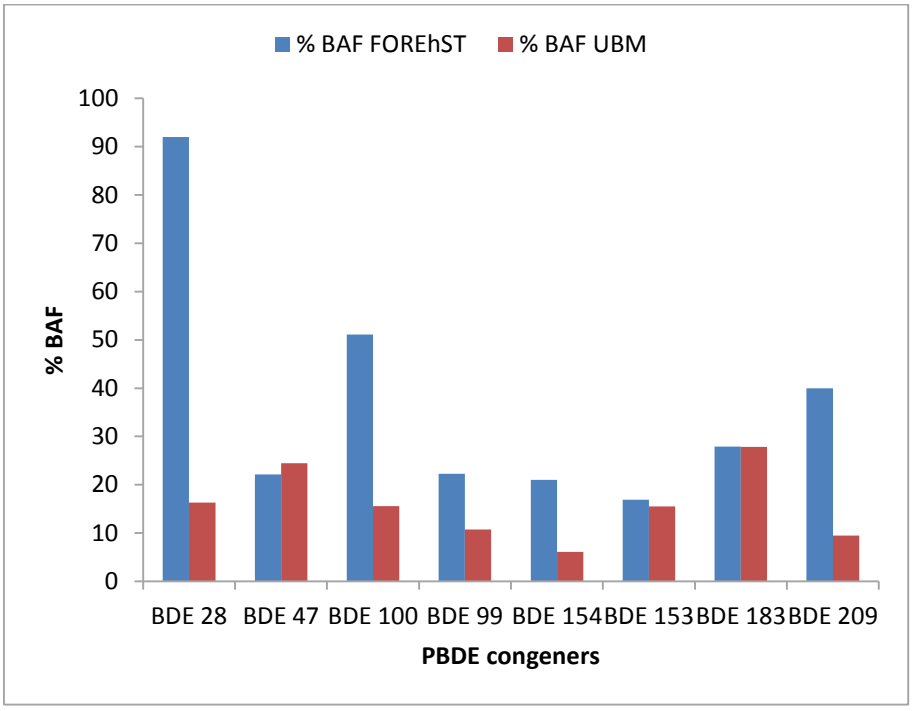

Figure 9.6 Comparison of the % BAF of PBDEs for the modified FOREhST and UBM extraction protocols in contaminated dust samples from the indoor environment of an e-waste recycling facility (site 1).

Unlike the observations with the SRM 2585 samples, there were no statistically significant differences (p value of 0.059) in the average % BAF of PBDEs by using the FOREhST and the UBM GIT protocols. This discrepancy could be attributed to the dust type and the concentrations of PBDEs in the heavily contaminated samples as opposed to those of SRM 2585. The average % BAF of BDE-209 following FOREhST extraction was approximately 40%. The % BAF of BDE-209 in this study is overwhelming, as no previous *in vitro* human GIT studies have shown BDE-209 to be this bioaccessible. Basically, the addition of the food component and the presence of bile salts in both the duodenal and bile fluid compartments enhanced the solubility of BDE-209; coupled with the prolonged incubation time of the modified GIT protocols in this study. The oral bioaccessibility of BDE-209 seems to be previously underestimated likely due to the GIT media compositions as well as GIT incubation time; as our study indicates a continual decrease in residual BDE-209 in the solute after 16 hours of incubation (Fig. 9.2). Similarly, different enzyme systems, inter-species differences, colon-residence time (16), nutritional status and the degree of contamination/administered dose play a significant role in the oral bioaccessibility/bioavailability of organic contaminants, hence it is unsafe to assume the bioavailability of POPs in animals to be the same as in humans.

Table 9.4 % BAF of PBDE congeners in dust samples collected from two contaminated e-waste recycling sites.

BDE Congener	Mean Concn. FOREhST Supernatant e-waste site 1 /ng g^{-1} (n = 3)	% RSD	%BAF	Mean Concn. FOREhST Supernatant e-waste site 2 /ng g^{-1} (n = 3)	% RSD	% BAF	Mean Concn. UBM Supernatant e-waste site 1 /ng g^{-1} (n = 3)	% RSD	% BAF
BDE 28	114	7	92	ND	ND	ND	20	8	16
BDE 47	656	5	22	98	19	38	726	13	25
BDE 100	532	6	51	107	0.2	105	162	3	16
BDE 99	1384	4	22	28	0.4	37	667	13	11
BDE 154	346	15	21	18	0.1	21	107	11	6.5
BDE 153	506	3	17	157	0.6	35	466	15	16
BDE 183	468	4	28	69	0.2	53	467	1	28
BDE 209	11001	14	40	3470	5.4	35	2610	7	9.5

9.3.5 Comparison of Batch and Sequential UBM in vitro GIT Extraction

Fig. 9.7 shows the % BAF of PBDE congeners in the three *in vitro* GIT media (gastric, duodenal and bile compartments) following the modified UBM extraction protocol and a comparison of batch extraction with sequential extraction of e-waste recycling site 1 dust. The data indicate the near consistent increase in the % BAF (Table 9.5) of the BDE congeners occasioned by the presence of pancrease, lipase and bile in the duodenal and bile fluid compartments.

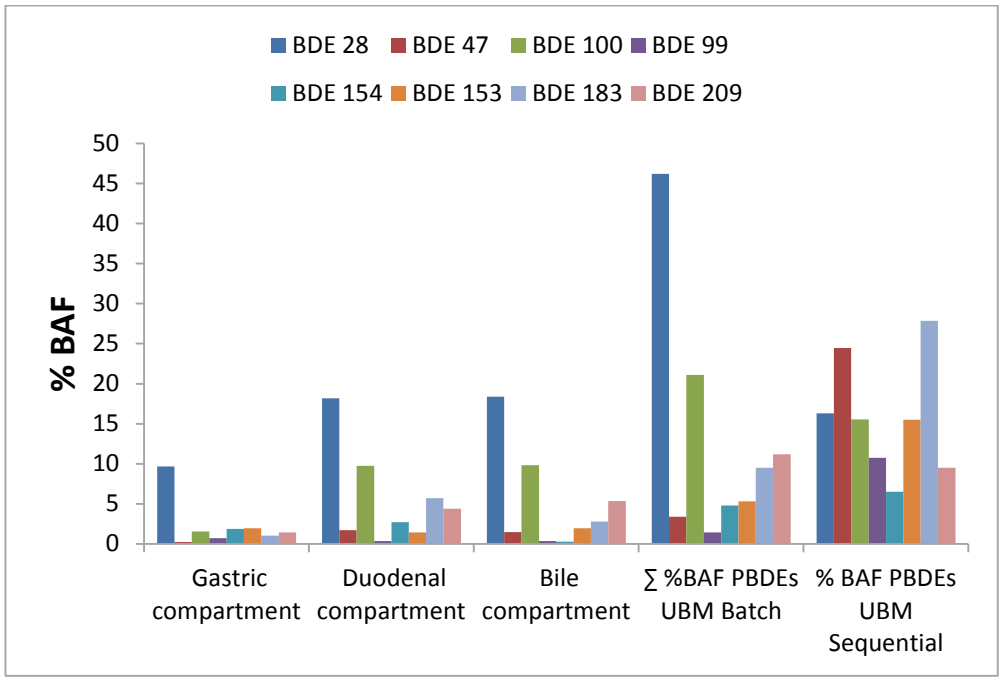

Figure 9.7 Percentage BAF of PBDEs in individual GIT media following batch extraction and comparison with the sequential UBM extraction format in e-waste sample site 1 dust.

Whilst the batch UBM protocol gave a better estimate of the % BAF of BDE-28 and BDE-209, the sequential UBM protocol favoured the % BAF of BDE-47, BDE-99, BDE-153, BDE-154 and BDE-183. Both the sequential and batch UBM protocol results were consistent, there was no statistically significant difference (p value of 0.48) in the average % BAF of the PBDEs by using both formats of extraction.

Table 9.5 % BAF of PBDEs in individual GIT compartments following batch and sequential UBM (fasted-state) extraction formats in e-waste recycling site 1 dust.

BDE Congener	% BAF PBDE in gastric compartment	% BAF PBDE in duodenal compartment	% BAF PBDE in bile compartment	∑ % BAF PBDEs UBM Sequential	% BAF PBDEs UBM Batch
BDE 28	9.65	18.18	18.37	46.20	16.30
BDE 47	0.21	1.69	1.46	3.36	24.47
BDE 100	1.54	9.75	9.82	21.11	15.55
BDE 99	0.72	0.36	0.33	1.41	10.72
BDE 154	1.84	2.68	0.26	4.79	6.49
BDE 153	1.93	1.42	1.96	5.31	15.51
BDE 183	1.04	5.69	2.77	9.50	27.87
BDE 209	1.43	4.39	5.35	11.18	9.48

9.3.6 Application of Modified Sequential FOREhST Protocol for Determination of the Oral Bioaccessibility of PBDEs in Dust Samples Collected from Two E-waste Recycling Sites

The modified FOREhST protocol in this study was applied to determine the oral bioaccessibility of PBDEs in dust samples from the indoor environment of two different e-waste recycling sites. Supplementary Material Table S9.12 shows the total concentrations of the individual PBDE congeners in the samples. The PBDE levels varied for both sites. Site 1 contained PBDEs in the range 123 ng g^{-1} – 27526 ng g^{-1} while the total PBDEs ranged from ND – 9897 ng g^{-1} for e-waste recycling site 2. The mean % BAF of PBDEs in these samples is shown in Table 9.4. The method responded well to both samples, as there were no statistically significant differences (p value of 0.79) observed for the average % BAF of each of the BDE congeners in the samples collected from the two e-waste recycling sites.

