A STUDY ON THE SOURCES AND FATE OF NOVEL FLAME RETARDANTS AND ORGANOPHOSPHATE ESTERS (OPEs) IN FOOD BY NON-TARGETED ANALYSIS

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Abstract

Flame retardants (FRs) are often added to commercial products to achieve flammability resistance. Examples of the FRs currently in use include organophosphate esters (OPEs) and novel brominated FRs (NBFRs). OPEs, specifically, are also applied as plasticizers, adhesives and coating agents in plastic packaging materials. These FRs are often added as additives, meaning that they are not chemically bonded to the polymers. Therefore, they can be easily released into the environment by wastewater discharge, atmospheric disposition, or runoffs during the production, use, disposal, and recycling processes. As these FRs are being classified as endocrine-disrupting chemicals, there are concerns regarding the exposure by humans. According to recent studies from various countries, OPEs and NBFRs are being detected in an increasing range of food, but the exact sources and fate are still unknown. The main objective of this thesis was to apply targeted and non-targeted analyses to detect OPEs and NFRs as additional sources of contamination in food; and to study the thermal degradation reactions of OPEs, to monitor the fate of these trace residues in food.

In Chapter 3, a literature review on the abiotic degradation of different classes of FRs summarized the stability of multiple classes of flame retardants in environmental and food matrices, including thermal and photodegradation. This highlighted the knowledge gap regarding the fate of degradation products and their toxicity is mostly unknown in the environment and food. It was also noted that there are very limited degradation studies using normal conditions, so the behaviour of FRs is unknown in these matrices.

In Chapter 4, the source of FRs in atmospheric air was investigated using honey as a bio-indicator. The FR profiles of honey samples collected from rural and urban regions were compared, and no significant difference was found. Also, a non-targeted screening workflow using an in-

house screening library was developed and validated to screen FRs in 200 honey samples at trace levels. This showed that a non-targeted workflow was effective in detecting unknown FR compounds present in the samples.

In Chapter 5, the additional source of FRs in food from plastic food packaging materials was investigated. A non-targeted screening was also applied to detect unknown FRs present in food packaging samples. Based on the occurrence study, it was found that thermal labels were the main source of FRs present in plastic food packaging materials, with tris(2-butoxyethyl) phosphate (TBOEP) showing a migration potential using a migration cell set-up. A controlled migration study was also done to understand the migration potential of OPEs from thermal labels into the fresh chicken meat samples. It was found that TBOEP could migrate into chicken meat, but it was below the specific migration limit.

In Chapter 6, the fate of these OPEs under cooking temperatures were investigated, in an aqueous model solution and various food matrices. The degradation products were identified by applying non-targeted analysis. Based on the results, it was found that the triester OPEs degraded into diesters in the model solution, most likely by hydrolysis. Under food matrices, including honey, salmon and chicken matrices, OPEs were spiked to the matrices and the degradation kinetics was studied and compared with the model solution. It was found the rates of degradation were hindered in these matrices, potentially by the specific interactions of OPEs with each matrix. Since the current exposure assessment neglected the unknown degradation products, and overestimated the dietary intake of OPEs, this study was able to provide more realistic data and refine OPE exposure assessment scenarios related to the diet. Also, this study demonstrated that a non-targeted analysis method was able to identify unknown compounds present in the samples.

Overall, this thesis was able to apply a non-targeted screening workflow to identify additional sources of FR contamination in food from the environment (atmospheric air) and industrial processing (packaging); and highlight the fate of OPEs detected from food under cooking conditions, by applying non-targeted analysis. These results suggested that the risk assessment should consider the additional sources of FRs, as well as cooked food for dietary intake analysis and unknown products for toxicity studies.

Résumé

Les retardateurs de flamme (RF) sont souvent ajoutés aux produits commerciaux pour obtenir une résistance à l'inflammabilité. Des exemples des RF actuellement utilisés incluent les esters organophosphorés (EOP) et les nouveaux RF bromés (NBRF). Les EOP, en particulier, sont également utilisés comme plastifiants, adhésifs et agents de revêtement dans les matériaux d'emballage en plastique. Ces RF sont souvent ajoutés en tant additifs, ce qui signifie qu'ils ne sont pas chimiquement liés aux polymères. Par conséquent, ils peuvent être facilement libérés dans l'environnement par les rejets d'eaux usées, la dispersion atmosphérique ou les écoulements lors des processus de production, d'utilisation, d'élimination et de recyclage. Comme ces RF sont classés comme perturbateurs endocriniens, des préoccupations existent concernant l'exposition humaine. Selon des études récentes menées dans divers pays, les EOP et les NBRF sont détectés dans un nombre croissant d'aliments, mais les sources exactes et les destins sont encore inconnus. L'objectif principal de cette thèse était d'appliquer des analyses ciblées et non ciblées pour détecter les EOP et les NBRF en tant que sources supplémentaires de contamination dans les aliments; et d'étudier les réactions de dégradation thermique des EOP, afin de surveiller le destin de ces résidus traces dans les aliments.

Dans le Chapitre 3, une revue de littérature sur la dégradation abiotique de différentes classes de RF a résumé la stabilité de multiples classes de retardateurs de flamme dans les matrices environnementales et alimentaires, y compris la thermodegradation et la photodégradation. Cela a mis en évidence le manque de connaissances concernant le destin des produits de dégradation et leur toxicité est principalement inconnue dans l'environnement et les aliments. Il a également été noté qu'il existe très peu d'études de dégradation utilisant des conditions normales, de sorte que le comportement des RF est inconnu dans ces matrices.

Dans le Chapitre 4, la source des RF dans l'air atmosphérique a été étudiée en utilisant le miel comme bio-indicateur. Les profils de RF des échantillons de miel prélevés dans des régions rurales et urbaines ont été comparés, et aucune différence significative n'a été trouvée. De plus, un flux de travail de dépistage non ciblé utilisant une bibliothèque de dépistage interne a été développé et validé pour dépister les RF dans 200 échantillons de miel à des niveaux de traces. Cela a montré qu'un flux de travail non ciblé était efficace pour détecter des composés RF inconnus présents dans les échantillons.

Dans le Chapitre 5, la source supplémentaire de RF dans les aliments à partir de matériaux d'emballage alimentaire en plastique a été étudiée. Un dépistage non ciblé a également été appliqué pour détecter les RF inconnus présents dans les échantillons d'emballage alimentaire. Sur la base de l'étude d'occurrence, il a été constaté que les étiquettes thermiques étaient la principale source de RF présents dans les matériaux d'emballage alimentaire en plastique, le phosphate de tris(2-butoxyéthyle) (TBOEP) montrant un potentiel de migration en utilisant un montage de migration. Une étude de migration contrôlée a également été réalisée pour comprendre le potentiel de migration des EOP à partir des étiquettes thermiques dans les échantillons de viande de poulet frais, il a été constaté que le TBOEP pouvait migrer dans la viande de poulet, mais qu'il était inférieur à la limite de migration spécifique.

Dans le Chapitre 6, les destins de ces EOP sous températures de cuisson ont été étudiés, dans une solution modèle aqueuse et diverses matrices alimentaires. Les produits de dégradation ont été identifiés en appliquant une analyse non ciblée. Sur la base des résultats, il a été constaté que les EOP triesters se dégradaient en diesters dans la solution modèle, très probablement par hydrolyse. Dans les matrices alimentaires, y compris le miel, les matrices de saumon et de poulet, les EOP ont été ajoutés aux matrices et la cinétique de dégradation a été étudiée et comparée à la solution

modèle. Il a été constaté que les taux de dégradation étaient entravés dans ces matrices, potentiellement par les interactions spécifiques des EOP avec chaque matrice. Étant donné que l'évaluation actuelle de l'exposition néglige les produits de dégradation inconnus, et surestime l'apport alimentaire en EOP, cette étude a permis de fournir des données plus réalistes et affiner les scénarios d'évaluation de l'exposition aux EOP liés au régime alimentaire. De plus, cette étude a démontré qu'une méthode d'analyse non ciblée était capable d'identifier des composés inconnus présents dans les échantillons.

Dans l'ensemble, cette thèse a permis d’appliquer un flux de travail de dépistage non ciblé pour identifier des sources supplémentaires de contamination RF dans les aliments provenant de l'environnement (air atmosphérique) et du traitement industriel (emballage); et mettre en évidence le destin des EOP détectés dans les aliments dans des conditions de cuisson, en appliquant une analyse non ciblée. Ces résultats suggèrent que l'évaluation des risques devrait prendre en compte les sources supplémentaires de RF, ainsi que les aliments cuits pour l'analyse de l'apport alimentaire et les produits inconnus pour les études de toxicité.

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Contributions of Authors

This thesis is presented in manuscript form and consists of seven chapters. Chapter 1 presents a general introduction to flame retardants as contaminants in food and the research objectives of the thesis. Chapter 2 is a literature review of novel flame retardants and organophosphate esters, including their occurrence in food, food safety concerns and toxicology. Chapters 3 to 6 are based on manuscripts and are arranged sequentially through connecting paragraphs. Chapter 3 has been published in *Food Additives & Contaminants: Part A*. Chapter 4 has been published in *Chemosphere*. Chapter 5 will be submitted for publication in the *Journal of Agricultural and Food Chemistry*. Chapter 6 will be submitted for publication in *Food Chemistry*. Chapter 7 presents an overall conclusion, a discussion of the contribution to original knowledge of this thesis and suggestions for future research.

The present author was responsible for the concepts, designs of experiments, experimental work, data acquisition, data analysis, and manuscript preparation of all chapters. Dr. Stéphane Bayen, the thesis supervisor, oversaw the progress of experimental work and data analysis, and was the co-author for all manuscripts. Dr. Melissa McKinney and Dr. Varoujan Yaylayan were the co-authors of Chapter 3. Dr. Lan Liu was involved in the LC-QTOF method development in Chapters 4 to 6. Dr. Lei Tian was involved in the LC-QTOF method development, data analysis and project administration in Chapter 4. Caren Akiki and Shaghig Bilamjian were responsible for collecting honey samples for Chapters 4 and 6. Ziyun Xu was responsible for packaging sample collection and sample preparation in Chapter 5. Dr. Barbara Hales was involved in the manuscript editing for Chapter 5. Dr. Cindy Goodyer was involved in the manuscript editing for Chapters 5 and 6.

Publications

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Conference Presentations

- Leung, G., Bayen, S. Mass Spectrometry Analysis of the Thermal Degradation of Organophosphate Esters (OPEs) in Water at 100 °C. 36th Annual Trent Conference on Mass Spectrometry, virtual, 11th-13th August 2020. (Oral)
- Leung, G., Bayen, S. Mass Spectrometry Analysis of the Thermal Degradation of Organophosphate Esters (OPEs). Macdonald Environmental Toxicology Research Cluster (METRC) Symposium, Montreal, Canada, 1st December 2020. (3-Minute oral)
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- Leung, G., Liu, L., Bilamjian, S., Bayen, S. A Study on the Thermal Degradation of Organophosphate Esters (OPEs) in Water and Food with Application of Non-Targeted Analysis. Macdonald Environmental Toxicology Research Cluster (METRC) Symposium, Montreal, Canada, 15th May 2024. (3-Minute oral)
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Abbreviations

BBOEP	Bis(butoxyethyl) phosphate
BDCIPP	Bis(1,3-dichloro-2-propyl) phosphate
BFR	Brominated flame retardants
CAS	Chemical Abstracts Service
CE	Collision energy
CEPA 1999	Canadian Environmental Protection Act, 1999
DPHP	Diphenyl phosphate
dw	Dry weight
EDC	Endocrine-disrupting chemical
EDI	Estimated daily intake
EFSA	European Food Safety Authority
EIC	Extract-ion chromatogram
ESI	Electrospray ionization
FDA	Food and Drug Administration
FR	Flame retardant
FWHM	Full width at half maximum
HBCDD	Hexabromocyclododecanes
HPLC	High-performance liquid chromatography
HRMS	High-resolution mass spectrometry
LC-MS	Liquid chromatography-mass spectrometry
LC-QTOF	Liquid chromatography-quadrupole time-of-flight

LD_{50}	Median lethal dose
LOD	Limit of detection
LOI	Limit of identification
LOQ	Limit of quantification
mOPE	OPE metabolite
MRLs	Maximum residue limits
MS/MS	Tandem mass spectrometry
NBFR	Novel brominated flame retardants
ND	Not detected
NFR	Novel flame retardants
NTA	Non-targeted analysis
OECD	Organization for Economic Co-operation and Development
OPE	Organophosphate ester
PBDE	Polybrominated diphenyl ether
PCA	Principal component analysis
POP	Persistent organic pollutant
PTFE	Polytetrafluoroethylene
QA	Quality assurance
QC	Quality control
RSD	Relative standard deviation
RT	Retention time
SD	Standard deviation

TBOEP	Tris(2-butoxyethyl) phosphate
TBP	Tributyl phosphate
TDCIPP	Tris(1,3-dichloro-2-propyl) phosphate
TIC	Total-ion chromatograms
TPHP	Triphenyl phosphate
UV	Ultraviolet
ww	Wet weight

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Chapter 1. General Introduction

1.1 General Introduction

Humans are exposed to a diverse range of chemicals, the majority from air, skin contact, food and water. Anthropological chemicals and their transformation products contribute to the majority of the human exposome [1], which refers to the totality of exposure to internal and external sources of chemical or biological agents over a lifetime [2]. Some of these exposures are unintentional and may pose health risks to the population. These sources of exposure can be found in everyday products, including textiles, building materials and polymers. During the processing of polymer products, it is common to add flame retardants (FRs) to achieve flammability resistance. Most of them are used as additives, which are blended into the polymer during processing but do not chemically react with the polymer [3]. Since they are not chemically bonded to the materials, these flame retardants can be easily released into the environment during the production, use, disposal and recycling processes [4].

Traditionally, polybrominated diphenyl ethers (PBDEs) were the most used FRs in the industry. However, it was found that these FR residues have become contaminants in the environment, and the concentrations in human tissue and breast milk increased from 1980s to 1990s [5]. These legacy FRs were also found to be hazardous to human health. In some toxicological studies, it was learned that PBDEs are neurotoxic, and the damage to the neuropsychological function of infants was associated with the increased concentration of PBDEs in breast milk [6]. These adverse effects were caused by the disruption of thyroid hormone, second messenger communication and alteration of neurotransmitter systems [5], for which these compounds were classified in a group of chemicals called endocrine-disrupting chemicals (EDCs).

In addition to the negative health effects on humans, several PBDEs were listed as Persistent Organic Pollutants (POPs) [7], leading to a decreasing usage over the past decade. As a response,

organophosphate esters (OPEs) became a popular alternative as flame retardants [8], and they can be used as additives in other consumer products.

Currently, different types of OPEs are used as flame retardants in a wide range of consumer products. For example, they are commonly used in building materials, electronic cables, furniture, and textures [9]. In addition, they can used as plasticizers, stabilizers, anti-foaming agents, wetting agents, or lubricants [10]. However, since these OPEs are also not chemically bonded to the materials during production, they are also easily released into the environment during their production cycle, by volatilization, leaching, abrasion and dissolution [11]. In recent studies, OPEs were found in a wide range of food, ranging from 0 to 80 ng g^{-1}, which is possibly due to the absorption of OPEs in crops and livestock [11]. Regarding the adverse health effects, there have been studies showing that OPEs can lead to hypertension, seizures, and damage to the cognitive function [12, 13].

