Analysis of Legacy and Emerging Flame Retardants, Plasticizers and their Metabolites in Food and Breast Milk

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ABSTRACT

On a daily basis, we are exposed to a wide variety of chemicals, such as flame retardants and plasticizers, that are incorporated into consumer products. Even at low doses, some of these chemicals have been found to disrupt the body's endocrine system, these are known as endocrine disrupting chemicals (EDCs). Due to the ubiquity of these substances, it is important to monitor our potential exposure to EDCs through diet. With this in mind, a multiclass extraction method was developed, optimized and validated for Polybrominated Diphenyl Ethers (PBDEs), Dechloranes, Organophosphate esters (OPEs) and their metabolites (mOPEs). The method uses sonication as an extraction technique and then fractionates the analytes based on their polarities; clean up steps are based on the physicochemical properties of the analytes. Samples were first lyophilized; the sample mass used for extraction was determined based on the lipid content of the matrix. Good recoveries are obtained for all analytes in all matrices (49-122%) with good repeatability (RSD 1-10%). The extracts were run on LC-QqQ-MS/MS for the separation and quantification of OPEs and mOPEs as well as GC-MS for the separation and quantification of PBDEs and Dechloranes. The chromatographic separation of Tri-tolyl phosphate (TCP) and isopropylphenyl diphenyl phosphate (ip-PDPP) isomers was achieved for the first time using LC-QTOF-MS. Method limits of quantification (MLOQs) ranged between 0.049 - 0.347 ng/g for PBDEs, between 0.13 - 0.91 ng/g for Dechloranes and between 0.36 - 1.54 ng/g for OPEs and mOPEs. The method was applied to various food samples including fish, chicken, vegetables, canned fish, bread and butter as well as breast milk. Several of the analyzed compounds were detected above MLOQ levels. Moreover, the current method is quicker, and less costly than other methods due to the reduction of required materials and processing steps. This is the first time PBDEs, Dechloranes, OPEs and mOPEs have been simultaneously analyzed in the same matrix.

Using the previously developed multiclass extraction method, the occurrence of 14 OPEs and mOPEs was investigated in 66 composite food samples purchased from the greater Montreal market. Also, 100 breast milk samples were collected from new mothers in Montreal. All OPEs analyzed were detected above the quantification limit in at least one matrix. Butter was the most contaminated food category with a total OPE concentration of 117 ng/g w.w. followed by bread (18 ng/g w.w.) and canned fish (14 ng/g w.w.). In breast milk, the ΣOPE concentration was 27 ng/g w.w., the highest level of OPE was Tris(2-ethylhexyl) phosphate (TEHP) at 8 ng/g w.w. followed by Tris(1-chloro-2-propyl) phosphate (TCIP) (6 ng/g w.w.) followed by ip-PDPP (4 ng/g w.w.). To investigate the effect of food packaging on the potential migration of OPEs and mOPEs into food, both packaged and non-packaged food samples were collected; however, there was no difference in the levels of OPEs or mOPEs in these samples ($P>0.05$). The lipid dependence of OPEs was also investigated by comparing the lipid content of the sample to the total levels of contaminants present; no trend was observed, thus, further suggesting the lipid independence of OPEs. The metabolism of OPEs was assessed by normalizing the detected OPE by the respective mOPE; most samples, including breast milk contained higher levels of OPE to mOPE. A notable exception is bread having higher levels for Tris(1,3-dichloro-2-propyl) phosphate to Bis(1-chloro-2-propyl) phosphate (TDCPP/BCIPP), Tris-tolyl phosphate to di-tolyl phosphate (TCP/DCP) and Tris(2-chloroethyl) phosphate to Bis(2-chloroethyl) phosphate (TCEP/BCEP); this may be due to the high temperature processing needed to bake bread.

Considering the daily average intake of food for the average Canadian, the dietary exposure to ΣOPE was estimated to be 12800 ± 1500 ng/day [183±22 ng/kg bw/day]. For individual OPEs, TEHP contributed on average 6094 ng/day. Several population groups were considered for dietary exposure including men, women, children (5-11 years) and young children (1-4 years). For all

groups, values of OPEs were several orders of magnitude lower than their corresponding health-based reference doses. mOPEs currently do not have reference dose values and therefore are not included in the exposure assessment. The exposure to OPEs through breast milk for young infants (3 months) was also considered. Both males and females as well as high and low consumption patterns were assessed. For individual OPEs, TEHP contributed on average 7300 ng/day (upper bound estimate). For all groups, values of OPEs were several orders of magnitude lower than their corresponding health-based reference doses.

By using the developed multiclass extraction method, it was possible to estimate the current dietary exposure levels of OPEs and mOPEs for Canadians. Based on this preliminary risk assessment, the Canadian population is exposed to a tolerable level of OPEs through diet, however, careful monitoring should continue and be expanded to other food groups in order to obtain a complete picture of dietary exposure to EDCs. With these data, policy makers can make more informed decisions with regards to replacement chemicals.

RÉSUMÉ

Tous les jours, nous sommes exposés à une grande variété de produits chimiques, tels que les retardateurs de flamme et les plastifiants, qui sont incorporés dans les produits de consommation. Même à faibles doses, il a été constaté que certains de ces produits chimiques perturbent le système endocrinien de l'organisme. C'est ce que l'on appelle des produits chimiques perturbant le système endocrinien (EDC). En raison de l'omniprésence de ces substances, il est important de surveiller notre exposition potentielle aux EDC dans le régime alimentaire. Dans cet esprit, une méthode d'extraction multi-classe a été développée, optimisée et validée pour les éthers diphényliques polybromés (PBDE), les déchloranes, les esters d'organophosphates (OPE) et leurs métabolites (mOPE). La méthode utilise la sonication comme technique d'extraction, puis fractionnent les analytes en fonction de leurs polarités. Les étapes d'enlèvement de lipide sont basées sur les propriétés physicochimiques des analytes. Les échantillons ont d'abord été lyophilisés; la masse d'échantillon utilisée pour l'extraction a été déterminée en fonction de la teneur en lipides de la matrice. De bonnes récupérations sont obtenues pour tous les analytes dans toutes les matrices (49-122%) avec une bonne répétabilité (RSD 1-10%). Les extraits ont été analysés sur LC-QqQ-MS/MS pour la séparation et la quantification des OPE et des mOPE, ainsi que par GC-MS pour la séparation et la quantification des PBDE et des Déchloranes. La séparation chromatographique des isomères du tri-tolyl phosphate (TCP) et de l'isopropylphényl diphényl phosphate (ip-PDPP) a été réalisée pour la première fois avec LC-QTOF-MS. Les limites de quantification de la méthode (MLOQ) variaient entre 0.049 - 0.347 ng/g pour les PBDE, entre 0.13 - 0.91 ng/g pour les Déchloranes et entre 0.36 et 1.54 ng/g pour les OPE et les mOPE. La méthode a été appliquée à divers échantillons d'aliments, notamment du poisson, du poulet, des légumes, du poisson en conserve, du pain et du beurre ainsi que du lait maternel. Plusieurs des composés analysés ont été

détectés au-dessus des niveaux de MLOQ, démontrant la robustesse de la méthode développée. De plus, la méthode actuelle est plus rapide et moins coûteuse que d'autres méthodes en raison de la réduction des matériaux et des étapes de traitement requis. C'est la première fois que les PBDEs, les déchloranes, les OPEs et les mOPEs sont analysés simultanément dans la même matrice.

À l'aide de la méthode d'extraction multi-classe mise au point précédemment, la présence de 14 OPEs et mOPEs a été étudiée dans 66 échantillons d'aliments composites achetés sur le marché du Grand Montréal. De plus, 100 échantillons de lait maternel ont été recueillis auprès de nouvelles mères à Montréal. Toutes les OPEs analysées ont été détectées au-dessus de la limite de quantification dans au moins une matrice. Le beurre était la catégorie d'aliments la plus contaminée avec une concentration totale en OPE de 117 ng/g l.w. suivi du pain (18 ng/g w.w.) et du poisson en conserve (14 ng/g w.w.). Dans le lait maternel, la concentration en OPE était de 27 ng/g w.w., le taux le plus élevé d'OPE était le phosphate de Tris (2-éthylhexyl) (TEHP) à 8 ng/g w.w. suivis par du phosphate de tris (1-chloro-2-propyle) (TCIP) (6 ng/g poids corporel) suivi par ip-PDPP (4 ng/g w.w.). Afin d'étudier l'effet du conditionnement des aliments sur la migration potentielle des OPE et des mOPE dans les aliments, des échantillons d'aliments emballés et non emballés ont été collectés; cependant, il n'y avait aucune différence dans les niveaux d'OPEs ou de mOPEs dans ces échantillons ($p > 0,05$). La dépendance vis-à-vis des lipides des OPE a également été étudiée en comparant la teneur en lipides de l'échantillon aux teneurs totales en contaminants présents; aucune tendance n'a donc été observée, suggérant ainsi l'indépendance des lipides des OPEs. Le métabolisme des OPEs a été évalué en normalisant les OPEs détectés par le mOPE correspondant; la plupart des échantillons, y compris le lait maternel, contenaient des taux plus élevés d'OPE dans mOPE. Une exception notable est le pain ayant des niveaux plus élevés de phosphate de tris (1,3-dichloro-2-propyle) en phosphate de bis (1-chloro-2-propyle) (TDCPP / BCIPP), de phosphate de

tris-tolyl en phosphate de di-tolyl (TCP/DCP) et du phosphate de tris (2-chloroéthyle) en phosphate de bis (2-chloréthyle) (TCEP/BCEP); cela peut être dû au traitement à haute température nécessaire pour cuire du pain.

Compte tenu de la consommation quotidienne moyenne d'aliments pour le Canadien moyen, l'exposition alimentaire aux OPE était estimée à 12800±1500 ng/jour [18322 ng/kg bw/jour]. Le TEHP a contribué en moyenne 6094 ng/jour pour les différents opérateurs. Plusieurs groupes de population ont été pris en compte pour l'exposition alimentaire, notamment des hommes, des femmes, des enfants (5 à 11 ans) et des jeunes enfants (1 à 4 ans). Pour tous les groupes, les valeurs des OPE étaient inférieures de plusieurs ordres de grandeur aux doses de référence correspondantes pour la santé. L'exposition aux OPEs par le lait maternel chez les nourrissons (3 mois) a également été prise en compte. Les hommes et les femmes ainsi que les modes de consommation élevés et faibles ont été évalués. TEHP a contribué en moyenne 7300 ng/jour (estimation de la limite supérieure) pour chaque OPE. Pour tous les groupes, les valeurs des OPEs étaient inférieures de plusieurs ordres de grandeur aux doses de référence correspondantes pour la santé.

En utilisant la méthode d'extraction multiclasse développée, il a été possible d'estimer les niveaux d'exposition alimentaire actuels des OPEs et des mOPEs pour les Canadiens. Sur la base de cette évaluation des risques, la population Canadienne est exposée à un niveau tolérable d’OPE. Cependant, une surveillance attentive devrait être poursuivie et étendue à d’autres groupes d’aliments afin d’obtenir une image complète de l’exposition alimentaire aux OPEs. Avec ces données, les décideurs peuvent prendre des décisions plus éclairées en ce qui concerne les produits chimiques de remplacement.

CONTRIBUTION OF AUTHORS

Mark Misunis was responsible for the development of the GC-HRMS method used for analysis of PBDEs and Dechloranes, Ken Heide was responsible for PBDE and Dechlorane data processing. Both Ken and Mark were responsible for PBDE and Dechlorane sample analysis.

Shabana Siddique and Gong Wang were responsible for LC-QqQ-MS/MS method development. Swaroopini Ramachandran was responsible for the lipid analysis of all food samples.

Section 4.2.1.2. was written by Samar Elzein, who was also responsible for the recruitment of volunteers for breast milk donation.

Jingyun Zheng and Lei Tian were responsible for food sample collection and processing.

Daniel Furlong, the author, was responsible for the experimental work and the writing of the thesis.

Dr. Stéphane Bayen, the MSc student's supervisor, Dr. Cariton Kubwabo, the MSc student's co-supervisor and Dr. Cindy Goodyer, the MSc student's advisor, guided all the research and critically revised the thesis prior to its submission.

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LIST OF ABBREVIATIONS

ASTM:	American Section of the International Association for Testing Materials
BDE:	Brominated Diphenyl Ether
BFR:	Brominated Flame Retardant
bw:	Body Weight
CAS:	Chemical Abstract Services
CEPA:	Canadian Environmental Protection Agency
CFR:	Chlorinated Flame Retardant
CRM:	Certified Reference Material
DP:	Dechlorane Plus
d.w.:	Dry Weight
ECNI:	Electron Capture Negative Ion
EDC:	Endocrine Disrupting Chemical
EPA:	Environmental Protection Agency
ESI:	Electrospray Ionization
EU:	European Union
FR:	Flame Retardant
GC:	Gas Chromatography
HPV:	High Production Volume
HRMS:	High Resolution Mass Spectrometer
IDL:	Instrument Detection Limit
ISTD:	Internal Standard
LC:	Liquid Chromatography
LOD:	Limit of Detection
LOQ:	Limit of Quantification
l.w.:	Lipid Weight
MDL:	Method Detection Limit
mOPE:	Organophosphate ester metabolite
MRM:	Multiple Reaction Monitoring
MS:	Mass Spectrometer
MS/MS:	Tandem Mass Spectrometer
ND:	Non Detect
NIST:	National Institute of Standards and Technology
OPE:	Organophosphate ester
PBDE:	Polybrominated Diphenyl Ether
PFK:	Perfluorokerosene
POPs:	Persistent Organic Pollutants
QC:	Quality Control
QqQ:	Triple Quadrupole
QTOF:	Quadrupole Time of Flight
RRF:	Relative Response Factor
RSD:	Relative Standard Deviation
RT:	Retention Time
SIM:	Single Ion Monitoring
SRM:	Standard Reference Material
w.w.:	Wet Weight

CHAPTER 1. INTRODUCTION

In modern life, we are surrounded by innumerable consumer objects that contain flame retardants and plasticizers. Generally speaking, the use of these chemicals has had beneficial impacts on our daily life by improving the safety of commercial products as well as their applicability. Flame retardants (FRs) can be added during processing of materials, such as plastics, or onto the finished products; the same is true for plasticizers [1-3]. Over time, there is the possibility that the added compounds leach out of the products they are applied to and migrate into the environment [4]. From there, the compounds can be ingested by humans through sources such as air [5], by dust ingestion [6-8], or through drinking water [4]. However, the contaminants can also be absorbed into food, diet can, therefore, play an important role in the ingestion of environmental contaminants [9]. If contaminants, such as FRs and plasticizers, are absorbed by breast feeding mothers, many of the contaminants can also be transferred to infants via breast milk [10-12]. It is important to understand the extent of this contamination. Since human milk is frequently the only or the major source of nutrition during infancy and early childhood and children are at a uniquely critical developmental phase [13-14].

It has previously been shown that many classes of FRs and plasticizers, both existing/legacy compounds and their emerging replacements, can be regularly detected in food and breast milk. Research has demonstrated that several of the studied compounds can be responsible for disrupting the hormone systems in humans; these are known as endocrine disrupting chemicals (EDCs) [15-16]. Even at low doses, EDCs can exert important biological effects during critical windows of susceptibility, such as during infancy [1-3, 15-17]. This has prompted governments around the world to

regulate the production, uses and importation of specific chemicals that have demonstrated the potential to disrupt the endocrine system or have other negative toxicological effects.

Legacy FRs, such as the polybrominated biphenyl ethers (PBDEs), have, as of 2008, been regulated in Canada and in many countries around the world [18]. Still, PBDEs are routinely detected in many food and breast milk. As a consequence of the regulations of PBDEs, novel FRs have emerged in order to fill the market "gaps". However, there is often little information on the degree to which we are exposed to these replacements or on their health impacts. Albeit, governments have already begun to place regulations on some of the new FRs; Dechloranes [19], TDCPP [20] and TCEP [21], for example, are regulated in Canada. However, there may still be many different replacement chemicals that have not been widely screened in food or breast milk samples. Moreover, the levels of the metabolites and breakdown products of these compounds in food have had very limited studies performed to date and none in Canada. For these reasons, it is important to be able to develop multi-residue extraction and analysis methods able to detect and quantify simultaneously legacy and emerging contaminants of concern in complex matrices.

PBDEs and Dechloranes can be extracted from food with the use of laborious techniques such as Soxhlet extraction [22], which is both time consuming and costly for routine analysis. Organophosphate esters (OPEs) or their metabolites (mOPEs) do not yet have official extraction methods in the published literature. By taking into account the physicochemical properties of these groups of chemicals, it should be possible to develop and validate robust extraction methods capable of extracting all groups of compounds [2]. To date, this has not been accomplished. For example, the studies using multi-residue analysis techniques have not reported any of the major mOPEs. This would lead to underestimations of total daily exposure to this group of compounds since OPEs are readily metabolized [17, 23-24].

From previous studies, it was determined that fish are generally the highest sources of contaminants in the food samples analyzed; this is true for both PBDEs and Dechloranes [25]. There is also an association between the levels of PBDEs detected and the lipid content of the fish. To date, there does not appear to be any conclusive evidence with regards to the concentrations of Dechloranes or OPEs and the lipid dependence for the food samples analyzed [23, 26]. For these reasons, the present study has chosen several different species of fish to represent a large range of lipid contents. Moreover, the fish chosen are marine and freshwater species giving a broad sample range. Furthermore, in order to be more representative of an actual diet, several other food groups were chosen, including chicken, leafy green vegetables, bread, butter and canned fish. Another topic that has not been extensively covered in the literature is the impact of food packaging and food contact materials on the levels of contaminants found in food. To gain some insights into this, both packaged and non-packaged food samples were collected when possible. Breast milk is a unique matrix that gives direct insights into the diet of infants and young children to properly assess the levels of legacy and emerging contaminants they are consuming.

The overall aim of the present project was to further our understanding of legacy and emerging EDCs and their metabolites in food and breast milk. More specifically, the objectives of the present thesis were: i) to develop, optimize and validate a multi class residue extraction method that uses less solvent, is simple, fast and less costly than what is currently available in the literature (**AIM 1**); ii) to analyze food and breast milk samples for OPEs and mOPEs which, to date, have not previously been reported in human milk samples (**AIM 2**); and iii) to assess the current dietary exposure to OPEs and mOPEs through diet for Canadians as well as the exposure for young infants through breast milk (**AIM 3**).

A literature review describing the current regulations, the physicochemical properties, metabolism and breakdown, occurrence in food and breast milk as well as the current state of analytical techniques of PBDEs, Dechloranes and OPEs is presented in **Chapter 2**. The optimized multi-class extraction procedure and description of the analytical instrumentation and methodologies is presented in **Chapter 3**. The results obtained from the collected food and human milk samples as well as a dietary exposure assessment for OPEs and mOPEs is discussed in **Chapter 4**. Finally, **Chapter 5** gives a general summary and conclusion for this study, and perspectives for future work are proposed.

CHAPTER 2. LITERATURE REVIEW

2.1. Introduction

Among the environmental contaminants of concern, flame retardants (FRs) represent an important group. With increasing usage, FRs have become ubiquitous contaminants in the environment and numerous studies show their impact on human health [1-3, 15, 27]. Moreover, the most commonly used FRs, the brominated FRs (BFRs) such as PBDEs, or chlorinated FRs (CFRs) such as polychlorinated biphenyls (PCBs), are being replaced by new classes of FRs because of these health concerns. FRs are chemicals that, when added to a combustible material, delay ignition and reduce flame spread with the resulting material when exposed to flame impingement [28]. In the modern world, there exist many applications for FRs in everyday products, buildings, vehicles and clothing. In plastics, FRs may be added as copolymers during the polymerization, mixed with polymers during a molding or extrusion process or applied as a topical finish. However, most FRs are not chemically bonded to the materials they are applied to. Therefore, it is possible for the FRs to migrate/leach out from the materials into the surrounding environment [2].

One group of such replacement FRs are the organophosphate esters (OPEs) and another are the Dechloranes, such as Dechlorane Plus (DP). However, there is still a possibility for these replacement chemicals to have similar persistence, bioaccumulation and toxicological properties in humans as the compounds they were designed to replace. In some instances, replacement chemicals have proven to be more toxic then the chemical they were designed to replace, for example the replacement of BPA by other analogues [29].

There exist two broad categories of FRs: halogenated (CFRs and BFRs) and non-halogenated (most OPEs). The halogenated FRs are generally more hydrophobic compounds, and thus are more

likely to be found at higher concentrations in non-polar matrices. On the other hand, the non-halogenated FRs are slightly more hydrophilic and therefore, are more likely to be present in water samples and other polar matrices. This is an important aspect to consider, since the study of one sample type may not give a complete picture for all groups of FRs.

Animal and human studies have provided evidence of certain detrimental health effects of these FRs: the major endpoints are neurotoxicity and endocrine disruption [15, 30-31]. Endocrine disrupting chemicals (EDCs) interfere with the endocrine systems by various modes of action. Any system in the body controlled by hormones can be "derailed" by endocrine disruptors. Specifically, endocrine disruptors may be associated with the development of learning disabilities, severe attention deficit disorders, cognitive and brain development issues, deformations of the body (including limbs), breast, prostate, thyroid cancers, and sexual development problems such as feminizing effects in males or masculinizing effects in females [15]. Furthermore, some studies have shown that halogenated FRs can be potentially more harmful for human health when compared to the non-halogenated FRs [2].

2.2. Current regulations

2.2.1 Regulations for PBDEs

A Screening Assessment on PBDEs, published in 2006 under the Canadian Environmental Protection Act (CEPA, 1999), concluded that TetraBDE (TeBDE), PentaBDE (PeBDE) and HexaBDE (HxBDE), which are brominated forms of PBDEs, (**Table 2.1**) meet the criteria for persistence and bioaccumulation under the CEPA. Based on the screening assessment carried out under CEPA 1999, the Canadian PBDE regulations prohibit the manufacture of all seven PBDE

commercial mixtures as well as the use, sale, offer for sale or import of TeBDE, PeBDE and HxBDE, HeptaBDE (HeBDE) and OctaBDE (OcBDE) mixtures, polymers and resins containing them [18]. However, the PBDE regulations do not prohibit the use, sale, offer for sale or import of Deca BDE (DeBDE) or mixtures, polymers and resins containing DeBDE [32].

Table 2.1 Composition of PBDEs in commercial mixtures [33]

Congener group	Acronym	CAS No.	No. of individual congeners
Tetrabromodiphenyl ether	TeBDE	40088-47-9	42
Pentabromodiphenyl ether	PeBDE	32534-81-9	46
Hexabromodiphenyl ether	HxBDE	36483-60-0	42
Heptabromodiphenyl ether	HeBDE	68928-80-3	24
Octabromodiphenyl ether	OcBDE	32536-52-0	12
Nonabromodiphenyl ether	NoBDE	63936-56-1	3
Decabromodiphenyl ether	DeBDE	1163-19-5	1

In 2003, the State of California also outlawed the sale of PeBDE and OcBDEs and products containing them (effective January 1, 2008) [34]. In 2008, the US environmental protection agency (US EPA) set safe daily exposure levels of 0.1, 0.1, 0.2, and 7 μg/kg body weight per day for PBDE congeners 47 (TeBDE), 99 (PeBDE), 153 (HxBDE), and 209 (DeBDE), respectively [35].

In 2009, as part of the Stockholm Convention on persistent organic pollutants (POPs), OcBDEs and PeBDEs were officially classified as POPs (SC-4/14, SC-4/18) by the United Nations Environmental Program (UNEP). This ruling has consequently urged the use of alternatives to PBDEs. As of 2009, TeBDE, PeBDE, HxBDE, HeBDE, and DeBDE are listed in Annex A of the Convention, which states that parties must take measures to eliminate the production and use of the chemicals listed [36].

2.2.2 Regulations for Dechloranes

In Canada, Dechloranes are currently not subject to any substance-specific risk management programs. Under CEPA, Dechloranes are listed on the domestic substance list (DSL).

In the US, Dechloranes are listed under the Toxic Substances Control Act as High Production Volume (HPV) chemicals within the HPV Challenge Program. According to this initiative, Dechlorane manufacturers and processors are "challenged" to publish data on the health and environmental effects of chemicals that are produced in or imported in the US in annual quantities of 450 tons or more. In the State of New York, Dechloranes are subject to water quality standards under the Environmental Conservation Law [37].

To date, Dechloranes have not been subject to a risk assessment process by the European Chemical Bureau and no legal restrictions exist for Dechloranes in the EU, and are currently not part of the Stockholm Convention for POPs.