Generally, site 2, which was the least contaminated site, had slightly higher % BAF for most of the PBDE congeners with the exception of BDE-100, 153, and 183. The % BAF of these three BDE congeners in site 2 are almost 100% higher than their corresponding % BAF in site 1. Interestingly, the total average concentrations of these BDE congeners (100, 153 and 183) in site 1 were over 100% higher than their total average levels in site 2. However, BDE-28, 154 and 209 showed a different behaviour; for these congeners, there was a consistent increase in % BAF with respect to corresponding increase in the total concentrations of the respective BDE congeners (Fig. 9.8). This influence which depicts the total concentration dependence of the modified FOREhST protocol can be attributed to the volume of GIT fluids used for incubation and the dust sample characteristics, consistent with the observations of Abdallah et al. (16).

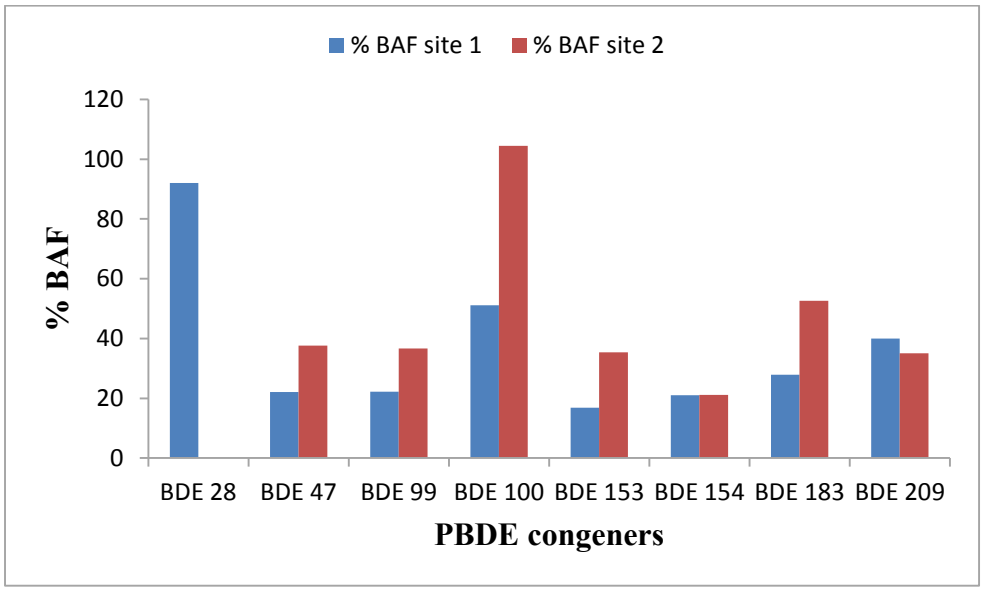

Figure 9. 8 Comparison of the % BAF of PBDE congeners obtained by the modified FOREhST protocol in samples collected from two different e-waste recycling sites.

9.3.7 Comparison of Modified FOREhST and UBM Protocols with Previously Reported In Vitro GIT Methods for PBDEs

Yu et al. (28) showed the % BAF of tri- to hepta-BDEs in natural dust samples from laboratories, offices and air conditioners to be in the range of 2.8 – 63% whilst the % BAF for spiked dust samples ranged between 17.3 – 59.9%. Similarly, a 5.0 – 34% range for BAF in chymes after intestinal fluid extraction of spiked grass carps has been reported (2). Lepom et al. (25) reported a % BAF of 27 – 42% for tri – to heptaBDEs and 10% BAF for BDE-209 in SRM dust samples. Abdallah et al. (16) have also reported a % BAF in the range 32 – 58% for tri- heptaBDEs and 14% for BDE-209 in dust samples collected from a United Kingdom home. Our modified UBM protocol showed a % BAF ranging from 4 - 30% for tri- to hepta-BDE congeners and 12% for BDE-209, whilst, the modified FOREhST protocol in this study resulted in % BAF ranging from 25 - 70% for tri- to hepta-BDEs and 22% for BDE-209 in SRM 2585 dust samples. For contaminated e-waste dust samples, the modified UBM protocol resulted in % BAF in the range 7 – 28% for tri- to hepta-BDEs and 9.5% for BDE-209. However, a % BAF ranging from 21 – 105 % for tri- to hepta-BDE and 35% for BDE-209 were observed in site 2; and % BAF ranging from 17 - 92% for tri- to hepta-BDE and 40% for BDE-209 in site 1 were obtained with the modified FOREhST protocol. Though our result for both protocols agrees with previously reported *in vitro* methods; the modified FOREhST protocol showed that BDE-209 can be bioaccessible to as much as 40% which is higher than previously reported.

9.4. Conclusions

The oral bioaccessibility of PBDEs in dust from e-waste recycling sites and SRM 2585 were measured by using two *in vitro* methods mimicking the human GIT. Both gastric pH condition and GIT extraction time were optimized for both methods. The use of the FOREhST (fed-state) GIT protocol resulted in higher % BAF for most of the PBDE congeners compared with the UBM (fasted-state) protocol. The % BAF of PBDEs following the UBM protocol correlated with the water solubility of the PBDEs. BDE-209 could be as high as 40% bioaccessible following the ingestion of food. No significant difference was obtained for % BAF of PBDEs for both the batch and sequential UBM GIT extractions. Results obtained for the modified UBM protocol were generally similar to reported % BAFs of the PBDEs in other studies. However, the prolonged GIT extraction time and the presence of food in the GIT system significantly increased the %BAF. A further replication of this preliminary experiment with more samples is required to confirm these findings, especially for the high BDE-209 bioaccessibility.

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Supplementary Material

Table S9.1 Analysis of SRM 2585.

BDE Congener	Measured Concentrations/ ng g^{-1} (n = 3)	Certified Values for PBDE congeners in SRM 2585/ µg kg^{-1}	% recovery	% RSD
BDE 28	48 ± 0.1	46.9 ± 4.4	103	0.2
BDE 47	480 ± 2.4	497 ± 46	97	0.5
BDE 99	854 ±1.0	892 ± 53	96	0.1
BDE 100	152 ± 0.2	145 ± 11	105	0.1
BDE 153	121 ± 0.1	119 ± 1	102	0.08
BDE 154	93 ± 0.2	83.5 ± 2	111	0.2
BDE 183	44 ± 0.04	43.0 ± 3.5	102	0.1
BDE 209	2806 ± 0.6	2510 ± 190	112	0.02

Table S9.2 Descriptive statistics for PBDEs concentrations (in ng g^{-1}) in supernatants of modified UBM protocol for SRM 2585 extracts.

BDE Congener	SRM 1 UBM Chymes	SRM 2 UBM Chymes	SRM 3 UBM Chymes	Mean	Std Dev	% RSD	Mean SRM value	% BAF	log K_{ow} (27)	% Recovery
BDE 28	3.06	1.43	1.43	1.97	0.94	47.69	48.12	4.10	5.94	94.01
BDE 47	85.96	89.33	84.35	86.55	2.54	2.94	480.04	18.03	6.81	79.86
BDE 100	19.37	18.88	18.19	18.81	0.59	3.15	152.25	12.36	7.24	42.37
BDE 99	96.83	93.66	96.83	95.77	1.83	1.91	853.97	11.22	7.32	44.16
BDE 154	6.44	6.35	6.44	6.41	0.05	0.81	92.74	6.91	7.82	69.62
BDE 153	29.46	45.05	35.54	36.68	7.86	21.42	121.22	30.26	7.9	91.15
BDE 183	11.4	11.29	9.60	10.76	1.01	9.37	43.99	24.47	8.27	73.60
BDE 209	319.96	384.72	308.96	337.88	40.94	12.12	2806.12	12.04	9.97	93.21

Table S9.3 Descriptive statistics for residual PBDEs concentrations (in ng g^{-1}) in solutes of modified UBM protocol for SRM 2585 extracts.

BDE Congener	Run 1	Run 2	Mean	STD DEV	% RSD
BDE 28	46	41	43	4	8.6
BDE 47	305	288	297	12	4.1
BDE 99	315	248	281	47	16.7
BDE 100	43	48	46	3.3	7.1
BDE 153	72	75	74	1.9	2.5
BDE 154	58	58	58	0.3	0.5
BDE 183	21	22	22	0.5	2.5
BDE 209	2359	2196	2278	115	5.1

Table S9.4 Descriptive statistics for PBDEs concentrations (ng g^{-1}) in supernatants of modified FOREhST protocol for SRM 2585 extracts.

BDE Congener	SRM 1 FOREhST supernatant/ng g^{-1}	SRM 2 FOREhST supernatant / ng g^{-1}	SRM 3 FOREhST supernatant/ ng g^{-1}	Mean	STD	Mean SRM value	% BAF	% SD	Max	Min	% Recovery
BDE 28	27.7	37.2	21.3	29.0	8.0	48.1	60	28.0	37.2	21.3	87
BDE 47	301.0	269.0	251.0	274.0	25.3	480.0	57	9.3	301.0	251.0	91
BDE 100	36.4	39.2	39.2	38.2	1.6	152.3	25	4.2	39.2	36.4	34
BDE 99	453.3	408.0	476.1	446.0	35.0	854.0	52	7.8	476.1	408.0	62
BDE 154	36.6	31.2	37.0	35.0	3.1	93.0	38	9.0	36.6	31.2	56
BDE 153	84.9	85.0	93.3	88.0	4.8	121.2	70	5.5	93.3	85.0	91
BDE 183	11.4	11.4	12.0	12.0	0.3	44.0	26	2.2	12.0	11.4	81
BDE 209	576.0	643.5	570.0	596.4	41.0	2806.1	22	6.7	643.5	570.0	70

Table S9.5 Descriptive statistics for residual PBDE concentrations (ng g^{-1}) in solutes of modified FOREhST protocol for SRM 2585 extracts.