In the current literature, the research on the detection of OPEs and their metabolites (mOPEs) in food has only been done in a few regions, but the food samples used were raw, such as meat products. The studies from the literature do not reflect the reality of food intake, where thermal processing was not taken into consideration. During the heating process, high temperatures also supply excessive energy to the OPE may lead to degradation, and this has not been addressed in the literature. Since the degradation products are unknown, it is not possible to assess the risk of exposure to these unknown chemicals from diet.

1.2 Research objectives

The main objective of this research was to apply targeted and non-targeted analyses to detect OPEs and NFRs as additional sources of contamination in food; and to study the thermal degradation reactions of OPEs, to monitor the fate of these trace residues in food. The specific objectives of this project were:

(1) To summarise the available information on the abiotic degradation of four classes of flame retardants (i.e. brominated, organophosphorus, mineral-based and nitrogen-based FRs) in the environment and food matrices.

(2) To screen novel organophosphorus FR (OPEs) and novel brominated FRs (NBFRs) with targeted and non-targeted approaches in honey samples.

(3) To study the occurrence of OPEs and NBFRs in plastic food packaging materials, using both targeted and non-targeted analysis.

(4) To study the degradation reaction of three target OPEs and their diester metabolites under heating in a model solution and different food matrices.

Chapter 2. Literature Review

2.1 Novel flame retardants (NFRs)

A flame retardant (FR) is a component that is used in combustible materials to slow down or hinder the ignition or combustion of that material [14]. It was estimated that the total consumption of flame retardants by the industry was 5 million metric tonnes in 2018, where metal hydroxides were the majority, followed by halogenated and non-halogenated FRs [8, 15]. Application of FRs in consumer products include textiles, cable wires, building materials, and electronic appliances. These flame retardants are classified either by their chemical nature – brominated, organophosphorus, nitrogen-containing or metal hydroxides, or by the means of their incorporation into the material – additive or reactive [16]. Additive FRs are not chemically bound to the chemical structure of the polymer, which can be released to the environment easily, by leaching, volatilization and dissolution [11], which will increase the risk of exposure to human, since the FR residue can travel a long distance through air, water, sediment and biota [8, 17].

Traditional FRs, such as polybrominated diphenyl ethers (PBDEs), have been used for commercial products since the 1970s, but were banned in Europe and California in 2003 due to their ubiquitous occurrence in the environment, persistence and bioaccumulation [18, 19]. In some toxicological studies, it was found that PBDEs are neurotoxic, and the damage to the neuropsychological function of infants was associated with the increased concentration of PBDEs in breast milk [6]. These adverse effects were caused by the disruption of thyroid hormone, second messenger communication and alteration of neurotransmitter systems [5], which these compounds were classified as endocrine-disrupting chemicals (EDCs). Exposure to PBDEs can also lead to developmental and reproductive problems, causing impaired spermatogenesis in males and disrupting thyroid hormone regulation in females [4]. Penta- and octa-BDEs were added to the list of persistent organic pollutants (POPs) and was eliminated from production and use in 2009 in the

Stockholm Convention; deca-BDEs was subsequently eliminated in 2017 [19, 20]. As a result, alternative flame retardants, such as OPEs, have been used as a replacement for these 'legacy' FRs since 2004 due to the phase-out of PBDEs [18, 19].

2.2 Organophosphate esters (OPEs)

OPEs are derivatives of phosphoric, phosphonic, or phosphonic acid. The common structure includes a phosphorus atom and a phosphoryl bond (P=O), with three side chains (Figure 2.1) [21]. The side chains (R_1, R_2 and R_3) can share the same or different structure, with aliphatic, aromatic, or halogenated carbon chains.

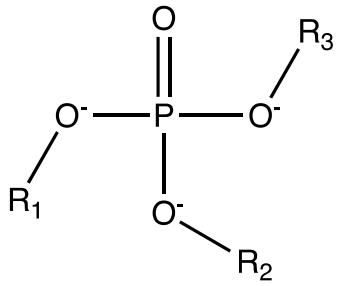


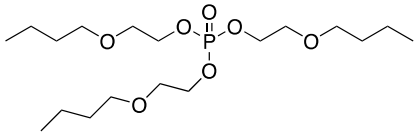
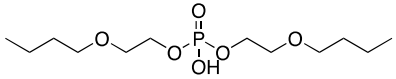
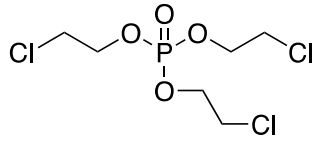
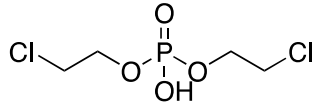
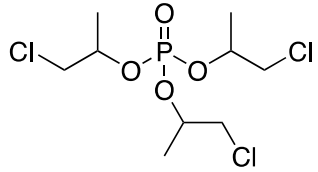
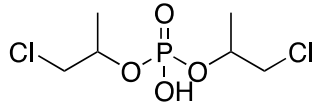
Figure 2.1 – General structure of organophosphate esters.

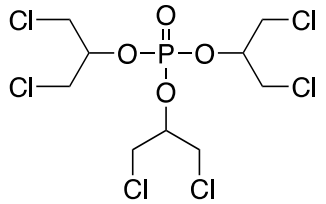
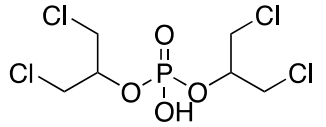
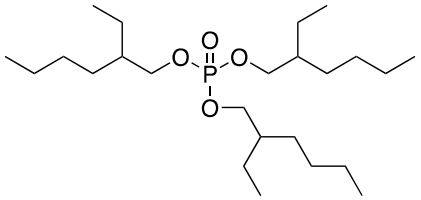
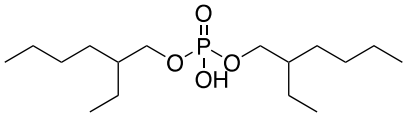
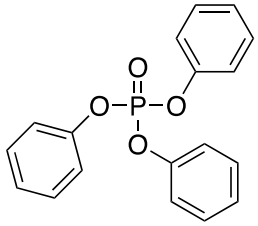
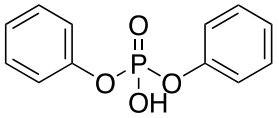
In 2013, the volume of OPEs used as flame retardants was 562,000 metric tons, and the global market share of OPEs was over USD 1.7 billion in 2017, with a growth rate of 8% up to 2025 [22]. OPEs account for 30% of the global demand, in particular, triphenyl phosphate (TPHP) and tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) have reached 23,000 metric tons in the United States [23]. According to the Organization for Economic Co-operation and Development (OECD), some OPEs including tributyl phosphate (TBP), tri-isobutyl phosphate, and diphenyl *p*-tolyl phosphate have been on the list of high-production volume chemicals since 2004 [24].

Halogenated OPEs are usually used to produce fire retardants, such as tris(1-chloro-2-propyl) phosphate (TCIPP) and tris-(2-chloroethyl)-phosphate (TCEP); whereas the non-halogenated OPEs are mostly used as plasticizers, such as TBP, TPHP [8, 25]. OPEs are also used in textiles (TNBP, TPHP, TCEP, triphenylphosphine oxide (TPPO)), electronic equipment (resorcinol-bis(diphenylphosphate) (RDP)), glue (TPHP), cellulose (TCEP, TEHP).

Some of the common types of OPEs are listed in Table 2.1, with information obtained from PubChem. In the table, the octanol-water partition coefficients (log K_{ow}) are listed, and the values range from 0.21 (least lipophilic) to 9.49 (most lipophilic). For most of these OPEs, the log K_{ow} values are around 3 to 5, which has a potential for accumulating in the fatty tissues [26]. In general, the presence of chlorine in the OPE chemical structures lowers the log K_{ow} values, whereas the sole presence of alkyl or aryl groups in the structures increases the lipophilicity of the OPEs. The lipophilicity is proportional to the size of the side chains (molecular weight).

Table 2.1 – List of common OPEs and mOPEs, including their molecular weight, log K_{ow} and molecular structures.

Acronym	Name	CAS number	Chemical formula	Molecular weight	log K_{ow}	Molecular structure
TBOEP	Tris(2-butoxyethyl) phosphate	78-51-3	$C_{18}H_{39}O_7P$	398.24	3.75	
BBOEP	Bis(butoxyethyl) phosphate	14260-97-0	$C_{12}H_{27}O_6P$	298.31	2.22	
TCEP	Tris(2-chloroethyl) phosphate	115-96-8	$C_6H_{12}Cl_3O_4P$	285.5	1.78	
BCEP	Bis(2-chloroethyl) phosphate	3040-56-0	$C_4H_9Cl_2O_4P$	222.99	0.21	
TCIPP	Tris(2-chloroisopropyl) phosphate	13674-84-5	$C_9H_{18}Cl_3O_4P$	327.6	2.59	
BCIPP	Bis(1-chloro-2-propyl) phosphate	789440-10-4	$C_6H_{13}Cl_2O_4P$	327.6	2.59	

TDCIPP	Tris(1,3-dichloro-2-propyl) phosphate	13674-87-8	$C_9H_{15}Cl_6O_4P$	430.9	3.65	
BDCIPP	Bis(1,3-dichloro-2-propyl) phosphate	72236-72-7	$C_6H_{11}Cl_4O_4P$	319.94	1.61	
TEHP	Tris(2-ethylhexyl) phosphate	78-42-2	$C_{24}H_{51}O_4P$	434.6	9.49	
BEHP	Bis(2-ethylhexyl) phosphate	298-07-7	$C_{16}H_{35}O_4P$	322.40	6.09	
TPHP	Triphenyl phosphate	115-86-6	$C_{18}H_{15}O_4P$	326.3	4.59	
DPHP	Diphenyl phosphate	838-85-7	$C_{12}H_{11}O_4P$	250.19	2.88	

2.3 Food safety issues associated with OPEs

Although OPEs were first introduced as a replacement for legacy fire retardants, recent studies on OPEs have shown that it is not completely safe and have raised concerns regarding food safety.

Firstly, OPEs used in the industry are only physically mixed, instead of chemically bonded in the material, which they can be easily released to the environment by volatilization, leaching, abrasion and dissolution [11]. There has been research detecting OPE residues in air, water, sediment and biota [8]. Some of these detections was found in remote region, implicating that the OPEs residues may travel in a long distance and persist in the environment [17]. The OPEs can be released to the environment and lead to exposure by human in different routes after entering the food chain. Studies have found that OPEs are absorbed readily by crops and ingested by livestock and fish [11, 27, 28].

The OPEs used in the industry can be released to the environment easily, and lead to exposure by human in different routes. OPEs evaporated in the air may disperse and transport in the atmosphere. Some of the OPEs are dissolved in rainwater and deposit in the aquatic environment, or directly deposit to the terrestrial ecosystem as dry dust. These OPEs in the environment can enter the food chain, as they are absorbed by crops and ingested by livestock and fish [11, 27]. These OPEs can be accumulated along the food chain and contaminated the food for human.

Apart from polluting the environment, human may be exposed to OPE residues in the diet. This can be resulted from the absorption of OPEs in the crops, or contamination during food processing [11]. Different types of OPEs have been detected in numerous food [29-31]. The estimated daily intake (EDI) of OPE from diet was found to range from 44.3 (median) to 75.8 (95th

percentile) ng kg bw^{-1} day^{-1} [7]. In comparison to the EDI from dust ingestion, it ranged from 9.24 and 155 ng kg bw^{-1} day^{-1} in average (or 23.3 and 390 ng kg bw^{-1} day^{-1} in the high-exposure group); while from dermal absorption, it ranged from 40.8 and 126 ng kg bw^{-1} day^{-1} in average-exposure (or 93.4 and 279 ng kg bw^{-1} day^{-1} in the high-exposure group) [32]. The EDI value from diet is comparable to the inhalation and dermal absorption in the normal cases, which makes it an important source of OPE exposure.

The most exposed OPEs from food differ by the region. For instance, 57% of OPEs exposure in Sweden population was from EDHPP, which has a larger portion than any other OPEs, while TPHP (45%) and TCIPP (18%) was the major source of OPE exposure in Belgium. The common major OPEs from different studies are TCEP, TCIPP, TPHP and TDCIPP [11]. However, comparing the EDI values with the reference dose (RfD) of OPEs, which ranges from 13,000 to 80,000 ng kg bw^{-1} day^{-1}, was much higher than that of the EDI by a magnitude of 100 to 1000 [11].

2.4 Types and concentrations of OPEs found in food samples

Common OPEs could be detected in various types of food, with samples originating from Australia [30], Belgium [33], Canada [34], China [35], Sweden [29] and the United Kingdom [36]. The data reported are listed in Table 2.2, mean concentrations of the detected OPEs, range of the mean concentrations from different studies, and the average reported detection frequency. Not all types of data are available for all the OPEs, as there have been only limited studies on the detection of OPEs in food samples. In general, most studies only targeted the parent OPEs (triester) but not their diester metabolites in all food matrices. For example, TDCIPP was detected in all groups of food matrices, while its metabolite, BDCIPP, was only detected in cereals, eggs, fruits, and

vegetables. Among the food matrices, the detection frequency in meat, fish and eggs was the highest, followed by fruits and cereals. However, the concentrations detected in cereals, oil and fish were generally higher than other foodstuff.

The detection of OPEs could result from other sources of contamination, such as the use of EHDPP in food packaging materials accounted for the detection in cereals [29], or the detection of TPHP could be due to the indoor dust concentration in the industrial facilities [11, 29]. These suggest that the industrial processes during food processing may have a risk of contaminating food with OPEs.

The limitation in this summary was the discrepancy in sampling size and the product types among food samples from different countries. This could also be a result of the difference in contamination level in the regions of food samples collection, or the methodology of OPE extraction, isolation, and separation was not standardized among the experiments conducted by different authors [11]. The data cited in Table 2.2 may not be representative of the samples collected in other countries, especially for North America and Western Europe, due to the fact that these places, and China, accounted for 60% of the global consumption of fire retardants [11]. Other countries that are reported to have rapid growth in the demand for OPEs are India and Thailand [11].

Table 2.2 – The concentrations (ng g^{-1} ww), range (ng g^{-1} ww) and average detection frequency of common OPEs found in different types of food.