2.2.3 Regulations for OPEs

In Canada, OPEs are regulated on a case-by-case basis and not as a “family” of compounds. To date, the only OPE to have undergone a full draft screening and risk management analysis in Canada is tris(chloro 2-propyl) phosphate (TCPP). This 2016 assessment concluded that TCPP is currently not subject to any substance-specific risk management program [20].

Environment Canada and Health Canada presented a risk management proposal for Ethanol, 2-chloro-, phosphate (3:1) (Tris(2-chloroethyl) phosphate) (TCEP), in 2009, with the objective to

reduce exposures by eliminating it from furniture, electronic products, adhesives, non-apparel textiles, upholstery, carpets, rubber, plastics, and paints and varnishes used in homes [21]. The final extent of the proposal will be determined upon further consultation and discussion with stakeholders.

In the State of California, a bill was passed requiring manufacturers of covered flexible polyurethane or upholstered furniture products subject to TB 117-2013 [38] to indicate whether the product contains added FRs at concentrations above 1000 ppm, by including a statement on the label; this also applies to OPEs.

In the EU, Commission Regulation (EC) No 506/2007 imposes testing and information requirements on the importers or manufacturers of certain priority substances, including tris(2-chloroisopropyl)phosphate (TCPP), in accordance with Council Regulation (EEC) No 793/93 on the evaluation and control of the risks of existing substances [39].TCPP is found on the list of undesirable substances issued by the EPA of Denmark [40].

In Australia, TCPP is subject to secondary notification conditions: under Section 65 of the industrial chemicals (Notification and Assessment) Act (1989), secondary notification of the chemical is required where a person becomes aware of any circumstances that may warrant a reassessment of its hazards and risks [41].

If Dechloranes or OPEs are to be used as replacements for PBDEs, it is important to avoid any compounds that could be equally or more persistent, bioaccumulative and harmful to the health of humans and/or to the environment. Therefore, it is critical to determine if Dechloranes or OPEs are a responsible replacement.

2.3. Physicochemical properties

2.3.1 Physicochemical properties of PBDEs

PBDEs are structurally analogous to PCBs, consisting of two halogenated aromatic rings with varying degrees of bromination. PBDEs are classified using the same International Union of Pure and Applied Chemistry (IUPAC) numbering system as PCBs (**Figure 2.1**), resulting in 209 possible congeners [42].

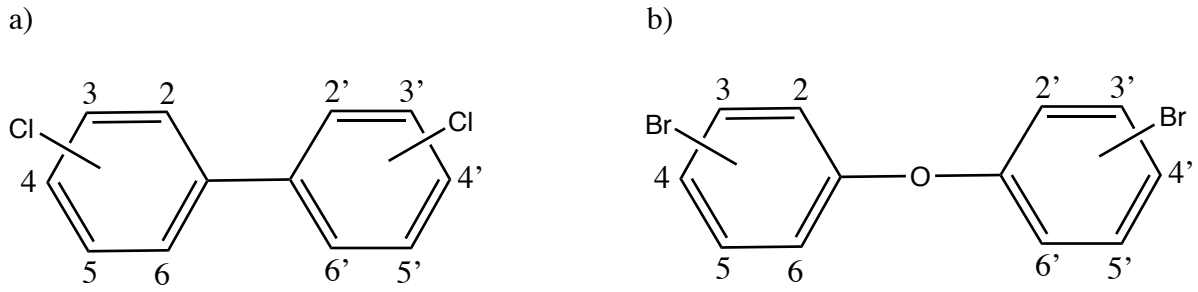


Figure 2.1 Structures of a) PCBs and b) PBDEs with IUPAC numbering system

With the exception of DeBDE, the commercially available PBDE products are not single compounds or even single congeners, but rather mixtures of congeners. The mixtures are blended for optimal flame retardation properties by the manufacturers [42]. As illustrated in **Table 2.1**, not all congeners are found in the commercially available mixtures. This is mainly due to the lack of stability and the potential for debromination of certain congeners, which could decrease the flame retardation potential [43]. The principally used mixtures are the commercial PeBDEs (primarily BDE-99 and BDE-100) and HxBDEs (primarily BDE-153 and BDE-154). The ratios of congeners can vary depending on the product and its final application, but generally it is roughly twice as much PeBDE to HxBDE. The commercial DeBDE mixture consists primarily of the fully brominated DeBDE congener in a concentration of 77-98 %, and smaller amounts of the congeners

of NonaBDE (NoBDEs) (0.3-22 %) and OcBDEs (0-0.04 %). PeBDEs have been steadily decreasing in commercial products due to a voluntary ban in 2003 and a formal ban in 2004 in the European markets [42].

The water solubility of PBDEs decreases with increasing bromine content. Another observable trend is the octanol-water partition coefficient (K_{ow}) values for PBDEs that increase with increasing bromine content [3] (**Table 2.2**). The higher brominated PBDEs are thus more likely to be in non-polar matrices and, the following can be hypothesized even with little information on the behavior of PBDEs in aqueous media: the lower brominated congeners, such as BDE-47 and BDE-99, are expected to be more water-soluble than the higher brominated congeners (e.g. BDE-209), and they are more mobile in water (i.e. higher migration potential). Moreover, one study reported that the higher brominated congeners can bind strongly to sediments, making them less mobile in aqueous media, thus reducing their potential uptake into marine animals [44].

Table 2.2 Structure and physicochemical properties of select PBDEs

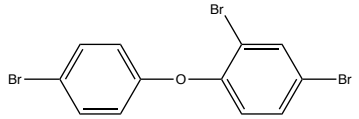
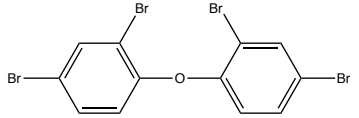
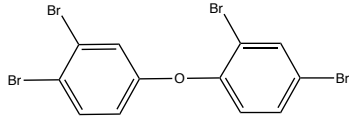
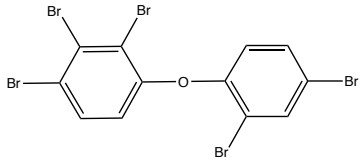
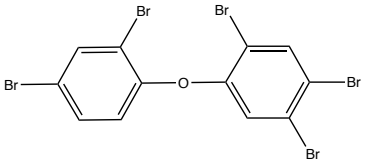
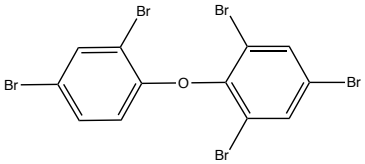
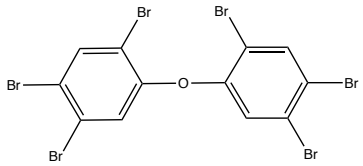
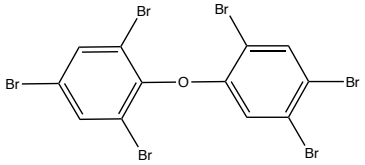
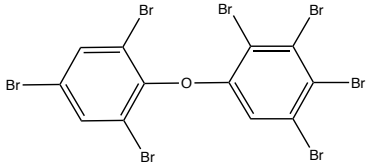
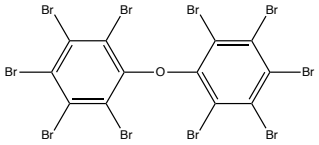
Name	CAS No.	Chemical Formula	Molecular Weight	log K_{ow}	Molecular Structure
BDE 28	41318-75-6	$C_{12}H_7Br_3O$	403.8046	5.5	
BDE 47	5436-43-1	$C_{12}H_6Br_4O$	481.7152	6.2	
BDE 66	189084-61-5	$C_{12}H_6Br_4O$	481.7152	6.2	
BDE 85	182346-21-0	$C_{12}H_5Br_5O$	559.6257	6.9	
BDE 99	60348-60-9	$C_{12}H_5Br_5O$	559.6257	6.9	
BDE 100	189084-64-8	$C_{12}H_5Br_5O$	559.6257	6.9	
BDE 153	68631-49-2	$C_{12}H_4Br_6O$	637.5362	7.6	

Table 2.2 *Continued…*

Name	CAS No.	Chemical Formula	Molecular Weight	log K_{ow}	Molecular Structure
BDE 154	207122-15-4	$C_{12}H_4Br_6O$	637.5362	7.6	
BDE 183	207122-16-5	$C_{12}H_3Br_7O$	715.4467	8.3	
BDE 209	1163-19-5	$C_{12}Br_{10}O$	949.1782	11.75	

2.3.2 Physicochemical properties of Dechloranes

Dechloranes are produced by the Diels-Alder reaction of two equivalents of hexachlorocyclopentadiene with one equivalent of cyclooctadiene (**Figure 2.2**) [4]. The *syn*- and *anti*- isomers of Dechlorane Plus are formed in an approximate ratio of 1:3 [45]. Dechloranes are characterized by very low water solubility, low to very low vapour pressure, and a very high K_{ow}. Other common chlorinated FRs include Dechlorane 602 (Dec 602) and Dechlorane 603 (Dec 603); their structures and details of their physicochemical properties are listed in **Table 2.3**. In general, Dechlorane additives are used as non-plasticizing FRs in polymeric systems and therefore do not bind to the materials they are applied to [4].

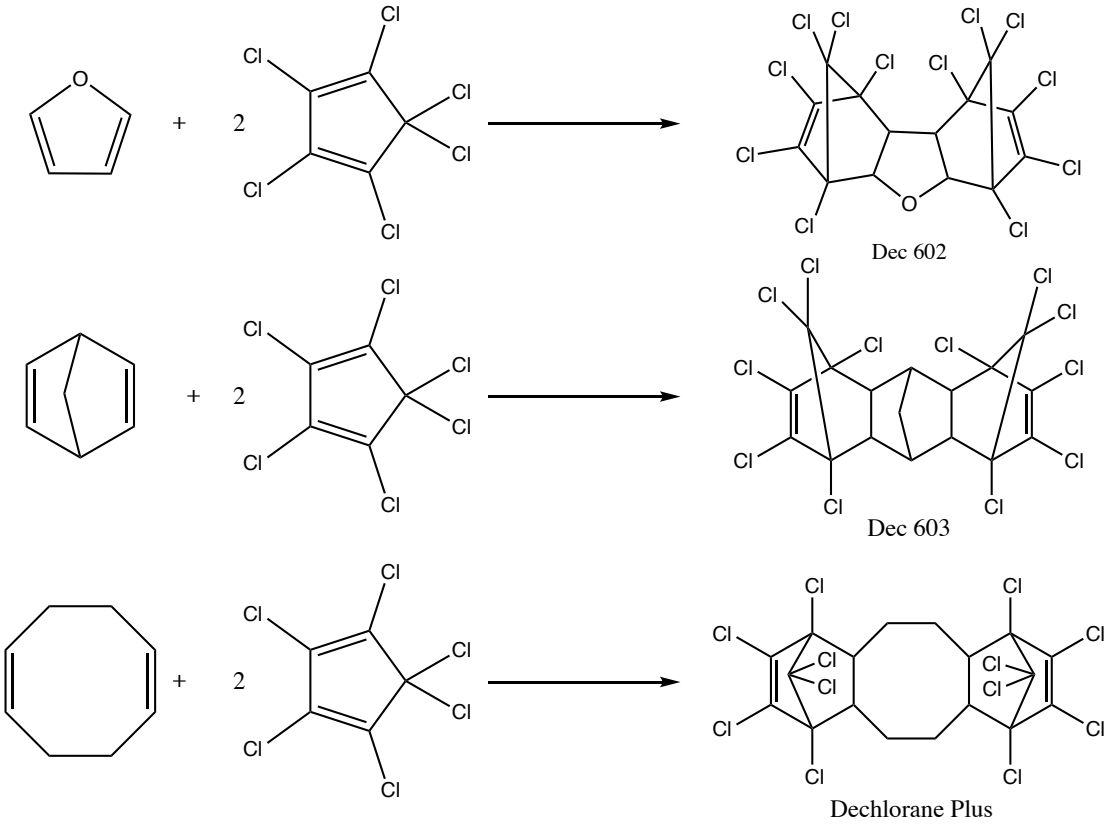
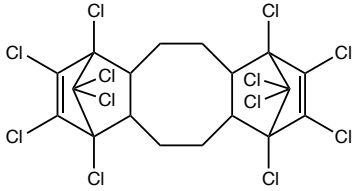
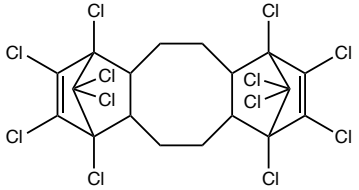
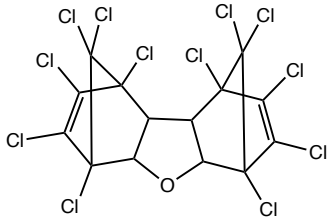
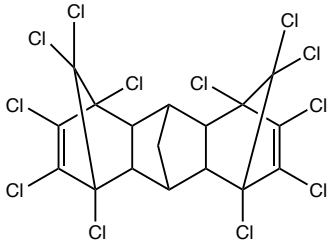


Figure 2.2 Diels-Alder reaction for synthesis of Dechloranes

Table 2.3 Structure and physicochemical properties of select Dechloranes

Name	CAS No.	Chemical Formula	Molecular Weight	log K_{ow}	Molecular Structure
Dechlorane Plus *syn*	135821-03-3	$C_{18}H_{12}Cl_{12}$	653.7240	9.51	
Dechlorane Plus *anti*	135821-74-8	$C_{18}H_{12}Cl_{12}$	653.7240	9.51	
Dechlorane 602	31107-44-5	$C_{14}H_4Cl_{12}O$	613.6170	8.38	
Dechlorane 603	13560-92-4	$C_{17}H_8Cl_{12}$	637.6810	8.24	

2.3.3 Physicochemical properties of OPEs

OPEs can be divided into three main groups; inorganic, organic and halogen-containing. Three different general structures can be further characterized: organophosphate esters, phosphonates, and phosphinates (**Figure 2.3**) [2]. The phosphinates are the most widely used group of halogenated OPEs. They have the properties of both the halogen atoms and the phosphorus components, hence improved flame retardant characteristics. The presence of halogen atoms can also increase the lifetime of the FR in the end-product by decreasing its mobility in the polymer [46]. Examples of OPEs and their metabolites are presented in **Tables 2.4** & **2.5**.

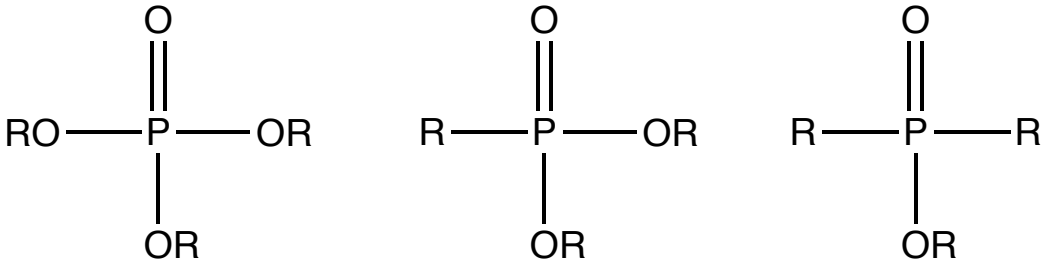


Figure 2.3 General structures of organophosphate esters, phosphonates, and phosphinates

Due to the variations in chemical structures, there is great deviation in physicochemical properties within the OPEs. Depending on the molecular masses and log K_{ow} values, the OPE can be either highly soluble in water or completely immiscible. There is a general trend showing a decrease in solubility with increasing molecular mass, as reported in a review by Van der Veen and de Boer [2]. The authors went on to hypothesize how the OPEs with lower masses are more likely to be found in the aquatic environment than those with higher molecular masses, which is then confirmed through other studies [2]. Most of the OPEs have a positive log K_{ow} value, meaning they are more lipophilic. However, the log K_{ow} values vary considerably between the different OPE groups, from 1.44 to 9.49.

Table 2.4 Structure and properties of select OPEs

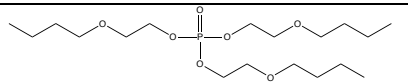
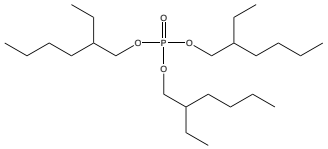
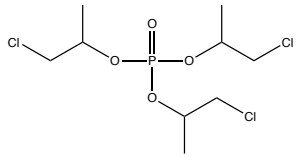
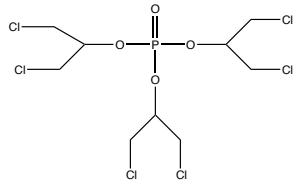
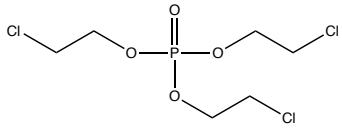
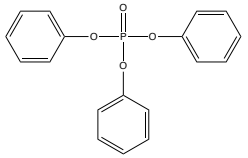
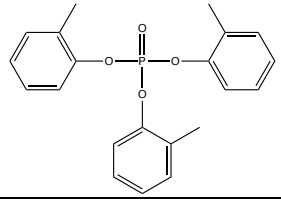
Acronym	Name	CAS No.	Chemical Formula	Molecular Weight	log K_{ow}	Molecular Structure
TBOEP	Tris (2-butoxyethyl) phosphate	78-51-3	$C_{18}H_{39}O_7P$	398.2433	3.75	
TEHP	Tris (2-ethylhexyl) phosphate	78-42-2	$C_{24}H_{51}O_4P$	434.3524	9.49	
TCIP	Tris (2-chloroisopropyl) phosphate	13674-84-5	$C_9H_{18}Cl_3O_4P$	326.0008	2.59	
TDCPP	Tris(1,3-dichloro-2-propyl) phosphate	13674-87-8	$C_9H_{15}Cl_6O_4P$	434.3524	3.65	
TCEP	Tris(2-chloroethyl) phosphate	115-96-8	$C_6H_{12}Cl_3O_4P$	284.9617	1.44	
TPHP	Triphenyl phosphate	115-86-6	$C_{18}H_{15}O_4P$	326.0707	4.59	
TOCP	Tri-o-cresyl phosphate	78-30-8	$C_{21}H_{21}O_4P$	368.1255	6.34	

Table 2.4 *Continued…*

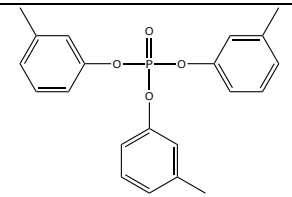
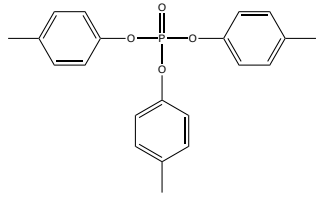
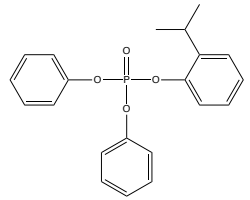
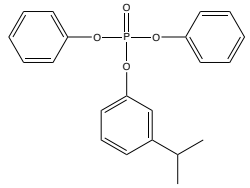
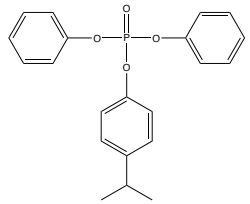
Acronym	Name	CAS No.	Chemical Formula	Molecular Weight	log K_{ow}	Molecular Structure
TMCP	Phosphoric Acid Tris (3-methylphenyl) Ester	563-04-2	$C_{21}H_{21}O_4P$	368.1255	4.00	
TPCP	Phosphoric Acid Tris (4-methylphenyl) Ester	78-32-0	$C_{21}H_{21}O_4P$	368.1255	3.16	
2-ip-PDPP	2-Isopropylphenyl diphenyl phosphate	64532-94-1	$C_{21}H_{21}O_4P$	368.1255	5.44	
3-ip-PDPP	3-Isopropylphenyl diphenyl phosphate	69515-46-4	$C_{21}H_{21}O_4P$	368.1255	5.44	
4-ip-PDPP	4-Isopropylphenyl diphenyl phosphate	55864-04-5	$C_{21}H_{21}O_4P$	368.1255	5.44	

Table 2.5 Structure and properties of select OPE metabolites

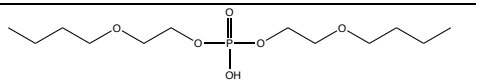
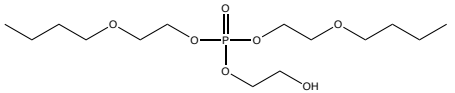
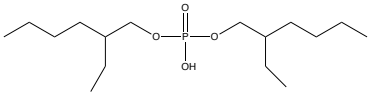
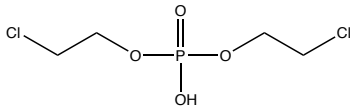
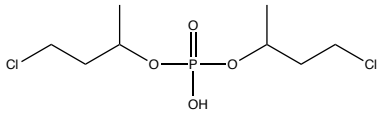
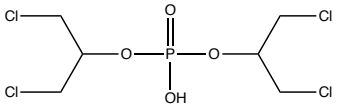
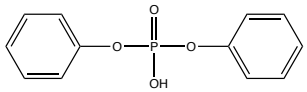
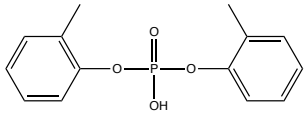
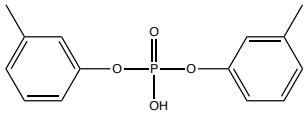
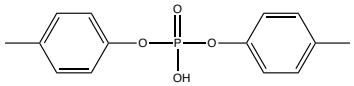
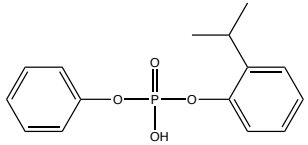
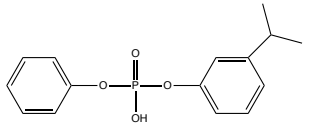
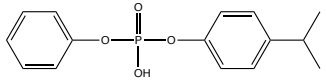
Acronym	Name	CAS No.	Chemical Formula	Molecular Weight	log K_{ow}	Molecular Structure
BBOEP	Bis (butoxyethyl) phosphate	14260-97-0	$C_{12}H_{27}O_6P$	298.3130	2.22	
BTBOEP	Bis (2-butohexylethyl) 2-Hydroxyethyl Phosphate Triester	1477494-86-2	$C_{14}H_{31}O_7P$	342.3700	ND	
BEHP	Bis (2-ethylhexyl) phosphate	298-07-7	$C_{16}H_{35}O_4P$	322.4210	6.09	
BCEP	Bis (2-chloroethyl) hydrogen phosphate	3040-56-0	$C_4H_9Cl_2O_4P$	222.9920	0.21	
BCPP	Bis (1-chloro-2-propyl) phosphate	789440-10-4	$C_6H_{13}Cl_2O_4P$	251.0450	0.91	
BDCP	Bis (1,3-dichloro-2-propyl) phosphate	72236-72-7	$C_6H_{11}Cl_4O_4P$	319.9350	1.61	
DPHP	Diphenyl phosphate	838-85-7	$C_{12}H_{11}O_4P$	250.1870	2.88	
DoCP	Dio-tolyl-phosphate	35787-74-7	$C_{14}H_{15}O_4P$	278.2400	2.26	
DmCP	Di-m-cresyl phosphate	36400-46-1	$C_{14}H_{15}O_4P$	278.2400	2.26	

Table 2.5 *Continued…*

Acronym	Name	CAS No.	Chemical Formula	Molecular Weight	log K_{ow}	Molecular Structure
DpCP	Di-p-cresyl phosphate	843-24-3	$C_{14}H_{15}O_4P$	278.2400	2.26	
o-ip-PPP	o-Isopropylphenyl phenyl phosphate	NA	$C_{21}H_{21}O_4P$	368.3630	5.44	
m-ip-PPP	m-Isopropylphenyl phenyl phosphate	NA	$C_{27}H_{33}O_4P$	452.5220	8.11	
p-ip-PPP	p-Isopropylphenyl phenyl phosphate	69415-02-7	$C_{15}H_{17}O_4P$	292.2700	NA	

2.4. Metabolism in the Environment

2.4.1 PBDE metabolism and breakdown

PBDEs are classified as persistent, however, they can break down in the environment when exposed to factors such as heat, light and UV rays, resulting in compounds such as hydroxylated-PBDEs (OH-PBDEs) and methoxylated-PBDEs (MeOH-PBDE) [47-49]. Anthanasiadou et al. (2008) reported a relationship between PBDEs and OH-PBDEs detected in human serum; the serum of children working at an e-waste disposal area in Nicaragua with high levels of PBDEs also had high levels of PBDE metabolites [47-49]. Nevertheless, metabolism and/or breakdown of PBDEs are rarely reported in the literature; there have been limited studies evaluating the presence of OH-PBDEs or MeOH-PBDEs in the environment, foodstuff and breast milk [48-51].