BDE congener	Run 1	Run 2	Mean	Std Dev	% RSD
BDE 28	12.2	13.7	14.0	1.1	8.6
BDE 47	174.5	156.8	166.0	12.5	7.5
BDE 99	87.5	76.7	82.1	7.7	9.4
BDE 100	14.4	13.5	14.0	0.6	4.4
BDE 153	22.5	21.7	22.1	0.6	2.7
BDE 154	17.5	17.1	17.3	0.3	1.6
BDE 183	21.2	27.1	24.2	4.2	17.3
BDE 209	1515.1	1230.7	1373.0	201.1	14.7

Table S9.6 Descriptive statistics for PBDE concentrations (in ng g^{-1}) in the supernatants of modified FOREhST protocol for contaminated e-waste sample site 1 extracts.

BDE Congener	Repeat 1 Supernatant	Repeat 2 Supernatant	Repeat 3 Supernatant	Mean SC Supernatant	STD DEV	%RSD	% BAF	% Recovery
BDE 28	96	117	128	122	16	13	99	99
BDE 47	676	621	671	656	31	5	22	103
BDE 100	508	567	522	532	30	6	51	61
BDE 99	1344	1424	-	1384	56	4	22	76
BDE 154	382	310	-	346	51	15	21	116
BDE 153	516	492	511	506	13	3	17	56
BDE 183	439	463	501	468	17	4	28	97
BDE 209	9497	11002	12505	11001	1504	14	40	72

Table S9.7 Descriptive statistics for residual PBDE concentrations (in ng g^{-1}) in solutes after modified FOREhST extraction of contaminated e-waste sample (site 1) extracts.

BDE Congener	Repeat 1	Repeat 2	Mean	SD	% residual fraction	%RSD
BDE 28	< LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
BDE 47	2440	2358	2399	58	81	2.4
BDE 100	101	101	101	0.1	10	0.1
BDE 99	3302	3377	3340	53	54	1.6
BDE 154	1424	1709	1567	201	95	13.0
BDE 153	1274	1101	1187	122	40	10.3
BDE 183	1270	1047	1158	157	69	14.0
BDE 209	8560	8883	8721	229	32	2.6

LOQ – limit of quantitation

Table S9.8 Descriptive statistics for PBDE concentrations (in ng g^{-1}) in supernatants of modified FOREhST protocol for contaminated e-waste sample (site 2) extracts.

BDE Congener	Repeat 1 supernatant	Repeat 2 supernatant	Repeat 3 supernatant	Mean	STD DEV	% RSD	% BAF
BDE 28	ND	ND	ND	-	-	-	-
BDE 47	113	83	-	98	21	19.7	38
BDE 100	104	111	192	107	5	0.2	104
BDE 99	31	26	107	28	4	0.4	37
BDE 154	19	17	56	18	1	0.1	21
BDE 153	162	153	400	157	7	0.6	35
BDE 183	70	67	173	69	2	0.3	53
BDE 209	3682	3410	3318	3470	189	5.5	35

Table S9.9 Descriptive statistics and % BAF for PBDE concentrations (in ng g^{-1}) in supernatants of modified UBM protocol for contaminated e-waste sample (site 2) extracts.

BDE Congener	Repeat 1 supernatant	Repeat 2 supernatant	Mean	Std dev.	%RSD	% BAF
BDE 28	19.0	21	20	1.7	8.3	16.3
BDE 47	661	792	726	92.4	12.7	24
BDE 100	159	165	162	4.1	2.5	16
BDE 99	605	729	667	88.2	13.2	11
BDE 154	98	115	107	12.0	10.8	65
BDE 153	415	516	466	71.8	15.4	15.5
BDE 183	463	472	467	5.8	1.3	27.87
BDE 209	2478	2743	2610	188	7.2	9.50

Table S9.10 Descriptive statistics for PBDE (concentration in ng g^{-1}) oral bioaccessibilities in contaminated dust samples from an e-waste recycling site (site 1) obtained by batch extraction via the modified UBM protocol.

BDE Congener	Gastric supernatant repeat 1	gastric supernatant repeat 2	Mean gastric fluid	Std dev	% RSD	duodenal chyme repeat 1	duodenal chyme repeat 2	Mean duodenal chyme	Std. Dev	% RSD	bile supernatant repeat 1	bile supernatant repeat 2	Mean Bile supernatant	Std Dev	% RSD
BDE 28	11.5	12.33	11.91	0.60	0.06	18.70	26.16	22.43	5.28	0.49	22.72	22.61	22.67	0.08	3.63
BDE 47	6.3	6.01	6.17	0.22	0.01	60.42	40.20	50.31	14.30	0.66	43.83	42.94	43.39	0.63	1.8
BDE 100	14.7	17.34	16.0	1.90	0.06	102.10	100.99	101.55	0.78	0.03	109.49	95.16	102.32	10.13	1.25
BDE 99	48.1	41.72	44.91	4.51	0.04	13.12	31.23	22.18	12.80	0.12	23.31	17.64	20.48	4.01	0.36
BDE 154	38.1	22.49	30.32	11.7	0.82	36.98	51.24	44.11	10.08	0.74	6.08	2.57	4.33	2.48	2.88
BDE 153	61.9	53.7	57.79	5.78	0.31	48.41	36.98	42.70	8.08	0.42	68.87	48.51	58.69	14.39	2.06
BDE 183	18.1	16.77	17.45	0.95	0.10	84.21	106.60	95.41	15.83	1.74	53.58	39.4	46.49	10.02	4.29
BDE 209	357.0	432.5	394.61	53.6	0.21	1193.3	1208.62	1200.97	10.82	0.04	1393.4	1552.6	1473	112.5	0.15

Table S9.11. Comparison of batch and sequential extraction protocol using a single factor ANOVA

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
∑ %BAF PBDEs UBM Sequential	8	94.45938	11.80742	190.5664
% BAF PBDEs UBM Batch	8	126.4024	15.8003	53.38239

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	63.77215	1	63.77215	0.522832	0.481542	4.60011
Within Groups	1707.641	14	121.9744			
Total	1771.414	15				

Table S9.12 Total PBDE concentrations (in ng g^{-1}) in the two e-waste recycling facilities.

	E-Waste Facility	
PBDE Congener	e-waste site 1	e-waste site 2
BDE 28	123	0
BDE 47	2968	260
BDE 99	6220	77
BDE 100	1042	103
BDE 153	1643	445
BDE 154	3000	83
BDE 183	1677	130
BDE 209	27526	9897

Chapter 10

Concentrations and oral bioaccessibility of phosphorus flame retardants in indoor dust of textile and polyurethane industries

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Abstract

Organophosphorus flame retardants (PFRs) are substitute flame retardants for brominated flame retardants (BFRs). To determine the widespread application of PFRs in South Africa and the subsequent risk associated with exposure to PFRs, we investigated indoor dust samples from a textile and a polyurethane industry. The concentrations of PFRs varied from 3.0 ± 0.2 – 5.8 ± 1.1 μg g^{-1} (TCEP), 29.2 ± 6.3 – 180.3 ± 12.9 μg g^{-1} (TCPP), 4.2 ± 1.0 – 97.4 ± 3.2 μg g^{-1} (TDCPP) and 1.0 ± 0.2 – 10.2 ± 1.4 μg g^{-1} (TPP) in the textile industry. PFR concentrations were highest in the finished fabrics storage area of the textile industry. Similarly, the concentrations of PFRs ranged from 3.2 ± 0.6 – 71.2 ± 8.4 μg g^{-1} (TCEP), 114.3 ± 18.9 – 2195.0 ± 126.0 μg g^{-1} (TCPP), 4.2 ± 1.3 – 212 ± 15.8 μg g^{-1} (TDCPP) and 2.4 ± 0.5 – 56.8 ± 6.3 μg g^{-1} (TPP) in the polyurethane industry. The average recovery of total PFRs after i*n vitro* gastrointestinal extraction was in the range 59.3 ± 9.0 % (TPP) to 110.0 ± 12.0 (TDCPP). The percentage bioaccessible fractions (% BAF) of PFRs were 39.8 ± 9.6 %, 26.3 ± 1.0 %, 0.9 ± 0.3 % and 19.6 ± 3.4 % for TCEP, TCPP, TDCPP and TPP respectively. The bioaccessibility of PFRs was independent of the log K_{ow} but depended strongly on the water solubility of the respective PFR. A strong correlation (r = 0.834) with a p value of 0.1657 was obtained for the % BAF and water solubility of the respective PFR. To our knowledge, this is the first study reporting PFRs in indoor dust of textile and polyurethane factories. Similarly, we report for the first time, the i*n vitro* oral bioaccessibility of PFRs in dust.

Keywords*: Organophosphorus flame retardants, in vitro, Gastrointestinal Tract, Bioaccessibility, Textile, Polyurethane, Industry*.

10.1 Introduction

Phosphorus flame retardants (PFRs) have been in use for over 150 years and are considered as suitable alternatives for brominated flame retardants (BFRs) (1). Before 1977, the most widely used additive flame retardant for children's sleepwear in the United States was tris(2,3-dibromopropyl) phosphate (tris-BP), a known mutagen and carcinogen in rats and mice (2). Federal regulations, such as the California Technical Bulletin (TB 117) for furniture, and the Underwriters'Laboratories 94 (UL 94), the standard for safety flammability of plastic materials for parts in devices and appliances (1) requiring children's sleepwear, mattresses, mattress pads and carpets meet flammability standards, have resulted in a decrease in the number of burn injuries and death (2).