OPE		Cereals	Meat	Fish	Oil/butter	Dairy products	Eggs	Vegetables	Fruits	Beverage	Major mOPEs
BBOEP	Mean	0.936	0.16	0.423	1.53	0.076	1.13	1.17	0.146	0.06	N.A.
	Range	(0.675, 1.2)	(0, 0.32)	(0.423, 0.423)	(1.53, 1.53)	(0.076, 0.076)	(1.13, 1.13)	(0.09, 2.24)	(0.146, 0.146)	(0.06, 0.06)	
	DF%	64.5	25	1.5	25	11.1	100	51.5	N.A.	10	
BCEP	Mean	0.72	N.A.	0.01	1.55	4.21	N.A.	0.0.1	N.A.	N.A.	N.A.
	Range	(0, 1.44)	N.A.	(0, 0.02)	(1.55, 1.55)	(4.21, 4.21)	N.A.	(0, 0.02)	N.A.	N.A.	
	DF%	7	N.A.	1.5	25	33.3	N.A.	7.5	N.A.	N.A.	
BCIPP	Mean	0.72	0.16	0.1	1.53	N.A.	N.A.	0.045	N.A.	N.A.	N.A.
	Range	(0, 1.44)	(0, 0.32)	(0, 0.02)	(1.53, 1.53)	N.A.	N.A.	(0, 0.09)	N.A.	N.A.	
	DF%	14.5	25	1.5	25	N.A.	N.A.	11.5	N.A.	N.A.	
BDCIPP	Mean	1.52	N.A.	N.A.	N.A.	N.A.	1.05	0.663	0.225	N.A.	N.A.
	Range	(1.52, 1.52)	N.A.	N.A.	N.A.	N.A.	(1.05, 1.05)	(0.663, 0.663)	(0.225, 0.225)	N.A.	
	DF%	83.3	N.A.	N.A.	N.A.	N.A.	66.7	20	33.3	N.A.	
BEHP	Mean	N.A.	0.0304	0.166	N.A.	0.203	1.64	0.16	0.499	1.15	N.A.
	Range	N.A.	(0, 0.0608)	(0.02, 0.312)	N.A.	(0.203, 0.203)	(1.64, 1.64)	(0.16, 0.16)	(0.499, 0.499)	(1.15, 1.15)	
	DF%	N.A.	12.5	29.3	N.A.	66.7	100	50.8	86.6	N.A.	
DBP	Mean	0.7	0.0416	0.448	N.A.	0.253	1.31	1.58	0.245	0.335	N.A.
	Range	(0.7, 0.7)	(0.0416, 0.0416)	(0.448, 0.448)	N.A.	(0.253, 0.253)	(1.31, 1.31)	(1.58, 1.58)	(0.245, 0.245)	(0.335, 0.335)	
	DF%	100	75	88.9	N.A.	100	100	100	86.6	50	

OPE		Cereals	Meat	Fish	Oil/butter	Dairy products	Eggs	Vegetables	Fruits	Beverage	Major mOPEs
DMPP	Mean	0.42	N.A.	2.7	N.A.	0.110	0.313	0.938	0.195	N.A.	N.A.
	Range	(0.42, 0.42)	N.A.	(2.7, 2.7)	N.A.	(0.110, 0.110)	(0.313, 0.313)	(0.938, 0.938)	(0.195, 0.195)	N.A.	
	DF%	91.6	N.A.	66.7	N.A.	18.1	100	60	13.3	N.A.	
DPHP	Mean	5.098	0.086	1.265	0.21	2.35	3.87	6.19	0.974	1.09	N.A.
	Range	(5.098, 5.098)	(0, 0.172)	(0.2, 2.51)	(0.21, 0.21)	(2.35)	(3.87, 3.87)	(0.17, 12.2)	(0.974, 0.974)	(1.09, 1.09)	
	DF%	50	50	40.4	13	100	100	57.5	86.6	70	
EHDPP	Mean	2.35	1.028	0.33	4.12	1.045	0.216	0.18	0.165	0.643	DPHP
	Range	(0, 5)	(0, 3.7)	(0, 0.064)	(0.14, 7.57)	(0, 3.76)	(0, 0.91)	(0, 0.58)	(0, 0.47)	(0, 1.5)	
	DF%	67.8	3.266	55.75	66.7	40.5	43	47.5	26.3	9	
TBOEP	Mean	3.157	0.723	3.07	0.285	3.94	N.A.	2.67	1.75	1.86	BBOEP
	Range	(0, 9.32)	(0, 2.55)	(0.112, 11.9)	(0.26, 0.31)	(1.07, 6.81)	N.A.	(0.120, 7.2)	(0.269, 3.23)	(0.111, 3.6)	
	DF%	24.3	3.266	33.2	9.5	9.8	N.A.	36.8	29.2	36	
TBP	Mean	0.566	2.58	0.41	3.36	2.76	0.122	0.466	0.659	1.155	DBP
	Range	(0.11, 0.93)	(0.09, 9.23)	(0.04, 0.56)	(0.1, 6.62)	(0.06, 10.2)	(0.08, 0.167)	(0.12, 0.95)	(0.11, 1.67)	(0.11, 2.2)	
	DF%	25	29.3	29.3	N.A.	34.7	25	21.9	17.9	5.57	
TCEP	Mean	0.766	0.165	0.148	1.78	0.163	0.222	0.177	0.249	0.2	BCEP
	Range	(0.05, 3.03)	(0.05, 0.26)	(0.06, 0.24)	(0.5, 4.31)	(0.05, 0.23)	(0.03, 0.5)	(0.01, 0.41)	(0.09, 0.52)	(0.17, 0.23)	
	DF%	56.1	55	50.1	62.5	63.2	56.8	87.4	63.3	36.1	

OPE		Cereals	Meat	Fish	Oil/butter	Dairy products	Eggs	Vegetables	Fruits	Beverage	Major mOPEs
TCIPP	Mean	1.12	0.493	0.863	10.9	0.769	0.301	0.630	0.788	0.405	BCIPP
	Range	(0, 3.65)	(0, 1.7)	(0.08, 1.41)	(0.52, 38.9)	(0.41, 1.10)	(0.1, 0.565)	(0.02, 1.91)	(0.11, 2.27)	(0.17, 0.846)	
	DF%	62.1	36.6	42.3	37.5	43.7	30.7	60.2	41.6	55.6	
TDCIPP	Mean	0.264	0.386	0.535	1.1	0.315	0.0767	0.186	0.298	0.480	BDCIPP
	Range	(0.16, 0.43)	(0, 0.8)	(0.055, 1.41)	(1, 1.2)	(0, 0.55)	(0, 0.18)	(0.01, 0.37)	(0.163, 0.44)	(0.231, 0.86)	
	DF%	52.1	23.75	42.3	54.3	33.4	16.7	69.9	44.4	82.3	
TEHP	Mean	0.1925	0.115	0.165	94.8	0.125	0.0567	(0.0525)	1.11	N.A.	BEHP
	Range	(0, 0.38)	(0, 0.35)	(0.055, 1.41)	(6.45, 183.2)	(0, 0.25)	(0, 0.15)	(0, 0.12)	(0.09, 2.12)	N.A.	
	DF%	21.75	16.8	32.8	33.3	N.A.	5.67	27.5	17.2	N.A.	
TMPP	Mean	0.1	0.072	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	BMPP
	Range	(0.1, 0.1)	(0.072, 0.072)	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
	DF%	33.3	8.3	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
TPHP	Mean	4.96	2.57	1.031	2.18	1.4	0.075	0.791	0.463	0.263	DPHP
	Range	(0.17, 26.14)	(0.137, 9.29)	(0.42, 2.53)	(0.78, 4.74)	(0, 3)	(0, 0.12)	(0.7, 2.01)	(0, 1.07)	(0.07, 0.47)	
	DF%	32.8	50.4	51.4	65.8	19.25	0	58.2	23.25	36.2	

2.5 Toxicokinetics of OPEs

2.5.1 Sources of absorption of OPEs

Apart from exposure through ingestion, OPEs can be absorbed by humans through dermal absorption and inhalation of dust, meaning all routes of entry are possible [37]. In dermal absorption, the OPEs that are moderately lipophilic are favourable (K_{ow} range from –2 to 2) to cross the epidermis and reach the circulation, and the general trend of the rate and extent of absorption is proportional to the log K_{ow} value [38]. The exposure of OPEs through inhalation of indoor dust was 9.24 to 155 ng kg bw^{-1} day^{-1}, and that of dermal absorption was 40.8 to 126 ng kg bw^{-1} day^{-1} [39]. This is in the same magnitude of ingestion from the diet, which ranged from 44.3 to 75.8 ng kg bw^{-1} day^{-1}, showing that diet is an equally important exposure route [7]. The details of the absorption of OPEs in the route of inhalation and absorption in the gut, based on bioavailability were not documented in the literature [11].

2.5.2 Distribution of OPEs in the body

After absorption into the bloodstream, the OPEs are distributed to the kidney and liver, and they are mainly metabolized in the liver. Some of them will be deposited at metabolically inactive tissues, such as adipose tissues and muscles [37]. However, the OPEs only have limited affinity with the lipid content in fish, meaning the deposition of OPEs involve more than the fatty tissues [37]. OPEs are also found in hair or nails in human, but it is unlikely to be distributed by blood due to rapid metabolism in the body. This could be attributed to the source of dermal contact [40].

2.5.3 Metabolic pathways for OPEs

The metabolic pathways of OPEs are differentiated by the types of OPEs, namely, chlorinated-, alkyl- and aryl-OPEs. The major steps of metabolism involve O-dealkylation, hydroxylation, oxidative dichlorination, oxidation and conjugation (Figure 2.2) [37].

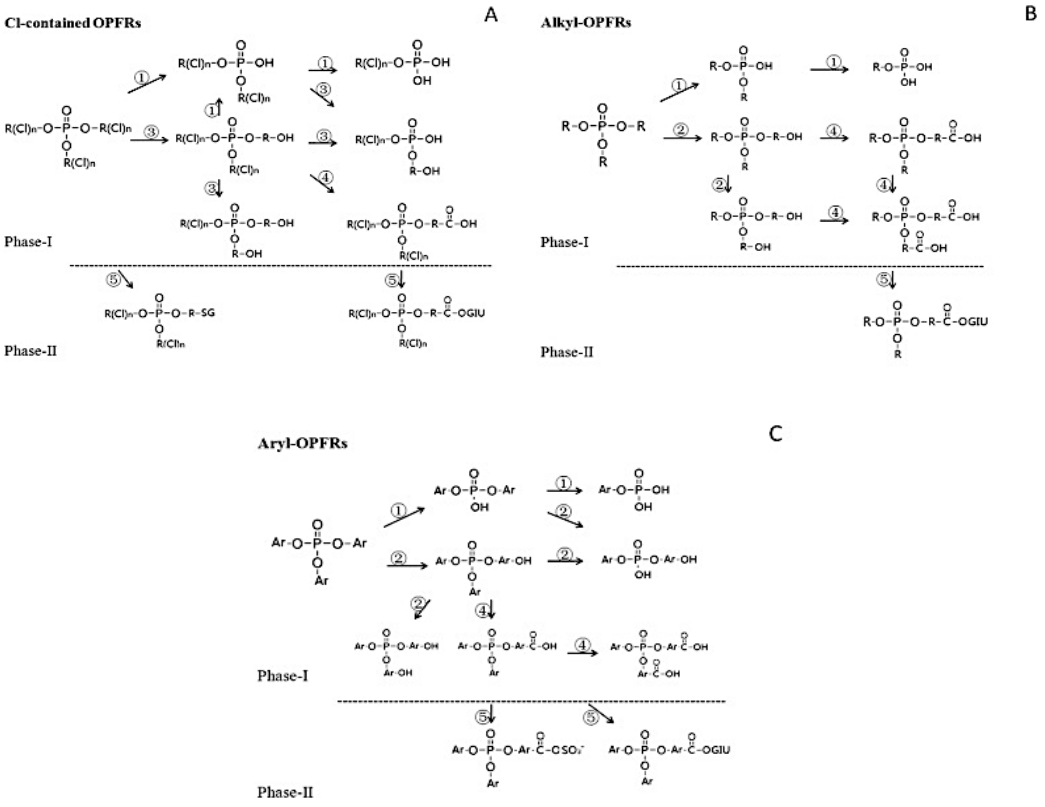


Figure 2.2 – Metabolic pathway of OPEs, (A) chlorinated OPEs, (B) alkyl-OPEs, (C) aryl-OPEs [37].

In the chlorinated OPEs, it first cleaves the ether bond by O-dealkylation and oxidative dehalogenation to form diesters and hydroxylated metabolites, and then glutathione conjugate substitutes the Cl atoms directly [37]. In alkyl-OPEs, dealkylation first occurs, followed by

hydroxylation to form diesters. Hydrocarboxylation or oxidative dealkylation may also occur. Similarly, in aryl-OPEs, the phenyl group first undergoes hydroxylation, dihydroxylation and carboxylation in phase I, followed by Glucuronide and sulfate conjugation in phase II (Hou et al., 2016) [37]. Major metabolites of OPEs are illustrated in Figure 2.3, where the metabolism involves cleavage or transformation of one of the three side chains in OPEs.

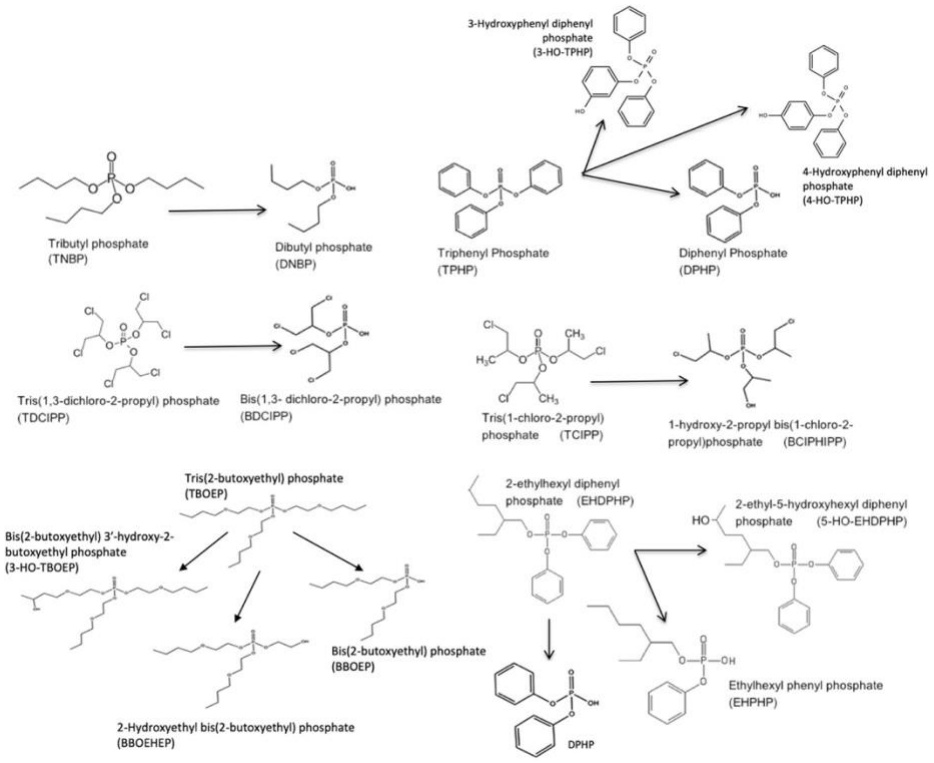


Figure 2.3 – Major metabolite of different OPEs [41].

For other organophosphates with P=S nomenclature, the oxon-analog can be activated by CYP450 mainly in the liver, but also in other extrahepatic sites, including the brain. This detoxification pathway is mediated by hepatic CYP450 by producing diethylthiophosphate and

3,5,6-trichloro-2-pyridinol (TCP) (Figure 2.4). The ratio between oxon-activation and detoxification depends on the chemical, species, gender, and age sensitivity to OPEs. Alternative detoxication can also be achieved by A-esterase or B-esterase [42].

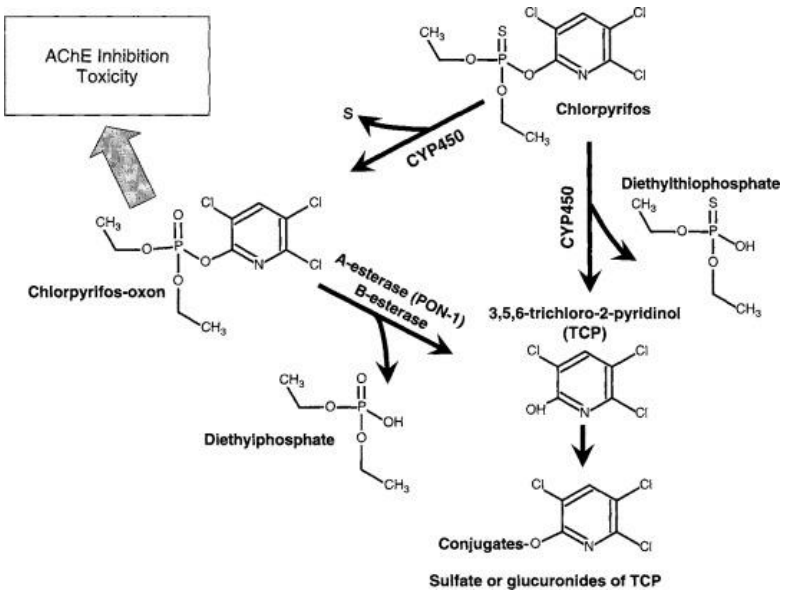


Figure 2.4 – Detoxification pathway of P=S analogue of OPEs [42].