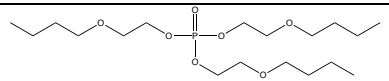
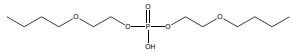
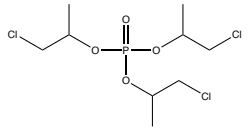
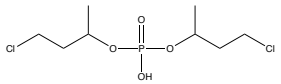
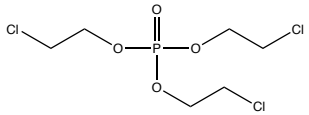
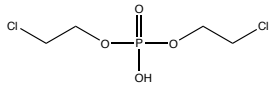
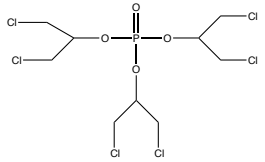
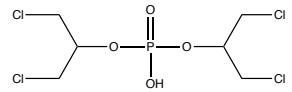
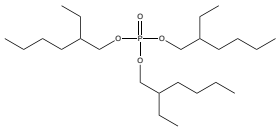
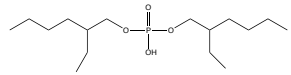
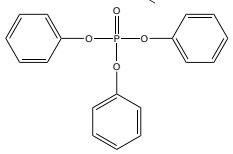
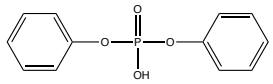
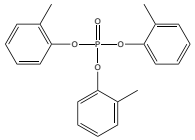
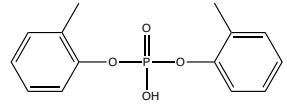
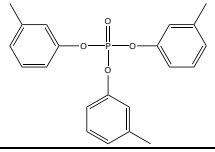
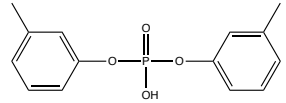
2.4.2 Dechlorane metabolism and breakdown

Currently, in the scientific literature, the data on biomonitoring of Dechloranes in the food web or in humans is not extensive. Studies have investigated the presence of Dechloranes in serum in Canada [52] and in Norway [53]; the highest levels were reported near e-waste recycling plants in China [54]. The results from the study conducted by Yan et al. (2012) [54], on Dechlorane levels in workers at an e-waste recycling plant, suggest isomer specific metabolites (i.e. a preferential metabolism of Dechlorane Plus *anti* over Dechlorane Plus *syn*). However, the mechanisms for the metabolism of Dechloranes have not yet been elucidated. Furthermore, the study was not able to positively identify the metabolites using authentic analytical reference standards, which leaves some uncertainty as to whether the metabolites of Dechloranes are, in fact, present in the samples. More studies are, thus, needed to determine if Dechloranes can be metabolized in the environment, or in humans, and if they can be absorbed by humans through food or by infants via breast milk [11].

2.4.3 OPE metabolism and breakdown

Based on the physicochemical properties of OPEs, they are subject to metabolism or breakdown. In laboratory studies, OPEs readily metabolize to their dialkyl or diaryl phosphates and to a variety of hydroxylation products [55] (**Table 2.6**). Several biomonitoring studies have been conducted on OPE metabolites (mOPEs) in house dust [55], urine [56] and food [23] as well as other matrices [57]. However, the parent to metabolite ratios have not been studied in detail. Moreover, it is not well understood if the mOPEs are due to environmental factors causing breakdown products (e.g. heat, light, UV rays) or by enzymatic reactions within the organism caused by metabolism. More research into the relationship between the parent OPE compounds and their metabolites is needed.

Table 2.6 Structure of OPE Parent compound and select OPE metabolite

Parent compound	CAS No.	Chemical Structure	Metabolite	CAS No.	Chemical Structure
TBOEP	78-51-3		BBOEP	14260-97-0	
TCIP	13674-84-5		BCIP	789440-10-4	
TCEP	115-96-8		BCEP	3040-56-0	
TDCIPP	13674-87-8		BDCP	3040-56-0	
TEHP	78-42-2		BEHP	789440-10-4	
TPHP	115-86-6		DPHP	838-85-7	
TOCP	78-30-8		DOCP	35787-74-7	
TMCP	563-04-2		DMCP	36400-46-1	

2.5. Occurrence of environmental contaminants in food and breast milk

There are various possible ways for humans to be exposed to environmental contaminants. Dietary exposure is a major route although dust, air and water are also important to assess. It is critical to assess food in order to position it within the total risk assessment context [58]. FRs may accumulate in food due to environmental exposure (contamination) or possibly through food processing and storage via food contact and/or packaging materials [59]. However, there is currently not enough evidence to derive any conclusions for the processing or storage of food being a source of FRs. It should be noted that the types of foods that are processed (e.g. breads, baked goods) tend to be higher in lipids than the less processed foods (e.g. vegetables) and, thus, would be more likely to contain lipophilic contaminants. The levels of contaminants in similar food products can vary both within and between studies and, for this reason, it is still difficult to make general conclusions on the levels of contaminants in specific food types. Future research should focus on a varied selection of foods ranging from low to high lipid content as well as the degree of processing.

Several studies have analyzed contaminants in breast milk, notably PBDEs; however, there is very little information with regards to Dechloranes, OPEs or their metabolites in this matrix.

2.5.1 Occurrence of PBDEs in food and breast milk

In 2002, Ohta et al. reported concentrations of PBDEs in fish, meat and vegetables sold in two food markets in the city of Hirakata, Japan. The authors also measured the concentrations of PBDEs in the breast milk of 12 primiparae nursing women and aimed to ascertain the relationship between the levels of PBDEs found in the diet and the levels found in humans [60]. The sum of

PBDE (∑PBDE) concentrations ranged from 21-1650 pg/g fresh weight in the edible tissues of five species of fish and one species of shellfish. The highest concentrations were measured in yellow-fin tuna, followed by short-necked clam, salmon, yellowtail, mackerel and young yellowtail. Interestingly, the difference in ∑PBDE concentrations between cultured mackerel samples from Japan and northern European waters was not statistically significant ($P<0.05$). ∑PBDE concentrations in beef, pork and chicken meat (6.25-63.6 pg/g w.w.) and in three different vegetables (38.4-134 pg/g w.w.) were lower ($P<0.05$) than the concentrations in fish. In human milk, ∑PBDE concentrations ranged from 668-2840 pg/g lipid weight basis, which is comparable to the levels of PBDEs reported in populations of nursing women in Sweden [61]. Ohta et al. reported a strong positive relationship between PBDE concentrations in human milk and dietary intake of fish and shellfish, which was established by responses of women to a questionnaire on dietary habits. The authors concluded that additional investigations of PBDEs in fish and in various food products are warranted to better understand the nature and extent of PBDE contamination in food[60].

A US-based market basket study was completed in 2004 by Schecter et al. that measured concentrations of 13 PBDE congeners in 62 food samples to estimate the levels of PBDE intake by the US population through dietary exposure [9]. The authors found that fish had the highest overall PBDE levels (median of 1725 parts per trillion (pg/g), range: 8.5-3078 pg/g, (wet weight) w.w.), followed by meat products (median of 283 pg/g, range: ND (0.2)-1373 pg/g), and dairy products (median of 31.5 pg/g, range: 0.9-679 pg/g). The range of PBDE concentrations in fish purchased in US supermarkets was significantly greater than meat samples (beef, pork, turkey and duck). One salmon fillet had the highest total concentration of PBDEs (3078 pg/g) of all food specimens, whereas tilapia fillets had the lowest level of total PBDEs (8.5 pg/g) in fish. Dairy products had even lower levels of PBDEs, ranging from 0.9 pg/g in evaporated milk to 679 pg/g

in cheese. The results illustrate that food of animal origin in the US has high levels of PBDEs. The authors further conclude that a large variation of PBDE levels in foods exists, even for the same types of food. Although this is a large PBDE food survey in the US, the authors could not claim that these data are a representative sampling of the US diet. As with other published surveys from other countries, the sample size should be increased and the samples should be representative of the diet(s) of the respective nations. Until this is done, uncertainty in estimates of food levels will persist and as a result, intake estimates will be imprecise.

A Belgian-based food market basket study was completed in 2007 by Voorspoels et al. that was representative of the diet for the general population, consisting of various meats, fish and dairy products, to analyze the PBDE content [62]. Additionally, fast food samples were investigated. Based on the measured PBDE levels, an average daily dietary intake estimate of PBDEs was calculated. Of the foods analyzed, fish had the highest average ∑PBDE levels (BDEs 28, 47, 99, 100, 153, 154, and 183; 460 pg/g w.w.), followed by dairy products and eggs (260 pg/g w.w.), fast food (86 pg/g w.w.) and meat products (70 pg/g w.w.). One fresh salmon fillet had the highest total concentration of PBDEs (2360 pg/g w.w.), whereas levels in steak and chicken breast were the lowest of all foods analyzed. BDE-209 was not found above the LOQ (800 pg/g w.w.) in any food sample analyzed. The authors reported that, although it is only a minor constituent of the Belgian diet, fish is a major contributor to the total daily PBDE intake, about 40%, while meat products account for about 30% of the total dietary intake of PBDEs. Dairy products and eggs contribute to a lesser extent. The authors conclude that, with the exception of fish, levels of PBDEs in the Belgian diet are low. The total intake was estimated between 23 and 48 ng/day.

The aforementioned studies have, for the most part, reported similar intake distributions in foodstuffs for PBDEs. PBDE congeners 47, 99, 100, 153, and 154, and in some cases 209, are

major contributors in foods. However, comparison of results between studies is complicated by the fact that there is no standardized manner of reporting results and the congeners included in the summation of PBDEs sometimes differ between studies. Comparison of the results obtained for meat is also difficult, since different foods were analyzed in each study and put under the umbrella term "meat", such as duck, turkey, and bacon. From the multiple studies that are available in the literature, it can be concluded that fish contain the highest levels of PBDEs.

2.5.2 Occurrence of Dechloranes in food and breast milk

Unlike PBDEs, Dechlorane exposure through diet has not been assessed through large market-basket studies. The studies to date have focused mainly on fish and oils. It is therefore difficult to make accurate exposure assessments given the current literature

Kakimoto et al. (2012) analyzed fish samples collected from Japanese markets for Dechlorane Plus (*syn*- and *anti*- isomers) [25]. DP was detected in 90% of the samples and ∑DP concentrations were <0.2-14.2 pg/g w.w.. Among the DP isomers, *anti*-DP was the dominant residue observed in this study. In fish landed near the East China Sea and the Sea of Japan, the authors detected relatively high concentrations of DP. This study illustrated that DP can be detected in fish marketed in Japan, despite there being no DP manufacturing facility in Japan. Concentrations of ∑DP were approximately one hundredth of those of ∑PBDEs previously reported in the same matrices.

Von Eyken et al. (2016) determined the levels of DP and Dechloranes in fish and vegetable oil samples from Catalonia (Spain); most of the oils were health supplements [63]. Dec 602 and Dec 603 were not detected in vegetable oil samples, but were found in most of the fish oil samples,

while Dec 604 was below the limit of detection (LOD) in all the studied samples. Both isomers of DP were found in all vegetable oils and mixtures of vegetable and fish oil samples. Generally, total ∑DP was higher in feed oils than in food oils. Values obtained for vegetable oils in this study are in the same order of those reported previously by Kim et al. (2014) for soy oil in Korea (3.32 pg/g for *syn*-DP and 15.81 pg/g for *anti*-DP) [64]. In 9 out of 12 oil samples where the mean and median isomer ratio values (fanti) were calculated by Von Eyken et al., the value was below the industrial rate, between 0.6 and 0.8 depending on the origin of the product, suggesting that some enrichment of the *syn*-isomer could be possible [63].

Dechlorane levels in human milk from two Canadian cities were studied by Siddique et al. in 2012 [11]. The levels of DP (mean value of 0.98 ng/g and a median value of 0.60 ng/g (l.w.)) were two to ten times lower than the currently measured levels of PBDEs, including BDE-209 [11]. The authors found that there was little difference in the levels of measured contaminants in milk samples (n=87) collected from the two cities. Furthermore, the fanti for DP in milk were 0.67 and 0.69, respectively, very similar to that of DP commercial products (0.75-0.80). This study was the first to report the levels of DP in human milk in perspective to those of PBDEs.

A later study (2014) by the same author analyzed five hexachloronorbornene-based FRs (DP, Dec 602, Dec 603, Dec 604 and hexachlorocyclopentadienyl-dibromocyclooctane (HCDBCO)) in human milk (n=105) [65]. Dec 602, Dec 603 and HCDBCO had detection frequencies over 60%, Dec 604 was only detected in 4.8% of milk samples while DP was present in 40–50%. The ratio of the two DP isomers found in human samples (fanti-DP = 0.8) remained similar to the ratio reported in the DP technical mixture (0.75-0.80). These biomonitoring results have provided baseline information about the presence of norbornene flame retardants in nursing women in Canada, which can be used for estimating human exposure to these chemicals. However, more

studies on the presence of these FRs in humans are needed to better understand the extent and nature of human exposure to these chemicals.

2.5.3 Occurrence of OPEs in food and breast milk

To date, there is very limited information available with regards to biomonitoring data for OPEs in food and breast milk [57]. Seeing that OPEs are the main candidate for PBDE replacement, and the production volume has increased tremendously in the past years, more information regarding these chemicals is essential.

Poma et al. (2017) investigated the occurrence of eight OPEs in 53 composite food samples from 12 food categories, collected in 2015 for a Swedish food market basket study [66]. EHDPHP, detected in most food categories, had the highest median concentration (9000 pg/g w.w., in pastries). It was followed by TPHP (2600 pg/g w.w., in fats/oils), TDCIPP (1000 pg/g w.w., in fats/oils), TCEP (1000 pg/g w.w., in fats/oils) and TCIPP (800 pg/g w.w., in pastries). The authors reported that the major contributor to the total dietary intake of OPEs is EHDPHP (57%), and the food categories which contributed the most to the total intake of OPEs were processed food, such as cereals (26%), pastries (10%), sugar/sweets (11%) and beverages (17%). The daily per capita intake of OPEs (TCEP, TPHP, EHDPHP, TDCIPP, TCIPP) from food ranged from 406-3266 ng/day (or 6-49 ng/kg bw/day). The authors concluded that the estimated human dietary exposure to OPEs may be equally important, if not more, than exposure via the ingestion of dust [59]. The percentage of non-detects varied between 55 and 100%, depending on the compound. TEHP, TNBP, and TBOEP were not detected in any of the analyzed food samples and, therefore, they were not included in the subsequent estimation of per capita intake. The high percentage of non-

detects could be due to a low accumulation/concentration of these compounds in biota and consequently in the human food chain. This lower accumulation/concentration of OPEs could also be due to their rapid metabolism/excretion [57]. Interestingly, the distribution of OPEs in the analyzed food categories show that cereals, pastries, fats/oils and sugar/sweets have the highest median OPE contamination, but not fish, which was the food type with the most positive detects for both PBDEs and Dechloranes. Cereals, pastries, fats/oils and sugar/sweets are more industrially processed compared to the other food groups, possibly suggesting that their contamination arises during food processing.

A more recent study, published in 2018, by Poma et al. investigated the occurrence of 14 OPEs in 165 composite food samples purchased from the Belgian market and divided into 14 food categories, including fish, crustaceans, mussels, meat, milk, cheese, dessert, food for infants, fats and oils, grains, eggs, potatoes and derived products, other food (stocks) and vegetables [59]. Seven OPEs (TnBP, TCEP, TCIPP, TDCIPP, TPHP, EHDPHP and TEHP) were detected at concentrations above quantification limits. Fats and oils were the most contaminated category, with a total OPE concentration of 84400 pg/g of w.w., followed by grains (36900 pg/g of w.w.) and cheese (20100 pg/g of w.w.). These results support the hypothesis that OPE contamination may occur during industrial processing and manipulation of food products (e.g. packaging, canning, drying, etc.). The authors used these data to estimate the dietary exposure for the model adult Belgian (15−64 years of age): ≤7500 ± 1550 ng/day (equivalent to 103 ± 21 ng/kg bw/day). For individual OPEs, TPHP contributed on average 3400 ng/day (46.6 ng/kg bw/day), TCIPP 1350 ng/day (18.5 ng/kg bw/day) and EHDPHP 1090 ng/day (15 ng/kg bw/day). The mean dietary exposure mainly originated from grains (39%), followed by fats and oils (21%) and dairy products

(20%). No significant differences between the intakes of adult men and women were observed by taking into account the differences in consumption of foods between sexes.

As well as testing urinary metabolites of organophosphate esters in Australian children, He et al. (2018) analyzed three breast milk samples [23]. The authors concluded that the estimated daily intakes (EDIs) via breastfeeding were 4.6, 26 and 76 ng/kg/day for TCEP, TBP and TEHP, respectively, and were higher than exposure via air and dust, suggesting higher exposure to OPEs through consumption of breast milk. Although this study only tested three samples, the results illustrate that breast milk is an important exposure route for young infants, before they are crawling in dust, which may then become a more important source. However, the sample size remains very limited and the diets of the woman who donated samples were not measured. This therefore does not give any insights on the impact of maternal intake of food on the concentration of OPEs in human milk.

2.6. Analytical methods for the determination of PBDEs, Dechloranes, OPEs and mOPEs in food and breast milk

2.6.1 Analytical detection methods for PBDEs in food and breast milk

PBDEs' lipid affinity requires non-polar to medium polarity solvents for their extraction from different matrices. Solvents commonly used for the extraction of PBDEs from food of animal origin are *n*-hexane (hxn), dichloromethane (DCM), acetone, ethyl acetate (EtAc), acetonitrile (ACN) or a mixture of these solvents. Common extraction methods used for other environmental contaminants, such as PCBs, dioxins or furans, may not be efficient for all of the PBDE congeners [22]. On reviewing the published literature, the most common extraction techniques for PBDEs are:

Soxhlet, pressurized liquid extraction (PLE), liquid-liquid extraction (LLE), assisted microwave extraction (MAE) and ultrasound extraction.

With the advance of extraction technologies, multi-residue methods to extract and clean up samples are appearing in more recent literature. The various techniques used for extractions often result in co-eluting interferences along with the desired analyte, hence clean-up is a necessary step prior to instrumental analysis, especially for trace level analysis. Acidified silica is often used as a sample clean up step to remove lipids and other interferences. In some cases, depending on the complexity of the matrix, further clean up steps must be applied such as the use of basic alumina columns and/or graphitized black carbon [22]. Since PBDEs are stable and non-polar compounds, they will not be destroyed by acids, even when using silica gel is acidified with sulfuric acid at 44% (w/v). Moreover, due to the lack of polarity, PBDEs will not be retained by highly polar clean up columns (such as aminopropyl silica) whereas any polar interferences will be.

Lyophilization (freeze drying) is an effective method of concentrating samples prior to extraction. This method also avoids later separation of organic and aqueous phases, which could cause problems during the drying steps and further on in the analysis [67].

Gas chromatography (GC) coupled to mass spectrometry (MS) is the technique of choice for the separation and quantification of PBDEs. PBDEs have low polarity, therefore the majority of selected GC columns used contain dimethylpolysiloxane with modifications from 1% to 50% phenyl. The highest resolution, stability and response for PBDEs were achieved when analytical columns with 1–5% phenyl additive were applied [68]. BDE-209 separation using columns longer than 30 m seems to be very challenging and in many cases not possible at all. This is due to the potential for higher brominated compounds to be debrominated at the elevated GC temperatures

[22]. Some methods in the literature make use of two columns, a shorter one (5–15 m) for unstable congener determination (OcBDE, NoBDE, DeBDE), and a longer column (30–60 m) for optimal separation of other PBDEs. Both the steep temperature gradient and fast mobile phase flow (2-3 ml/min) can help reduce BDE-209 degradation in the GC column prior to detection [68].

High resolution MS (HRMS) techniques are able to achieve LOD values below 100 pg/g lipid weight (l.w.) for PBDE congeners with nine or fewer bromine atoms. Triple quadrupole GC tandem MS instruments GC-MS/MS (QqQ) have also performed well for PBDE analysis.

Magnetic sector HRMS is still characterized by the highest specificity and precision in PBDE analysis and is considered to be a reference technique for PBDE analysis [68].

2.6.2 Dechloranes in food and breast milk

Similar to PBDEs, Dechloranes require non-polar to medium polarity solvents for their extraction from different matrices. The same methods used in Section 2.6.1 are used for Dechlorane extraction from food and breast milk samples. Since Dechloranes are stable and non-polar compounds, they will not be destroyed by acids, even when using silica gel acidified with sulfuric acid at 44% (w/v). Moreover, due the lack of polarity, Dechloranes will not be retained by highly polar clean up columns (such as aminopropyl silica) whereas any polar interferences will be.

For the determination of Dechloranes, the majority of studies performed so far used electron ionization (EI)-MS(/MS) or electron capture negative ionization (ECNI)-MS(/MS) with the need of an additional injection, separated from the one used to separate PBDEs [69]. Similar to PBDEs, the analysis of Dechloranes can benefit from selecting more specific transitions coming from the

molecular ion by using softer ionization sources. Several studies have investigated the different fragmentation of *anti*- and *syn*-DP isomers under variable ECNI source temperatures, either in full-scan or in selected ion monitoring (SIM) experiments, but not yet in multiple reaction monitoring (MRM) experiments. Selectivity problems are often encountered when single quadrupole MS systems are used for detection of Dechloranes, while the application of MS/MS based techniques significantly reduces the probability of interference. However, selectivity issues may be still encountered in ultra-trace analysis of POPs using MS/MS methods. Therefore, the GC-HRMS technique is still recognized as the "gold standard" in the analysis of POPs at ultra-trace levels [68].

2.6.3 Analytical detection methods for OPEs in food and breast milk

Prior to extraction, samples are often lyophilized in order to concentrate OPEs and to remove any moisture which facilitates extraction [67]. Non-polar to polar solvents such as hxn, DCM, EtAc and ACN can be used for extraction. Some of the successfully employed extraction methods for OPEs are: Soxhlet, PLE, LLE, MAE and ultrasound extraction. The difficulty for extraction of OPEs from foodstuff lies in the removal of lipids and other non-polar interfering substances since OPEs are degraded easily by strong acids [2]. Therefore, several column cleanup methods have been tried, such as basic alumina or modified QuEChERS techniques [70-71]. For the most part, all food samples (including breast milk) are treated similarly for lipid removal [44, 59, 72-73]. However, few studies have analyzed fruits and vegetables, which could have potential interferences if pigments are not properly removed; in such cases, another sample cleanup step either before or after lipid removal may be necessary. There are no official methods in place, as of yet, for the extraction of OPEs from foodstuff.

One area of research that has not been explored in depth is the extraction and analysis of the metabolites of OPEs. Van den Eede et al. (2013) analyzed mOPEs in human liver tissue samples [74]. Recently, He et al. (2018) extracted OPEs along with their metabolites from various food matrices [23]. Interestingly, the mOPEs follow the same extraction pathway as their parent compounds. It can be rationalized that since the mOPEs are equally, if not more, polar than their parent compounds, they will be co-extracted. However, to positively confirm this, isotopically labelled surrogates are required. To date, there are few chemical standard suppliers that offer these labeled surrogates and most mOPEs are custom synthesized. This, therefore, adds a challenge to the analysis of OPEs.

For the instrumental analysis of OPEs, several techniques, such as GC–Flame Photometric Detector (FPD), GC–Nitrogen Phosphorus Detector (NPD), GC–Atomic Emission Detector (AED), GC–MS and LC–MS/MS are currently applied [2]. Many recent studies have reported good results (i.e. recovery, accuracy and precision) using LC-MS/MS, such as the work done by Santin et al. which used LC-quadrupole-linear ion trap MS (LC-MS-qLIT) for simultaneous determination of 16 OPEs and plasticizers in fish [73]. A study by Giulivo et al. (2017) developed an online sample purification and analysis technique using LC-MS for OPEs [44]. The trend in analysis for OPEs leans towards the use of LC-MS/MS systems although many studies have obtained acceptable results for a wide range of OPEs using GC-MS/MS. For future work studying metabolites of OPEs, LC uses a less destructive separation technique than the GC systems. Thus, for complete analysis of OPEs and their metabolites, it seems LC-MS/MS may be the preferred analytical method.