Of the 465000 tonnes of flame retardants consumed in Europe in 2006, PFRs accounted for 20 % (1). Unfortunately, some fraction of the many million tonnes of flame retardants produced will find their way into people. *"The chemical are rubbing off on children's skins, may be inhaled from furniture, rugs, car seats and tents and may eventually end in the food chain on disposal into the environment*" (2). Little is known on the toxicity of PFRs, however, studies have shown tris(2-chloroethyl) phosphate (TCEP) to be a neuro and reproductive toxin as well as a carcinogen (3). Other, studies have indicated tris(chloropropyl) phosphate (TCPP) as potentially carcinogenic and tris(1,3 dichloro-2-propyl) phosphate (TDCPP) as a carcinogen. Triphenyl phosphate (TPP) has been associated with altered hormone levels and decreased sperm concentrations (4). PFRs have been detected in various environmental media including air (3, 5-9); surface and drinking waters and sediments (10-15); biota (16, 17) and indoor dust of various microenvironments in several locations worldwide (6, 18-23).

Total contaminant concentrations are frequently used in risk assessment of contaminated sites to human health (24). Such assessment, though advantageous for precautionary measures, may lead to overestimation of the amount of contaminant absorbed by humans (24). These overestimations have significant implications for cost and sustainability of brownfield remediation; hence, the use of bioaccessible and bioavailaible fractions of contaminants for site specific risk assessment are very important parameters. The idea of bioaccessibility and oral bioavailability are essential for quantifying the risks that are associated with oral exposure to environmental contaminants (25). The use of gastrointestinal tract (GIT) extraction systems, such as the Universal Bioaccessibility Method (UBM), is a valuable tool in assessing the human health risk of persistent organic pollutants. These extraction processes tend to imitate the process of the human digestive system to determine the bioacessibility of accidentally or intentionally ingested contaminants (26). Though, a close association between dust concentrations of PFRs and household items such as foams and furniture, the sources of PFRs in the indoor environment, are still unclear. No direct regulations are currently in place in South Africa with regards to production and use of flame retardant chemicals; hence the use and

applications of flame retardants in various industrial sectors are unknown. The paucity of data on phosphorus flame retardants in the African continent motivated the present study which was aimed to investigate the levels of PFRs in indoor dust of industries with a history of flame retardant use by their international counterparts. Specifically, we investigated the indoor dust collected from a textile and a polyurethane industry in South Africa. As a second objective, we studied for the first time, the oral bioaccessibility of PFRs following the UBM *in vitro* gastrointestinal test for accurate risk assessment.

10.2 Materials and methods

10.2.1 Chemicals

Reagents used for the GIT extraction procedure were purchased from different reagent suppliers in South Africa or otherwise as stated: $CaCl_2.2H_2O$ from NT Laboratory Supplies (Pty) Ltd, HCl and KCl from Promark Chemicals, KSCN from Merck Laboratory Supplies (Pty) Ltd, $MgCl_2.6H_2O$ from Saarchem Pty Ltd, NaCl from SMM Instruments, NaOH, Na_2SO_4 from Associated Chemical Enterprise, NH_4Cl from PAL Chemicals, $NaHCO_3$ from Merck Laboratory Supplies (Pty) Ltd, and $NaH_2PO_4.2H_2O$ from May and Baker Ltd, Dagenham, England. α-Amylase from porcine pancreas, uric acid, urea, pepsin from porcine gastric mucosa, D-glucuronic acid, glucosamine hydrochloride (Fluka), D(+) – glucose, pancreatin, bile salts (Fluka Analyticals), and lipase were obtained from Sigma Aldrich, South Africa; bovine serum albumin was purchased from Melford, United Kingdom and gastric mucin was purchased from Hangzhou Dayangchem Co, Ltd. HiPP organic creamy oats porridge was purchased from a local retail store. All organic solvents were high performance liquid chromatography grade from Sigma Aldrich; water was from a millipore Elix system with a resistance of 18 Ω.

10.2.2 Sample Collection

A total of five dust samples were collected from the indoor environments of polyurethane and a textile factory. In the polyurethane industry, dusts were collected from two plants. Polyurethane samples 2 and 3 were collected on two different days from the indoor environment of an existing plant were both flexible and rigid polyurethane are produced. Polyurethane sample 1 was collected from a plant which was decommissioned in 2011. One sample each was collected from both the preparation and finished textile storage locations of the textile industry. Dust samples from the textile industry were collected with a LG 1600 W vacuum cleaner fitted with a dust unit which could easily be removed and emptied after each collection. Between each collection it was cleaned with a disposable cloth wetted with *iso*-propanol. Because, the authors and research team were not allowed access to the polyurethane plants, samples from this industry were collected from the vacuum cleaners of the industry after instructions regarding study and sampling protocol were given to the management of the industry. Samples were transported in an ice chest to the laboratory and stored at -10 ºC in a cold room. The polyurethane industry sample 2 was employed for oral bioaccessibility studies.

10.2.2 Extraction and clean-up for total PFR in dust

Approximately 1.0 g of sample was quantitatively weighed into a glass test tube and spiked with 10 µg $^{13}C_{12}$ PCB-209 as the internal standard. A volume of 12 mL *n*-hexane:methanol (1:3 v/v) was added. Samples were mixed in an orbital shaker for 15 mins and then extracted in an ultrasonic water bath at 40 °C for 30 mins. The mixing and extraction was repeated for a second time without addition of fresh solvent. The samples were then centrifuged at 3500 rpm for 10 mins and the supernatants were stored at <4 °C prior to clean-up. Replicate analyses were carried out for all samples. Silica gel was activated at 130 °C for 16 hours and anhydrous sodium sulfate was baked at 450 °C for 5 hours before use. Silica gel and anhydrous sodium sulfate were subsequently cooled in a desiccator. A 30 cm × 1 cm glass column was packed with 4 g of deactivated silica gel. Each column was topped with 2.0 g of anhydrous sodium sulfate and then 30 mL of the extraction solvent was passed through the column. Extracts were loaded onto columns just before the exposure of the sodium sulfate layer. PFRs were eluted with 30 mL *n*-hexane. This was kept as Fraction 1; columns were further eluted with 30 mL of diethyl ether/*n*-hexane (50:50 v/v) and kept as Fraction 2; both fractions were mixed together. Finally, columns were eluted with 30 mL acetone:dichloromethane (1:1 v/v) as Fraction 3. Eluates were reduced to approximately 1 mL in a rotary evaporator at 50 °C and stored in 1.5 mL amber glass GC/MS vials. The column flow rates were maintained at 0.5 mL min^{-1}. All extracts were stored at <4 °C until instrumental analysis.

10.2.3 Preparation of Gastrointestinal Fluids

10.2.3.1 Simulated saliva fluid

Simulated saliva fluid was prepared by adding 146 mg of α-amylase, 56 mg mucin and 18 mg uric acid to a 1 L Duran bottle. Separately, inorganic saliva components were prepared by adding a 900.30 mg KCl, 900.90 mg NaH_2PO_4. $2H_2O$, 207.20 mg KSCN, 581.10 mg Na_2SO_4, 300.0 mg NaCl and 1.8 mL 1.0 M HCL into a 500 mL Duran bottle and made up to the mark with Millipore water. For the organic saliva component, 222 mg urea was added into a 500 mL Duran bottle and made to the mark with Millipore water. The entire contents of the inorganic and organic saliva components were simultaneously poured into the 1 L Duran bottle. The entire content of the bottle was thoroughly mixed in a shaker. The pH of the simulated saliva fluid was measured and when necessary adjusted to be within 6.50 ± 0.5. The pH was adjusted with either 1.0 M NaOH or 10.32 M HCl.

10.2.3.2 Simulated gastric fluid

Gastric fluid was prepared by adding 1123 mg bovine serum albumin (BSA), 3018 mg mucin and 1017 mg pepsin to a 1 L Duran bottle. Then, 827 mg KCl, 276 mg $NaH_2PO_4.2H_2O$, 401 mg $CaCl_2.2H_2O$, 314 mg NH_4Cl, 2740 mg NaCl and 8.30 mL 10.32 M HCl were added to a 500 mL Duran bottle and made up to the mark with Millipore water to form the inorganic gastric components. To a second 500 mL Duran bottle, 664 mg D (+) glucose, 28 mg D-glucuronic acid, 88 mg urea and 328 mg glucosamine

hydrochloride were added and made up to the mark with Millipore water to form the organic gastric components. The inorganic and organic gastric components were simultaneously poured into the 1 L Duran bottle. The entire content of the bottle was thoroughly mixed in a shaker. The pH of the gastric fluid component was measured and adjusted when necessary to be within 0.9 – 1.0. The pH was adjusted with either 1.0 M NaOH or 10.32 M HCl.

10.2.3.3 Simulated duodenal fluid

The duodenal fluid was prepared by adding 216 mg $CaCl_2$, 1061 mg BSA, 3013 mg pancreatin and 500 mg lipase into a 1 L Duran bottle. Then, the inorganic duodenal component was prepared by adding 578 mg KCl, 88 mg KH_2PO_4, 58 mg $MgCl_2.6H_2O$, 5629.7 mg $NaHCO_3$, 7092.0 mg NaCl and 180 µL of 10.32 M HCl into a 500 mL Duran bottle and made up to the mark with Millipore water. For the organic duodenal component, 115.7 mg urea was added into a 500 mL Duran bottle and made up to the mark with Millipore water. Simultaneously, the inorganic and organic duodenal components were added into the 1 L Duran bottle and the contents were mixed thoroughly in a shaker. The pH of the duodenal fluid component was measured and adjusted when necessary to be within 7.31 ± 0.2. The pH of the solution was adjusted with either 1.0 M NaOH or 10.32 M HCl.