It is worth noticing that the metabolite of organophosphate compounds can be the same as some of the OPEs. As the P=S nomenclature will be converted to the oxon-analogue before further metabolism, the presence of some mOPEs may not be fully originated from its parent OPE compound, instead, interconverted from a greater family of organophosphorus compounds.

2.5.4 Excretion of OPEs

The major excretion route of OPEs is through urination. The major excreted metabolites: resorcinol, DPHP, OH-DPHP, and hydroxylated RDP isomers (OH-RDP, di-OH-RDP). A minor portion is excreted by feces and expired gas [37]. The excretion of BDCIPP (metabolite of TDCIPP), in particular, has a slow metabolite rate and can be excreted out by urination directly [37]. Based on this fact, urinary mOPEs have been used for monitoring the exposure of OPEs in children and pregnant women, DPHP, BDCIPP and diethyl phosphate (DEP) are the most common biomarkers used [43].

2.6 Adverse health effects and their mechanisms of OPEs (Toxicodynamics)

OPEs have been used in numerous studies to investigate their toxicity effect as endocrine-disrupting chemicals. The major threat of OPEs is that they can bind to acetylcholinesterase (AChE) in red blood cells thus inactivating them. This causes an overstimulation by the overabundance of acetylcholine within synapses and neuromuscular junctions [13]. Acute symptoms include hypertension, sweating, tachycardia, miosis, confusion, drowsiness, emotional lability, seizures, hallucinations, headaches, insomnia, memory loss and circulatory or respiratory depression [13].

In the long term, the chronic adverse health effects of OPE exposure include affected psychomotor function, cognitive function, balance and nerve function [12]. In some studies with animals, it was found that TPHP and TDCIPP could induce neurotoxicity in Chinese rare minnows (*Gobiocypris rarus*), developmental and reproductive toxicity and cardiotoxicity in zebrafish [11]. The use of zebrafish to model human toxicity has become more common in the past decade. As OPEs can be found as a contaminant in the environment, using zebrafish as a model can provide information on the etiologies and mechanisms of diseases resulting from environmental exposure

[44]. Since the gene, molecular processes and gene programming in the early life stage are conserved in the animal phyla, the zebrafish can be used as an alternative model from rodents to elucidate the toxic effect in humans [44, 45]. In other studies, BDCIPP, TDCIPP and TPHP were found to be associated with adverse developmental behaviour in children, when the mothers were exposed to these OPEs [46].

In a recent study, it was found that metabolites of OPEs are associated with higher oxidative stress in pregnant women, especially for BDCIPP and DPHP. This is caused by affecting antioxidant enzyme activities or altering the gene transcription [47]. Another recent study on this topic using human ovarian cells showed that OPEs could increase their oxidative stress drastically, which is linked to increased expression of biomarkers for oxidative stress and DNA damage in pregnant women, these could result in impaired oocyte quality and decreased successful implantation [48].

Aside from the immediate toxic effect, the intake of OPEs (and other organophosphates) can lead to glucose intolerance. The OPEs and OPs can be metabolized into short-chain fatty acids, such as acetic acid, by the gut microbiota and absorbed by the intestine. The acetic acid transported to the liver would eventually be converted to glucose by gluconeogenesis and lead to diabetes [49].

2.7 Non-targeted analysis

2.7.1 Introduction to non-targeted analysis

In traditional mass spectrometry, targeted analysis has been applied to focus on selected compounds in the samples in the study of environmental and food samples. Scientists have been focusing on the known harmful compounds, however, not all the toxic compounds found in the environment are known, as there is a large number of chemicals released to the environment every day [50]. In the evaluation of chemical safety, targeted analysis cannot account for other unknown compounds, which limits the holistic assessment of the risk of exposure to chemicals in the environment [51].

When analyzing environmental and food samples, applying targeted analysis relies on the use of standards for expected compounds, which may be sufficient as some compounds may not have their standards available. For example, the thermal degradation products in food could be a diverse set of compounds, but not all of these degradation products are known to scientists [52], therefore, the lack of reference standards will be insufficient for the study. This also fails to address the overall picture of the degradation pathways.

This is where non-targeted analysis (NTA) (also known as non-targeted screening (NTS)) comes into the picture and becomes an essential technique in analytical chemistry. According to the European Food Safety Authority (EFSA), non-targeted analysis is defined by the following:

> '*An analytical method, mainly based on mass spectrometry followed by data mining and elaboration, aimed at the acquisition of undefined information from a sample ("profiling"). Information about "known" and "unknown" analytes can be obtained in the post-acquisition data elaboration.*' [53]

In the early stage of development, the application of non-targeted analysis identified a range of unknown contaminants, resulting from legacy pollutants, namely polycyclic aromatic hydrocarbons, chlorinated pesticides, flame retardants, alkylphenols [50]. In this analysis, the analytes are either unknown, in which there is no information on the compound, or putatively characterized, in which the analyte class of the compounds is classified according to their chemical properties. It complements the targeted analysis to uncover the complete spectrum of extractable chemicals in the samples [52]. In the case of non-targeted screening or studying the degradation products, it is expected to analyze all extractable degradation products, with data acquired from high-resolution mass spectrometry (HRMS), in favour of understanding the entire degradation pattern and pathway. Nevertheless, it requires a database for authentication for the results [54], and sometimes the characterized compounds remain "known unknown" [52].

2.7.2 General outline for non-targeted analysis

The general workflow of non-targeted analysis is presented in Figure 2.5 [50]. The process begins with sampling, followed by suitable extraction methods. The treated sample is then analyzed by LC coupled with HRMS. Afterwards, the results are processed and hence identify the structure unknown compound.

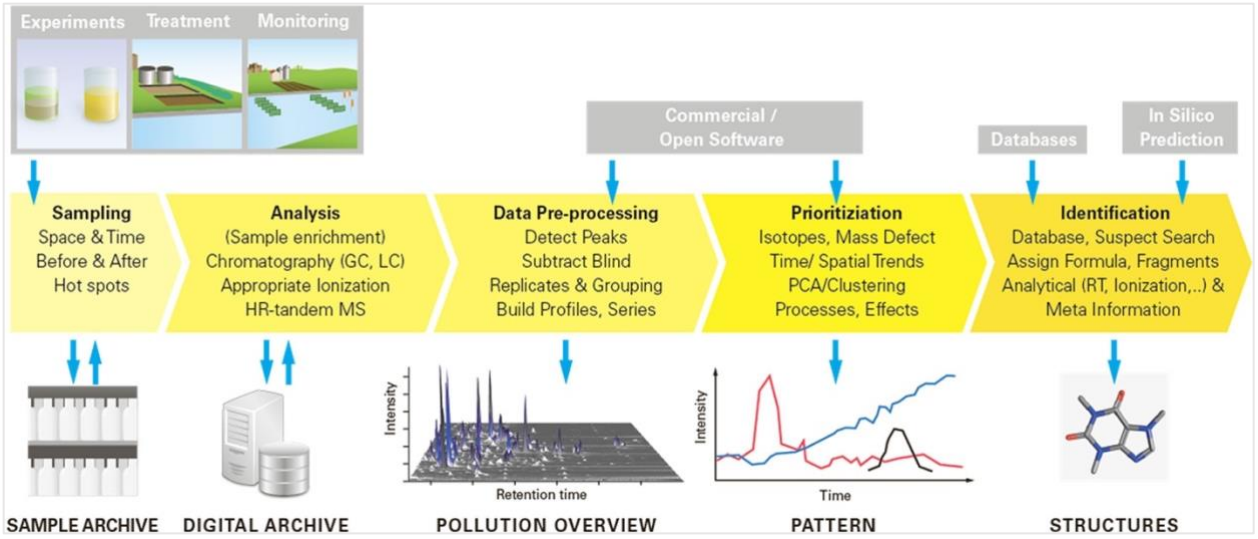


Figure 2.5 - General workflow for non-targeted analysis [50].

The characteristic of non-targeted analysis is the use of HRMS. In HRMS, more advanced instruments, such as orbitrap and time-of-flight (TOF) are commonly used. The major advantages are the acquisition of full-scan spectra with high sensitivity, high resolving power (up to 100,000 FWHM), high accuracy in mass measure (<5 ppm) and record of a broad range of *m*/*z* simultaneously [55]. In the data treatment, it first finds the masses of interest by prioritization and filtering of chemical features, followed by the identification of compounds based on the exact mass, isotope, adduct and fragmentation information [56]. Since there can be a large number of signals generated, the prioritization can be approached by selection based on frequency and signal intensity of masses, characteristics isotopic patterns (such as Cl and Br), specific functional groups or based on predicted products [50]. Chemometrics techniques, such as principal component analysis (PCA), clustering and regression can also aid the prioritization of compounds of interest, by grouping the data into different sets according to their similarities of the compounds [50, 55].

The final identification can be done by gathering information from the MS or MS/MS spectra, such as molecular ion, isotope patterns, fragmentation patterns, and database. Meta information can also be useful, for instance, consider the environmental context and the possible source of the compound. Validation of the compound should be done with standards to ensure the identification is correct [50].

In the process of structural elucidation, recent developments in artificial intelligence software could provide more reliable results. For instance, *SIRIUS* is a machine software that could propose possible structures of unknown compounds, based on MS/MS spectra [57]. From each MS/MS spectrum, a fragmentation tree is proposed by annotating MS/MS spectra by modelling the fragmentation and assigning a molecular formula to each fragment ion peak [58]. The peaks were correlated to a probable sub-structure (fingerprint) of the compound. This fingerprint would be searched against various online spectral database, and suggest possible structures that would best explain the MS/MS spectra [57]. The incorporation of *SIRIUS* in the non-targeted workflow has been reported in the literature, for instance, a study was done to elucidate the unknown metabolites of ricinine from castor cake fertilizer [59]

2.7.3 Applications of non-targeted analysis

Another non-targeted workflow has been developed by Tian et al. [60] Figure 2.6. Briefly, the samples were first prepared and divided equally into half. First, half the samples were spiked with known standards, followed by LC-MS analysis. The parameters in the instruments were adjusted and optimized so that the spiked standard could be correctly found. Then, these optimized parameters are used to analyze the other half of the samples. The identity of the non-targeted compounds could be found by a high matching score according to the Agilent Technologies

MassHunter software, which is based on the mass, isotopic abundance and isotopic distribution. The false-positive candidates could be screened out by predicting the retention time in the LC by using the log K_{ow} values of suspected compounds. Further confirmation can be done by acquiring targeted MS/MS spectra of the identified compound and repeating the experiment by using the respective standard for validation [60].

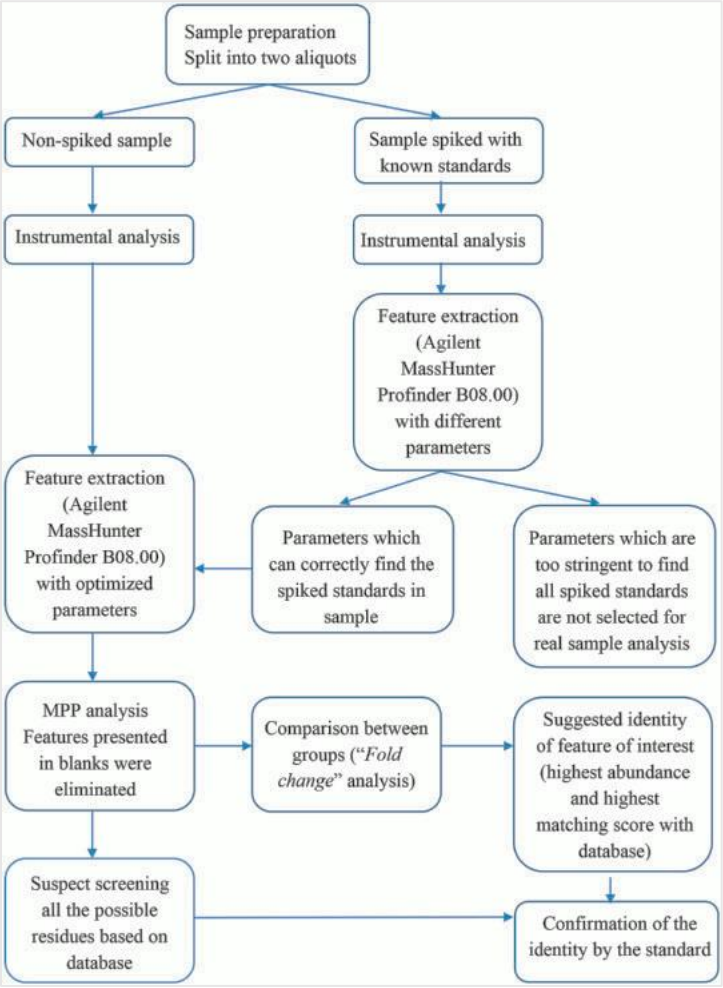


Figure 2.6 – Example workflow of non-targeted analysis [60].

Non-targeted analysis has been used to detect OPEs from samples in the literature. Non-targeted screening was a common technique to identify biotransformation products and metabolites of OPEs. For example, metabolites based on the previously reported metabolite pathways could be used to create a screening library, and applied to screen for their presence in the samples. This strategy was applied for the identification of biotransformation products of TPHP [61] and metabolites of other aryl-OPEs [62].

Non-targeted screening was also applied to other types of samples. The technique was recently used to screen for the presence of OPEs in plastic packaging materials, with an in-house screening library as well as structural elucidation by comparing the characteristic fragment ions for online database search [63]. In environmental samples, non-targeted screening has been applied to analyze seawater samples to study the pollutants in the Arctic sea, including TCIPP and TDCIPP [64]. The OPE profiles in soil sediments have also been studied, combined with of targeted analysis and function group-dependent analysis, a comparison of OPE profiles in different depths of the sediment could be made, and different dominant types of OPEs were observed [65].

Overall, non-targeted analysis could be useful to determine unknown or unreported compounds in the samples and has been applied in different matrices. The workflow proposed in the literature could also provide a framework of the non-targeted analysis used for this research.

2.8 Conclusion

OPEs and other NFRs have been used to replace legacy FRs for their bioaccumulative and persistent properties, but these OPEs have similar disadvantages. In the current literature, there are still limited studies on the occurrence of OPEs in food, due to their recent introduction in the industry, as well as the application of targeted analysis with a small number of targets. The current findings may also suggest that this replacement would require corresponding policy changes to address similar food safety and environmental concerns as the legacy FRs.

In this review, several knowledge gaps have been identified. This includes the limited knowledge of a standardized non-targeted workflow for the identification of FRs in food, limited studies on the additional source of FRs from the environment in food, and from plastic packaging materials in fresh food, as well as scarce information on the thermal degradation of OPEs under typical cooking temperatures. This information is essential to the ongoing risk assessment of FRs through diet. With the recent development of non-targeted analysis, including machine-learning software to analyze HRMS data, this project can contribute to a better estimation of the exposure in a realistic scenario by the general population, and identify unexpected FR contaminants in food.