2.7. Conclusion

There have been many useful studies and reviews of the various groups of FRs on topics such as toxicity screening, risk assessment, analysis in biological, food and environmental samples as well as their physicochemical characterization. These studies have been invaluable for improving public health by identifying harmful chemicals. However, "gaps" still exist in the current literature. In addition, studying both legacy and emerging contaminants in parallel will better inform everyone, from manufacturers of products containing FRs to policy makers and consumers, about the need to find responsible replacements.

As mentioned earlier, select PBDE mixtures, especially the lower brominated mixtures (e.g. TeBDE, PeBDE, HxBDE), have been banned in many countries. However, in the current toxicity studies, only select PBDE congeners have been evaluated and analyzed. Many higher brominated PBDEs are still in use [5]. Generally, PBDEs are applied to products as commercial mixtures, therefore characterizing the toxicity of only single PBDE mixtures has left an incomplete dataset and should be further investigated while considering the metabolic pathways of the compounds of interest [75]. This is also true for the Dechloranes. Few studies have been conducted on a wide epidemiological basis and the toxicity studies have been conducted mainly in fish and chickens. This creates very high uncertainty from a risk assessor's point of view for translating data to humans. As for the OPEs, not every compound has been evaluated with regards to toxicity, but as screening technology develops, it will become easier to test multiple environmental contaminants in one analysis. In addition, the question of mixtures of OPEs and mOPEs and their health effects remains unanswered: these FRs are generally used in combination (e.g. Firemaster® 550) (**Table 2.7**) due to their varied mechanisms of flame retardancy and their additive effects. Thus, it can be

assumed that mixtures of these compounds will be found in samples as opposed to a single OPE. Their combined toxicological effects have, to date, not been assessed in great detail.

Table 2.7 Main chemical composition of Firemaster® 550

Compound	Acronym	CAS No.
Isopropylated triphenol phosphate	ip-PDPP	68937-41-7
2-ethylhexyl 2,3,4,5-tetrabromobenzoate	TBB	183658-27-7
Triphenyl phosphate	TPHP	115-86-6
Bis(2-ethylhexyl) 3,4,5,6-tetrabromophthalate	TBHP	26040-51-7
1,3-Isobenofurandione, 4,5,6,7-tetrabromo-, reaction products with 2-ethyl-1-hexanol	NA	219632-53-8

In terms of occurrence, PBDEs and Dechloranes have both been reported to be ubiquitous contaminants and are both persistent in the environment and capable of long range transport; they have been found in samples from the Arctic to Antarctica [45]. However, there have not been any major studies quantifying these compounds in the Canadian food market; the same is true for OPEs. These data can serve to help policy makers determine which compounds can be produced, sold and imported into the Canadian market. They can also be useful to determine how replacements of chemicals should be carried out in the future on a national or international basis.

On the analytical detection side, there has been progress for combined extraction methods for PBDEs, Dechloranes and OPEs. However, the extraction process is laborious and complicated due to the wide range in chemical properties for each family of compounds. Method optimization and validation for all food matrices has not yet been accomplished. There have been a few recent studies that reported the metabolites of OPEs in food, urine and breast milk. It would be very interesting to monitor the fate of the OPEs from "farm-to-fork", including the potential degradation

during processing and/or cooking of the food product. No studies have evaluated any effects of processing foods and the degradation of OPEs, PBDEs or Dechloranes using mass labelled surrogates for their respective metabolites. Many studies have only evaluated the parent OPE compounds, and this could be underestimating the actual amount of OPEs present in our food supply. This is especially true for matrices such as breast milk since it is very likely that some of the OPEs will be metabolized by the mother and transferred to the child through breast feeding. This may also lead to studies that evaluate the effect of pasteurization on the contaminants in breast milk. Since breast milk donation banks Pasteurize the milk that is donated, it would be useful to understand the thermal degradation of these compounds. Furthermore, it may be possible that some of the metabolites are produced during freeze-thaw cycles when storing breast milk over long periods of time; however, this has yet to be evaluated.

From the instrumentation standpoint, with the advancement of analytical technology and HRMS instruments, several methods have been established that are able to detect the chemicals of interest at very low levels. Increasingly, there are more studies that are including multiple FRs in their analysis to get a more complete picture and a better understanding of the environmental contamination in foods and its potential role in exposure for humans. However, quantifying all the analytes along with their metabolites (in some cases it can be more than one metabolite per parent compound) becomes challenging in a single analysis. Using isotopically labelled surrogates throughout the analysis has become the norm even with the high cost associated with this technique. However, it has not been established if the metabolites will behave the same way as their parent compounds either in terms of extraction properties or analysis. Further studies are required to develop simplified and robust methods of analysis that can be used for routine

monitoring of all major classes of OPEs and mOPEs in different matrices and relate these metabolites to their parent compounds.

Overall, there are several challenges that we need to overcome in order to better understand the effects that legacy and emerging contaminants may have on human health. This can be accomplished by developing robust extraction methods that simplify the extraction efficiency of all compounds of interest from food and biological matrices. Moreover, by screening key food items and breast milk, it is possible to get a first look into the levels of legacy and emerging contaminants that could be consumed by Canadians. These data can be a driving force in shaping policy and changing international regulations regarding the assessment of emerging chemicals.

CONNECTING STATEMENT I

Chapter 2 presents a literature review on the current state of legacy and emerging contaminants including PBDEs, Dechloranes and OPEs. The review covers the current regulations in Canada, the US and internationally, the physicochemical properties of PBDEs, Dechloranes and OPEs, their metabolism and breakdown in the environment, their occurrence in food and breast milk and the current analytical detection methods used for their quantification.

Chapter 3 presents a novel multi-class extraction method as well as the instrumental analysis that has been developed, optimized and validated for PBDEs, Dechloranes, OPEs and mOPEs. The analytical parameters including recoveries, accuracy, precision, MDLDs and MLOQs as well as critical considerations of the method are discussed in detail. Separation of TCP and ip-PDPP isomers is presented and discussed. Lastly, several food samples including fish, chicken, vegetables, canned fish, bread and breast milk were analyzed.

CHAPTER 3. PBDE, DECHLORANE, OPE & mOPE METHOD DEVELOPMENT

3.1. Introduction

In recent years, OPEs and Dechloranes have emerged as a prevalent class of flame retardants and plasticizers to replace legacy compounds such as PBDEs due to their global phase-out [1, 5-6]. PBDEs have been shown to meet all the criteria for POPs such as persistence in the environment, long range transport, bioaccumulation and toxicity [3, 15]. Unfortunately, some of the emerging replacement chemicals exhibit similar detrimental properties [19-20, 76]. Moreover, many biomonitoring studies have shown OPEs and their metabolites to be present in food [23, 44, 59, 66, 72-73, 77-78], dust [5-6, 79-80], water [1, 57, 81], urine [55-56] and breast milk [1, 12]. The presence of both legacy and emerging contaminants in breast milk is especially concerning for infants since this is a uniquely sensitive period of development for humans [13-14, 82].

Therefore, it is important to develop robust and sensitive methods capable of monitoring a large range of environmental contaminants, both legacy and emerging. Biomonitoring studies should be designed in such a way as to be able to detect and quantify both parent compounds and metabolites of chemicals of concern in order to properly assess their daily intake. Such an analytical method must be amenable to a variety of matrices with varying degrees of complexity. In the present study, we developed a novel multiresidue method for simultaneous extraction of polar and nonpolar contaminants (i.e. OPEs/mOPEs, PBDEs/Dechloranes; log K_{ow} ranging from XX to YY) from food and breast milk. The method is inexpensive, quick and scalable. It relies on the fractionation of the nonpolar PBDEs and Dechloranes from the polar OPEs and mOPEs. Cleanup steps were determined according to the physicochemical limitations of each analyte [2] and final extracts were

reconstituted in appropriate solvents for GC and LC separation. This is the first time PBDEs, Dechloranes, OPEs and mOPEs are simultaneously extracted from food and breast milk samples. The samples selected for analysis included breast milk and a number of foods varying in their lipid, protein, water, carbohydrate and pigment contents. This has led us to demonstrate the robustness of the developed multiclass extraction method for the determination of legacy and emerging contaminants.

It is important to efficiently separate different chemical isomers from each other for risk assessment purposes. Indeed, among the three TCP isomers, only the *ortho* isomer (TOCP) demonstrates a relatively higher toxicity[83]. Due to TOCP's toxicological effects in humans, its commercial production has been reduced significantly by favouring the *meta* and *para* isomers (TMCP and TPCP) during synthesis [2]. Recently, ip-PDPP, one of the main constituents of the commercial mixture Firemaster® 550, has also illustrated acute neurotoxicological effects [31]. Moreover, a study by Doherty et al. (2019) found a correlation between measured levels of ip-PDPP in children and cognitive development [27]. To date, studies have not evaluated the link between specific ip-PDPP isomers (2-, 3-, 4-ip-PDPP) and potential endocrine disruption and/or toxicity. Thus, the method was further refined to separate TCP and ip-PDPP isomers in food and breast milk.

3.2. Materials and methods

3.2.1 Chemical standards

Analytical standards 2-ip-PDPP, BDCP, TEHP, BDCPP-d12, TCEP-d12, TDCPP-d15, TPP-d15, BDE-MXF (mixture of BDE-28, 47, 66, 85, 99, 100, 153, 154, 183 congeners), BDE-209, $^{13}C_{12}$-

BDE-28, 47, 99, 100, 153, 154, 183, 209, BDE-ISS (mixture of $^{13}C_{12}$-BDE-79, 139, 180, 206 congeners) were purchased from Wellington Laboratories (Guelph, Canada). 3-ip-PDPP, 4-ip-PDPP, BCEP, BCIPP, DmCP, DoCP, DpCP, m-ip-PPP, o-ip-PPP, p-ip-PPP, BBOEP, BEHP, BTBOEP, TMCP, TPCP, TDCPP, ip-ppp-d7, DPHP-d10, BCIPP-d12, DpCP-d14, DmCP-d14, DoCP-d14, BBOEP-d8, BDEP-d10, DBHPT-d4, BEHP-d34, DmCP-d7, TEHP-d51, DP-*anti*, DEC-602 and DEC-603 were purchased from Toronto Research Chemicals Inc. (Toronto, Canada). DPHP, TCEP, TOCP, TBOEP, TPHP were purchased from Sigma Aldrich (St. Louis, Missouri, United States). TCIP, DP-*syn*,$^{13}C_{10}$-DP-*anti*, $^{13}C_{10}$-DP-*syn*, $^{13}C_{10}$-DEC 602 were purchased from Cambridge Isotope Laboratories (Tewksbury, Massachusetts, United States).

3.2.2 Solvents and materials

All solvents used during the extraction steps were pesticide or MS grade. Acetonitrile, DCM, ethyl acetate, iso-octane, isopropyl alcohol, *n*-hexane, methanol, toluene, water, ammonium acetate (Optima™ LC/MS Grade), Formic Acid (99.5+%, Optima™ LC/MS Grade), anhydrous sodium sulfate (pesticide grade, granular, 10-60 mesh), concentrated sulfuric acid (trace metal grade) and mini centrifuge tubes (Axygen™ MaxyClear Snaplock Microtubes, 2.0mL) were purchased from Fisher Chemical (Ottawa, Canada). Anhydrous silica gel (high-purity grade, pore size 60 A, 70-230 mesh) was purchased from Sigma-Aldrich (St. Louis, Missouri, United States). Nitrogen gas for drying was purchased from Praxair (Montreal, Canada).

3.2.3 Material preparation

The risk of background contamination from laboratory environment and equipment is high when working at ultra-trace levels [3, 70]. Therefore, it is imperative to take every possible precaution to avoid contamination. All equipment used during sample processing was solvent rinsed and then baked at 320˚C for 8 hours prior to use. If baking was not possible, equipment was thoroughly cleaned and rinsed first with *n*-hexane then with DCM before use. All bench tops and workstations were covered in aluminum foil and were wiped with solvents to avoid any potential contamination from the work surfaces to the samples. Plastics were avoided at every stage of processing as much as possible. Aluminum foil was placed between the lid of the jar and the samples to avoid any potential migration from the lid liners into the samples.

3.2.4 Acidified silica gel column cleanup

Anhydrous silica gel was mixed with concentrated sulfuric acid (44% w/v) and stored in a 1 L glass bottle with a tight fitting cap. Acidified silica gel columns were prepared using Agilent Bond Elut empty 12 mL SPE cartridges with two pre-inserted PTFE frits (Agilent, Santa Clara, California, USA) filled with 10 g of the acidified silica gel mixture. A PTFE frit was placed at the top and was packed down. Columns were prepared and used the same day.

3.2.5 Collection and processing of food samples

Food samples, including fish, chicken, bread, butter and leafy green vegetables, were collected in October 2017 from grocery stores in Montreal. The fish and chicken samples were passed through a stainless steel meat grinder until homogenized before placing them into a 250 mL amber glass jar certified for semi-volatile organics, pesticides, PCBs and metals analyses (Fisher Scientific Catalogue No. 05-721-102). The bread, butter and vegetables were placed directly into a 250 mL amber glass jar certified for semi-volatile organics, pesticides, PCBs and metals analyses (Fisher Scientific Catalogue No. 05-721-102). After lyophilisation, all samples were crushed into a fine powder. For all matrices, moisture content was determined gravimetrically after lyophilisation. Samples were stored at -80 ˚C until chemical analysis.

3.2.6 Collection of breast milk samples

Five volunteers donated breast milk samples of ~100 mL each. The samples were pooled together to make a mixture to be used for method development and validation. The pooled milk samples were freeze dried (Labconco FreeZone Cascade Benchtop Freeze Dry Systems) and was stored at -80˚C until chemical analysis.

3.2.7 Multi-residue Extraction method

Several variations of the extraction method first reported by Xu. et al. (2015) [67] were tested in a preliminary work. The method employs a filtration step at the beginning of the extraction as well

as ACN as the extraction solvent. In this work, the filtration step was deemed unnecessary since the SPE cartridges provided adequate filtration throughout the procedure. Also, samples with high fat content, such as butter, tend to clog the filters and limit the amount of solvent that can pass through. Moreover, ACN, as an extraction solvent, did not extract the PBDEs or Dechloranes with satisfactory recoveries; OPEs, PBDEs and Dechloranes were all below an acceptable level (<50%) for the food matrices tested. Finally, ACN was too slow to evaporate for the process to be considered quick.

Hence, the above method was modified by removing the filtration step, and the d-SPE step was attempted both immediately after extraction or after the amino-propyl silane (NH_2) SPE cartridge. These modifications improved PBDE and Dechlorane recoveries, however OPE and mOPE recoveries were still too low to be deemed acceptable (<50%). The low OPE recoveries could be due to co-extracted interferences; thus, the cleanup step was also modified. Both C-18 and primary secondary amine (PSA) sorbents, as well as Supel™ QuE Z-Sep+ were employed; however, these modifications did not significantly improve recoveries.

It was therefore necessary to use different extraction solvents in an attempt to improve the initial extraction. In other studies, *n*-hexane and acetone have both been successfully used to extract PBDEs, Dechloranes and OPEs [68]. These solvents could also be used for mOPEs due to the physicochemical properties of the analytes [2]. However, using hexane and acetone resulted in more lipids being co-extracted during the initial step, unlike ACN, these solvents do not select against lipids and other interfering substances. To overcome this, the PBDE and Dechlorane cleanup step was modified to include more acidified silica. It was hypothesized that, by omitting further cleanup steps for OPEs (i.e. NH_2 SPE cartridge and/or d-SPE) and simply diluting and precipitating out interfering substances by freezing the final extract, higher recoveries would be obtained. Reducing

the number of steps help in avoiding potential losses of analytes from transfer steps and the possibility of analytes being "trapped" by the SPE and/or d-SPE sorbents. After attempting this method for various food samples as well as breast milk, acceptable recoveries were obtained for all analytes (**Table 3.5, Appendix Table 1 & 2)**.

The final, optimized extraction method is presented in **Figure 3.1**. The required amount of lyophilized sample was weighed into a 15 mL centrifuge tube, spiked with internal standards (10 ng of each compound) and left overnight to equilibrate. The amount of sample per analysis varied from 0.5 to 2.0 g of sample (detailed masses listed in **Appendix Table 3**) to keep the amount of lipids below 500 mg. To the spiked samples, 5 mL of hexane:acetone (9:1, v/v) was added. The samples were vortexed for 2 minutes and sonicated for 15 minutes. After sonication, the samples were centrifuged at 3500 rpm for 5 minutes, and the solvent layer was transferred to a borosilicate test tube. The entire step was repeated twice for a total of 15 mL of extraction solvent. The extract was then concentrated to 0.5 mL under a gentle flow of nitrogen (99% purity).

For the fractionation, a Florisil SPE cartridge (Agilent Bond Elut Florisil cartridge, 500 mg, 6 mL) was preconditioned with 6 mL of ethyl acetate followed by 6 mL of *n*-hexane:dichloromethane (9:1, v/v). The concentrated sample was loaded onto the SPE cartridge and Fraction A (**FA**), containing the nonpolar analytes, was eluted with 15 mL of *n*-hexane:dichloromethane (9:1, v/v). Fraction B (**FB**), containing the relatively more polar analytes, was subsequently eluted with 15 mL of ethyl acetate.

FA was concentrated to 0.5 mL under a gentle stream of nitrogen. **FA** was loaded onto an acidified silica gel column (44%, 10 g, preconditioned with 6 mL *n*-hexane:dichloromethane (1:1, v/v)) and eluted from the silica column with 15 mL of *n*-hexane:dichloromethane (1:1, v/v). The extract was

then concentrated to near dryness under a gentle stream of nitrogen and reconstituted in 100 µL of iso-octane, and transferred to a GC vial for PBDE and Dechlorane analysis.

FB was concentrated to near dryness under a gentle flow of nitrogen and reconstituted in 500 µL of 70% methanol in water and transferred to a mini-centrifuge tube. The centrifuge tubes were capped and placed at -20˚C for at least one hour to precipitate out any lipids and/or proteins and then centrifuged at 1250 rpm for 3 minutes. The supernatant was transferred to a GC vial for OPE and mOPE analysis.

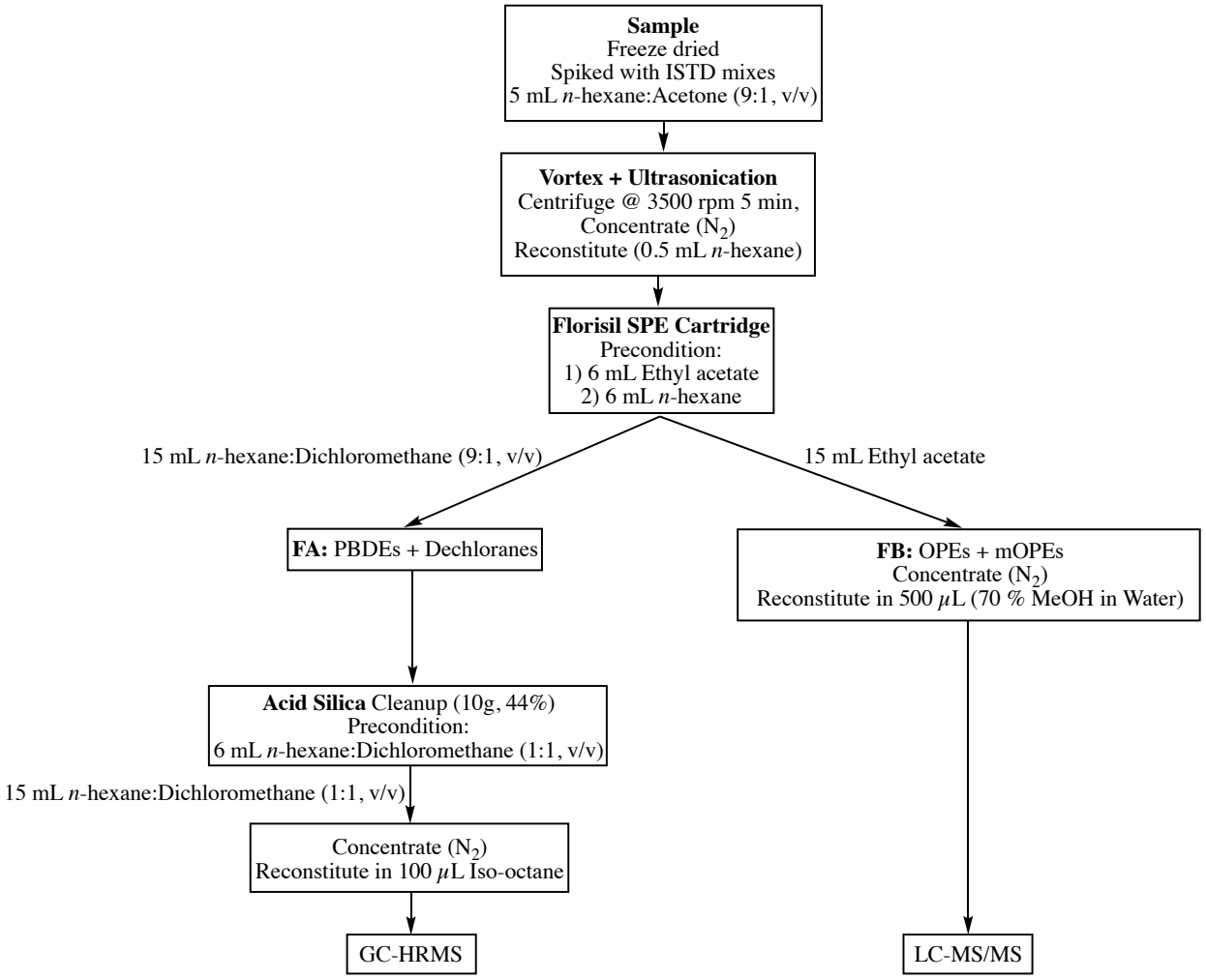


Figure 3.1 Flow chart for the developed multiclass extraction method

3.2.8 Instrumental analysis

3.2.8.1 LC-QqQ-MS/MS

Chromatographic analysis of OPEs and mOPEs was performed on a Waters Acquity Ultra Performance Liquid Chromatography (UPLC) system (Milford, MA, USA) coupled to a Waters Xevo TQD mass spectrometer (Milford, MA, USA) operated in ESI positive and negative mode. Separation of analytes was performed at 40 °C using an Acquity UPLC BEH C_{18} column from Waters (1.7 μm, 2.1 mm × 50 mm) fitted with a Waters Van Guard BEH C_{18} pre-column (1.7 μm, 2.1 × 5 mm).

The mobile phase consisted of (A): 10 mM ammonium acetate in water and (B): methanol. The gradient programming was as follows: initial gradient 5% (B) hold for 0.1 min, increase to 90% (B) in 2.5 min, to 95% (B) in 1.75 min, hold for 3.0 min, and 5.0 min equilibrate to 5% (B). The injection volume was 1.5 μL (full loop) and the flow rate set at 0.22 mL/min.

Quantifiers and qualifiers of multiple reaction monitoring (MRM) transitions of the target analytes and surrogate standards used as well as associated collision energies are presented in **Appendix Table 4**. Source temperature, desolvation temperature, and desolvation gas flow were set at 150 °C, 350 °C and 650 L/h, respectively. Extractor voltage was set at 3 V. Capillary voltages were set at 1.64 kV and 2.23 kV for positive and negative modes, respectively. Instrument performance for LC-QqQ-MS/MS are listed in **Table 3.1**.

Table 3.1 Instrument performance for LC-QqQ-MS/MS

Compound	ESI	RT (min)	LOD (ng/ml)	LOQ (ng/ml)	Linear range (ng/ml)	Linearity (R^2)
OPE						
TPP	+	3.46	0.11	0.36	0.25-500	0.9997
TEHP	+	5.85	0.03	0.10	0.25-100	0.9936
TDCPP	+	3.44	0.18	0.58	0.25-500	0.9999
TCP (isomers)	+	3.80	0.22	0.74	0.75-750	0.9959
TCIP	+	3.22	0.70	2.22	2.5-250	0.9986
TCEP	+	2.79	0.48	1.54	2.5-500	0.9999
TBOEP	+	3.66	0.34	1.08	0.25-500	0.9997
ip-PDPP (isomers)	+	3.76	0.19	0.64	0.5-500	0.9990
mOPE						
DPHP	-	2.65	0.24	0.78	0.25-500	0.9996
BEHP	-	3.78	0.25	0.83	0.25-500	0.9999
DCP	+	2.97	0.24	0.78	0.75-1500	0.9998
BCIPP	+	2.46	0.17	0.58	0.25-500	0.9996
BCEP	+	1.81	2.26	7.55	2.5-500	0.9987
BTBOEP	+	3.19	0.28	0.95	0.25-500	0.9999
BBOEP	+	2.99	0.26	0.88	0.25-500	0.9991
ip-PPP (isomers)	+	3.10	0.21	0.71	0.75-300	0.9968

3.2.8.2 LC-QTOF-MS

Chromatographic analysis of TCP and ip-PDPP isomers was performed using an Agilent 1290 Infinity II LC system. LC separation was performed with an InfinityLab Poroshell 120 EC- C_{18} (3.0 × 100 mm, 2.7 μm) column fitted with an InfinityLab Poroshell 120 Phenyl Hexyl (3.0 × 5 mm, 2.7 μm) guard column, both from Agilent Technologies. The LC system was coupled to a 6545 series QTOF from Agilent Technologies equipped with a Dual AJS ESI ion source operating in positive (ESI+) and negative ionization (ESI-) mode.