10.2.3.4 Simulated bile fluids

The simulated bile fluid was prepared by adding 246.8 mg $CaCl_2$, 1807.9 mg BSA and 6007.6 mg bile into a 1 L Duran bottle. Separately, 385.4 mg KCl, 5771.6 mg $NaHCO_3$, 5299.1 mg NaCl and 180 µL of 10.32 M HCl were added into a 500 mL Duran bottle and made up to the mark with Millipore water. The organic bile component was prepared by adding 251.9 mg urea into a 500 mL Duran bottle and made up to the mark with Millipore water. Both inorganic and organic bile components were simultaneously added to the 1 L Duran bottle. The content of the bottle was mixed thoroughly in a shaker. After complete dissolution of solid reagents, the pH of the solution was measured and where possible adjusted to be within 8.03 ± 0.1. The pH was adjusted with either 1 M NaOH or 10.32 M HCl.

10.2.4 Gastrointestinal (GIT) extraction

The GIT extraction was carried out by quantitatively weighing 0.9842 g – 1.0034 g of the polyurethane industry dust sample 2 into a 45 mL BIOLOGIX tube, followed by the addition of 2.45 mL Millipore water. Then 4.5 mL of simulated saliva fluid was added and the solution was rotated in an end-over-end shaking water bath for 5 minutes at approximately 35 rounds per minute kept at 37 ± 2 ºC. Then, 9 mL of simulated gastric fluid was added to the solution by pipette; samples were incubated in the shaking water bath under the same conditions for a further 2 hours. After the extraction, the pH of the solution was measured (ca. pH 0.99 ± 0.1 for extraction blank and 2.43 ± 0.1 for polyurethane dust). Then, 9 mL of duodenal fluid and 9 mL of bile fluid were added. The solution pH was checked and adjusted when necessary to be within the tolerance of

an intestinal pH of 6.0 ± 0.5 (typically 6.15 ± 0.2). The solution was incubated in the shaking water bath under the same conditions for a further 8 hours. Then the solution pH was checked to be within 6.0 ± 0.5. The solutions were centrifuged at 3500 x g for 10 minutes prior to liquid–liquid extraction of the supernatant.

10.2.4.1 Liquid- liquid extraction of supernatants after GIT extraction

A 5 mL aliquot of the supernatant solution from the GIT extraction was first extracted with 30 mL diethyl ether:n-hexane (50:50, v/v) mixture, followed by extraction with 10 mL of the same extraction solvent in a separating funnel. Both extracts were pooled and filtered through a 0.45 µm hydrophilic PVDF syringe filter (Millipore Millex-HV). Extracts were reduced to approximately 1 mL in a rotary evaporator and stored in amber GC-MS vials. Extracts were ready for GC-MS analysis without further clean-up. Also, residual PFRs in solutes after GIT extraction were extracted with a previously optimized ultrasonic-assisted extraction method [see chapter 6 (27)]. All analyses were carried out in triplicate.

10.2.5 GC-EI/MS Analysis

An Agilent 6890 gas chromatograph fitted with a Restek Rtx® – 1614 fused silica (5% diphenyl, 95% dimethyl polysiloxane) capillary column (15 m × 250 µm × 0.1 µm) coupled to an Agilent 5973N series mass spectrometer was used for the separation, detection and quantitation of all PFRs. Injections were made in the pulsed splitless mode with the injector temperature set at 250 ºC. The injection volume was 2 µL. The GC oven temperature programme started at 90 ºC (held for 2 mins), then was increased at 20 ºC min^{-1} to 270 ºC and held for 1 min, and finally increased at 10 ºC min^{-1} to 290 ºC and held there for a minute. Helium was used as carrier gas at a flow rate of 1.2 mL min^{-1} and a constant linear velocity of 37 cm s^{-1}. For the MS, the ion source and transfer line temperatures were 230 ºC and 350 ºC, respectively; and the ionization energy was 70 eV. PFR mass spectra were obtained in full scan mode to select prominent ions that were utilized in the selected ion monitoring (SIM) mode. OPEs were quantified by monitoring m/z 205, 249 for TCEP; m/z 201, 277 for TCPP; m/z 321, 381 for TDCPP and m/z 326, 325 for TPP in the SIM mode.

10.2.6 Statistics

The distribution of OPE concentrations in the different microenvironments was tested with the Shapiro-Wilk test of normality. Descriptive statistics such as sum, mean, median, minimum, maximum and parametric statistics such as t-test and analysis of variance (ANOVA) were calculated by using Microsoft Excel® 2010. Non-parametric statistics such as Wilcoxon Signed-Ranks test, Kendall tau test and Spearman rank correlation were performed with Analyse-it® software in Microsoft Excel® 2010. The Kruskal-Wallis test was employed to test for differences in location by using XLSTAT 2014 software. Limits of detection (LOD) and quantitation (LOQ) were estimated following Thomsen et al. (28). Samples below the detection limit were treated as zero throughout the statistical analysis.

Calculation of Bioaccessibility

Bioaccessibility is defined as the fraction of PFRs detected in the supernatant of the centrifuged GIT extracts. This was calculated as the percentage of the average amount of each PFR (all experiments were performed in triplicate) in the supernatant of the respective UBM medium to the average amount of each target PFR originally present in the dust sample extracted (Table 10.1) as follows:

$$\text{Bioaccessibility}(\%) = \frac{\textit{Average amount of each PBDE in the supernatant of GIT medium}}{\textit{Average amount of each PBDE originally present in extracted dust}} \times 100 \ldots\ldots \text{ Eqn.1}$$

10.2.7 Validation and quality control

Recoveries for PFRs in dust were determined from spiked anhydrous sodium sulphate at different spike concentrations (Table 10 2). Samples were left to stand for at least 21 days at -10 °C. Spiked samples were extracted and cleaned-up following the procedure for real samples. Method blanks (n = 3) were analysed with samples. For the method blank, dust samples were replaced with anhydrous sodium sulfate and passed through all the analytical procedure carried out for real samples. Solvent blanks were injected after the analysis of at most three samples. All glassware was cleaned with laboratory wash solutions, rinsed with distilled water and then with organic solvents. Non-volumetric glassware was oven-dried prior to use. Direct ultraviolet light and plasticware was avoided throughout the analysis. Equation 2 gives a mass balance exercise carried out by using both spiked anhydrous sodium sulphate and contaminated dust from the polyurethane industry to determine the recovery of each of the PFR following *in vitro GIT* extractions.

$$\%\, recovery = \frac{\begin{array}{c}\textit{Average mass of PFR in supernatants of}\\ \textit{GIT fluid+average mass of PFRs in}\\ \textit{solute after GIT extraction}\end{array}}{\textit{Average mass of PFR in the extracted dust}} \ldots\ldots\ldots\ldots\ldots\ldots\text{Eqn. 2}$$

10.3 Results and discussion

The PFRs were measured in all dust samples and the oral bioavailable fractions of the total concentrations have been shown following an in vitro GIT model.

10.3.1 Distribution of PFRs in dust

The concentrations of all PFRs measured in the indoor dust of both the textile and the polyurethane industry are presented in Figs. 10.1-10.4.

10.3.1.1 Tris(2-chloroethyl) phosphate (TCEP)

TCEP was present in all samples collected from the two industries. The concentrations of TCEP ranged from 2.96 to 5.81 μg g^{-1} and 3.18 – 71.2 μg g^{-1} for the textile and polyurethane industries respectively. TCEP is both a solid and gas phase additive flame retardant used in a variety of consumer products (1). The concentrations of TCEP in these samples were not surprising as we had earlier measured high levels of TCEP in

various indoor microenvironments in Durban, South Africa (see Chapter 8). The dust samples from the polyurethane industry contained higher TCEP levels compared with the levels found in the dust from the textile industry. Within the textile industry, higher TCEP concentrations were found in dust collected from the "finished products (i.e. processed fabrics)" storage location in comparison with levels found in the "preparation area" of the industry. Little or no data are available in the literature on TCEP levels in fabrics or textiles. Recent studies have mainly focused on household consumer products such as polymers, electronics, foams and furniture as sources of TCEP in the indoor environment (1, 22). The result of the present study shows that fabrics and textiles (i.e. clothing) could be a significant source of TCEP in the indoor environment. However, we cannot exclusively conclude that TCEP concentrations found in dust from the textile industry were directly released from the processed fabrics owing to the small sample size as well as the fact that no fabric sample was analyzed in the current study. The flammability of textiles varies greatly with each type, presenting different problems in reducing flammability. Cotton- and cellulose-based fibers are flame retarded by impregnating cellulose phosphate esters formed by direct esterification of the cellulose molecule with a phosphate of the flame retardant compound (Blum and Ames, 1977).

Table 10.1 Concentrations of PFRs in dust from the various locations in the textile and polyurethane industries (n = 3)

	Mean Concentration/μg g^{-1}			
Location	TCEP	TCPP	TDCPP	TPP
Textile site 1	2.96 ± 0.22	29.2 ± 6.32	4.18 ± 1.01	0.89 ± 0.18
Textile site 2	5.81 ± 1.08	180 ± 12.8	97.4 ± 3.22	10.2 ± 1.41
Polyurethane sample 1	6.07 ± 0.15	863 ± 14.8	16.9 ± 3.52	4.29 ± 0.69
Polyurethane sample 2	3.19 ± 0.61	114 ± 18.9	4.21 ± 1.27	2.41 ± 0.49
Polyurethane sample 3	71.2 ± 8.38	2195 ± 126	212 ± 15.8	56.8 ± 6.33

10.3.1.2 Tris(1-chloro-2-propyl)phosphate (TCPP)

The concentration of TCPP varied from 29.2 – 180 μg g^{-1} and 114 – 2195 μg g^{-1} for the textile and polyurethane industries respectively. Similar to TCEP, TCPP were highest in the "finished product" storage area of the textile industry. The highest concentrations of TCPP in the polyurethane industry were found in samples collected from an existing plant in which both flexible and rigid polyurethane foam (PUF) are blended. However, TCPP concentrations as high as 863 μg g^{-1} were found in a plant which was decommissioned in 2011 in this polyurethane industry. TCPP is known to be one of the replacements for penBDEs in polyurethane foams (18). The use of TCPP dates back to the mid-1960s and is used as replacement for TCEP. TCPP as high as 2.2% by weight has been reported in foams (18). The enormous level of TCPP in the dust samples from the polyurethane industry is indicative of wide volume usage of TCPP in both flexible

and rigid foams in South Africa. No regulations are currently in place in South Africa to control and monitor the use of flame retardant chemicals in consumer products; however, South Africa is party to the Stockholm Convention on persistent organic pollutants. The levels of TCPP in these industries could have significant implications for overall indoor human exposure to this organophosphate flame retardant chemical.