Connecting Paragraph

Chapter 2 described the general use and sources of FRs, with a focus on OPEs. More specifically, the occurrence and food safety concerns of OPEs were summarized. The concept of non-targeted analysis was also introduced and will be applied to the studies conducted in this thesis. As these FRs were also detected in the environment as contaminants, it is also important to understand their fate. Chapter 3 will provide a summary of the current findings of abiotic degradation of FRs in both environmental and food matrices for four classes of FRs, and suggest knowledge gaps on the degradation studies for these FRs. Chapter 3 has been published in *Food Additives & Contaminants: Part A*: Leung, G., McKinney, M. A., Yaylayan, V., & Bayen, S. (2024). Abiotic degradations of legacy and novel flame retardants in environmental and food matrices - a review. *Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment*, 1–22.

Chapter 3 – Abiotic Degradations of Legacy and Novel Flame Retardants in Environmental and Food Matrices – A Review

Abstract

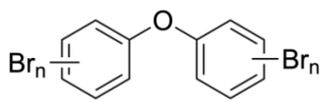
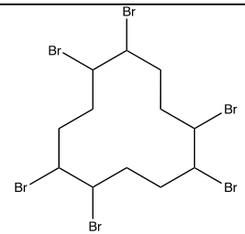
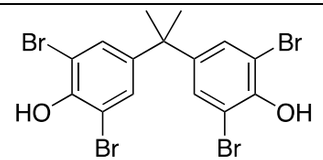
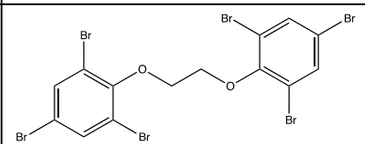
Flame retardants (FRs) are commonly added to commercial products to achieve flammability resistance. Since most of them are not chemically bonded to the materials, they could be leached to the environment during the production and disposal cycle. These FRs were categorised based on their chemical nature, including brominated, organophosphorus-, mineral- and nitrogen-based. This review summarised the abiotic degradation reactions of these four classes of FRs, with a focus on thermal and photodegradation reactions in environmental and food matrices. Only 25 papers have reported related information on abiotic degradation reactions that could be useful for predicting possible degradation pathways, and most focused on brominated FRs. Most studies also investigated the thermal degradation of FRs under high temperatures (>400 °C), which exceeds the normal cooking temperature at 100 to 300 °C. For photodegradation, studies have used up to 5 times the energy typically used in UV radiation during food processing. It is recommended that future studies investigate the fate of these FRs in foods during more realistic processing conditions, to provide a more comprehensive picture of the estimated consumption of FRs and their degradation products from foods, and facilitate a better risk assessment of the use of these novel FRs.

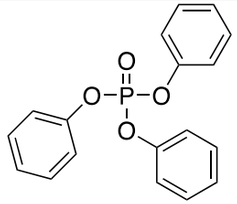
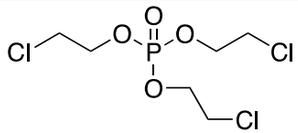
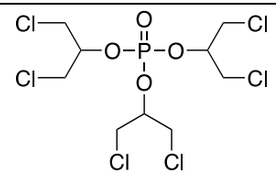
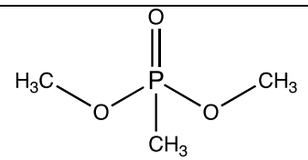
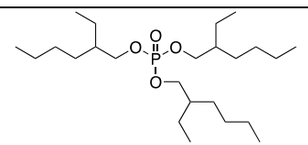
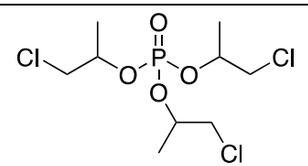
Keywords: novel flame retardants; abiotic degradation; risk assessment

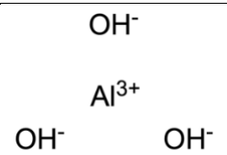
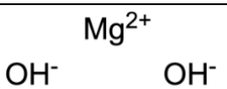
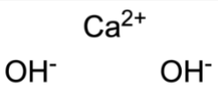
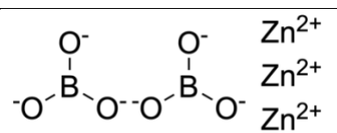
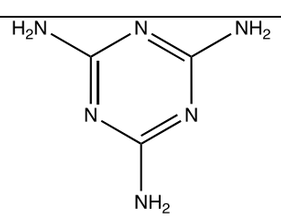
3.1 Introduction to flame retardants (FRs)

Flame retardants (FRs) are chemicals added to combustible materials to slow down or hinder the ignition or combustion of that material [1]. Traditionally, brominated flame retardants, such as polybrominated biphenyls (PBBs), polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCDD) have been widely used in commercial products. But as more extensive studies have revealed their toxicity in humans and persistence in the environment, these 'legacy' flame retardants have been phased out under the Stockholm Convention [2] and novel FRs have been employed as alternatives. These current-use FRs have been grouped based on their chemical nature – brominated, organophosphorus-, mineral and nitrogen-based, or by the means of their incorporation into the material – additive or reactive [3]. Unlike reactive FRs, additive FRs are not chemically bound to the polymers and are thus easily released to the environment by leaching, volatilisation and dissolution [4], which increases the risk of exposure to humans, since FR residues can travel long distances through the air, water, and biota [5, 6]. Application of FRs in consumer products includes textiles, cable wires, building materials, and electronics. It was estimated that the global industry's total consumption of flame retardants was 5 million metric tonnes in 2018, of which aluminum trihydroxides were the majority (38% share consumption), followed by organophosphorus (18% share consumption) and brominated FRs (17% share consumption) [7]. The information and structures of FRs mentioned in this review are summarised in Table 3.1, their properties, including chemical formula, molecular weight, melting point, boiling point, vapor pressure, water solubility and log K_{ow} value were obtained from PubChem [8].

Table 3.1 – Summary of FR compounds mentioned in this review, in four classes, namely brominated, organophosphorus, mineral-based and nitrogen-based FRs.

Name	Acronym	FR class	CAS	Chemical formula	Molecular weight	Chemical structure	Melting point (°C)	Boiling point (°C)	Vapor pressure (Pa, 25 °C)	Water solubility (mg L^{-1} at 25 °C)	Log K_{ow}
Polybrominated diphenyl ethers	PBDEs	Brominated	N.A.	$C_{12}H_{(10-x)}Br_xO$	N.A.		305 (Decabromo-)	425 (Decabromo-)	N.A.	Not solutble (Decabromo-)	4.8 (Dibromo), ~ 9.97 (Decabromo-
Hexabromocyclododecanes	HBCDDs	Brominated	134237-50-6 / 134237-51-7 / 134237-52-8	$C_{12}H_{18}Br_6$	641.7		N.A.	N.A.	N.A.	N.A.	α: 7.1 β: 7.1 γ: 7.1
Tetrabromobisphenol A	TBBPA	Brominated	79-94-7	$C_{15}H_{12}Br_4O_2$	543.9		179	316	6.23×10^{-6}	1.26 (pH 7)	6.8
1,2-Bis(2,4,6-tribromophenoxy)ethane	BTBPE	Brominated	37853-59-1	$C_{14}H_8Br_6O_2$	687.6		222	N.A.	2.52×10^{-6}	2.23×10^{-4}	7.7

Triphenyl phosphate	TPHP	Organophosphorus	115-86-6	$C_{18}H_{15}O_4P$	326.3		49	370	2.67×10^{-4}	1.9	4.6
Tris (2-chloroethyl) phosphate	TCEP	Organophosphorus	115-96-8	$C_6H_{12}Cl_3O_4P$	285.5		–55	330	2.20×10^{-3}	5 (at 20 °C)	1.78
Tris (1,3-dichloro-2-propyl) phosphate	TDCIPP	Organophosphorus	13674-87-8	$C_9H_{15}Cl_6O_4P$	430.9		N.A.	236	3.81×10^{-5}	7	3.65
Dimethyl methylphosphonate	DMMP	Organophosphorus	756-79-6	$C_3H_9O_3P$	124.1		<50	181	128	insoluble	–0.7
Tris(2-ethylhexyl) phosphate	TEHP	Organophosphorus	78-42-2	$C_{24}H_{51}O_4P$	434.6		–74	220	<1	Insoluble	9.49
Tris(1-chloro-2-propyl) phosphate	TCIPP	Organophosphorus	13674-84-5	$C_9H_{18}Cl_3O_4P$	327.6		N.A.	235	12.3	1.6×10^3	2.59

Aluminium trihydroxide	ATH	Mineral-based	21645-51-2	$Al(OH)_3$	78.0		N.A.	N.A.	N.A.	N.A.	N.A.
Magnesium hydroxide	MDH	Mineral-based	1309-42-8	$Mg(OH)_2$	58.3		350	N.A.	N.A.	Insoluble	N.A.
Calcium hydroxide	N.A.	Mineral-based	1305-62-0	$Ca(OH)_2$	74.1		N.A.	N.A.	N.A.	N.A.	N.A.
Zinc borate	N.A.	Mineral-based	10361-94-1	$B_2O_6Zn_3$	313.8		980	N.A.	N.A.	N.A.	N.A.
Melamine	N.A.	Nitrogen-based	108-78-1	$C_3H_6N_6$	126.1		345	N.A.	6.7 (at 20 °C)	3.23×10^3	–1.37

N.A.: Not available

The release of FRs into the environment can occur during the manufacturing process, consumer use, or after disposal. Workers in the primary and secondary industries for consumer products manufacturing can be at high risk of exposure to FRs; for example, the organophosphorus FR, triphenyl phosphate (TPHP), was found to have concentrations in air of up to 7,170 ng m^{-3} at a chemical manufacturing site in the United States [9]. The FRs released from industrial facilities and landfills can travel long distances without proper waste management to contain the release [10]. For instance, FRs leached from landfills can enter waterways unless a leachate collection system is built or if the collected leachate sent to wastewater treatment plants (WWTPs) is not equipped to remove these contaminants, hence concentrating in biosolids [11]. With the detection of organophosphate FRs in air, snow and seawater in the North Sea and over the Pacific, Indian, Arctic, and Southern Oceans, it is clear that these FRs can travel long distances by air or water from source regions, also known as long-range transport [12-14].

In addition to polluting the environment, leached FRs can enter human food chains, as well as accumulate in wildlife. FR contaminants (such as tetrabromobisphenol A (TBBPA), and HBCDDs) have been reported in food, including fish, vegetables, meat, and dairy products [3, 15]. The novel FRs, including 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) and HBCDDs, have been detected in eggs of herring gulls at the Laurentian Great Lakes [16] and glaucous gulls in the Norwegian Arctic [17]. These FRs can be highly persistent, and due to their lipophilic nature, can bioaccumulate in organisms [18]. Concentrations of PBDEs in ivory gull eggs from the Canadian Arctic Ocean increased from 1979 to 2004, but there was no further increase afterwards, mostly due to the legislative regulations posed on the use of PBDEs in North America [19, 20]. This coincides with the timeline of voluntary reduction and restriction regulations at the time, as most PBDEs were phased out of production in North America in 2004 [19]. In terms of bioaccumulation

and biomagnification, multiple PBDE congeners showed the highest concentrations at the higher trophic levels in the Lake Erie [21]. This is similar to HBCDDs, for which concentrations were found to increase along the food chain, and at high concentrations were found to be in lipid-rich organs and tissues, such as the liver, in aquatic mammals and fish [22]. Thus, the FRs leached into the environment could eventually be harmful to human health when contaminated foods are being consumed.

Regarding human exposure, the concentration of PBDEs in human blood, tissue and breast milk increased exponentially from the 1980s to 1990s in Europe, Japan and North America [23, 24]. The occurrence of organophosphate esters (OPEs), which are replacements for PBDEs since 2004, shared a similar detection level in human blood, breast milk, hair, and nails. The median detected levels from studies done between 2001 to 2021 were above 40 ng g^{-1} lipid, 150 ng g^{-1} lipid, 1500 ng g^{-1} dry weight (dw) and 500 ng g^{-1} dw respectively in China, where the trend could reflect the pattern of usage in the industry and the exposure from the participants' occupation [25].

The high levels of detection of certain FRs in humans have sparked concerns over the safety of such wide usage in various commercial products. For instance, exposure to PBDEs can lead to developmental and reproductive problems [26], they are classified as endocrine-disrupting chemicals (EDCs). HBCDDs and TBBPAs can cause disruption of thyroid homeostasis and neurobehavioral alterations [22, 27]. Similarly, organophosphate esters (OPEs) are carcinogenic, neurotoxic, nephrotoxic, and hepatotoxic, and can cause metabolic disruption [28, 29]. As a result, safety guidelines have been put in place to limit human exposure to FRs, notably through diet. For example, the tolerable daily intake (TDI) for tris(2-chloroethyl) phosphate (TCEP) is 0.002 mg kg bw^{-1} day^{-1} [30]. However, such guidelines are not available for all the current-use FRs in the industry, unlike for other chemicals, such as pesticides, for which there are clear guidelines and

legislation in Canada. Health Canada establishes the regulation for pesticides, namely the maximum residue limits, identified in both raw and processed foods before they are allowed to be sold in the market to consumers, and the Canadian Food Inspection Agency enforces these regulations [31]. These regulations provide a basis to assess the risks associated with usage, but since there are no specific regulations for FRs, it is difficult to know the safety level of these chemicals for consumption.

FRs can undergo degradation reactions, which break down their complex structures into simpler structures. Under abiotic conditions, FR degradation in the environment can occur via oxidation-reduction, hydrolysis, thermal degradation, or photolysis (a.k.a. photodegradation). Although FRs are intended to provide flame resistance, some of them are not stable under high energy and will degrade accordingly. Current studies on testing FR products in environmental and food samples tend to focus on the parent compounds (i.e. the original compound). However, in some although not all cases, the degradation products may possess similar or even more toxicity than the parent compounds [32]. For example, certain PBDEs (commonly BDE-47, -99, -100, -153, -209 congeners) can degrade to partially debrominated degradation products, with thus higher toxicity by environmental transformations, such as photodegradation [33]. These less brominated products are also prone to bioaccumulation and biomagnification in the food chain [34] and more cytotoxic [35]. For other compounds, the toxicity of the degradation products is still unknown, but since some transformation reactions leave the active moiety intact, there could be an additive effect versus only the parent compound [36]. Therefore, it is relevant to investigate the transformation and degradation of these emerging contaminants in the environment, which could be subsequently detected in food. These complex degradation products can pose a challenge when assessing the risk of consumption, especially if these unknown transformation products pose a potentially

similar or even higher toxicity, persistence, mobility, and bioaccumulative potential than the parent compound [37], therefore, it is necessary to take them into consideration during the risk assessment process.

Currently in Canada, the government conducts risk assessments for chemicals under the Canadian Environmental Protection Act, 1999 (CEPA 1999), for their impact on human health and the environment [38]. For flame retardants, the Canadian government has listed HBCDDs and PBDEs in the List of Toxic Substances, and is considering adding other flame retardants including organophosphate esters [38, 39]. In the current risk assessment process, the Canadian government concludes with information on the concentrations, environmental fate, hazards and exposure of the chemicals. These results could be from studies found in the literature or from government researchers [40]. From the current results, the risk assessment is mostly focused on the parent compounds. Since these FRs could undergo degradation in the environment, it is crucial to take into account the exposure of degradation products.

This article will first summarise the available data on abiotic degradation processes and conditions of the four classes of flame retardants (i.e. brominated, organophosphorus, mineral-based and nitrogen-based FRs) in the environment and food production systems, and then will discuss degradation product toxicity reported from the literature. More specifically, the intent of this review is to confirm whether (i) the thermal, photolytic and hydrolytic stability of the various families of flame retardants have been studied in both environmental and food models (ii) the associated degradation kinetics and mechanisms are affected by the matrix (environmental or food) (iii) the resulting degradation/transformation products have been identified and are relatively less or more hazardous than the parent compounds.