For compounds run in ESI+, the mobile phase consisted of (A): 0.1% formic acid in water and (B): 0.1% formic acid in methanol. The gradient programming was as follows: initial gradient 70% (B) hold for 1.00 min, increase (B) to 82% in 2.00 min then increase (B) to 85% in 7.00 min, at 10.00

min, increase (B) to 100% in 1.0 min, hold for 1.00 min. Equilibrate column to starting conditions for 3 minutes prior to next injection. The total run time for this method was 15.00 minutes. For the first 0.50 min and last 1.0 minutes of the run, the flow was directed from the LC column to waste.

For compounds run in ESI-, the mobile phase consisted of (A): 5 mM of ammonium acetate in water and (B): 5 mM ammonium acetate in methanol. The gradient programming was as follows: initial gradient 70% (B) hold for 1.00 min, increase (B) to 82% in 5.00 min then increase (B) to 85% in 2.00 min, at 8.00 min, increase (B) to 100% in 1.00 min, hold for 2.00 min. Equilibrate column to starting conditions for 2 minutes prior to next injection. The total run time for this method was 13.00 minutes. For the first 0.60 min and last 1.00 minutes of the run, the flow was directed from the LC column to waste.

For both ionization modes, samples were kept at 4°C in the multisampler compartment, the injection volume was 10 µL, the flow rate was set at 0.5 mL/min and the column was kept at 20˚C throughout the analysis. Once the chromatographic method was developed, the parameters of the ESI source were optimized for both positive and negative ionization modes; most notably, the drying gas flow rate and gas temperature to accommodate the increased flow rate. The optimized ESI source parameters are presented in **Table 3.2**. The acquisition range used was from *m/z* 50-750 with an acquisition rate of 2 spectra/sec, acquisition time of 500 ms/spectrum and 4089 transients/spectrum, the collision energy was set to 10 V. Instrument performances for LC-QTOF-MS are presented in **Table 3.3**. Details on the monitored quantitative ions are presented in **Appendix Table 5**.

Table 3.2 Optimized ESI source parameters for LC-QTOF-MS

ESI Parameter	ESI +	ESI -
Nozzle Voltage (V)	2000	2000
Capillary Voltage (V)	4500	4000
Drying Gas (l/min)	10	10
Gas Temperature (˚C)	350	325
Skimmer Voltage (V)	75	75
Fragmentor Voltage (V)	150	150
Sheath Gas Temperature (˚C)	375	400
Sheath Gas Flow (l/min)	10	12
OCT 1 RF Vpp (V)	750	750
Nebulizer (psi)	30	20

Table 3.3 Instrument performance for LC-QTOF-MS

Compound	ESI	RT (min)	LOD (ng/mL)	LOQ (ng/mL)	Linear Range (ng/mL)	Linearity (R^2)
OPE						
TCEP	+	1.38	0.65	2.16	1.0-250	0.99946
DCP	+	1.44	3.58	11.94	5.0-250	0.99985
BBOEP	+	1.51	0.69	2.29	1.0-250	0.99965
BTBOEP	+	2.23	0.41	1.36	0.5-250	0.99960
TCIP	+	2.35	0.81	2.71	1.0-250	0.99961
TDCIPP	+	3.50	0.58	1.92	1.0-250	0.99990
TPHP	+	3.59	0.47	1.56	0.5-250	0.99980
TBOEP	+	4.67	0.35	1.18	0.5-250	0.99910
2-ip-PDPP	+	4.97	0.60	2.00	1.0-250	0.99992
3-ip-PDPP	+	5.21	0.64	2.13	1.0-250	0.99995
4-ip-PDPP	+	5.35	0.89	2.97	1.0-250	0.99984
TOCP	+	5.42	0.66	2.21	1.0-250	0.99902
TMCP	+	5.59	1.10	3.66	1.0-250	0.99954
TPCP	+	5.59	1.10	3.66	1.0-250	0.99954
TEHP	+	12.04	4.20	13.98	5.0-250	0.99912
mOPE						
BCEP	-	1.05	0.38	1.28	0.5-250	0.99379
BCIPP	-	1.09	0.82	2.74	1.0-250	0.99548
DPHP	-	1.12	0.82	2.74	1.0-250	0.99737
BDCP	-	1.35	1.78	5.94	2.5-250	0.99984
BEHP	-	6.40	0.50	1.67	0.5-250	0.99928

3.2.8.3 GC-MS

Determination of PBDEs and Dechloranes was performed with a 8890 GC (Agilent, Santa Clara, CA, USA) coupled to a 5977B MS (Agilent, Santa Clara, CA, USA) using electron impact (EI), the MS was operated in SIM mode. A capillary DB-5HT column (15 m × 0.25 mm i.d., 0.25 μm) (Agilent) was used, with helium at a constant flow of 1.8 mL/min as the carrier gas. The oven temperature program started at 190 °C (held for 1.00 min), ramped at 20 °C/min to 340 °C (held for 2.00 min). Ion source and transfer line temperatures were both 340 °C. Instrument performances for GC-MS are presented in **Table 3.4**. The monitored quantitative ions are presented in **Appendix Table 6**.

Table 3.4 Instrument performance for GC-MS

Compound	RT (min)	LOD (ng/mL)	Linear Range (ng/mL)	Linearity (R^2)
PBDE				
BDE-28	6.42	0.0149	0.25-100	0.999994
BDE-47	7.27	0.0486	0.5-200	0.999999
BDE-99	8.16	0.0858	0.5-200	0.999993
BDE-100	7.94	0.0717	0.5-200	1.000000
BDE-153	9.27	0.0948	0.5-200	0.999997
BDE-154	8.87	0.0623	0.5-200	0.999966
BDE-183	10.61	0.0729	1.0-400	0.999991
BDE-209	16.93	0.1043	2.5-1000	0.999998
Dechlorane				
DEC-602	8.13	0.0992	0.5-200	0.999997
DEC-603	10.18	0.2718	0.5-200	0.999974
DP-*anti*	12.45	0.1580	0.5-200	0.999996
DP-*syn*	11.94	0.0402	0.5-200	0.999993

3.2.9 Data analysis

LC-QqQ-MS/MS data was analyzed using Waters Targetlynx Application Manager software (Waters Corporation, Milford, Massachusetts, USA).

Data treatment for LC-QTOF-MS and GC-MS data was done using the Agilent MassHunter Qualitative Analysis software (Version B.07.00 Service Pack 2/ Build 7.0.7024.29) and Agilent MassHunter Quantitative Analysis software (Version B.07.01 Service Pack 1/ Build 7.1.524.1 for QTOF) (Agilent Technologies, Santa Clara, CA, USA).

For both LC and GC instruments, the targeted compounds were identified through comparison with the retention time of the standards. A leniency of ± 0.15 minutes was accepted for retention times. Limits of detection were assessed as the concentration giving a signal equal to three times the baseline noise. The method limit of quantification of an analyte was determined by multiplying a Student's t-value designated for a 99% confidence level with standard deviations in replicate analyses (n=8). For quantitative analysis, relative response factors (RRF) were used to determine the concentrations of the analytes present in samples (*Equation 3.1*).

$$\mathrm{RRF} = \frac{\left(\mathrm{Peak\ Area}_{\mathrm{Analyte}} / \mathrm{Concentration}_{\mathrm{Analyte}}\right)}{\left(\mathrm{Peak\ Area}_{\mathrm{ISTD}} / \mathrm{Concentration}_{\mathrm{ISTD}}\right)}$$

Equation 3.1

3.2.10 Quality control/quality assurance

For method performance, three aliquots of blank material (anhydrous sodium sulfate) and sample homogenates were spiked with two levels of each analyte, 10 ng and 20 ng. Extraction, cleanup and instrumental determination were performed according to the previously described methods.

Procedural blanks (sodium sulphate) were included in each batch of samples to monitor background contamination from the laboratory environment. Accuracy was calculated as the mean bias between theoretical and experimental concentrations of each compound in the spiked samples normalized by the spiking concentration (*Equation 3.2*).

$$\text{Accuracy (\%)} = \frac{\text{Concentration prespiked extract} - \text{Concentration unspiked extract}}{\text{Theoretical spiking concentration}} x\ 100$$

Equation 3.2

Four standard reference materials (SRMs) were used to evaluate the accuracy of the developed method for PBDEs. WMF-01: Reference Fish Tissue for Organic Contaminant Analysis (Wellington Laboratories, Guelph, Ontario, Canada), EDF-2524: clean fish reference material, EDF-2525: contaminated fish reference material (Cambridge Isotope Laboratories, Inc. Cambridge, Massachusetts, USA) and SRM 1953 - Organic Contaminants in Non-Fortified Human Milk (National Institute of Standards and Technology, Gaithersburg, Maryland, United States). Currently, there are no CRMs for Dechloranes or OPEs available on the market.

Precision was expressed for each analyte as the relative standard deviation of the three spiked samples results divided by the average concentration calculated in the same samples (*Equation 3.3*).

$$\text{Precision (\%)} = \frac{\text{Standard deviation concentration prespiked extract (triplicate)}}{\text{Average Concentration prespiked extract (triplicate)}} x\ 100$$

Equation 3.3

Linearity was calculated for each compound as the relative standard deviation of the response factor in all the calibration curve standards ($R^2 > 0.99$ for all analytes) [63].

Absolute recovery values were determined by measuring prespiked analyte response to the calibration curve without internal standard correction (*Equation 3.4*).

$$\text{Absolute recovery (\%)} = \frac{\text{Area prespiked extract} - \text{Area unspiked extract}}{\text{Area post spiked extract} - \text{Area unspiked extract}} \; x \; 100$$

Equation 3.4

Matrix Factor values were determined by measuring the area for each analyte in pure solvent normalized to the area obtained for the same concentration in an extract before and after spiking (*Equation 3.5*).

$$\text{Matrix factor} = \frac{\text{Area STD (x ng/mL)}}{\text{Area extract post spiked (x ng/mL)} - \text{Area extract unspiked}} \; x \; 100$$

Equation 3.5

To check the instrument performance, QC samples containing all the monitored analytes were injected between samples. If the levels (area counts) of analytes decreased by more than 20% for two consecutive injections, maintenance was performed, the column, both analytical and guard, were flushed with pure solvents. Another QC sample was run to determine if the column had returned to normal conditions. If not, the guard column was replaced, equilibrated and another QC sample was run and evaluated. Albeit an arduous process, this method has run up to 100 samples without requiring column maintenance.

3.3. Method validation

To test the applicability of this method, various food matrices including, fish, chicken, leafy green vegetables, butter and breast milk were tested. These matrices contain varying degrees of lipids, proteins, pigments and other interferences after lyophilization. **Table 3.5** shows OPE and mOPE recoveries and other method performances for food samples. Method detection limits for OPEs

and mOPEs for each matrix are listed in **Table 3.6**. Good recoveries were observed for most analytes tested. All internal standards met the recovery requirements according to the U.S. EPA for all tested matrices [84]. Notable differences in overall recoveries between some compounds (i.e. TEHP & BEHP) indicated that matrix affected recoveries to a certain extent. This was mainly true for mOPEs. Since the matrix effect using LC is always a challenge, the isotopically labeled internal standards corrected for signal enhancement or suppression by using relative response ratios.

Table 3.5 LC-QqQ-MS/MS method performances for food samples

Compound	Accuracy (%) (n=8)	Precision (%) (n=8)	Absolute Recovery (%) (n=8)[a]
OPEs			
TPHP	107.04	96.89	81 ± 19
TEHP	99.00	99.90	89 ± 45
TDCPP	110.61	96.82	85 ± 40
TCP (isomers)	95.11	90.41	81 ± 40
TCIP	111.07	90.33	101 ± 28
TCEP	100.84	88.78	93 ± 50
TBOEP	96.65	95.85	97 ± 20
ip-PDPP (isomers)	88.72	86.63	70 ± 29
mOPEs			
DPHP	102.06	92.85	87 ± 47
BEHP	92.74	92.97	69 ± 23
DCP	108.11	93.55	91 ± 27
BCIPP	115.97	95.41	101 ± 16
BCEP	91.34	89.91	95 ± 4
BTBOEP	103.55	89.29	85 ± 13
BBOEP	118.78	96.71	87 ± 26
ip-PPP (isomers)	86.71	90.64	84 ± 48

[a]Recovery calculated for spiked food samples, including fish, chicken and vegetables.

Table 3.6 Method detection limits for LC-QqQ-MS/MS (ng/g d.w.)

	TPHP	TEHP	TDCPP	TCP	TCIP	TCEP	TBOEP	ip-PDPP	DPHP	BEHP	DCP	BCIPP	BCEP	BTBOEP	BBOEP	ip-PPP
Low fat fish[a]	0.028	0.008	0.045	0.055	0.175	0.120	0.085	0.048	0.060	0.063	0.060	0.043	0.565	0.070	0.065	0.053
High fat fish[b]	0.055	0.015	0.090	0.110	0.350	0.240	0.170	0.095	0.120	0.125	0.120	0.085	1.130	0.140	0.130	0.105
Vegetables[c]	0.055	0.015	0.090	0.110	0.350	0.240	0.170	0.095	0.120	0.125	0.120	0.085	1.130	0.140	0.130	0.105
Bread	0.037	0.010	0.060	0.073	0.233	0.160	0.113	0.063	0.080	0.083	0.080	0.057	0.753	0.093	0.087	0.070
Butter	0.110	0.030	0.180	0.220	0.700	0.480	0.340	0.190	0.240	0.250	0.240	0.170	2.260	0.280	0.260	0.210
Canned tuna in water	0.028	0.008	0.045	0.055	0.175	0.120	0.085	0.048	0.060	0.063	0.060	0.043	0.565	0.070	0.065	0.053
Canned tuna in oil	0.055	0.015	0.090	0.110	0.350	0.240	0.170	0.095	0.120	0.125	0.120	0.085	1.130	0.140	0.130	0.105
Chicken	0.028	0.008	0.045	0.055	0.175	0.120	0.085	0.048	0.060	0.063	0.060	0.043	0.565	0.070	0.065	0.053
Breast Milk	0.055	0.015	0.090	0.110	0.350	0.240	0.170	0.095	0.120	0.125	0.120	0.085	1.130	0.140	0.130	0.105

[a] Includes sole, cod, tilapia, haddock and hake (<2% lipid content)

[b] Includes rainbow trout, salmon, halibut and basa (>2% lipid content)

[c] Includes spinach, romaine lettuce, arugula and watercress

In general, recoveries of PBDEs and Dechloranes were better than those of the OPEs (**Table 3.7**). The physicochemical properties of these compounds likely allow for more complete extraction from the matrix and cleanup was more thorough when large amounts of acidified silica gel were used, resulting in better overall recovery [22]. All analytes are within the acceptable criteria defined by the US EPA for method performances [84].

Table 3.7 GC-MS method performances for food samples

	Recovery (%)				Accuracy	Precision
	Vegetables (n=3)	**Bread (n=3)**	**Fish (n=3)**	**Breast Milk (n=3)**	**(%)**	**(%)**
PBDE						
BDE-28	106 ± 23	104 ± 41	111 ± 28	104 ± 25	94.3	79.65
BDE-47	103 ± 21	100 ± 14	94 ± 23	101 ± 16	89.11	82.93
BDE-66	90 ± 8	109 ± 65	90 ± 16	88 ± 26	89.11	82.93
BDE-99	93 ± 25	82 ± 11	87 ± 14	92 ± 25	101.63	89.24
BDE-100	93 ± 39	98 ± 11	92 ± 13	96 ± 9	116.22	82.94
BDE-153	106 ± 16	95 ± 31	108 ± 20	102 ± 10	67.63	78.55
BDE-154	136 ± 6	111 ± 28	89 ± 6	85 ± 18	27.00	94.76
BDE-183	83 ± 36	98 ± 4	102 ± 29	94 ±18	87.56	82.35
BDE-209	48 ± 16	36 ± 19	31 ± 16	76 ± 13	11.67	20.53
Dechlorane						
DEC 602	81 ± 8	ND	87 ± 23	97 ± 27	82.25	63.32
DEC 603	86 ± 40	68 ± 13	84 ± 20	93 ± 42	84.75	87.13
DP-*anti*	101 ± 30	99 ± 21	93 ± 23	110 ± 14	76.75	66.57
DP-*syn*	101 ± 8	86 ± 40	100 ± 22	103 ± 18	78.75	75.74

3.4. Results and Discussion

3.4.1 Application to food and breast milk samples

The developed method was applied to several food and breast milk samples. All OPEs were detected in at least one sample (**Table 3.8**). The levels for OPEs in food and breast milk are in line with previously reported values for the same sample types [12, 23, 57, 59, 66, 78, 85]. TEHP was the OPE detected in the highest concentration, in one butter sample at 108.38 ng/g w.w.. This OPE is a

commonly used plasticizer and its detection can most likely be attributed to its ubiquitous use in PVC manufacturing [1]. With the exception of BBOEP, all of the mOPEs were detected in breast milk. BCIPP was quantified at 10 ng/g in one breast milk sample. Sundkvist et al. (2010) previously analyzed breast milk in Sweden for OPEs [12]; the levels reported are similar to this study, but this is the first report of mOPEs in the same sample.

Table 3.8 Water content (%), lipid content (%) and concentration of analytes in selected matrices (reported as ng/g w.w.)

	Fish (n=15)	**Chicken (n=2)**	**Vegetables (n=7)**	**Bread (n=4)**	**Butter (n=4)**	**Breast Milk (n=5)**
Water Content	82	76	93	37	9	78
Lipid Content	2.68	1.12	0.21	0.02	76.12	5.39
OPEs						
TEHP	ND - 19	ND - 0.18	ND - 0.37	ND	77.68- 108.3	0.02 - 8.0
TBOEP	ND - 2.25	ND	ND - 3.20	ND	ND	ND - 4.0
TDCPP	0.1 - 13	ND - 0.56	0.10 - 0.37	ND-0.18	ND - 2.30	ND - 1.6
ip-PDPP	ND	ND	ND - 0.1	0-0.03	ND - 0.18	ND
TCP	ND - 0.075	ND	ND - 0.04	ND-0.56	ND	ND - 0.075
TCIP	1 - 7.5	ND	ND - 12.9	ND	ND - 10.12	1.5 - 17.5
TPP	ND - 7.0	ND - 1.38	0.07 - 0.27	ND	ND - 1.46	0.05 - 1.5
ip-PPP	ND - 2.0	ND	ND	ND	ND	ND
TCEP	0.05 - 0.5	ND - 0.41	ND - 0.14	ND	ND - 0.70	0.05 - 0.75
mOPEs						
DCP	ND - 4.5	ND	ND - 0.21	ND	ND	ND - 0.75
BCIPP	0.05 - 5.75	ND - 0.64	ND - 0.21	ND	ND	0.05 - 10
BCEP	ND - 4.0	ND	ND - 0.15	ND	ND - 10.37	0.05 - 0.5
BBOEP	ND	ND - 0.64	ND - 0.34	ND	ND	ND
BTBOEP	ND - 4.5	ND	ND - 1.28	ND	ND	0.05 - 0.5
DPHP	0.02 - 0.1	ND	ND - 1.57	ND	ND - 0.02	ND - 0.075
BEHP	0.025 - 0.05	ND	ND - 1.46	ND	ND	0.025 - 0.05

A food study in Queensland Australia by He et al. (2018) found similar levels of mOPEs in the analyzed samples [23]. As with this study, most mOPEs were below the MLOD and those that were quantified were in the low-ppb range. As other authors have hypothesized, the low detection for OPEs may be due to the rapid metabolism of these compounds in organisms such as food of animal origin [59]. The rapid metabolism of OPEs may also be true for human milk. This, therefore, raises the concern that the OPEs and mOPEs are transferred to the nursing infants.

Although lower levels of these OPEs are found in food and breast milk compared to dust, the exposure via diet may not be negligible, especially for breast milk since this is the only food source for developing infants [14]. Therefore, it is necessary to develop robust and sensitive analytical methods capable of monitoring multiple contaminants in a broad range of matrices. Moreover, it is critical to continuously monitor both emerging and legacy contaminants in order to assess changes observed over time due to factors such as voluntary removal of chemicals by manufacturers, concern from consumers, environmental contamination or government regulations.

3.4.2 ip-PDPP & TCP isomer separation

One of the challenges associated with this analysis was the separation of two sets of isomers (TOCP, TMCP, TPCP, 2-ip-PDPP, 3-ip-PDPP, 4-ip-PDPP). The analysis of these OPEs was performed using LC-QTOF-MS. Since these isomers differ only in their arene substitution patterns, there are no significant spectral changes that can be used between isomers, thus there is no substitute for good chromatography. For this reason, the solvent gradient programming and flow rate had to be optimized to increase separation while at the same time, not hindering separation of other analytes. The flow rate was set to 0.5 mL/min to efficiently "push" the

compounds out of the column and facilitate separation. Slower flow rates did not obtain adequate separation. The solvent gradient programming was adjusted to the flow rate to ensure optimal separation of all other analytes. The compounds were well separated using a C_{18} analytical column. Improved separation was obtained using a phenyl hexyl guard column, more so than a C_{18} guard column. Counterintuitively, lower column compartment temperatures obtained the best separation. The separation of the isomers is shown in **Figure 3.2**, the elution of the analytes is as follows: 2-ip-PDPP (RT: 5.65 min) followed by 3-ip-PDPP (RT: 5.97 min), followed by 4-ip-PDPP (RT: 6.10 min), followed by TOCP (RT: 6.27 min), lastly TMCP and TPCP (6.51 min). It was not possible to separate TMCP from TPCP while maintaining good separation of the other analytes, they are therefore quantified together. Most importantly TOCP is separated from the other analytes since this is the most toxic compound and great care should be taken to determine its presence in samples [83]. Currently, TMCP and TPCP are produced in equal molar ratios on an industrial scale while limiting the amount of TOCP production. It is therefore reasonable to quantify both TMCP and TMCP together since they have similar toxicological effects, which are much less serious when compared to TOCP.

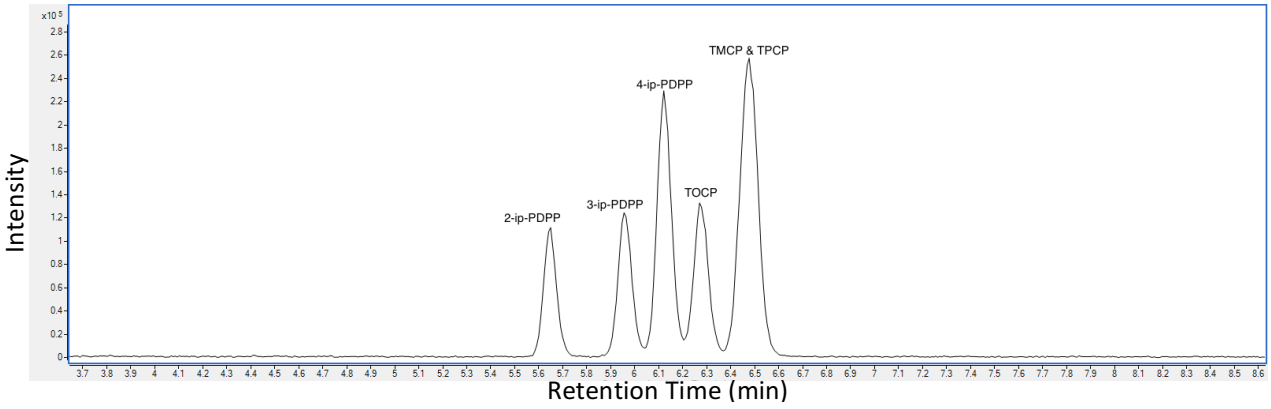


Figure 3.2 TCP & ip-PDPP isomer separation in pure solvent

In food and breast milk samples analyzed, it was possible to obtained good separation of the isomers even with potential interferences from the matrices. for example, Figure 3.3 shows a spinach extract that did not contain any of the analytes and was then spiked with 10 ng of the isomers. From this figure it can be observed that good separation is obtained and there appears to be roughly double the peak area for TMCP and TPCP.