10.3.1.3 Tris(1,3-dichloro-2-propyl)phosphate (TDCPP)

TDCPP was detected in all samples. The concentrations of TDCPP ranged from 4.18 – 97.4 μg g^{-1} and 4.21 – 212 μg g^{-1} in the textile and polyurethane industries respectively. TDCPP was highest in a dust sample collected from an existing plant in the polyurethane industry. TDCPP is used as substitute flame retardant for pentaBDE in polyurethane foam and has been detected up to 5% by weight in furniture foam from the United States (18). Although no study has reported TDCPP concentrations from a textile industry, TDCPP was reported in nine of fifty fabrics from children pyjamas in the United States (29). Despite the fact that TDCPP has since 1977 been voluntarily withdrawn in the US for use in children's sleepwear because of its structural similarity to tris(2,3-dibromopropyl)phosphate (tris-BP) (30), alarming concentrations of TDCPP were found in the dust samples collected from this textile industry. However, TDCPP is still being used in millions of tonnes per year as a flame retardant in flexible and rigid polyurethane foams, textile backcoatings, and adhesives (30), hence may account for the higher concentrations of TDCPP in dust from the polyurethane industry (30).

10.3.1.4 Triphenyl phosphate (TPP)

The concentrations of TPP ranged between 0.89 – 10.2 μg g^{-1} and 2.41 – 56.8 μg g^{-1} in the textile and polyurethane industries respectively. Similar to the chlorinated organophosphorus flame retardants, TPP was highest in dust from the polyurethane industry. TPP is added to PUF in combination with the halogenated mixtures. It is applied as a by-product in Firemaster 550, an alternative flame retardant for pentaBDE used in PUF (18). Similarly, TPP is used in the technical mixture of resorcinol bis(diphenylphosphate) (PBDPP) and bisphenol A bis(diphenylphosphate) (BPA-BDPP), both are alternative flame retardants for decaBDE. TPP is a gas-phase additive flame retardant, which is an effective flame retardant for many polymers (1).

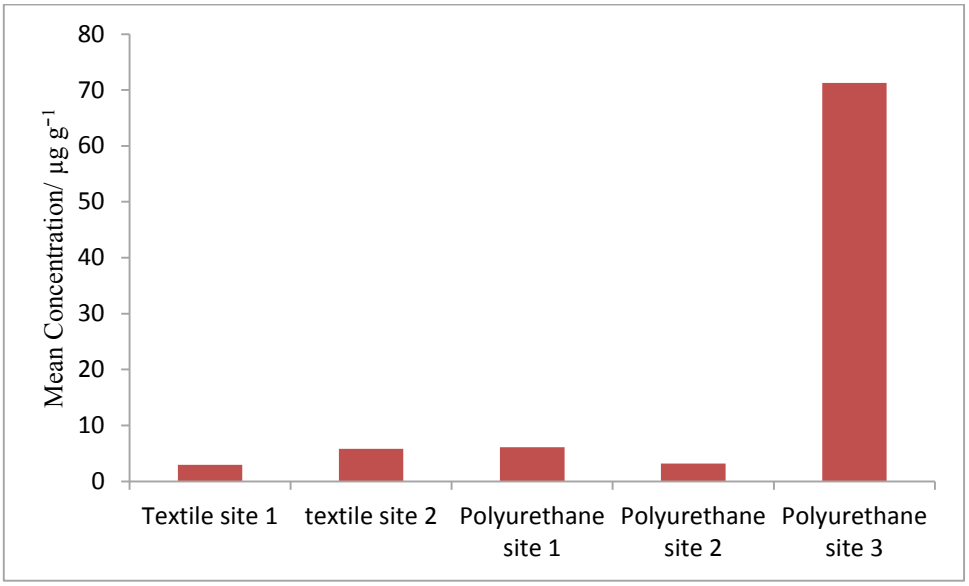

Figure 10.1 Distribution of TCEP in the dust from the two industries.

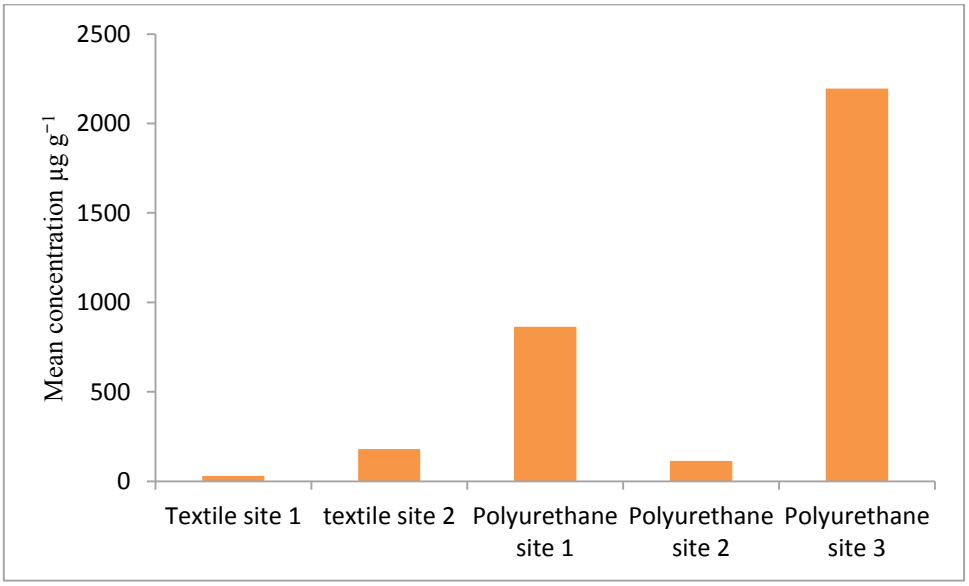

Figure 10. 2 Distribution of TCPP in the dust from the two industries.

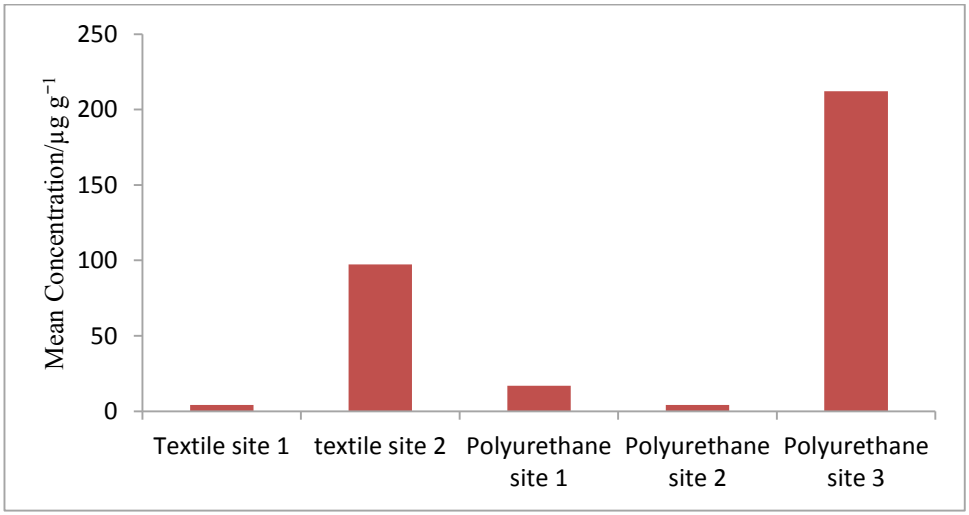

Figure 10.3 Distribution of TDCPP in the dust from the two industries.

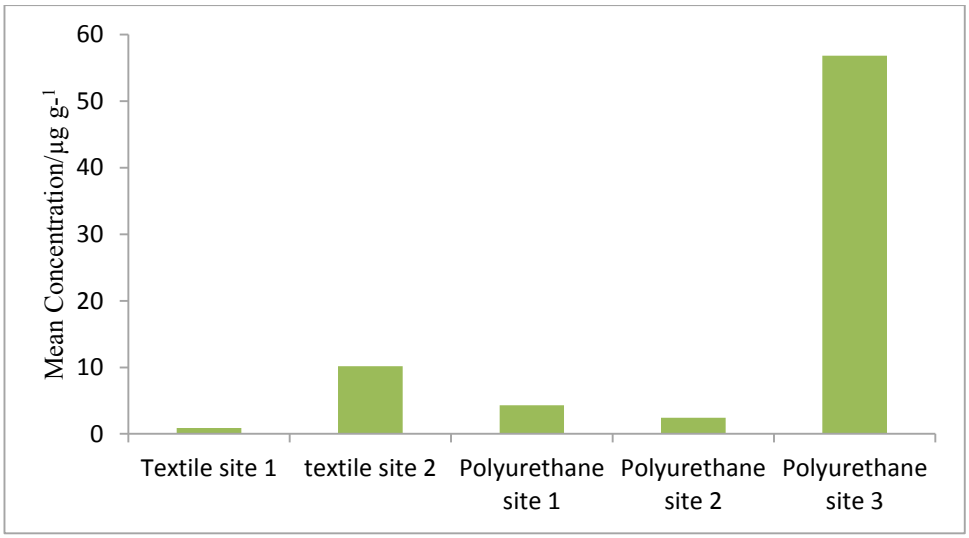

Figure 10.4 Distribution of TDCPP in the dust from the two industries.