The information search for this review was performed using the Web of Science search engine. Keyword search included the names of the FR classes (i.e. brominated, organophosphorus, mineral-based and nitrogen-based FRs) or the names of major FRs (such as PBDEs, and TBBPA). Both ‘degradation’ and ‘transformation’ were used for the search process. From the results, studies involving biotic degradation conditions, such as microbial degradations, were eliminated. Only 24 reports satisfied the criteria of abiotic degradation or transformation of flame retardants in environmental or food matrices.

3.2 Abiotic degradation of flame retardants during food production

Apart from the abiotic transformation, some of these flame retardants can undergo biodegradation from microorganisms, but the toxicity of the degradation products could be higher than its precursor compound. For example, TBBPA could be O-methylated by *Rhodococcus* sp. strain 1395 under aerobic conditions to form a more lipophilic and bioaccumulative product [41]. While there are plenty of studies done on biodegradation [42, 43], abiotic degradation of these flame retardants may be equally important to the safety of foods for consumption.

This section will mainly focus on the thermal, photolytic and hydrolysis degradation processes of the four major families of FRs because they are the main source of abiotic degradation in the environment and food processing, as heat and light are the two major sources of energy supplied to the foods. The sources of abiotic degradation in food processing and the environment are illustrated in Figure 3.1. In food processing, an example would be pasteurisation, which applies heat or UV radiation to inactivate pathogenic microorganisms in milk to prevent spoilage. In thermal treatment, the usual range of heating is around 60 to 160 °C, but it can be up to 190 °C for deep-frying. For UV radiation treatment, the intensity of the UV can be from 1 to 3 mW cm^{-3}. In the environment, thermal degradation could be caused by heat from soil remediation or sunlight, and photodegradation could occur in the air or during the water treatment process. These sources of energy can be a potential trigger of different degradation reactions of FRs in food during processing. FRs could also degrade in water by hydrolysis, for instance, during food processing, pasteurisation or storage (such as canned food).

Several studies have examined the levels of organic contaminants before and after cooking, including PBDEs [44], OPFRs [45], PCBs and dioxins [46]. These studies showed that the levels of these contaminants, as well as their bioaccessibility, were reduced greatly after cooking [47].

While the reduction in concentrations could be due to lipid removal during the cooking process [48], it could also be due to the transformation of the parent compounds. For example, debromination of BDE-209 occurs to form BDE-206, -199 and -196 after heating at 200 °C for 15 minutes [44].

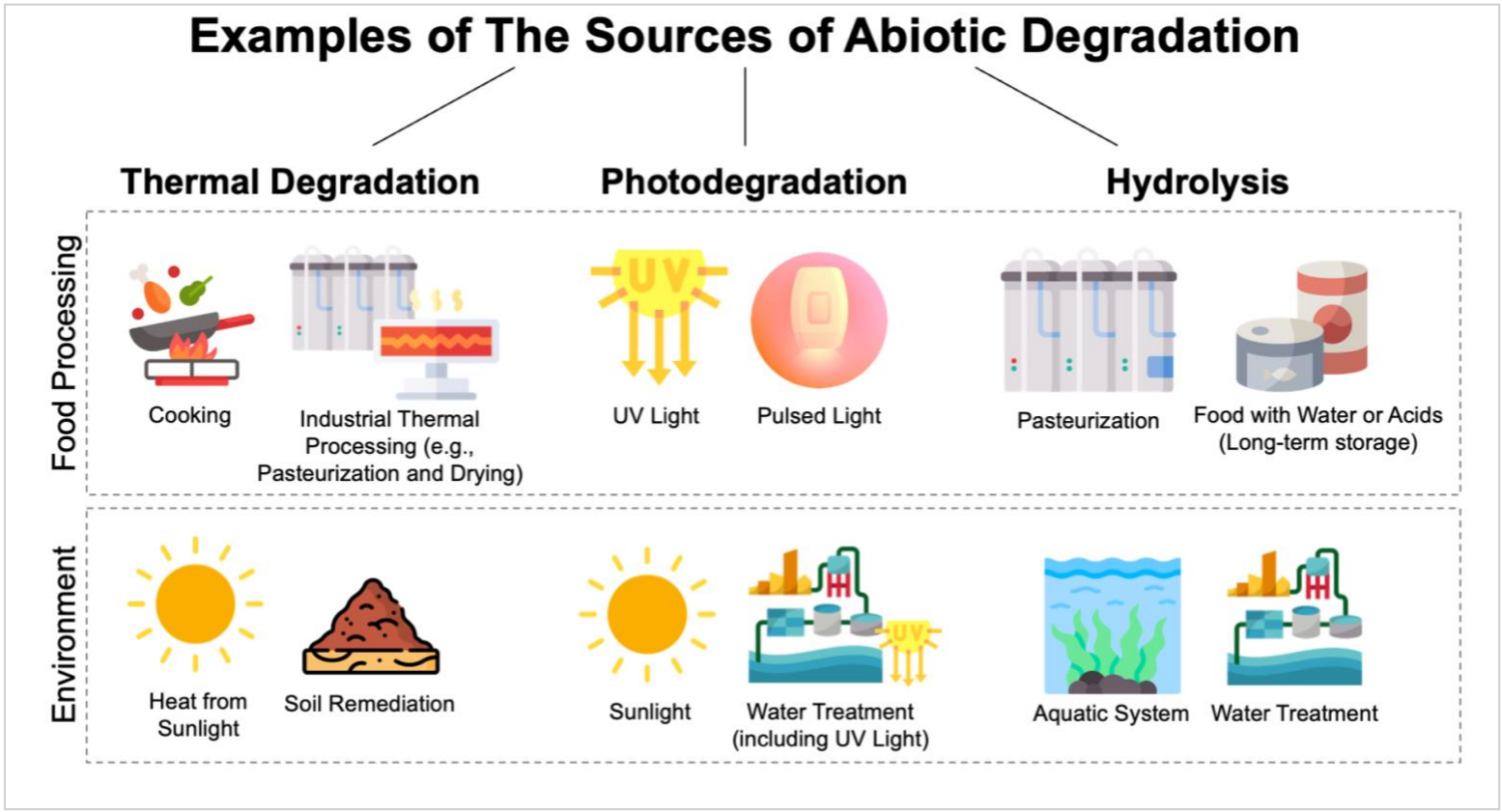


Figure 3.1 – Examples of sources of abiotic degradation reactions in food processing and the environment.

Credits on the illustration: Cooking, pasteuriser, UV radiation, pulsed light, soil, seaweed, and infra-red lamp icons made by Freepik from www.flaticon.com; Canned food icon made by surang from www.flaticon.com; Sunny icon made by kosonicon from www.flaticon.com; Water treatment icon made by mynamepong from www.flaticon.com.

3.2.1 Brominated FRs (BFRs)

Brominated FRs (BFRs) are the earliest class of FRs used in the industry, having entered the environment decades ago, and are now recognised as legacy contaminants. The main principle of these FRs to quench the fire is by replacing the high-energy radicals responsible for flame propagation with the and Br· radicals generated, which carry less energy. Key examples include polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs), and tetrabromobisphenol A (TBBPA). PBDEs, in particular, were listed as Persistent Organic Pollutants (POPs) under the Stockholm Convention; consequently, these legacy FRs have been substituted with emerging brominated FRs by the industry [49].

3.2.1.1 PBDEs

PBDEs were the largest class of FRs used, with 7,100 tons of the Penta product used in North America, representing about 95% of its global use; 24,500 tons of the Deca product was used in North America and about 23,000 tons was used in Europe, representing about 44% and 41%, respectively, of global consumption in 2001 [50]. They were later found in, e.g., human breast milk, foods, and wildlife and showed effects on endocrine systems and neurotoxicity, leading to phase-outs and inclusion under the Stockholm Convention (POPs. int). Although levels have declined in recent years, due to their persistence, they are still ubiquitously detected in water [51, 52], outdoor air [53, 54] and soil [55, 56].

3.2.1.1.1 Thermal degradation of PBDEs

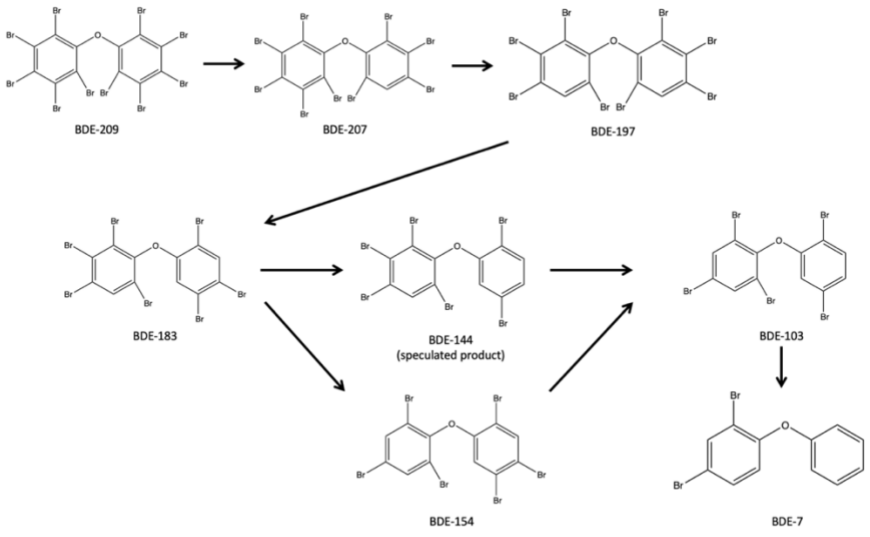
Several studies have focused on the degradation of PBDEs. Food processing involves elevated temperatures, although not as high as most previous studies have used, it remains

unknown what the effects of typical cooking temperatures are on the degradation of PBDEs in food before consumption. Most studies on PBDE degradation have used temperatures over 100 °C, some up to 400 °C, on various matrices, mostly of environmental relevance. For instance, the study by Li & Yang [57] used Fe_3O_4 micro/nano-material as a catalyst, which is typically used for environmental remediation, to test the thermal degradation kinetics of BDE-209, as opposed to other methods, such as photolytic degradation and anaerobic microbial debromination. At 300 °C, the amount of highly brominated PBDE homologues decreased steadily over time, while that of low brominated PBDE homologues increased, showing that these highly brominated PBDEs degrade at high temperatures by reductive hydro-debromination reactions. According to the study the predicted degradation pathway is as follows: (

Figure 3.2)

BDE-209 → BDE-207 → BDE-197 → BDE-183 → BDE-144 and BDE-154

→ BDE-103 → BDE-7



Li, Q., F. Yang, G. Su, L. Huang, H. Lu, Y. Zhao, and M. Zheng, *Thermal degradation of polybrominated diphenyl ethers over as-prepared Fe3O4 micro/nano-material and hypothesized mechanism*. Environmental Science and Pollution Research, 2016. 23(2): p. 1540-1551.

Figure 3.2 – Degradation pathway of BDE-209 by hydrodebromination [57].

Although a different type of study, another study was done on soil remediation, where BDE-209 treated soil samples were heated up to 450 °C to remediate the contamination [58]. The results showed that thermal treatment at 250 °C for 30 minutes led to less than half of the BDE-209 remaining in the sample. The removal was more efficient at even higher temperatures, with no detectable BDE-209 at 450 °C. For temperatures under 200 °C, the removal efficiency was limited, for instance, the removal was about 90% at 150 °C. Although the study did not include elucidation of the degradation products, it shows that at least some BDE-209 could degrade efficiently at increasing temperatures. This trend was also found in the incineration process, that at higher temperature of 740-820 °C, Br_{10}-DE could generate gaseous products, such as HBr and Br_2 from a mixture of BFRs, including PBDEs, TBBBPA and HBCDDs [59]. Another study on the incineration done at 500-900 °C was also able to identify polybrominated dibenzo-*p*-dioxins (PBDDs) as a thermal degradation product, but this was found in resins containing a mixture of both PBDEs and TBBPA (which will be discussed in Section 3.2.1.2.1) [60].

Another experiment carried out at high temperatures also traced possible transformation products by Roszko et. al. [61]. In this study, multiple PBDE congeners were heated from 100 to 400 °C in a medium (dichloromethane or aqueous hydrochloric acid) with 250 µM of chlorine. At a lower temperature, single substitution reactions of Br atoms were observed, while multiple substitution or full substitution was observed at higher temperatures. To extend the study, pork meat was used as a food matrix to study the transformation of PBDEs by spiking a known concentration and heating the samples via grilling (at 180 °C) [61]. However, the levels of only BDE-183, but not BDE-99 or -209, were statistically different after treatment. The results were the same when repeated in rapeseed oil, possibly due to the excess oil molecules compared to the aryl

radical generated or because the oil medium does not initiate the PBDE chlorination process. In either case, the experiment showed that oil medium enhances the stability of PBDEs under thermal processing.

In addition to the experiment on pork and rapeseed oil, PBDE thermal degradation has also been studied in salmon [44]. This study showed the major degradation pathway was hydrodebromination, where lower brominated congeners were detected at increasing levels along the course of the two-hour heating period, while the level of parent BDE-209 decreased continuously. The major degradation products from this study were BDE-196, -199, -206, -207 and -208 congeners, confirmed with GC/ECNI-MS by examining characteristic ring fragments and abundance of Br_6-, Br_7-, Br_8- and Br_9DEs. In the salmon cooking experiment, the samples were spiked with decaBDE, and then heated at 200 °C for two hours. This was also confirmed by spiking Br_6- to Br_8DEs in the salmon samples in the same study, where Br_7- and Br_8DEs were reduced continuously to Br_6DEs, and no Br_5DE or less brominated-DEs were detected. Though the conditions used in this study were more drastic than actual cooking conditions, it was also noted that there is an issue of PBDEs and other compounds transforming, as these compounds could behave differently in food and the environment.

3.2.1.1.2 Photodegradation of PBDEs

PBDEs can undergo photodegradation by direct photolysis or photocatalytic degradation. Direct photolysis occurs when PBDEs absorb light at wavelength 280 to 400 nm, turning into an electronically excited state, leading to decomposition into intermediates [62]. During photocatalytic degradation, PBDEs degrade in the presence of high-performance semiconductor

photocatalysts, such as TiO_2, and undergo photocatalytic reductive debromination and oxidative degradation [62].

According to Shih and Wang [63], the photodegradation kinetics of PBDEs mainly follow a pseudo-first-order reaction model. Other factors influencing the kinetics include initial concentration, light intensity, and the particular PBDE congener. From their studies, the initial PBDE concentration did not affect the degradation rate, but light intensity showed a positive correlation to the rate of photodegradation. Regarding the type of PBDE congener, the same study showed that highly brominated congeners have a higher degradation rate than lower brominated congeners; this trend is the same for the dichlorination process of highly chlorinated aliphatic compounds.

Roszko, Szymczyk and Jędrzejczak [61] considered photodegradation and thermal degradation of PBDEs. In their study, BDE-99 was found to degrade mainly by debromination in both hexane and rapeseed oil under irradiation using a 4 W mercury lamp at 254 nm and radiation intensity at 16 mW cm^{-2}. Similar to the thermal degradation results from the same study, photodegradation in rapeseed oil was hindered in the oil matrix, mostly because the UV radiation was absorbed instead by the oil molecules. There was also a distinct difference in the debromination products when using rapeseed oil, compared to water or other organic solvents, such as methanol or toluene. Regarding the degradation products, BDE degradation products from decaBDE were measured over time. In the initial phase, nonaBDEs were the most abundant congeners, but they decreased continuously, whereas a gradual increase of tri- to hepta-BDEs was shown over the radiation time of 60 minutes. This trend indicated that the loss of a single bromine atom is the first step of the degradation, and that BDE-209 continues to lose bromine atoms to form lower-brominated BDE congeners.