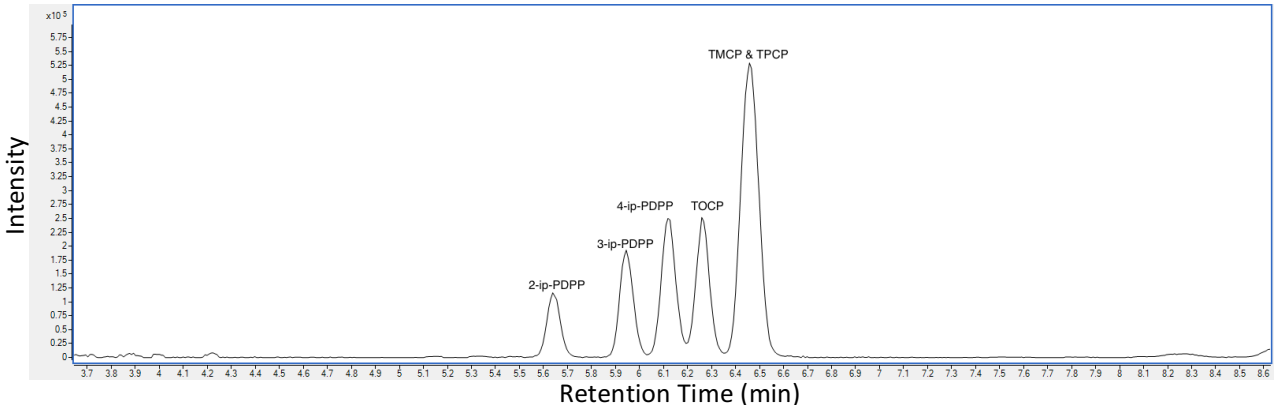


Figure 3.3 TCP & ip-PDPP isomer separation in spiked spinach extract

Although the TCP and ip-PDPP isomers were separated chromatographically, their detection frequencies were low in the analyzed samples. There was no difference ($P>0.05$) between the multiple food types and isomeric distribution within the samples. This may be due to the high non detect percentages of all TCP and ip-PDPP isomers in the analyzed samples. Nonetheless, due to the high toxicity of TOCP, there should be continuous monitoring for this analyte. If levels are above a certain threshold, there should be a "red flag" raised for samples. It is also important to have a reliable method for separating the ip-PDPP isomers since recent studies have identified potential toxicity [27, 31, 86], but have not identified isomer-specific toxicity. Using this analytical method to analyze food and breast milk samples allows for risk assessor to appropriately judge

toxicity on an isomer specific basis. Moreover, the isomeric profiles may allow for toxicologists to design toxicity studies with real world isomer profiles.

3.5. Critical considerations

Several drying steps were performed throughout the analysis, and due to the semi-volatility of some OPEs and lower brominated BDEs, extracts should never be dried to complete dryness [2-3, 22]. Moreover, a very gentle flow of nitrogen should be used along with minimal heating (<30˚C) to reduce any possible degradation of OPEs during chemical analysis. This precaution increased drying time, but reduced the potential for degradation of parent OPEs into their respective metabolites.

As mentioned previously, an LC guard column should be used and monitored periodically to avoid contamination of the analytical column, a GC guard column was also appropriate. The GC guard column can prevent non-volatile residues, such as lipids, pigments or carbohydrates from being put on the analytical column. Non-volatile residues on the analytical column reduce sensitivity, degrade peak shape and result in an overall decrease of column performance. The inlet liner cleanliness has an important impact on the quality of the analysis since any residues on the liner will decrease performance for higher brominated congeners. During the method development process, most food samples' extracts were slightly dirty after extraction, thus large batches of samples could not be injected on the GC without extensive maintenance. Increasing the amount of acid silica as well as firmly packing a PTFE frit on top of the sorbent resulted in cleaned extracts with high recoveries for Tri-Deca BDEs and Dechloranes. These modifications allowed for a larger number of sample injections without need for liner replacement or column maintenance.

Lastly, blank contamination is ubiquitous and every possible precaution should be taken to minimize background interferences. This includes baking of all glassware, rinsing working surfaces with solvents and using the highest grade solvents and materials available. Such steps add

cost and time to the analysis; however, they are necessary in order to ensure low level detection for the analytes of interest.

The newly developed extraction method was time consuming due to the multiple drying steps, but these are unavoidable in any method in the current literature. However, the method mentioned above was less time consuming than traditional methods such as Soxhlet, or methods that use more SPE cartridges and/or d-SPE. The reduced amount of equipment, SPE cartridges, d-SPE sorbents and solvents make the method a more economical option than other methods in the current literature. A disadvantage with this method is that the OPE extracts were not fully "cleaned". Therefore, the LC guard column prior to the analytical column should be replaced more often than normally required for other methods and the ESI source should periodically be maintained according to manufacturers specifications.

Overall performance of the developed multiclass extraction method was on par or better than other methods. This method has several key advantages. First, it is less time consuming than previously reported methods due to the reduction of steps throughout the procedure. Second, the cost is reduced from the amount of materials, solvent and time needed for the extraction. Finally, due to the reduction of steps, mOPEs can be readily extracted from food and breast milk. Although other methods have successfully extracted mOPEs from food, they have not extracted nonpolar analytes such as PBDEs and Dechloranes from the same samples. For this reason, the above mentioned method is a better alternative to the methods commonly reported in the literature. As mentioned above, there are also key disadvantages: such as the use of large amounts of acidified silica gel which destroys any acid labile compounds, therefore the nonpolar analytes of interest must be acid resistant. Also, since **FB** is simply diluted, potential instrument contamination must be properly addressed and care should be taken when running large batches of samples.

3.6. Conclusion

A multiclass extraction method for the simultaneous determination of PBDEs, Dechloranes, OPEs and mOPEs was successfully achieved by LC-QTOF-MS and GC-MS. A robust methodology is capable of extracting various environmental contaminants using sonication as the extraction technique and fractionation of compounds based on their polarities using SPE is presented. The optimized multiclass extraction method is more rapid and cost-effective than the current methods in the literature. The optimized methodology was applied to fish, chicken, bread, butter, vegetables and breast milk collected in Montreal in October 2017. This is the first time mOPEs have been detected in breast milk. Moreover, the chromatographic separation of TCP and ip-PDPP isomers was achieved.

These emerging contaminants were widespread in various food commodities and, most importantly, in breast milk. Their concentrations and detection frequencies demonstrate the need for robust methodologies capable of analyzing both legacy and emerging contaminants in complex matrices. Further studies should be performed to analyze more samples to better understand the daily exposure to these chemicals from the Canadian diet.

CONNECTING STATEMENT II

The efficiency of the multi-class extraction method for PBDEs, Dechloranes, OPEs and mOPEs was evaluated and applied to various food and breast milk samples in **Chapter 3**. Good recoveries were obtained for all analytes in all matrices.

In **Chapter 4**, the developed multi-class extraction method is applied to food samples (n=66) and breast milk samples (n=100) collected in Montreal in October 2017 and May 2018. The levels and detection frequencies of these contaminants are assessed. Moreover, dietary exposure assessments are made for adults and children as well as the implications for breast feeding young infants.

CHAPTER 4. ASSESSMENT OF OPEs & mOPEs IN FOOD AND BREAST MILK FROM MONTREAL, CANADA

4.1. Introduction

Flame retardants and plasticizer chemical additives are applied to a broad range of consumer products in order to meet regulatory requirements and industry standards. Due to concern for both environmental and human toxicity, some of these chemicals have been voluntarily removed or formally regulated and banned in Canada, the United States and other countries [5]. Since 2009, some of the polybrominated diphenyl ethers (PBDEs) have been regulated in accordance with the Stockholm Convention [36]. To meet the technical requirements and replace legacy chemicals of concern, emerging replacement chemicals are being used more frequently. One such class of replacement chemicals are Organophsophate esters (OPEs). Some OPEs are already considered as high production volume chemicals [87].

Previously, studies have analyzed the presence and fate of emerging chemicals, such as OPEs, in water, soil, food, dust, urine and breast milk [57], however there still exist large data gaps with regards to the metabolites of the OPEs (mOPEs). To obtain a complete picture of emerging chemicals in food and breast milk, as well as a better understanding of the parent and metabolite relationship, a complete screening of OPEs should be analyzed from the same sample.

Prompted by this lack of exposure data, twelve OPEs and their respective metabolites were monitored in food samples including fish, chicken, vegetables, bread, butter and canned fish as well as breast milk. Food samples were collected on two different occasions from various grocery stores and markets in Montreal, Canada during the period of November 2017 and May 2018; to

assess the effect of food packaging, both packaged and non-packaged varieties of foods were purchased. Breast milk samples were obtained from women 4-6 weeks post-partum during the same chronological time frame. This is the first time that OPEs and mOPEs were analyzed together in food and breast milk. The results provide a preliminary insight for exposure to OPEs and mOPEs through food and breast milk.

4.2. Materials and methods

4.2.1 Sample collection, processing and storage

4.2.1.1 Food collection

Sampling of food was done in two phases (November 2017 and May, 2018) from grocery stores and markets in Montreal, Quebec, Canada in November 2017 and again in May 2018. The rationale behind the food sampling patterns was to coincide with the collection of breast milk samples from volunteers during the same time period. It can be assumed that this sampling patter is representative of the foods the participating mothers could have consumed. It was also critical to select a broad range of food samples in order to be able to assess the potential uptake of environmental contaminants through dietary sources. The selected foods also represented a wide array of matrices that varied in protein, carbohydrate, lipid, pigment and water content as well as in the degree of processing (**Appendix Table 7**). In the fish category, several species were selected in order to target marine and fresh water fish with both high and low lipid contents. The rationale was similar for the leafy vegetables. Boneless, skinless chicken breasts were chosen to represent chicken samples since this is a common way of purchasing chicken, moreover, it will also facilitated processing and extraction steps by avoiding further handling in the laboratory. For bread, both white and brown were assessed since these are generally consumed equally; both salted and non-

salted butter were chosen for the same reason. Bread and butter can also be considered as processed commodities and, thus, can give more insight into the effects of food processing on the levels of the monitored environmental contaminants.

The sampling of packaged and non-packaged food items was done to determine whether the measured contaminants could come from the packaging, or if the contaminants are present in the food itself. Also, the advantage of sampling the same food items in both packaged and non-packaged forms is that a direct comparison can be made on the potential migration of environmental contaminants from food packaging materials into the foodstuff. In the case of bread and butter, core samples were taken from the same samples used for analysis. This reduces the possible variation since it is possible to purchase non-packaged bread from a small bakery, however it would not be the same sample used for the packaged bread. As it is impossible to purchase non-packaged butter, core samples were the only option. After purchase, the non-packaged foods were wrapped in aluminum foil to avoid potential exposure to contaminants through other routes.

4.2.1.2 Breast milk collection

Women were recruited 24 hours post partum from two Montreal hospitals for breast milk donations. To be eligible to participate in this study, women had to be at least 18 years old, had given birth to a single baby, had the intention to breast feed, speak English and/or French, had lived in Montreal for the past year and were willing to have two study visits at home. Women who met all the above criteria were consented and their contact information obtained. Four to five weeks later, the women were visited at their homes and given a collection kit and instructions on how to

manually express hindmilk directly into the containers (250 mL amber glass jar certified for semi-volatile organics, pesticides, PCBs and metals analyses (Fisher Scientific Catalogue No. 05-721-102)) provided to them. They were instructed to preserve the collected milk in their fridge (4 ˚C) during the collection day and then to store the samples in their freezer (-20 ˚C) by the end of the day. The samples were then collected one week after the first visit, brought to the laboratory and stored at -20 ˚C until further processing. The study protocol was approved by the McGill University Health Centre Research Ethics Board (MP-37-2018-3730) and the analysis of OPEs and mOPEs was approved by Health Canada Research Ethics Board.

4.2.2 Sample processing

The risk of background contamination from laboratory air and equipment is high when working at trace levels for environmental contaminants [3, 70]. It is imperative to take every possible precaution to avoid potential contamination. All equipment used during processing was solvent rinsed, first with *n*-hexane then with dichloromethane and then baked at 320˚C for 8 hours prior to use. If baking was not possible, equipment was thoroughly cleaned and rinsed with solvents before use. All bench tops and workstations were covered in aluminum foil and were rinsed with solvents to avoid any potential contamination from the work surfaces to the samples. Plastics were avoided at every stage of processing whenever possible. Aluminum foil was placed between the lid of the jar and the samples to avoid any potential migration from the lid liners into the samples. Aluminum foil was previously tested and found to be free from all target analytes.

For all matrices, moisture content was determined gravimetrically after lyophilisation, then stored at -80 ˚C until chemical analysis.

4.2.2.1 Food processing

Depending on the food type, different levels of pre-treatment were needed. For bread, butter, leafy vegetables and canned fish, the foods were weighed in equal amounts and placed into a 250 mL amber glass jar certified for semi-volatile organics, pesticides, PCBs and metals analyses (Fisher Scientific Catalogue No. 05-721-102). For fish and chicken samples, the foods were first homogenized using a stainless steel meat grinder and then weighed into a 250 mL amber glass jar. Composite samples were prepared for each food type. Composite samples consist of one to six samples of a single food type purchased from different locations (**Appendix Table 8**). All containers were then placed into a freeze dryer (Martin Christ Gamma 2-16 LSC plus) until completely lyophilized. Once lyophilized, the samples were ground into a fine powder using a mortar and pestle. Ground composite samples were then stored at -80 ˚C until chemical analysis.

4.2.2.2 Breast milk processing

Breast milk samples were placed in a lyophilizer (FreeZone Cascade Benchtop Freeze Dry Systems, Labconco, Kansas City, USA) in the same jars used for collection. Once lyophilized, the samples were broken down into a fine powder and homogenized in their collection jars by using a baked and solvent-rinsed spatula. All samples were stored at -80 ˚C until chemical analysis.

4.2.3 Lipid analysis

An aliquot of the lyophilized food composite samples was used to determine the lipid content based on the USDA method CLG-FAT.03 [88]. Briefly, 3 g was placed in a Soxhlet extraction

apparatus and extracted with *n*-hexane for 40 cycles or roughly 4 hours. The solvent was then evaporated using a rotary evaporator and the lipid content was determined gravimetrically.

For breast milk samples, 0.5 g of the lyophilized material was combined with 10 mL of hexane, shaken for 2 minutes and then vortexed for 2 minutes. An aliquot of 1 mL was removed and placed onto a pre-weighed aluminum tray. The solvent was evaporated and the lipid content was determined gravimetrically [12]. The moisture and lipid contents of the samples are listed in **Appendix Table 7 & 8**.

4.2.4 Extraction method

Detailed information on the extraction method is presented in chapter 3.

4.2.5 Instrumentation

Detailed information on instrumentation is presented in chapter 3.

4.2.6 Data treatment

Detailed information on data treatment is presented in chapter 3.

4.2.7 QA/QC

Detailed information on QA/QC is presented in chapter 3.

4.2.8 Statistical Analysis

For data analysis, Microsoft Excel and IBM SPSS Statistics 24 were used. For exposure assessment, the non-detects were substituted with half the quantification limits [<LOQ = ½ X

LOQ, i.e. medium bound (MB)] [59]. Statistical analysis was performed at the 95% confidence interval for all tests. The statistical significance between two groups (i.e. packaged vs. non-packaged) was determined using a t-test. For the statistical difference between multiple groups (i.e. food type) was determined by analysis of variance (ANOVA), depending on whether data followed a normal distribution or not. A value for which the probability is lower than 0.05 ($P < 0.05$), and the corresponding value of F is lower than that of F_{crit}, was considered as statistically significant.

4.3. Exposure doses and risk assessment

The dietary exposure of Canadians was estimated in order to approximate the magnitude of dietary exposure to OPEs and mOPEs and to determine the relative importance of dietary exposure to overall flame retardant and plasticizer exposure. After the determination of the levels of OPEs and mOPEs in food, their average daily doses (DI) through dietary consumption were calculated for adults and children. The food consumption was based on the Reasonable Daily Food Consumption (median, g/d) for Canadians [89].

For breast milk, the daily dose was calculated for infants from 0-3 months since this is the age range at the time of breast milk collection. The average consumption of breast milk for infants in the 0-3 months age category is between 590-900 mL [14], therefore, these values were used as lower and upper bounds.

$$DI = \frac{\sum(c_i \text{ X } CF_i)}{BW}$$

Equation 4.1

Where DI is the dietary intake of a specified compound (ng/kg bw/day), c_i is the median concentration of the compound in the *i*th food item, CF_i is the daily consumption (median value) of the *i*th food item, and BW is based on an average body weight of 70 kg for the general population, 60 kg for women, 26.4 kg for children 5-11 years of age, 14.4 kg for children 1-4 years of age. For breast milk consumption, 6 kg and 5.4 kg were used as BW for male and female three month olds, respectively.

The exposure risks of each compound were estimated according to the US EPA reference doses. Hazard quotient (HQ) was used to evaluate the exposure risks as follows:

$$\mathrm{HQ} = \frac{\mathrm{DI}}{\mathrm{RfD}}$$

Equation 4.2

Where RfD is a reference dose for each compound obtained from previous studies; in the case of conflicting values, the most conservative method was applied. An HQ of <0.2 for any given pathway is often considered acceptable [90].

4.4. Results and discussion

4.4.1 Concentrations of OPEs & mOPEs in foodstuff

All target OPEs and mOPEs were detected in at least one food sample. In general, the detection frequency was low. Studies that have analyzed similar matrices for OPEs and mOPEs also report low detection frequencies [23, 66, 85]. **Table 4.1** reports the detection frequencies as well as the detected levels for all samples analyzed in the present study.

Butter had the highest levels of OPEs, with a mean ΣOPE concentration of 110 ng/g w.w. followed by bread (16 ng/g w.w.), canned fish (6 ng/g w.w.), fish and chicken (5 ng/g w.w.) and finally vegetables (3 ng/g w.w.) (**Figure 4.1**). These values are in line with a previous study by Poma et al., who analyzed similar food categories in Belgium for 14 OPEs [59], and another study by the same author for OPEs in food from Sweden [66]. The concentrations of mOPEs, when compared to the values reported in Australia by He et al. are also within the same order of magnitude for all tested food groups [23]. It is interesting to note that for all studies, including this one, grain products are the group with the highest detection frequencies for OPEs as well as the highest median concentrations.

When individually considered, the distribution of OPEs greatly varied among the food groups. For example, TBOEP was highest in canned fish up to a concentration of 13 ng/g, TEHP in butter at 78 ng/g, TDCPP in canned fish at 4.30 ng/g, TCP isomers in bread up to 0.56 ng/g, TCIPP and TPHP in fish at 20 ng/g and 13 ng/g, respectively, ip-PDPP in vegetables at 0.71 ng/g.

The sources of individual OPEs may be difficult to pinpoint due to their widespread usage in commercial applications as well as the variations between the food types. The presence of TPHP, which is used both as a FR and plasticizer, can potentially derive from its application in electrical industrial equipment [2]. Usually, high concentrations of TPHP can be detected in indoor dust [8], but the source of the other OPEs remains unclear.

Table 4.1 Concentrations of OPEs and mOPEs (ng/g w.w.) in food and breast milk

			TBOEP	TEHP	TDCPP	TCP	TCIP	TPP	ip-PDPP	TCEP	DCP	BCIPP	BCEP	BBOEP	BTBOEP	BEHP	DPHP
Fish (n=33)	Range	Min	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		Max	3.19	1.86	3.71	0.09	19.53	12.7	0.12	1.70	ND	0.56	0.68	0.56	9.10	0.61	0.67
	Mean		0.15	0.23	0.53	0.01	1.43	1.29	0.01	0.24	0.00	0.02	0.02	0.02	0.66	0.02	0.02
	Std. Dev		0.61	0.41	0.74	0.02	3.80	2.30	0.02	0.41	ND	0.10	0.12	0.10	1.93	0.11	0.12
	Percentile	25	ND	ND	0.03	ND	ND	0.12	ND	ND	ND	ND	ND	ND	ND	ND	ND
		50	ND	ND	0.27	ND	ND	0.63	ND	ND	ND	ND	ND	ND	ND	ND	ND
		75	ND	0.29	0.80	0.02	0.48	1.71	0.02	0.36	ND	ND	ND	ND	ND	ND	ND
		95	2.07	1.34	2.20	0.06	11.81	6.08	0.06	1.32	ND	0.17	0.21	0.17	6.24	0.18	0.20
	% Detect		10	48	81	32	32	87	42	42	ND	3	3	3	10	3	3
Vegetables (n=15)	Range	Min	ND	ND	0.10	ND	ND	0.03	ND	ND	ND	ND	ND	ND	ND	ND	ND
		Max	3.20	0.37	1.02	0.04	12.90	0.27	0.71	0.23	0.08	0.61	0.15	0.61	1.28	1.46	1.57
	Mean		0.67	0.12	0.29	0.01	0.89	0.11	0.12	0.07	0.01	0.09	0.02	0.09	0.18	0.16	0.17
	Std. Dev		0.90	0.12	0.24	0.01	3.32	0.07	0.19	0.06	0.02	0.18	0.05	0.18	0.38	0.43	0.47
	Percentile	25	ND	0.00	0.11	0.00	ND	0.04	0.00	0.02	ND	ND	ND	ND	ND	ND	ND
		50	0.35	0.10	0.17	0.01	ND	0.08	0.08	0.06	ND	ND	ND	ND	ND	ND	ND
		75	0.89	0.18	0.44	0.02	ND	0.16	0.11	0.13	ND	0.19	ND	0.19	0.15	ND	ND
		95	2.32	0.35	0.70	0.03	5.45	0.21	0.47	0.18	0.03	0.45	0.13	0.45	1.00	1.14	1.24
	% Detect		62	77	100	85	15	100	92	77	8	23	15	23	23	15	15
Bread (n=7)	Range	Min	ND	ND	0.06	ND	ND	ND	ND	ND	16.5	ND	ND	ND	ND	ND	ND
		Max	ND	0.19	0.75	0.56	ND	0.60	0.03	0.36	29.2	4.39	10.11	4.39	6.56	ND	ND
	Mean		ND	0.04	0.16	0.12	ND	0.17	ND	0.05	10.4	1.20	1.44	1.20	0.94	ND	ND
	Std. Dev		ND	0.07	0.26	0.22	ND	0.29	0.01	0.14	13.5	2.05	3.82	2.05	2.48	ND	ND
	Percentile	25	ND	ND	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		50	ND	ND	0.06	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		75	ND	0.07	0.18	0.25	ND	0.57	ND	ND	27.1	4.02	ND	4.02	ND	ND	ND
		95	ND	0.12	0.41	0.37	ND	0.59	0.01	0.14	28.0	4.16	4.04	4.16	2.62	ND	ND
	% Detect		ND	29	86	43	ND	43	14	14	43	29	14	29	14	ND	ND