10.3.2 Oral Bioaccessibility of Organophosphate Flame Retardants

The analytical performance of the UBM method was evaluated by performing a mass balance exercise by using the polyurethane dust sample. The recovery of the analytes after GIT extraction was calculated by using Equation 2. Good recoveries ranging from 59 – 110 % were obtained for all target compounds (Table 10.2).

Following the UBM method, the percentage bioaccessible fractions (% BAF) ranged from 0.9 – 40.0 %. The known human carcinogen, TCEP, had the highest observed bioaccessible fraction (40.0 %) whilst TDCPP exhibited the least oral bioaccessibility (0.9 %) of the studied PFRs. A % BAF of 26.3 % and 19.6 % were observed for TCPP and TPP respectively. The bioacessibility of PFRs was independent of the log K_{ow}. The % BAF showed a negative correlation (p value of 0.2000) with the log K_{ow} of the respective PFR. However, a strong dependent relationship was observed for the % BAF

of PFRs and their water solubility. The % BAF exhibited a strong positive correlation (r = 0.834, p value of 0.1657) with the water solubility of the PFRs.

Table 10. 2 Concentrations (μg g^{-1}) of PFRs in supernatant and solute of GIT fluids (n = 3 for supernatants, and n = 2 for solute).

GIT Media	TCEP	TCPP	TDCPP	TPP
Mean concentration in supernatant	1.27 ± 0.31	30.1 ± 1.12	0.04 ± 0.01	0.47 ± 0.08
Mean concentration in solute	1.70 ± 0.23	41.4 ± 2.00	4.58 ± 0.49	0.96 ± 0.13
% Recovery[a]	93.0	63.0	110	59.3
%BAF[b]	40.0	26.3	0.87	19.6
log Ko_w[c]	1.44	2.59	3.80	4.59
Water solubility/$mg\ L^{-1}$	7000	1600	1.5	1.9

[a] % recovery of PFRs following the balance equation; [c,d] van der Veen and de Boer (1)
[b] % bioaccessible fractions of PFRs

10.4 Conclusions

The present study has indicated high volume application of PFRs in both the textile and polyurethane industry in South Africa. The implication is that, contrary to widespread belief and reports that sources of PFRs in the indoor environment are primarily from household products such as electronics, carpets, foams and furniture; textile materials and other clothing materials may contain a greater amount of PFRs and they can be released during the products' useful life, hence contaminating the indoor environment. There would be a greater risk of exposure to PFRs from clothing, since PFRs are known to rapidly absorb through the skin. We have also shown that PFRs particularly the carcinogenic tris(2-chloroethyl) phosphate is bioaccessible to as high as 40.0 % whilst the most abundant PFR found in South African homes and other indoor environments, namely TDCPP is weakly bioaccessible (0.87%). Generally, less than 40% of PFRs ingested from contaminated dust samples would potentially be available for absorption in the human gastrointestinal tracts. Contrary to the abundance of TDCPP and TCEP in South African homes, offices, automobiles and computer laboratories, TCPP was the most abundant PFR found in these South African industries.

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Supplementary Material

Table S10.1. Concentrations (μg g^{-1}) of PFRs in supernatant and solute of GIT fluids from the UBM protocol for the polyurethane industry sample 2.

GIT Media	TCEP	TCPP	TDCPP	TPP
Supernatant repeat 1	0.92	30.5	0.03	0.53
Supernatant repeat 2	1.48	31.0	0.03	0.51
Supernatant repeat 3	1.41	29.0	0.05	0.38
Mean ± Standard deviation	1.27 ± 0.31	30.1 ± 1.12	0.04 ± 0.01	0.47 ± 0.08
Solute repeat 1	1.54	42.8	4.93	0.86
Solute repeat 2	1.86	40.0	4.23	1.05
Mean ± Standard deviation	1.70 ± 0.23	41.4 ± 2.0	4.58 ± 0.49	0.96 ± 0.13
% Recovery[a]	93.0	63.0	110	59.3
%BAF[b]	40.0	26.3	0.87	19.6
log K_{ow}[c]	1.44	2.59	3.80	4.59
Water solubility/$mg\ L^{-1}$[d]	7000	1600	1.5	1.9

[a] % recovery of PFRs following the mass balance equation

[b] % bioaccessible fractions of PFRs

[c,d] van der Veen and de Boer (1)

Chapter 11

Conclusions

Flame retardants (FRs) are a group of chemicals used to flame-retard a wide range of consumer products including construction materials, polyurethane foams, textiles and fabrics, furniture and foams, car interiors and carpets, electronic equipment such as printed circuit boards, and high impact polystyrene and acrylonitrile butadiene-styrene (1). The widespread application and use of FRs has resulted in a growing scientific concern in studying the potential effects of these chemicals on humans and the environment. Evidence from several studies shows that FRs do not remain bound to products but can migrate into various environmental compartments including air, dust, water, soil, sediments and sludge during various stages of manufacture, use, repair, recycling and disposal. These chemicals are global contaminants of concern as they are persistent, can bioaccumulate, biomagnify and have potential for long-range atmospheric transport. Most FRs are toxicants of particular concern to human health since they affect thyroid hormones, endocrine systems and neurobehavioural development and are possibly carcinogenic. Given the above, the main objective of this thesis were to examine three classes of FRs – PCBs, BFRs and PFRs, in various residential and workplace indoor environments in Durban, South Africa and to shed light on the pathways and magnitude of human exposure to these FR chemicals. The major findings of this thesis are summarized Section 11.1:

11.1 Summary of Major Findings

- Different sample preparation steps, including extraction, clean-up and derivatization methods, were developed/modified and validated and were successfully applied for the determination of the target compounds in dust and simulated GIT fluids. Analytical methods based on GC-EI/MS (PBDEs, PCBs, PFRs and TBBPA) and LC-ESI-MS/MS (TBBPA) were developed/and or validated for BFRs, PCBs and PFRs (see Chapters 3, 4 and 6).
- The relationship between PBDE levels and automobile manufacturer and model were established. Levels of PBDEs in automobiles significantly correlated between vehicles produced by Toyota ($r = 0.98$), Audi ($r = 0.76$) and Honda ($r = 0.63$). Automobiles manufactured prior to 2010, contained significantly higher PBDE levels compared with automobiles manufactured after 2010, indicating a decline in the usage of PBDEs as flame retardants for automobile applications (see Chapter 3).
- All dust samples collected from three other indoor microenvironments (homes, offices and computer laboratories) contained PBDEs. BDE-209 was the most prevalent in dust from homes and offices and accounted for 43% and 45% of the total PBDEs ($n = 8$)

congeners, in homes and offices respectively. BDE-153 was the most abundant congener and accounted for 38% of the total PBDEs present in dust from the computer laboratories. The abundance of BDE-153 in this microenvironment is similar to reports from other South African outdoor environments (2, 3). This observation indicates the use of the commercial pentaBDE formulation in products in the country. Generally, BDE-28 seemed depleted in all South African indoor microenvironments studied (see Chapter 6).

- The distributions of the PBDE congeners were statistically different ($p = 0.011$) in the three microenvironments. The levels of PBDEs reported in these indoor microenvironments fall within the ranges measured in Toronto, Canada (4) and in Shanghai, China (5). However, the levels are much lower than concentrations of PBDEs observed in the USA and the United Kingdom, but not as low as values reported in Germany (6), New Zealand (4), Vietnam (7) and Pretoria, South Africa (8, 9), for example. Variability in the distribution of PBDEs was associated with size and degree of industrialization of Durban; as well as differences in particle size fractions and methods employed for analytical data management (see Chapter 6).
- Typical household items were implicated as reservoirs and sources of PBDEs in the indoor environment. The levels of BDE-47 were positively correlated ($r = 0.77$) with mattresses, foams and furniture as did BDE-209 levels with electronic appliances ($r = 0.54$) in homes; and BDE-183 with electronic equipment ($r = 0.54$) in offices. The observation is in agreement with those of de Wit et al. (10) in Sweden (see Chapter 6).
- PCB-180 dominated the three microenvironments studied. It accounted for 79 %, 81 % and 75 % of total PCBs measured in homes, computer laboratories and offices, respectively. The concentrations of PCBs in homes and offices were similar to levels reported in the USA (11) and Kuwait (12). The observed levels of PCBs in computer laboratories were within the ranges reported by in homes near New Bedford harbor monitored during dredging of PCB contaminated harbour sediments Vorhees et al. (13).
- The elevated levels of PCBs found in homes were independent of home characteristics. There was no statistical relationship ($p = 0.0625$) between PCB concentrations in homes and the building construction year. Similarly, PCB concentrations were independent of the type of floor, that is, either carpeted, bare or tiled floor. This is contrary to the observations of other studies (13-15). This disagreement may not be unconnected with the limited sample size utilized for source characterization in the present study (see Chapter 6).
- PBDE concentrations correlated positively ($r = 0.60$) with the concentrations of PCBs in homes but not with those in offices and computer laboratories, hence indicating similar sources but compound-specific differences in the indoor fate and transport of these persistent organohalogens (see Chapter 6).
- In Durban e-waste dismantling/recycling facilities, BDE-209 was the dominant congener in the indoor dust and comprised 76% of total PBDEs ($n = 8$) congeners, this was followed by BDE-99 which accounted for 7.3% of total PBDEs in the e-waste dust samples. PBDE levels in Durban e-waste sites were similar in profile to levels reported

in Guiyu, China (16) and much lower than levels reported in recent Chinese studies (17, 18) but higher than levels reported in Vietnam (7). The pentaBDE profile in Durban e-waste dust samples resembled those of the commercial formulation DE-71 and Bromka 70-5DE (see Chapter 7).