3.2.1.1.3 Hydrolysis of PBDEs

In the environment, it is common for chemicals to be transformed by nucleophilic attack, including water [64]. PBDEs can be hydrolyzed into HO-PBDEs, which are abundantly found in the environment. There is a larger concern over HO-PBDEs since they are considered to be more toxic than PBDEs, they can disrupt thyroid hormone homeostasis, and oxidative phosphorylation, and are neurotoxic [65]. However, most studies have mainly focused on the formation of HO-PBDEs in biological systems. For example, in red alga [66], rat [67], and human plasma [68].

These studies show that the major degradation products of PBDEs are the lower brominated congeners of PBDEs. Since the chemical properties of these degradation products are similar to those of the parent compounds, the transformation products may show similar toxicity. Humans can be exposed to these compounds through oral intake via food, inhalation of dust or contact with skin [69]. Apart from being EDCs, lower-brominated PBDEs (debromination products), e.g., are also carcinogenic and toxic to reproductive health. Toxicity testing on rats has shown that exposure to PBDE-47 led to cancer of the thyroid, pituitary gland and uterus (in females), which was caused by the disruption of hormone levels and oxidative damage from reactive oxygen species to the DNA [70]. There is also evidence that these compounds can have epigenetic effects in humans, interfering with DNA methylation and chromatin dynamics, and leading to neurodevelopmental disorders as a consequence [69].

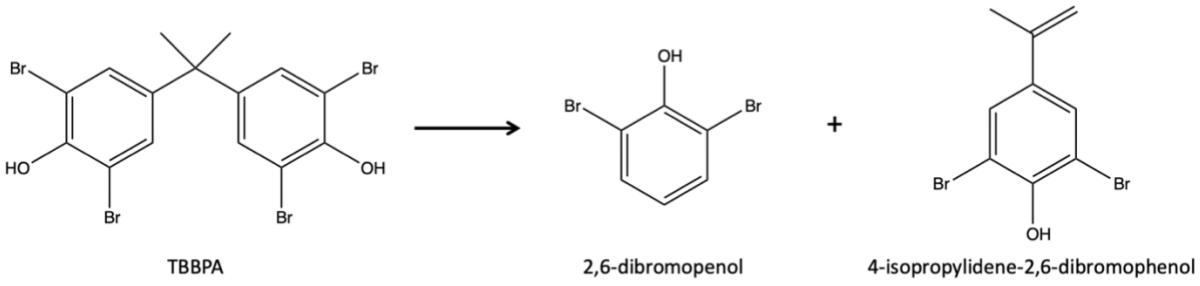
3.2.1.2 TBBPA

Tetrabromobisphenol A (TBBPA) is the most widely used as BFRs [71]. Due to its high thermal stability, it is widely used in electronics, textiles, building materials, etc. [72]. It has the highest production volume in the BFR category [73, 74], with the total production volume from

China, USA and the Middle East estimated at 241,352 tonnes in 2016, and production is expected to increase continuously in the coming years [75]. In previous food studies, TBBPA was found predominantly in fish and seafood, ranging from <0.001 to 5.8 ng g^{-1} wet weight (ww), and in other food groups, including meat (<LOD-1.386 ng g^{-1} fat), dairy products (<LOD-0.848 ng g^{-1} fat), and eggs (<LOD-0.892 ng g^{-1} fat) [76]. Toxicity studies in animals, done in both *in vivo* and *in vitro*, have shown that TBBPA can exert hepatic, renal, neural, cardiac, and reproductive toxicities, but the low exposure level and rapid metabolism in the general population could mean that TBBPA is generally safe in humans (except early stages of development) [75].

3.2.1.2.1 Thermal degradation of TBBPA

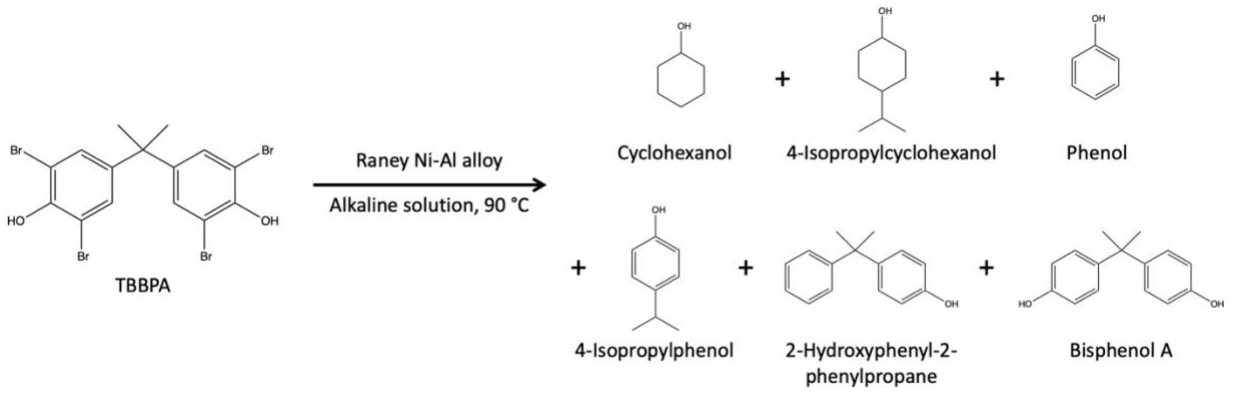
Thermal degradation of TBBPA has been investigated with thermogravimetric analysis (TGA) [77]. TBBPA showed a sharp 60% drop in mass from 200 to 290 °C, and a more gradual 20% drop from 290 to 500 °C. Infrared (IR) spectroscopy and gas chromatography-mass spectrometry (GC-MS) were used to show that HBr, which indicates dehalogenation of TBBPA, was only detected after 270 °C. This means that the homolytic bond cleavage of Ar–Br takes place at 270 °C, while the cleavage of the phenyl–isopropenyl (Ar–C) bond takes place above 300 °C, forming degradation products 2,6-dibromopenol and 4-isopropylidene-2,6-dibromophenol (Figure 3.3).



Luda, M.P., A.I. Balabanovich, A. Hornung, and G. Camino, *Thermal degradation of a brominated bisphenol a derivative. Polymers for Advanced Technologies*, 2003. 14(11-12): p. 741-748.

Figure 3.3 – Thermal degradation of TBBPA at 300 °C [77].

With the use of a metal alloy and basic conditions, debromination of TBBPA can occur at lower temperatures. The study done by Liu et al. [78] used Raney Ni–Al alloy in water, with alkaline solutions (potassium hydroxide (KOH), cesium hydroxide (CsOH) and sodium hydroxide (NaOH) each at 1% (%weight)) and heating at 90 °C for debromination. The major degradation product was bisphenol A (BPA), with other dehydrobrominated minor products including cyclohexanol, 4-isopropylcyclohexanol, phenol, 4-isopropylphenol and 2-hydroxyphenyl-2-phenylpropane (Figure 3.4). It was also found that BPA served as the common intermediate, that would continue the degradation and give rise to the same degradation products as TBBPA with 1% KOH or NaOH. However, no brominated phenols were detected as they could be readily reduced to their respective phenols and cyclohexanols.



Liu, G.-B., L. Dai, X. Gao, M.-K. Li, and T. Thiemann, *Reductive degradation of tetrabromobisphenol A (TBBPA) in aqueous medium*. Green Chemistry, 2006. 8(9): p. 781-783.

Figure 3.4 – Thermal degradation of TBBPA under alkaline conditions [78].

3.2.1.2.2 Photodegradation of TBBPA

Similar to PBDEs, photodegradation of TBBPA can occur by both direct and indirect mechanisms. When TBBPA enters an excited electronic state by irradiation, it can react with hydroxyl radicals (·OH) or singlet oxygen ($^1O_2$), which are readily available in the environment, such as from water or organic matter [79]. To investigate the active reagent in the reactive oxygen species (ROS) induced photo-transformation of TBBPA, a study was done by inducing phototransformation with ROS initiators and trappers [80]. According to this study, TBBPA could absorb light and react with $^1O_2$ as ROS, or it can act as its own singlet oxygen sensitiser to initiate its own degradation. When it is at an excited state, it generates a phenoxy radical for C–C bond cleavage and C=C bond formation for two radical intermediates (Figure 3.5). One of the intermediates can further undergo H abstraction to form 2,6-dibromophenol; the other will be further hydrolyzed to form 4-(2-hydroxyisopropyl)-2,6-dibromophenol (to be discussed further in Section 3.2.1.2.3). From the same study, it was also found that the rate of photolysis under a

nitrogen atmosphere was only slightly lower than that under an air atmosphere, meaning that direct photolysis of TBBPA could also occur in a reductive debromination pathway, without the presence of $^1O_2$ or dissolved oxygen.

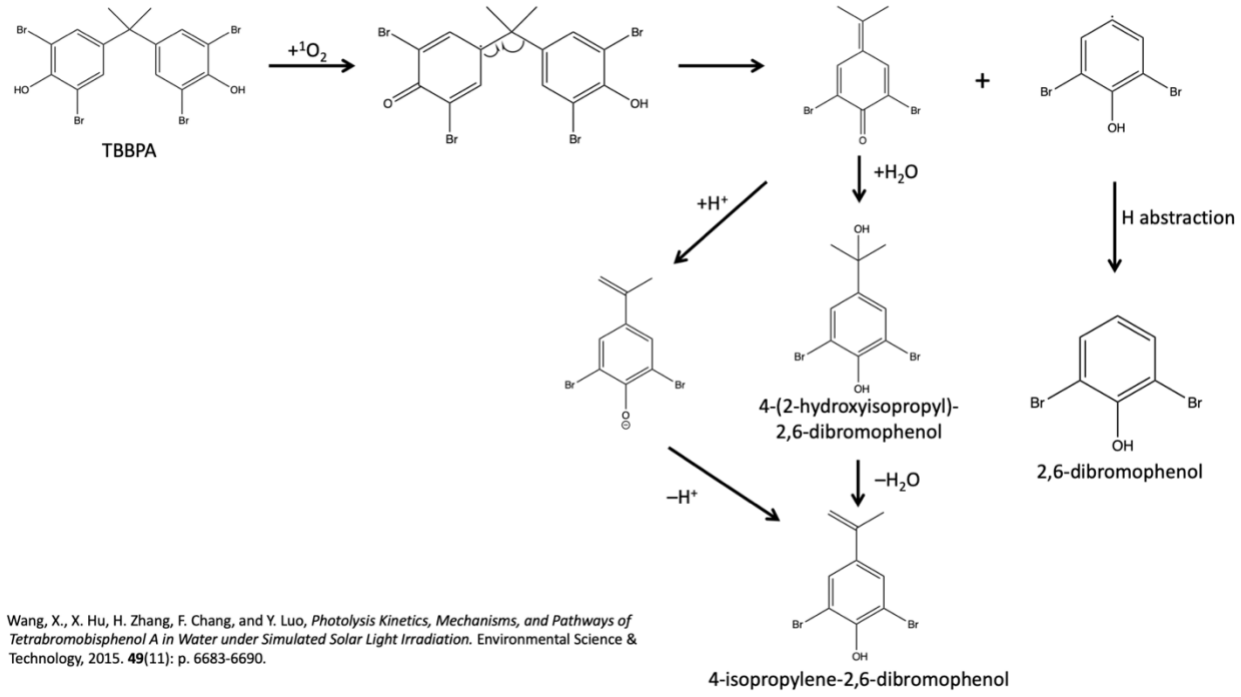


Figure 3.5 – Photodegradation and hydrolysis pathway of TBBPA with $^1O_2$ [80].

Another study of photodegradation of TBBPA by ROS was conducted in water, detecting degradation products by GC-MS [81]. From this study, the degradation products included 4-hydroxy-2,6-dibromophenol, 2,6-dibromophenol, 4-(2-hydroxyisopropyl)-2,6-dibromophenol, 4-isopropylene-2,6-dibromophenol, 2,6-dibromo-4-isopropylphenol. The formation of these products can be explained by the mentioned two processes. For direct photodegradation, debromination occurs by cleavage of the C–Br bond, or cleavage of the C–C bond to form two

phenols; while for indirect photodegradation, the phenol H would first abstract $^1O_2$ before the C–C bond cleavage and produce two phenols [79]. Under conditions where there is no sufficient supply of ROS, it was found that TBBPA did not degrade under LED radiation (white light, $\lambda >$ 400 nm) because the absorption wavelength of TBBPA is < 350 nm [82]. Xiong, Li, Peng, Gelman, Ronen and An [82] also noted that the addition of humic acid in an aquatic solution can serve as a precursor or weak sensitiser to generate ROS, such as $^1O_2$, $O_2^{\bullet-}$ or H_2O_2. In addition to the degradation products mentioned earlier, the same study also observed an oxidative skeletal rearrangement pathway of TBBPA with humic acid under irradiation, where 2,6-dibromo-4-(1-methoxy-ethyl)-phenol can be generated.

3.2.1.2.3 Hydrolysis of TBBPA

A few studies have reported the hydrolysis of TBBPA in an abiotic setting. Hydrolysis of TBBPA can be in the same pathway as photodegradation. TBBPA reacted with $^1O_2$ could form a phenoxy radical, which is responsible for C–C bond cleavage and C=C bond formation for the formation of the reaction intermediate. This intermediate can be further hydrolyzed and generate 4-isopropylene-2,6-dibromophenol by losing a water molecule, or it can be formed by deprotonation directly (Figure 3.5) [80]. The same study also examined the occurrence of a magnetic isotopic effect (MIE) during hydrolysis, where TBBPA can undergo photoexcitation to generate radical pairs in its singlet state. By C–Br bond cleavage, the hydrolysis reaction of TBBPA can displace Br atoms by water [80]. Another study by Xiong, Li, Peng, Gelman, Ronen and An [82] also noted the hydrolysis debromination reaction of TBBPA, where produce hydroxyl-TBBPA (also known as [1-(3-bromo-4,5-dihydroxyphenyl)-1-methylethyl]-phenol) as a

degradation product. This reaction was a subsequent reaction of the photoexcitation of humic acid, acting as a sensitiser in the system.

3.2.2 Organophosphorus FRs

Organophosphorus flame retardants (OPFRs) are another class of FRs increasingly used to replace phased-out FRs, namely, PBDEs [83]. This class can be further divided into phosphinates, phosphonates, and phosphate esters. The most common ones are triphenyl phosphate (TPHP), tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), and dimethyl methylphosphonate (DMMP). The production volume of OPFRs has increased and now represents a larger portion of the FR market. In 2007, the production volume reached 70,000 tonnes, and it was estimated to be increasing by 15% annually [84], and from 2013-2018, the consumption of OPFR was 18%, which is comparable to BFRs at 17% [7].

3.2.2.1 Thermal degradation of OPFRs

There is only limited work on the thermal degradation of OPFRs to date. One OPFR, DMMP, was found to degrade at high temperatures, above 500 °C. The final products of this thermal degradation process were methanol, formaldehyde, and phosphine oxide. The same study also investigated the intermediates that generate phosphoryl radicals along the degradation pathway. The results of the thermal degradation showed a diverse pattern, such as methanol, formaldehyde, and methoxy methylene oxophosphorane [85]. Even though OPFRs were meant to be safer alternatives, these diverse compounds may pose a risk to the environment, in part because they are not chemically bound to the polymers in the commercial products in which they are used.