Table 4.1 *Continued…*

			TBOEP	TEHP	TDCPP	TCP	TCIP	TPP	ip-PDPP	TCEP	DCP	BCIPP	BCEP	BBOEP	BTBOEP	BEHP	DPHP
Butter (n=8)	Range	Min	ND	77.68	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		Max	0.90	378.9	2.55	ND	10.12	2.21	0.18	1.89	ND	8.44	10.37	8.44	9.86	0.03	1.20
	Mean		0.26	183.2	1.12	ND	3.65	1.05	0.02	0.52	ND	1.53	1.55	1.53	1.23	ND	0.21
	Std. Dev		0.38	126.5	1.19	ND	4.17	0.93	0.06	0.67	ND	3.09	3.63	3.09	3.49	0.01	0.43
	Percentile	25	ND	88.60	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		50	ND	105.2	0.92	ND	2.32	1.26	ND	0.33	ND	ND	ND	ND	ND	ND	ND
		75	0.64	313.2	2.29	ND	7.41	1.90	0.01	0.84	ND	2.83	1.54	2.83	ND	ND	0.34
		95	0.82	356.8	2.47	0.00	9.24	2.12	0.12	1.54	ND	6.80	7.46	6.80	6.41	0.02	0.94
	% Detect		38	100	63	13	50	63	25	50	ND	25	25	25	13	13	38
Chicken (n=2)	Range	Min	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		Max	ND	0.18	0.56	ND	ND	1.38	ND	0.41	ND	0.64	ND	0.64	ND	ND	ND
	Mean		ND	0.09	0.28	ND	ND	0.69	ND	0.21	ND	0.32	ND	0.32	ND	ND	ND
	Median		ND	0.09	0.28	ND	ND	0.69	ND	0.21	ND	0.32	ND	0.32	ND	ND	ND
	% Detect		ND	50	50	50	ND	50	ND	50	ND	50	ND	50	ND	ND	ND
Canned fish (n=4)	Range	Min	ND	ND	0.05	0.02	ND	0.15	ND	ND	ND	ND	ND	ND	ND	ND	ND
		Max	12.69	1.15	4.30	0.12	0.90	1.14	ND	0.91	ND	ND	ND	ND	9.14	0.31	0.04
	Mean		3.17	0.48	1.52	0.05	0.23	0.60	ND	0.41	ND	ND	ND	ND	2.28	0.09	0.01
	Median		ND	0.38	0.87	0.03	ND	0.54	ND	0.37	ND	ND	ND	ND	ND	0.02	ND
	% Detect		25	50	100	100	25	100	ND	75	ND	ND	ND	ND	25	50	25
Breast Milk (n=100)	Range	Min	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		Max	12.72	89.41	21.80	5.87	40.98	27.0	39.54	2.54	11.6	9.88	18.55	9.88	20.69	6.33	7.01
	Mean		0.13	8.11	0.47	0.57	6.42	1.60	4.66	0.10	1.51	0.92	1.49	0.38	0.66	0.19	0.21
	Std. Dev		1.27	14.05	2.19	1.14	5.43	4.79	8.29	0.34	2.35	2.07	3.16	1.57	2.70	0.98	1.06
	Percentile	25	1.27	14.05	2.19	1.14	5.43	4.79	8.29	0.34	2.35	2.07	3.16	1.57	2.70	0.98	1.06
		50	ND	ND	ND	ND	3.15	0.03	0.13	ND	ND	ND	ND	ND	ND	ND	ND
		75	ND	2.12	0.15	ND	5.14	0.21	0.90	ND	0.47	ND	0.26	ND	ND	ND	ND
		95	ND	10.32	0.32	0.43	7.99	0.49	4.60	0.07	1.90	0.84	1.25	0.01	ND	ND	0.02
	% Detect		16	55	75	42	98	84	85	32	53	34	57	27	14	25	38

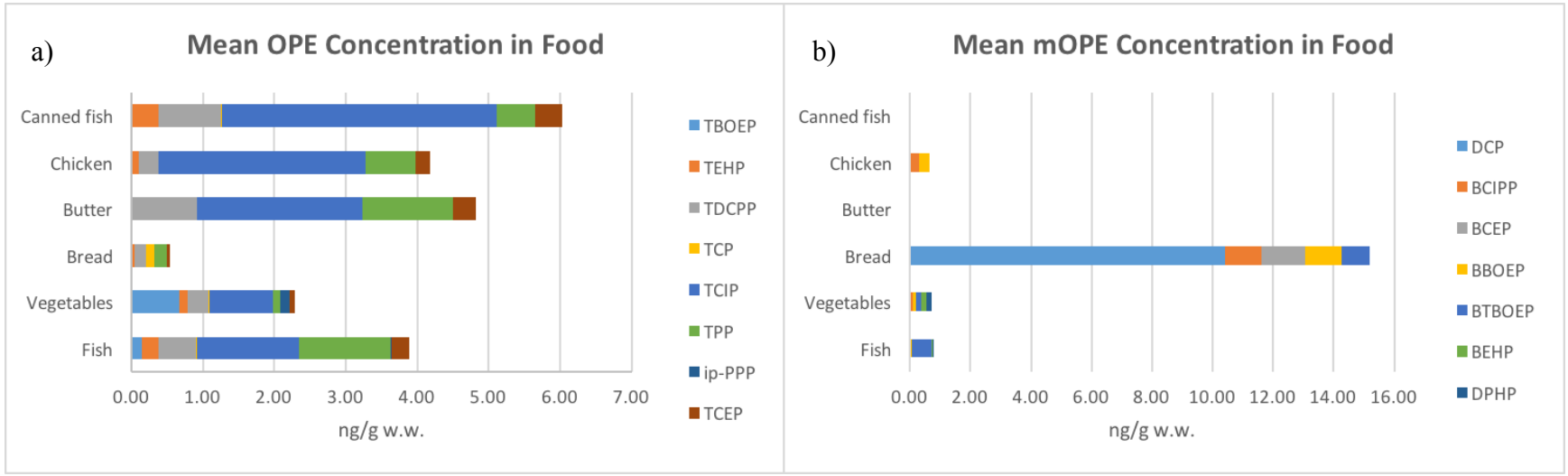


Figure 4.1 Mean OPE and mOPE concentration in different food groups and contributions of individual a) OPEs and b) mOPEs to the overall contamination

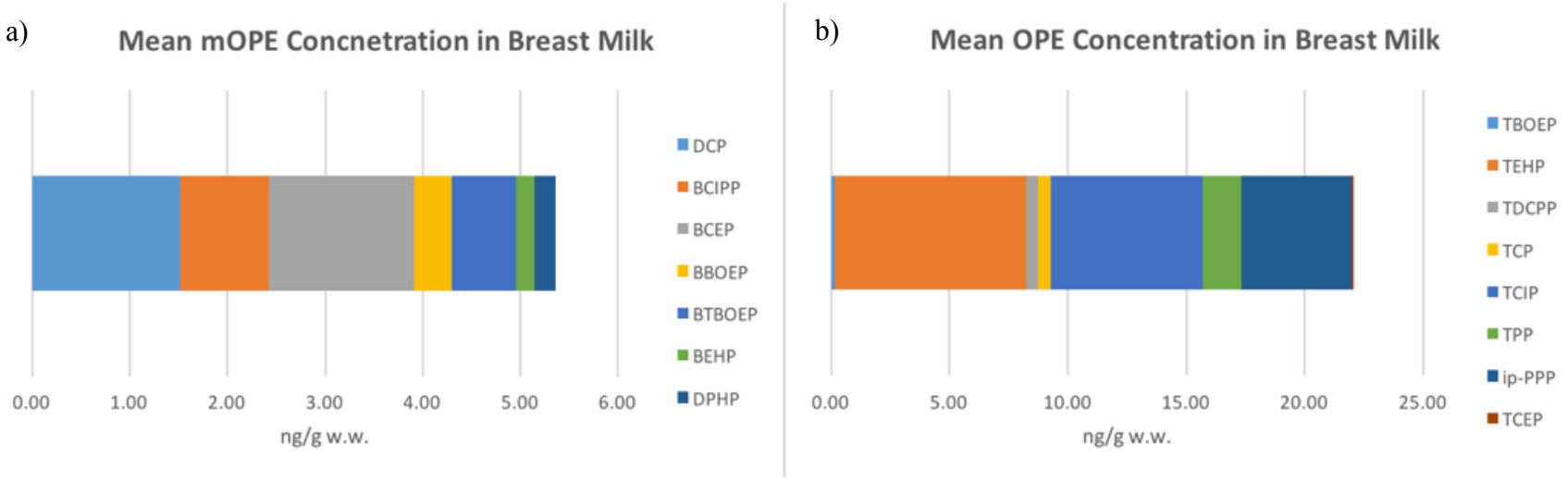


Figure 4.2 Mean OPE and mOPE concentration in breast milk and contributions of individual a) OPEs and b) mOPEs to the overall contamination

As previously reported, OPEs do not show lipid dependent migration, meaning foods with high lipid contents do not necessarily correlate to having high concentration of OPEs [26]. One of the possible reasons for lipid independent migration, proposed by Ding et al. (2018), was the relatively fast degradation of OPEs compared to hydrophobic organic pollutants. A notable exception is butter, which contained the highest mean levels of TEHP (78 ng/g), TCEP (2 ng/g), BCIPP (8.44 ng/g), BCEP (10 ng/g), BBOEP (8.44 ng/g) and BTBOEP (9 ng/g). Other studies have also found samples with high lipid contents (e.g. butter & oil) to contain higher levels of OPEs compared to other food types [23, 26, 59, 85]. Poma et al. reported that processed foods had significantly higher levels of OPEs compared to non-processed foods. This could be one of the reasons for butter having higher levels of the above mentioned analytes [59]. However, it remains unclear if the presence of OPEs in processed foods is from the processing of the foods or from the ingredients typical of processed foods (i.e. grains, fat, oil, meat, etc.). To further investigate the lipid independence of OPEs, the fish samples were individually ranked based on their lipid profiles (i.e. at the 5^{th}, 25^{th}, 50^{th}, 75^{th}, 95^{th} percentiles of lipid content) and compared; no statistically significant difference ($P>0.05$) was observed for any analyte at any lipid content.

When samples of different types were compared within food groups, there was no statistically significant differences ($P>0.05$) for any of the analytes. Also, marine fish were compared to freshwater fish species for differences in levels of OPEs, again, no statistically significant difference was observed ($P>0.05$). For this reason, samples were grouped together in broad categories (i.e. fish, vegetables, canned fish, chicken, bread and butter). The concentration of OPEs between most food groups varied significantly (**Table 4.1**), but not within food groups. The influence of packaging was also negligible; Xu et al., hypothesized that food packaging may play a major role for the migration of OPEs into food [67], but this was not the case in the present study.

When non-packaged samples were compared to packaged samples, there was no statistically significant differences ($P>0.05$) for any of the analytes and any of the food matrices. For further statistical evaluation, packaged and non-packaged samples were combined. With no statistically significant differences, this may illustrate that the environment the food is from or the industrial processing of the food may play a more important role on the total concentration of OPEs. To date, there are no other studies that have compared packaged and non-packaged foods for differences in levels of OPEs.

Other authors have hypothesized the low concentrations of OPEs in fish and meat are due to the rapid metabolism of these compounds by the organism [26]. To test this hypothesis, OPEs and their respective metabolites were quantitated. Only samples containing both the parent and respective metabolite were normalized. The mean ratios of parent to metabolite, are presented in **Table 4.2.** From these data, it can be noted that the ratio is generally >1, meaning there is more parent than metabolite present in the sample. A notable and interesting exception is bread, which has ratios of 0.137 (TDCPP/BCIPP), 0.011 (TCP/DCP) and 0.035 (TCEP/BCEP). This may illustrate the effect of processing on foodstuff and the potential degradation or metabolism of OPEs. Since bread is baked at elevated temperatures, it may be hypothesized that this might cause the metabolism or breakdown of OPEs. A possible reason for other food types having ratios >1 could be due to mOPEs reactivity within the matrix causing them to bind to components such as proteins, carbohydrates, lipids, etc.; and, thus, the analytical method used was not optimized to detect these adducts. Further work may use non-targeted workflows in an attempt to identify these adducts or other potential metabolites and breakdown products.

Table 4.2 Ratio of median concentration of OPE to mOPE in food and breast milk

	TBOEP/ BTBOEP	TEHP/ BEHP	TDCPP/ BCIPP	TCP/ DCP	TCIP/ BCIPP	TPP/ BEHP	TCEP/ BCEP
Fish	>1	>1	>1	>1	>1	>1	>1
Vegetables	>1	0.739	>1	>1	>1	0.667	>1
Bread	>1	>1	0.137	0.011	>1	>1	0.035
Butter	>1	>1	>1	>1	>1	>1	>1
Chicken	>1	>1	0.875	>1	>1	>1	>1
Canned fish	>1	>1	>1	>1	>1	>1	>1
Breast Milk	0.356	>1	0.515	>1	>1	>1	>1

4.4.2 Concentrations of OPEs & mOPEs in breast milk

All target compounds tested were detected in at least one breast milk sample. The range of concentrations of OPEs within and between samples is large, as illustrated by the standard deviation for each compound (**Tables 4.1** & **Appendix Table 9)**. Most OPEs were not detected above the MDL in breast milk. Of the samples with positive detections, one sample contained TEHP at a concentration of 89 ng/g w.w.. **Figure 4.3** illustrates how DCP and TCIP account for the majority of the total mean percent distribution of OPEs in breast milk. The values for OPEs are within the same order of magnitude as those reported by Sundkvist et al.

To further study the lipid independence of OPEs, breast milk samples were grouped according to their lipid content (i.e. at the 5^{th}, 25^{th}, 50^{th}, 75^{th}, 95^{th} percentiles). At no point was there a statistically significant difference for any of the analytes due to the lipid content of the samples, even with a range of 1.6 – 14.2% lipids.

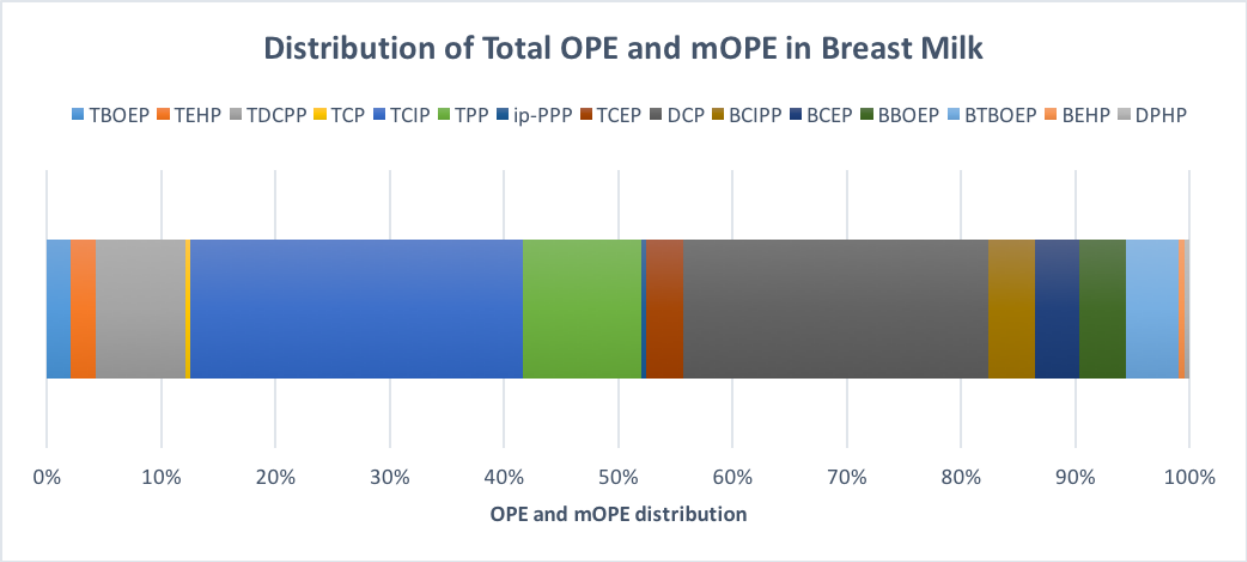


Figure 4.3 Distribution of total OPE and mOPEs in breast milk samples (n=100)

Sundkvist et al. reported similar detection frequencies for OPEs in breast milk [12], however, mOPEs have not been previously measured in this particular matrix. With the exception of BCEP, all other mOPEs have lower detection frequencies than their parent OPEs (**Table 4.1**). The ratios of OPE parent to metabolite are presented in **Table 4.2.** The ratios are generally greater than one (>1), with the exception of TBOEP/BTBOEP and TDCIPP/BCIPP. These data show that most OPEs are not fully metabolized before excretion in breast milk and, thus, the infants are primarily exposed to the parent OPEs. Urine biomonitoring studies have shown a similar trend when incomplete metabolism of OPEs occurred [24].

Even with the non-metabolized OPEs, the mean concentration of the parent OPE is in the low or even sub-ppb levels (on a w.w. basis). Nonetheless, careful monitoring of emerging contaminants should continue in order to assess whether these substances are detected more in the future.

4.4.3 Estimation of human dietary exposure to OPEs and mOPEs

Estimation of the dietary exposure of the Canadian population to OPEs plus mOPEs was based on the measured concentrations (median levels, 50th percentile) of the targeted compounds in 66 composite food samples. It should be noted that the foods collected only represent a fraction of the average Canadian diet [89] and therefore should not be treated as an accurate assessment, nevertheless it does provide a first look into the dietary intake for Canadians. The total dietary intake of OPEs and mOPEs was estimated to be 12800 ± 1500 ng/day [183±22 ng/kg bw/day] (**Table 4.3**). This is most likely an overestimation of the total dietary ingestion of OPEs for these foods due to substituting values <MLOQ by half of the MLOQ. Also, TEHP in butter represents a daily exposure of 5997 ng/day, which is roughly half the overall dietary intake. This is unlikely to be the case since many people do not consume butter and/or they use other fats and oils that have not been evaluated in this study. If the estimate of dietary exposure is done without including the result for TEHP in butter, the value is estimated to be 6800±500 ng/day [98±7 ng/kg bw/day]. Poma et al. also noted that fat contributed much more to the daily OPE intake than any other food group. The values calculated for the present study were higher than those reported by Poma et al. for the Belgian population [7500±1500 ng/day] [85]. It is necessary to point out that the study by Poma et al. did not take into account the mOPEs, which, in the present, study account for a daily exposure of 4275±604 ng/day, roughly a third of the total dietary intake mentioned above. In addition, the consumption habits for Canadians are different than those of the Belgian population[89].

The relative contribution from each food group to the total daily consumption of OPEs is such that vegetables represents 4%, fish 7%, chicken 17%, bread 41%, butter (without TEHP) 10% and

canned fish 21%, **Figure 4.4**. The contribution from each food group is similar to those reported by Poma et al. [59], with grains contributing the most to the overall ingestion of OPEs.

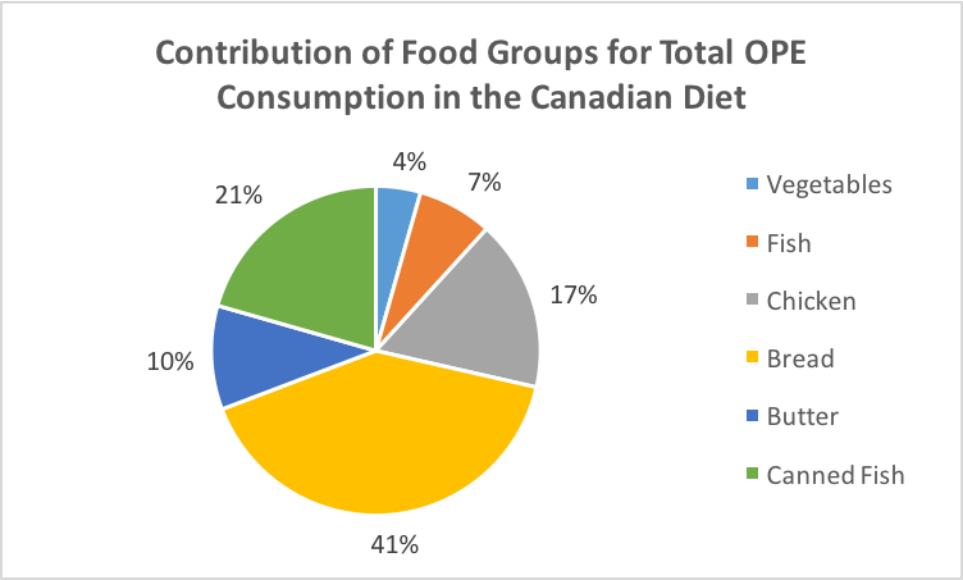


Figure 4.4 Contribution of Selected food groups on total OPE consumption based on the Canadian diet

For young infants, the dietary exposure was based solely on breast milk since this is the only source of nutrition they are receiving. Due to the variations between both the weight in males and females and the uncertainty in consumption of breast milk, lower and upper bound calculations were under taken. At the lower bound estimate, the dietary intake of OPEs was determined to be 13029 ± 1879 ng/day [2171 ± 313 ng/kg bw/day and 2413 ± 348 ng/kg bw/day for males and females, respectively]. The upper bound estimate was calculated to be 19874 ± 2866 ng/day [3312 ± 478 ng/kg bw/day and 3680 ± 531 ng/kg bw/day for males and females, respectively] (**Table 4.4**). Both these estimates were greater than the dietary exposure of the general Canadian population illustrating that young infants can be exposed to relatively high levels of OPEs through breast milk consumption.

Table 4.3 Exposure Assessment for food based on the Canadian diet (values reported as ng/day)

	TBOEP	TEHP	TDCPP	TCP	TCIP	TPP	ip-PDPP	TCEP
Vegetables	67	12	29	1	89	11	12	7
Fish	15	23	53	1	143	129	1	24
Chicken	54	9	28	37	291	69	32	21
Bread	81	6	25	18	167	25	48	8
Butter	31	5997	52	21	132	72	18	19
Canned Fish	317	48	152	5	23	60	32	41
Total (ng/day)	565	6094	339	83	844	365	144	119
Total (ng/kg bw/day) General (70 kg)	8	87	5	1	12	5	2	2
Total (ng/kg bw/day) Women (60 kg)	9	102	6	1	14	6	2	2
Total (ng/kg bw/day) 5-11 yrs (26.4 kg)	21	231	13	3	32	14	5	5
Total (ng/kg bw/day) 1-4 yrs (14.4 kg)	39	423	24	6	59	25	10	8
RfD (ng/kg bw/day)	$1.5x10^{4}$	$3.5 x10^{4}$	$1.5 x10^{4}$	$4.0 x10^{3}$	$8.0 x10^{4}$	$7.0 x10^{4}$	$1.5 x10^{4}$	$2.2 x10^{4}$
HQ (General)	0.0005	0.0025	0.0003	0.0003	0.0002	0.0001	0.0001	0.0001
HQ (Women)	0.0006	0.0029	0.0004	0.0003	0.0002	0.0001	0.0002	0.0001
HQ (5-11 yrs)	0.0014	0.0066	0.0009	0.0008	0.0004	0.0002	0.0004	0.0002
HQ (1-4 yrs)	0.0026	0.0121	0.0016	0.0014	0.0007	0.0004	0.0007	0.0004

Table 4.4 Exposure assessment for young infants consuming breast milk

	TBOEP	TEHP	TDCPP	TCP	TCIP	TPP	ip-PDPP	TCEP
Breast milk (ng/day)	79	4786	279	336	3790	946	2751	61
Lower bound[a] (ng/kg bw/d) Male 3 months (6 kg)	13	798	46	56	632	158	458	10
Upper bound[b] (ng/kg bw/d) Male 3 months (6 kg)	20	1217	71	86	964	241	699	16
Lower bound (ng/kg bw/d) Female 3 months (5.4 kg)	15	886	52	62	702	175	509	11
Upper bound (ng/kg bw/d) Female 3 months (5.4 kg)	22	1352	79	95	1071	267	777	17
RfD (ng/kg bw/d)	$1.5x10^{4}$	$3.5\ x10^{4}$	$1.5\ x10^{4}$	$4.0\ x10^{3}$	$8.0\ x10^{4}$	$7.0\ x10^{4}$	$1.5\ x10^{4}$	$2.2\ x10^{4}$
Lower bound HQ (Males)	0.0009	0.0228	0.0031	0.0140	0.0079	0.0023	0.0306	0.0005
Upper bound HQ (Males)	0.0013	0.0348	0.0047	0.0214	0.0120	0.0034	0.0466	0.0007
Lower bound HQ (Females)	0.0010	0.0253	0.0034	0.0156	0.0088	0.0025	0.0340	0.0005
Upper bound HQ (Females)	0.0015	0.0386	0.0052	0.0238	0.0134	0.0038	0.0518	0.0008

[a] Based on a consumption of 590 mL of breast milk per day
[b] Based on a consumption of 900 mL of breast milk per day

4.4.4 Risk assessment of OPEs in food and breast milk

As defined by the US EPA, the reference dose (RfD) is an estimate of the daily oral exposure in the human population that is likely to be without an appreciable risk of deleterious effects during a lifetime. The estimated dietary intakes of OPEs were compared using the available RfDs, from previously published studies [23, 26, 59, 66, 78, 91]. The calculated HQ for each compound was several orders of magnitude lower than the HQ value of 0.2 and therefore within the acceptable range [89]. This is true for all groups, even the 1-4 year group; however, by using the same food consumption patterns as the general population for this group, it greatly overestimates the total hazard and is therefore a conservative estimate.

For breast feeding young infants, all calculated HQ values are below the threshold of 0.2 by several orders of magnitude. This shows that there should not be any adverse effects from OPE ingestion through breast milk based on the current toxicological literature.

On the basis of these preliminary results, the Canadian population has a low exposure to OPEs compared to the toxicological thresholds and should not suffer adverse effects from OPE ingestion through diet. Currently, there are no RfDs for any of the mOPEs and, thus, the HQ could not be assessed.