- In the same light, PCB-180 was most prevalent in Durban e-waste recycling site dust samples and composed 57% of the total PCB congeners studied. The levels reported here are lower than concentrations reported in hair of e-waste dismantling site workers and in dust around an e-waste dismantling site in China (19).
- Occupational exposure to PBDEs and PCBs via dust ingestion showed the relevance of indoor dust as an exposure pathway of e-waste recyclers and dismantlers to organic flame retardant chemicals. The average daily exposure dose of both PBDEs and PCBs for recyclers/dismantlers in these e-waste facilities was lower than the EPA's reference dose for the respective PBDE or PCB congeners. Dermal absorption of these flame retardants accounted for approximately 21% of the total exposure dose of PBDEs and PCBs in these sites (see Chapter 7).
- A significant reduction in concentration profiles and subsequent human exposure magnitude of PBDEs and PCBs was observed following a routine e-waste recycling/dismantling site cleaning (see Chapter 7).
- TBBPA was detected in high amount in the indoor dust of the two e-waste recycling sites. Although, there is currently no suitable data available worldwide for comparison, the levels of TBBPA found in the recycling sites falls within the range reported by Yu and Hu (20) in dust from inside of computers (see Chapter 4).
- Similarly, TBBPA was detected above the LOQ in 71%, 100%, 86% and 86% of home, computer laboratory, office and automobile dust samples respectively. The levels of TBBPA were lowest in computer laboratories but TBBPA was detected in all samples collected from this microenvironment. No statistical difference was observed in the TBBPA levels from the four microenvironments. Concentrations of TBBPA found in automobile dust were higher than levels reported in United Kingdom cars (21). No association was, however, found between TBBPA concentrations and automobile model and year of manufacture. The implication is that an external contamination source may be responsible for the high TBBPA levels in automobiles (see Chapter 5).
- TBBPA concentrations found in homes and offices resemble levels reported in other parts of the world (21-24). No statistical relationship was found between TBBPA and BDE-183 levels in the studied microenvironments; however, the levels of TBBPA were at least an order of magnitude greater than BDE-183 levels in most of the microenvironments (see Chapter 5 and Chapter 7).
- On average, the South African population is exposed to 0.08, 0.08 and 0.60 ng kg^{-1} bw day^{-1} of TBBPA for adults, teenagers and toddlers, respectively. These doses are higher than the average dietary intake of 0.04 ng kg^{-1} bw day^{-1} TBBPA of the Dutch population. This result further implicates dust ingestion as a principal human exposure pathway of

TBBPA, similar to the observation for the United Kingdom population (21) (see Chapter 5).

- Organophosphate flame retardants (OPEs) were detected in all samples collected from homes in Durban, with TDCPP being the dominant OPE present in this microenvironment followed by TCEP. Distribution of OPEs in Durban home dust are similar in profile to reported profiles in Swedish homes (25), German homes (26) and Egyptian homes (27). The levels of TCEP and TCPP in this microenvironment strongly correlated ($r = 0.82$), suggesting source similarities and/or use pattern. Statistical differences ($p = 0.022$) were observed in the distribution pattern of all studied OPEs in Durban homes (see Chapter 8).
- In these homes, the concentrations of ∑OPEs in dust showed positive correlations ($r = 0.22$, p value of 0.4862) with electronics and also correlated ($r = 0.522$, p value of 0.0675) with foams and furniture present in this microenvironment. Thus, this implicates household electronics, foams and furniture as reservoir and sources of PFRs indoors (see Chapter 8).
- In Durban offices, PFRs were detected in 100% of the samples with TCEP dominating the OPE profile. The level of TCEP in these samples was much higher than levels reported in a Swedish office (28). Since the use of TCEP has been restricted in consumer products by various regulations, the high levels of TCEP in this microenvironment were linked to the flame retardant V6 which is a reportedly known important source of TCEP in the environment (29) or a possibility of non-compliance with international regulations. Similarly, TCEP was the dominant PFR detected in dust from computer laboratories, whilst TDCPP was most abundant in automobile dust samples. TDCPP levels in automobiles were similar to levels reported in cars from the Netherlands (30) and Germany (26). Overall, TCEP seemed to be in continual use in products in South Africa (see Chapter 8).
- No relationship was found between TDCPP levels and automobile age, contrary to the observation of Brommer et al. (26), in which TDCPP levels were associated with older cars. OPE concentrations varied widely among automobiles manufactured by Honda and Audi. However, TCEP and TCPP levels strongly correlated ($r = 0.995$) in Toyota products. Interestingly, higher OPE concentrations were found in automobiles manufactured post-2004, which signifies the transition from traditional pentaBDE formulations for use in foams and furniture. The distribution and profile of OPEs in Durban indoor environments, is reflective of large volume usage of these FRs in consumer goods, probably in response to international regulations on the use of alternative FRs as opposed to traditional BFRs such as PBDEs (see Chapter 8).
- The worse-case scenario daily human exposure dose of TDCPP is at par with the toxicological reference dose of 30000 ng d^{-1} TDCPP. The prevalence of two chlorinated OPEs – TDCPP and TCEP – in indoor dust in Durban, may pose a significant health challenge, particularly among toddlers who are exposed to higher magnitudes of FR chemicals due to frequent hand-to-mouth activity and inadvertent dust ingestion.

- In industries, alarming levels of organophosphorus flame retardants (PFRs) were found in the indoor dust of a textile and a polyurethane industry. TCPP dominated all samples from the two industries. In the polyurethane industry, TCPP in excess of 2195 $\mu g\ g^{-1}$ was found in a sample. These observations further confirm the current use of PFRs in a wide range of consumer products ranging from textiles and fabrics to foams and furniture, amongst others in South Africa (see Chapter 10).
- Factors responsible for the oral bioaccessibilities of PBDEs and PFRs were elucidated in this study. Gastric pH, extraction/incubation time, dust physiochemistry and levels of contaminants in dust, as well as state of the GIT (fed or unfed), are major factors responsible for oral bioaccessibility of these FR chemicals (see Chapter 9 and Chapter 10).
- Finally, the oral bioaccessibilities of PBDEs and PFRs were assessed following a developed and/or modified Universal Bioaccessibility Method (UBM) and the Fed Organic Estimation human Simulation (FOREhST) procedures. Following the FORhEST protocol, the bioaccessibilities of BDE congeners 28, 47, 99, 100, 153, 154, 183 and 209 were 92, 22, 22, 51, 31, 12, 28 and 40% respectively, in dust samples collected from an e-waste recycling/dismantling site. Whilst, by using the UBM (fasted-state) GIT protocol, the overall bioaccessibilities of the BDE congeners were 16, 25, 11, 16, 16, 7, 28 and 10% respectively in the same dust sample. The bioaccessibility of PBDEs in e-waste dust following the fasted-state conditions correlated ($r = 0.62$) with the respective log K_{ow} of each of the PBDE congeners. Statistically significant differences were obtained in the % BAF of PBDEs following the fed- and the fasted-state conditions. However, no differences were observed in the % BAF of PBDEs by using either the sequential or batch extraction formats (see Chapter 9).
- For PFRs in dust from the polyurethane industry, the overall bioaccessibility (% BAF) were 40, 26, 0.9 and 20% respectively for TCEP, TCPP, TDCPP and TPP, following the fasted-state GIT extraction. Unlike PBDEs, the % BAF of PFRs are independent ($p = 0.2000$) of log K_{ow}, but depend strongly ($r = 0.834$, p value of 0.1657) on the water solubility of each PFR (see Chapter 10).
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11.2 Research Gaps and Future Perspectives

The continuous scientific, industry and policy interest in flame retardants, particularly BFRs, PCBs and PFRs, has resulted in an expanding database relevant to their environmental fate, behaviour and human exposure to these classes of chemicals. However, salient research gaps still exist and further research is required to:

- Monitor levels of legacy and emerging FRs in a country-wide range of indoor environments including homes, vehicles and workplace indoor/outdoor environments, e-waste recycling/dismantling sites, as well as industries involved in the production of consumer products such as electronics, textiles, fabrics, carpets, foams and furniture. In order to create a database of legacy, current and emerging FRs in use in South Africa, and possibly, a continent-wide database.

- Elucidate the mechanisms of release of FRs from treated products to the indoor environments and further understand the cause of variability in FR levels in indoor dust. Controlled chamber experiments as well as advanced analytical techniques such as environmental scanning electron microscopy (ESEM) and mathematical models may be used to achieve this.
- Monitor the levels of PFRs and other emerging FRs in cloths and fabrics as they are possible overlooked sources of human exposure to FRs.
- Monitor other possible sources of human exposure such as dietary intake of these FRs and provide more information on non-dietary intakes including dust ingestion, air inhalation, dust dermal absorption and incidental soil contacts.
- Relate indoor levels of FRs to human body burdens of the FRs. This can be achieved by carrying out human biomonitoring assessment of these FRs or by using toxicokinetics and/or physiologically based pharmacokinetic/pharmacodynamics modelling.
- Elucidate the factors and extent of *in vitro* human bioaccessibility/bioavailability of FRs via different exposure routes including air inhalation, dust/food ingestion and dermal absorption.
- Investigate the relationship between the intake of organic FRs via multiple exposure routes and human body burdens to understand the relative influence of each exposure route. This also emphasizes the need to explore effective alternatives to non-invasive bioindicators, for example, human scalp hair, fingernails, urine and faeces, sweat and saliva for assessing human exposure to FRs, most importantly for infants, toddlers, young children, pregnant women and physically challenged individuals.
- Understand the human health implications of exposure to a mixture of PCBs, BFRs PFRs vis-à-vis other emerging and legacy flame retardants and persistent organic pollutants.

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