4.5. Conclusion

The presence of OPEs and mOPEs in food and breast milk has been assessed. Based on the analysis of packaged and non-packaged food items, no observable difference ($P>0.05$) was found between

these groups in terms of their overall OPE or mOPE contamination. In addition, the types of food analyzed show significantly different distribution patterns although there are no differences within food groups. OPEs and mOPEs illustrated lipid independence in both food and breast milk, with no statistically significant differences between high and low lipid content samples. After assessing the dietary exposure for OPEs and mOPEs, there is currently a tolerable level of these compounds in all sample types [89]. The samples collected in the present study are from the greater Montreal area and may not be representative of the current levels for OPEs and mOPEs across Canada. Also, diet represents only one possible exposure route, for total OPE and mOPE exposure other sources such as dust, air and water should be included. Finally, it should be noted once again that the samples analyzed do not represent a typical Canadian diet and therefore more samples should be included to give a more representative assessment in future studies. This is the first exposure and risk assessment for OPEs in food from the Canadian market. Also, this is the first exposure and risk assessment for OPEs in breast milk.

CHAPTER 5. GENERAL SUMMARY AND CONCLUSION

The development of a novel multiclass extraction method for PBDEs, Dechloranes, OPEs and mOPEs using sonication as the extraction technique and fractionation using SPE SPE was validated for food and breast milk. Several variations of novel and traditional extraction methods in the literature were tested, however the method presented in this study is rapid and cost effective. The optimized methodology was applied to fish, chicken, canned fish, bread, butter and vegetables collected in Montreal in November 2017 and May 2018 and breast milk collected between November 2017 and June 2018. Good recoveries were obtained for all compounds analyzed. Analytical approaches, including LC-QqQ-MS/MS, LC-QTOF-MS and GC-MS, were optimized and validated. In addition, using LC-QTOF-MS, the isomers of TCP and ip-PDPP were separated chromatographically for the first time.

A screening for OPEs and mOPEs in 66 composite food samples and 100 breast milk samples for OPEs and mOPEs was performed. The levels of OPEs and mOPEs in food were consistent with the results from other published studies [23, 59, 66]. As of now, only one other study has analyzed OPEs in breast milk[12]; this is, therefore, the first report of mOPEs in breast milk. We found that there was no significant migration of contaminants from the packaging materials. The types of food showed significantly different distribution patterns of the target analytes, but there was no statistically significant difference within the food groups. In food and breast milk, OPEs and mOPEs showed lipid independence with no statistically significant differences between high and low lipid content samples. In food and breast milk, it was observed that, generally, there were more OPEs to mOPEs. One notable exception to this was bread, which had higher metabolite to parent ratios than any other food group. A possible reason for this could be the high temperature

processing needed to bake bread thus causing degradation of OPEs to mOPEs. In breast milk, having mostly higher OPE to mOPE ratios might suggest that complete metabolism of the compounds did not take place within the mammary gland of the mother and that the young infants were receiving primarily OPEs through ingestion of breast milk.

Dietary estimates of OPEs and mOPEs through consumption of food for four population groups [men, women, children (5-11 years) and young children (1-4 years)] were assessed. Based on the HQ values, there is a tolerable level for OPEs and mOPEs via dietary exposure, even when overestimating the consumption of the food. It should be noted, however, that the samples analyzed do not represent a typical Canadian diet; different sample types should be included in future studies to give a more representative assessment for dietary exposure and also while considering the values for risk assessment other potential routes of exposure (i.e., dust, air, water, more hand-to-mouth activity for toddlers) should be considered.

Dietary exposure to OPEs and mOPEs by young infants was assessed for 3 month old males and females at low and high levels of breast milk consumption. For both lower and upper bound consumption estimates, the HQ is several orders of magnitude lower than 0.2, suggesting that there is a tolerable level of OPEs and mOPEs in breast milk.

The detection of these emerging contaminants illustrates their widespread presence in various food commodities and, most importantly, in breast milk. The concentrations and detection frequencies of these compounds demonstrate the need for robust analytical techniques capable of analyzing both legacy and emerging contaminants as well as their metabolites in complex matrices. Further studies should be performed to analyze different sample types to better understand the daily exposure to these chemicals from the Canadian diet.

As legacy chemicals are replaced by formal bans or voluntary withdrawal, new alternatives will emerge to fill the market gaps; such is the case with OPEs. Future work may expand food types and investigate the effectiveness of various food preparation procedures to decrease exposure to OPEs and mOPEs. Non-target analysis techniques may be employed to better understand possible degradation products that are not accounted for using targeted analysis and to screen for a wider array of unknown contaminants by using large databases. Future studies would also benefit from the use of non-targeted analysis to investigate the potential migration of OPEs through food contact materials such as food packaging.

It should be noted that the present study analyzed 14 of the most commonly used OPEs however, there are many other emerging OPEs that are produced in high volumes that merit being screened for in future studies. By providing toxicologists with adequate information for emerging chemicals it will allow for assays that are representative of the current levels of contaminants in the diet. Ultimately, this flow of information will lead to more informed and responsible policies that can be put into place to avoid the potential replacement of chemicals with more hazardous substitutes.

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APPENDIX

Table 1 Method performance for LC-QTOF-MS

Compound	Matrix factor	Accuracy (%) (n=8)	Precision (%) (n=8)	Recovery
OPE				
TCEP	11.44	91.62	88.07	74-116
TCIP	2.95	97.70	91.13	74-116
TDCIPP	5.38	85.26	91.2	74-116
TPHP	5.94	95.22	76.7	31-86
TBOEP	7.55	93.64	85.6	74-116
2-ip-PDPP	3.58	144.40	93.08	51-147
3-ip-PDPP	0.50	88.86	92.9	51-147
4-ip-PDPP	1.27	88.86	90.64	51-147
TOCP	2.39	78.93	95.65	51-147
TMCP	1.56	95.90	93.54	51-147
TPCP	1.56	95.90	93.55	51-147
TEHP	1.01	91.24	85.03	4.1-12.9
mOPE				
DCP	2.32	93.89	86.04	83-96
BBOEP	1.13	97.99	86.47	83-96
BTBOEP	2.28	74.97	85.56	51-147
BCEP	1.35	85.17	77.89	27-89
BCIPP	1.00	142.8	97.14	27-89
DPHP	3.62	96.12	82.67	131-172
BDCP	5.40	95.21	96.63	27-89
BEHP	0.20	94.85	97.12	2.3-4.2

Table 2 Method performance for internal standards with LC-QTOF-MS

Compound	ESI mode	IDL (ng/L)	MDL (ng/L)	Absolute recovery (%)
TCEP-d12	+	0.57	1.89	74-116
DCP-d14 (isomers)	+	1.09	3.63	83-96
DBHPT-d4	+	0.34	1.13	51-147
TDCPP-d15	+	1.64	5.47	30-60
TPHP-d15	+	0.44	1.47	31-86
DMCP-d7	+	1.12	3.74	20-79
TEHP-d51	+	2.13	7.10	4.1-12.9
DPHP-d10	-	0.71	2.35	131-172
BBOEP-d8	-	0.58	1.93	47-63
BDCP-d10	-	0.54	1.80	27-89
BEHP-d34	-	0.66	2.19	2.3-4.2

Table 3 Food sample description and mass used for extraction

Sample Type		Sample Description	Mass Used (g)
Fish[a]	Fresh Water Fish: Low Fat	Tilapia	2.0
		Hake	2.0
	Fresh Water Fish: High Fat	Rainbow Trout	1.0
		Basa	1.0
	Marine Fish: Low Fat	Haddock	2.0
		Cod	2.0
	Marine Fish: High Fat	Salmon	1.0
		Halibut	1.0
Leafy Vegetable		Spinach	1.0
		Arugula	1.0
		Romaine Lettuce	1.0
		Watercress	1.0
Bread		White loaf	1.5
		Brown loaf	1.5
Butter		Salted	0.5
		Unsalted	0.5
Canned Foods		Canned Tuna in water	2.0
		Canned Tuna in oil	1.0
Chicken		Breast, skinless, boneless	2.0
Breast Milk		NA	1.0

[a]Low fat refers to samples with <2% lipid content, high fat refers to fish with >2% lipid content

Table 4 LC-QqQ-MS/MS MRM parameters

Compound	Parent Ion[a]	Daughter Ion[a]	Cone (V)[b]	Collision (eV)[c]	ESI[d]
Analytes					
BCEP	222.93	98.86	32	18	+
BCIPP	253.00	98.81	25	20	+
DpCP	279.21	91.13	46	30	+
TCEP	287.10	99.01	36	24	+
m-ip-PPP	293.30	77.08	46	36	+
BBOEP	299.28	45.05	34	18	+
TPP	327.18	77.13	56	38	+
TCIP	329.18	99.01	32	20	+
BTBOEP	343.15	44.88	22	18	+
TmCP	369.26	91.13	68	38	+
4-ip-PDPP	369.26	215.15	64	36	+
TBOEP	399.37	45.04	38	22	+
TDCPP	431.04	99.01	36	22	+
DPHP	249.03	93.03	56	24	-
BDCP	318.64	34.42	18	8	-
BEHP	321.01	78.88	58	32	-
TEHP	435.53	99.00	18	16	+
Internal Standards					
ip-PPP-d7	224.18	176.10	24	8	+
BCIPP-d12	264.98	100.88	26	14	+
DpCP-d14	293.3	97.25	56	30	+
TCEP-d12	299.17	67.10	40	26	+
BBOEP-d8	307.33	49.01	32	18	+
TPHP-d15	342.19	81.82	64	40	+
BTBOEP-d4	347.17	44.95	32	18	+
TBOEP-d27	426.42	208.09	36	16	+
TDCPP-d15	446.14	102.00	40	26	+
TEHP-d51	486.85	102.19	28	22	+
DPHP-d10	259.15	98.04	54	26	-
BDCP-d10	328.71	34.74	18	8	-
BEHP-d34	355.41	227.26	60	24	-

[a] *m/z*

[b] Voltage

[c] Electro Spray Ionization

Table 5 Analytical details for LC-QTOF-MS

Compound	Formula	Monoisotopic Mass[a]	Accurate Mass[a]	Mass Error[b]	ISTD
OPEs					
TBOEP	$C_{18}H_{39}O_7P$	399.2511 $[M+H]^+$	399.2525	3.34	TPHP-d15
TCEP	$C_6H_{12}C_{l3}O_4P$	284.9617 $[M+H]^+$	284.9611	2.13	TCEP-d12
TCIP	$C_9H_{18}C_{l3}O_4P$	327.0086 $[M+H]^+$	327.0085	0.48	TCEP-d12
TDCIPP	$C_9H_{15}C_{l6}O_4P$	428.8917 $[M+H]^+$	428.8920	0.60	TDCPP-d15
TEHP	$C_{24}H_{51}O_4P$	435.3603 $[M+H]^+$	435.3603	0.05	TEHP-d51
TPHP	$C_{18}H_{15}O_4P$	327.0786 $[M+H]^+$	327.0794	2.38	TPHP-d15
2-ip-PDPP	$C_{21}H_{21}O_4P$	369.1255 $[M+H]^+$	369.1261	1.43	DBHPT-d4
3-ip-PDPP	$C_{21}H_{21}O_4P$	369.1255 $[M+H]^+$	369.1264	2.24	DMCP-d7
4-ip-PDPP	$C_{21}H_{21}O_4P$	369.1255 $[M+H]^+$	369.1256	0.27	DMCP-d7
TMCP	$C_{21}H_{21}O_4P$	369.1255 $[M+H]^+$	369.1263	1.97	DMCP-d7
TOCP	$C_{21}H_{21}O_4P$	369.1255 $[M+H]^+$	369.1260	1.16	DMCP-d7
TPCP	$C_{21}H_{21}O_4P$	369.1255 $[M+H]^+$	369.1263	1.97	DMCP-d7
mOPEs					
BBOEP	$C_{12}H_{27}O_6P$	299.1623 $[M+H]^+$	299.1640	5.68	BBOEP-d8
BTBOEP	$C_{14}H_{31}O_7P$	343.1885 $[M+H]^+$	343.1888	0.68	DBHPT-d4
BCEP	$C_4H_9C_{l2}O_4P$	222.9693 $[M-H]^-$	222.9694	0.45	DPHP-d10
BCIPP	$C_6H_{13}C_{l2}O_4P$	251.0006 $[M-H]^-$	251.0007	0.32	BDCP-d10
BDCP	$C_6H_{11}Cl_4O_4P$	316.9070 $[M-H]^-$	316.9071	0.32	BDCP-d10
BEHP	$C_{16}H_{35}O_4P$	321.2194 $[M-H]^-$	323.2354	0.86	BEHP-d34
DPHP	$C_{12}H_{11}O_4P$	249.0316 $[M-H]^-$	249.0317	0.40	DPHP-d10
DmCP	$C_{14}H_{15}O_4P$	277.0629 $[M-H]^-$	279.0779	2.59	DCP-d14
DoCP	$C_{14}H_{15}O_4P$	277.0629 $[M-H]^-$	279.0778	2.95	DCP-d14
DpCP	$C_{14}H_{15}O_4P$	277.0629 $[M-H]^-$	279.0783	1.16	DCP-d14
m-ip-PPP	$C_{15}H_{17}O_4P$	291.0786 $[M-H]^-$	293.0937	1.96	DBHPT-d4
o-ip-PPP	$C_{15}H_{17}O_4P$	291.0786 $[M-H]^-$	293.0934	2.98	DBHPT-d4
p-ip-PPP	$C_{15}H_{17}O_4P$	291.0786 $[M-H]^-$	323.2354	0.86	DBHPT-d4

[a] *m/z*

[b] Mass Error (ppm) =[(Accurate mass-Monoisotopic mass)/monoisotopic mass] X 10^6

Table 6 Scan descriptors, levels of bromination and chlorination, m/z's and BDEs and Dechloranes monitored by GC-HRMS

Function and bromine or chlorine level	*m/z*	*m/z* type	Substance
Fn-1, Br-3	405.8027	M+2	TrBDE
	407.8002	M+4	TrBDE
	417.8429	M+2	$^{13}C_{12}$ TrBDE
	419.8409	M+4	$^{13}C_{12}$ TrBDE
	442.9728	Lock	PFK
Fn-2, Br-4	483.7132	M+2	TeBDE
	485.7111	M+4	TeBDE
	497.7514	M+2	$^{13}C_{12}$ TeBDE
	499.7493	M+4	$^{13}C_{12}$ TeBDE
	492.9691	Lock	PFK
Fn-3, Br-5	563.6216	M+4	PeBDE
	565.6196	M+6	PeBDE
	575.6619	M+4	$^{13}C_{12}$ PeBDE
	577.6598	M+6	$^{13}C_{12}$ PeBDE
Fn-4, Br-6	641.5322	M+4	HxBDE
	643.5302	M+6	HxBDE
	653.5723	M+4	$^{13}C_{12}$ HxBDE
	655.5704	M+6	$^{13}C_{12}$ HxBDE
Fn-5, Br-7	721.4406	M+6	HpBDE
	723.4386	M+8	HpBDE
	733.4809	M+6	$^{13}C_{12}$ HpBDE
	735.4788	M+8	$^{13}C_{12}$ HpBDE
Fn-6, Br-8	799.3511	M+6	OcBDE
	801.3491	M+8	OcBDE
	811.3914	M+6	$^{13}C_{12}$ OcBDE
	813.3893	M+8	$^{13}C_{12}$ OcBDE
Fn-7, Br-9	879.2596	M+8	NoBDE
	881.2575	M+10	NoBDE
	891.2998	M+8	$^{13}C_{12}$ NoBDE
	893.2978	M+10	$^{13}C_{12}$ NoBDE
	804.9505	Lock	PFK
Fn-8, Br-10	957.1701	M+8	DeBDE
	959.1680	M+10	DeBDE
	971.2083	M+10	$^{13}C_{12}$ DeBDE
	973.2063	M+12	$^{13}C_{12}$ DeBDE
Fn-9, Cl-10	271.8102	M+2	DEC 602, DP *anti* & *syn*
	273.8072	M+4	DEC 602, DP *anti* & *syn*
	260.8599	M+2	DEC 603
	262.8570	M+4	DEC 603
	276.8597	M+2	$^{13}C_{10}$ DP *anti*
	278.8240	M+4	$^{13}C_{10}$ DP *anti*
	276.8597	M+2	$^{13}C_{10}$ DEC 602
	278.8240	M+4	$^{13}C_{10}$ DEC 602

Table 7 Details for food samples collected in Montreal

Food Composites	Moisture content (%)	Fat content (%)	Type	Year	No. of Samples/Composite
Tilapia	78	3.38	Packaged	1	6
Rainbow Trout	74	7.27	Packaged	1	6
Basa	82	6.1	Packaged	1	5
Haddock	80	0.35	Packaged	1	2
Cod	82	0.07	Packaged	1	6
Salmon	72	3.76	Packaged	1	6
Halibut	80	0.04	Packaged	1	1
Sole	79	0.5	Packaged	1	3
Spinach	91	0.09	Packaged	1	2
Arugula	96	0.08	Packaged	1	4
Romaine lettuce	94	0.39	Packaged	1	6
Watercress	92	0.29	Packaged	1	1
Canned tuna in water	79	4.17	Packaged	1	6
Canned tuna in oil	52	21.7	Packaged	1	6
White loaf	33	0.53	Packaged	1	6
White loaf	33	1.22	Packaged	1	6
White loaf	42	0.53	Core Samples	1	6
White loaf	42	0.53	Core Samples	1	6
Brown loaf	37	0.02	Packaged	1	6
Brown loaf	37	0.02	Packaged	1	6
Brown loaf	44	0.02	Core Samples	1	6
Brown loaf	54	0.02	Core Samples	1	6
Butter (salted)	5	76.12	Packaged	1	6
Core pieces:	9	76.12	Core Samples	1	6
Butter (unsalted)	6	70.51	Packaged	1	6
Core pieces:	9	70.51	Core Samples	1	6
Chicken breast	76	1.12	Packaged	1	6
Cod	82	0.06	Non-Packaged	1	4
Salmon	84	4.52	Non-Packaged	1	5
Rainbow Trout	71	7.27	Non-Packaged	1	4
Sole	82	2.15	Non-Packaged	1	5
Tilapia	75	2.94	Non-Packaged	1	4
Halibut	81	0.04	Non-Packaged	1	1
Haddock	79	0.43	Non-Packaged	1	3
Arugula	91	1.19	Non-Packaged	1	6
Watercress	93	0.51	Non-Packaged	1	6
Spinach	88	0.01	Non-Packaged	1	6
Romaine lettuce	94	0.39	Non-Packaged	1	6
Tilapia	78	1.73	Packaged	2	6
Rainbow trout	73	9.73	Packaged	2	6
Basa	85	0.93	Packaged	2	6
Haddock	83	0.231	Packaged	2	6
Cod	81	0.003	Packaged	2	6
Salmon	71	7.36	Packaged	2	6
Halibut	81	0.24	Packaged	2	1
Hake	77	1.3	Packaged	2	5
Sole	85	0.32	Packaged	2	6
Spinach	92	0.2	Packaged	2	6
Arugula	94	0.26	Packaged	2	6
Romaine lettuce	94	0.19	Packaged	2	6
Watercress	94	0.01	Packaged	2	6
Canned tuna in oil	49	26.44	Packaged	2	6

Table 7 *Continued…*

Food Composites	Moisture content (%)	Fat content (%)	Type	Year	No. of Samples/Composite
Canned tuna in water	77	0.99	Packaged	2	6
White loaf	32	1.27	Packaged	2	6
White loaf	37	1.27	Core Samples	2	6
Brown loaf	32	1.27	Packaged	2	6
Brown loaf	44	1.27	Core Samples	2	6
Salted butter	10	61.38	Packaged	2	6
Salted butter	13	70.46	Core Samples	2	6
Non-salted butter	13	78.7	Packaged	2	6
Non-salted butter	17	83.08	Core Samples	2	6
Chicken breast	72	0.98	Packaged	2	6
Cod	82	0.25	Non-Packaged	2	6
Salmon	69	7.14	Non-Packaged	2	6
Rainbow trout	73	4.32	Non-Packaged	2	6
Basa	82	0.59	Non-Packaged	2	6
Tilapia	78	0.79	Non-Packaged	2	6
Haddock	77	0.18	Non-Packaged	2	6
Sole	84	0.15	Non-Packaged	2	6
Watercress	93	0.14	Non-Packaged	2	6
Spinach	88	0.07	Non-Packaged	2	6
Romaine lettuce	95	0.03	Non-Packaged	2	6

Table 8 Moisture and lipid content for breast milk samples

Human Subject Number	Moisture content (%)	Fat content (%)
1001	81.0	5.3
1003	82.2	3.9
1010	81.0	4.2
1007	79.8	4.6
1009	81.3	3.2
1011	78.4	5.9
1025	82.4	2.7
1016	80.9	5.6
1021	82.4	6.9
1024	79.4	6.2
1020	82.4	2.5
1026	80.0	4.5
1027	82.5	5.0
1029	81.9	4.0
1031	81.5	2.7
1037	79.7	5.6
1019	78.8	4.7
1036	81.3	2.8
1039	77.4	4.8
1041	76.8	9.3
1044	82.8	3.8
1046	79.2	5.8
1030	51.6	9.1
1048	84.0	1.7
1035	84.5	3.5
1053	78.9	6.3
1045	74.9	3.3
1051	78.5	2.8
1055	81.9	4.5
1052	83.6	2.5
1054	79.2	6.1
1065	80.1	3.7
1063	79.7	3.7
1059	80.6	3.2
1061	80.5	6.1
1066	75.1	10.9
1058	77.7	5.2
1073	79.7	5.9
1075	77.4	4.1
1062	82.1	1.6
1072	77.2	4.8
1082	77.6	6.5
1077	84.9	2.9
1079	77.2	5.2
1083	77.3	6.0
1087	78.2	5.7
1084	81.5	2.1

Table 8 *Continued…*

Human Subject Number	Moisture content (%)	Fat content (%)
1088	71.2	14.2
1080	79.4	2.9
1096	80.4	6.3
1093	55.6	9.3
1094	79.1	6.2
1090	71.4	5.4
1091	80.7	4.8
1101	83.7	1.7
1103	76.2	7.2
1104	69.2	8.0
1106	78.1	6.8
1107	77.4	7.1
1115	81.2	3.7
1118	79.8	1.7
1119	80.7	3.6
1114	80.2	5.1
1121	81.0	3.7
1120	79.3	5.7
1123	78.0	5.2
1105	56.8	N/A
1110	64.5	6.1
1127	76.1	6.5
1111	81.2	4.7
1124	76.8	6.8
1128	79.2	6.2
1138	78.8	9.4
1129	80.1	6.5
1126	80.8	4.0
1134	81.3	3.2
1139	80.0	5.1
1135	80.4	4.4
1130	80.2	3.3
1133	80.0	7.8
1145	76.6	7.3
1142	78.8	7.7
1149	80.3	5.1
1147	81.0	6.0
1152	80.2	5.8
1074	77.8	9.1
1146	80.9	4.1
1150	78.1	7.3
1153	80.9	5.5
1154	72.3	5.5
1155	81.0	5.2
1156	81.4	5.4
1161	80.1	4.3
1164	73.9	9.6

Table 8 *Continued…*

Human Subject Number	Moisture content (%)	Fat content (%)
1165	77.3	9.6
1166	76.9	9.9
1168	79.5	4.6
1171	77.3	9.6
1174	81.9	3.4
1175	82.0	5.0
1176	77.6	4.7
1177	73.5	5.8

Table 9 Concentrations of OPEs and mOPEs in breast milk (ng/g l.w.)

	Breast Milk l.w. (n=100)							
	Range		SD	Percentile				% Detect
				25th	50th	75th	95th	
TBOEP	ND	4.89	0.72	ND	ND	ND	0.74	16
TEHP	ND	328.42	51.79	ND	ND	ND	132.29	55
TDCPP	ND	95.62	13.35	ND	2.65	7.28	33.13	75
TCP	ND	76.11	14.43	ND	ND	2.67	42.03	42
TCIP	ND	302.42	41.94	ND	ND	ND	90.50	98
TPP	ND	794.38	109.67	ND	3.42	12.55	214.65	84
ip-PDPP	ND	42.32	7.17	ND	ND	2.85	20.26	85
TCEP	ND	149.52	16.34	ND	ND	1.51	13.64	32
DCP	ND	44.20	6.85	ND	ND	ND	16.25	53
BCIPP	ND	38.94	7.17	ND	ND	ND	22.19	34
BCEP	ND	37.53	6.21	ND	ND	3.91	12.29	57
BBOEP	ND	38.94	3.92	ND	ND	ND	0.62	27
BTBOEP	ND	106.99	14.19	ND	ND	ND	3.37	14
BEHP	ND	3.94	0.80	ND	ND	ND	2.83	25
DPHP	ND	8.71	1.31	ND	ND	0.24	2.36	38