Therefore, OPFRs may readily leach out during production and use, leading to exposure to these chemicals in humans [86].

3.2.2.2 Photodegradation of OPFRs

In the environment, some OPFRs can be broken down by photodegradation. Under the presence of ·OH radicals produced by photonic activation of TiO_2 or ZnO catalysts, the acid ester group can be readily removed [87]. The possible products from the study exhibited a complex degradation pattern under different conditions, except for direct cleavage of side chains. The cleaved side chains can also undergo reactions with themselves or other degradation products which complicates the situation. As a result, the actual degradation pattern in food samples, which are composed of complex matrices and trace levels of metal ions, may have a similarly complex pattern. Nonetheless, the photodegradation of OPFRs in food matrices has yet to be reported.

For the OPFRs present in the atmosphere as dust, they are susceptible to oxidation by ·OH radicals. TPHP, TDCIPP, and tris(2-ethylhexyl) phosphate (TEHP) were found to be reactive towards ·OH radicals when they were coated on $(NH_4)_2SO_4$. Specifically, in the degradation of TPHP, the heterogenous oxidation produced a $(C_6H_5O)_2PO(OC_6H_5O)^+$ (addition of oxygen atom to one of the phenyl rings) and a fragmentation of $(C_6H_5O)PO(OC_6H_5O)^+$ by a radical addition reaction [88]. Another OPFR, TCIPP, can undergo H-abstraction, and OH-addition that would prolong its persistence in the atmosphere. However, the abstraction of Cl, CH_3 and CH_2Cl are not thermodynamically favourable by ·OH [89].

Overall, the knowledge on the abiotic degradation of OPFRs is very limited from the literature. Some of the OPFRs, such as TPHP and TDCIPP, have been found to affect psychomotor

function, cognitive function, balance and nerve function [90], which are the same safety concerns as posed by the legacy BFRs. While there is insufficient information regarding their degradation products and routes, it is difficult to assess the safety of using these chemicals in consumer products due to the possible presence of these degradation products with unknown toxicities. Further studies on their degradation reactions can aid in developing the technology to safely remove hazardous OPFR residues from the environment.

3.2.3 Mineral FRs

Mineral FRs were introduced to the industry as halogen-free FRs, since BFRs were found to be unsafe for human health and the environment. Common mineral FRs include aluminum trihydride (ATH), magnesium hydroxide (MDH), magnesium carbonate, zinc borates, zinc hydroxystannate, and calcium carbonate. These alternatives were applied mostly in polymeric products, such as building materials and cable wires. Unlike BFR, mineral FRs work by forming a char layer as an insulator to the flame [91]. ATH and magnesium hydroxide were classified as high-production volume chemicals by the EU (production volume exceeding 1,000 tons per year) [92]. ATH in particular was the largest single FR, representing 35% of FR production from 2013-2018 [7] with a production volume of over 700,000 tons from 2007-2012 [3]. Based on the current studies, it is unknown if these minerals FRs could enter the food chain like the other FR groups.

3.2.3.1 Thermal degradation of mineral FRs

ATH is the most common FR of the inorganic FRs. Similar to another metal hydroxide, MDH, ATH absorbs heat at high temperatures and decomposes into metal oxide and water through an endothermic reaction. The inert vapour then becomes a barrier against oxygen and heating to

stop combustion from spreading [93]. Hull, Witkowski and Hollingbery [91] summarised the thermal decomposition onset temperature for other mineral flame retardants. For ATH, the decomposition begins from 180-220 °C, which is the second lowest onset temperature, followed by Nesquehonite ($MgCO_3 \cdot 3H_2O$) at 70-100 °C. The other mineral FRs, including calcium hydroxide ($Ca(OH)_2$), magnesium carbonate, and aluminum oxide hydroxide (AlO(OH)) have an onset temperature of 300 °C; and calcium hydroxide and huntite ($Mg_3Ca(CO_3)_4$) has an onset temperature above 400 °C.

Zinc borate has a similar working principle as ATH and MDH as flame retardants. In addition, zinc borate can produce aliphatic hydrocarbon cross-linkages with the polymer to further suppress smoke formation during burning [94]. Its stability temperature is up to 290 °C, which is higher than the majority of mineral FRs. A study on the thermal degradation of zine borate was carried out, with degradation products characterised by pyrolysis gas chromatography (Py-GC). A range of organic gases was released, such as benzene, aniline and quinone. It was also found that zine borate can react with ammonium polyphosphate (APP) to form zinc pyrophosphate and borophosphate, which can further react with melamine to produce melam, nitrile and a charring network [94].

3.2.3.2 Photodegradation of mineral FRs

Regarding the photodegradation of these mineral FRs, no such transformation has been reported previously in the literature. UV radiation has not been reported in the reactions related to metal hydroxide or carbonates. These degradation studies from the literature have only considered the application in FRs, hence, there is a knowledge gap to evaluate their impact on the environment and human health. For instance, these degradation studies can provide a guide for toxicity testing

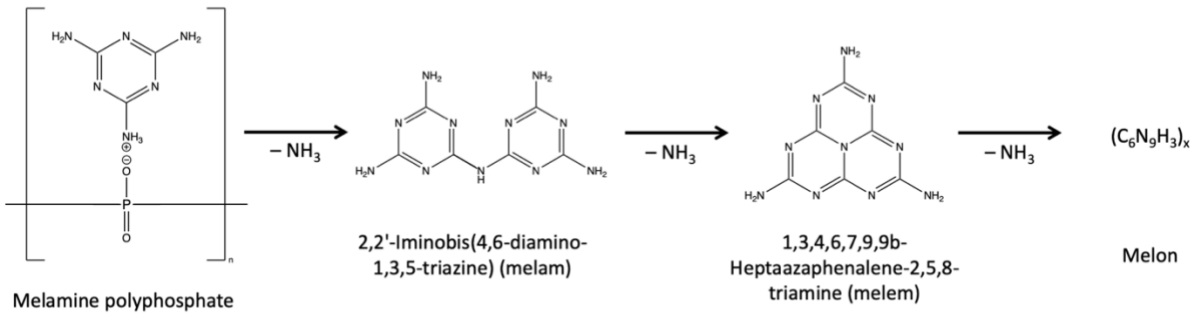
for newly discovered chemicals. Research in this area could help to better understand the impact on human health from exposure to these degradation products, since they are branded as an alternative and replacement to BFRs.

3.2.4 Nitrogen-based FRs

Nitrogen-based FRs are often combined with other inorganic materials to form flame retardants, for instance, melamine mixed with phosphoric acid to produce melamine phosphates (MP). This class of FR is especially useful for polymers, such as polyolefins, and other applications including intumescent paints, textiles and wallpapers [95]. A major feature of nitrogen-based FRs is that they are considered to be more environmentally friendly, since the elemental compositions of nitrogen and phosphorus are similar to that of the polymeric materials; the waste disposed of is also similar to that of fertilisers in terms of chemical composition [96]. Melamine has been detected in food samples [97], however, there is no known explanation if or how these FRs could enter the food chain.

3.2.4.1 Thermal degradation of nitrogen-based FRs

In general, flame retardants made with melamine and its polycondensation products have high thermal resistance. For instance, melamine polyphosphate (MPP) can resist heat up to 300 °C [98]. Under heating, melamine undergoes progressive endothermic condensation, which releases ammonia and other by-products, namely 2,2'-Iminobis(4,6-diamino-1,3,5-triazine) (melam), 1,3,4,6,7,9,9b-Heptaazaphenalene-2,5,8-triamine (melem) and melon (Figure 3.6). These side products are thermally more stable than the melamine precursor, which can resist heat up to 350 °C, 450 °C and 600 °C respectively [99].



Liu, X., J. Hao, and S. Gaan, *Recent studies on the decomposition and strategies of smoke and toxicity suppression for polyurethane based materials*. RSC Advances, 2016. 6(78): p. 74742-74756.

Figure 3.6 – Thermal degradation of melamine polyphosphate (MPP) at 300 °C [99].

Since melamine is often mixed with other constituents in the production of flame retardants, there have been investigations on interactions between melamine and other materials. In particular, when MPP is mixed with aluminum phosphinate (AlPi-MPP) and organo-modified clay, the decomposition temperature is lowered, but the degradation products remain the same. Meanwhile, the decomposition of AlPi-MPP is catalyzed by Lewis acid-base interactions, in which the phosphinates are converted into alumino-phosphate below 425 °C [100].

3.2.4.2 Photodegradation of nitrogen-based FRs

Regarding the effect of light, not much information has been published. Based on one of the few studies, melamine FRs can be mineralised into cyanuric acid by oxidation. This photolysis process is catalyzed by TiO_2, but the extent of the photolysis is hindered under the presence of oxygen in the solution. The experiment also found that the absence of H·, OH·, or molecular oxygen can lead to a distinctive degradation pathway [101]. Since this study was conducted in solution, it may not

be applicable to infer the degradation reactions of melamine FRs exposed to air or food matrices. More information is required to fully assess if harmful products are formed with other reactants that may be present.

3.2.5 Summary of FRs degradation from literature

Degradation conditions, products and reaction kinetics by each FR class are summarised in Table 3.2. It is noted that BFRs, mostly PBDEs, are most susceptible to degradation in both thermal and photolytic conditions in the environment, at temperatures above 180 °C. Information from the literature is limited regarding degradation in food samples, with most studies focused on the FRs degradation in the environment or within an isolated system. Although there is much evidence of FRs leaching into the food chain, there is no direct study of the degradation of FRs within food samples. This missing information could be crucial to assessing the risk of consuming these residual FRs via the diet, as most food will be processed or cooked before intake by humans, this could improve the accuracy of estimating the exposure of FRs via diet.

The only class of FRs that have been reported in food samples are BFRs (mostly PBDEs) and OPFRs. For PBDEs, concentrations ranged from 39 to 1400 pg g^{-1} ww in meat, from 10 to 3700 pg g^{-1} ww in fish, and from 80 to 2800 pg g^{-1} ww for other food items [102]. For TBBPA, concentrations ranged from non-detected (ND) to 0.23 ng g^{-1} ww in meat, ND to 1.8 ng g^{-1} ww in fish, and ND to 1.1 ng g^{-1} ww in dairy products [15]. For OPFRs, concentrations ranged from 0.01 to 3.6 ng g^{-1} ww for meat, 0.12 to 5.8 ng g^{-1} ww for fish and seafood items, and 0.02 to 62 ng g^{-1} ww for vegetables [4]. For mineral and nitrogen-based FRs, to the best of our knowledge, studies have not been done to detect their levels in any foods to date, and there is no known source or pathway for the potential contamination of foodstuffs by these FRs. For BFRs and OPFRs, as their

concentrations in foods were determined from the raw food items, there may be a discrepancy between these concentrations and the exposure actual concentrations after food processing (i.e., cooking).

Based on the reviewed literature on thermal, photolytic, and hydrolytic degradation of FRs in relation to typical food processing conditions, it is possible that BFRs and OPFRs may be degraded via food processing, especially from heat. This is due to the low degradation threshold in terms of temperature, as food processing can easily reach these temperatures. For the mineral and nitrogen-based classes of FRs, since most studies were based on high-temperature thermogravimetric analysis, transformations are less likely as such high temperatures will not normally be reached during typical food processing. Therefore, no conclusive prediction can be drawn for the risk of degradation of most of the flame retardants via food processing, except for some PBDE congeners that have been studied in pork and rapeseed oil [61]. The limited number of studies in the food matrices is illustrated in Figure 3.7, which shows that most of the degradation studies were done in the environmental context, with only two studies that reported degradation in food for PBDEs. While food matrices are vastly different from environmental matrices, the studies done on environmental matrices could provide insights into future studies in food matrices, in terms of the degradation kinetics and reaction of different FRs.

Future studies should be designed to simulate the temperature and light intensity that food is exposed to during processing to understand the actual degradation reactions happening in food samples. Furthermore, as OPFRs are increasingly used in the industry for consumer products, and are now listed as high production volume chemicals by the Organisation for Economic Co-operation and Development [103] as of 2004, the usage has been rising ever since [103]. Therefore, it is vital to study the behaviour of OPFRs in food during food processing to provide a better

evaluation of their safety for consumption by humans. Nonetheless, a different model should be applied to environmental matrices, as they have distinctive compositions. Soil, for example, is composed of both organic and inorganic matter, including silicate and metal hydroxides [104]. Whereas the food matrix is referred to part of the microstructures of foods, such as starch granules, tissues or microorganisms [105].

Table 3.2 – Summary of degradation studies of FRs from literature, including the experimental conditions, degradation products, order of reaction and degradation constant.

Family of FRs	Matrix	Degradation type	Conditions	Degradation products	Order of reaction /Model	Degradation constant *k*	Ref.
BFRs	Environment	Thermal	BDE-209 in Fe_3O_4 micro/nanomaterial	Various debrominated homologs	Pseudo-first-order kinetics	0.15 min^{-1}	[57]
			BDE-209 in soil sample at 450 °C	*Not reported in literature*	*Not reported in literature*	*Not reported in literature*	[58]
			BDE congeners in chlorine medium in 100 to 400 °C	Multiple or fully chlorine substituted products	*Not reported in literature*	*Not reported in literature*	[61]
			TBBPA in 200 to 500 °C	2,6-dibromopenol and 4-isopropylidene-2,6-dibromophenol	*Not reported in literature*	*Not reported in literature*	[77]
			TBBPA with Raney Ni–Al alloy in water (with alkaline solution)	Bisphenol A and other dibrominated products (cyclohexanol, 4-isopropylcyclohexanol, phenol, 4-isopropylphenol and 2-hydroxyphenyl-2-phenylpropane)	*Not reported in literature*	*Not reported in literature*	[78]
			Mixture of PBDEs, TBBPA and HBCDDs in incinerator under 740 to 820 °C	Numerous gaseous products, such as HBr and Br_2	*Not reported in literature*	*Not reported in literature*	[59]
			Mixture of PBDEs, TBBPA in resin in incinerator under 500 to 900 °C	PBDDs	*Not reported in literature*	*Not reported in literature*	[60]

		Photolysis	BDE-209 under UV irradiation	Less brominated BDEs	Pseudo-first-order kinetics	0.051 ± 0.0026 h^{-1} at 350–400 nm, 0.11 ± 0.0075 min^{-1} at 300–330 nm, and 0.050 ± 0.0081 min^{-1} at 300 to 400 nm	[63]
			TBBPA under 20 mW cm^{-2} simulated solar irradiation	Various hydroxylated and dibrominated products	Pseudo-first-order kinetics	0.0093 min^{-1}	[80]
			TBBPA in UV irradiation	4-hydroxy-2,6-dibromophenol, 2,6-dibromophenol, 4-(2-hydroxyisopropyl)-2,6-dibromophenol, 4-isopropylene-2,6-dibromophenol, 2,6-dibromo-4-isopropylphenol	First-order kinetics	0.07×10^{-3} s^{-1}	[81]
	Food	Thermal	BDE-99, 183, 209 were grilled at 180 °C in pork and vegetable oil samples, until sample reach 80 °C	Various debrominated products	*Not reported in literature*	*Not reported in literature*	[61]
			BDE-209 was heated in salmon sample, at 200 °C for 120 minutes	Various debrominated products	First-order kinetics	−1.14% min^{-1}	[44]
		Photolysis	BDE-99, 154 were radiated under UV with irradiation density of 16 mW cm^{-2} (4 W mercury lamp, 254 nm wavelength	Various debrominated products	*Not reported in literature*	*Not reported in literature*	[61]

OPFRs	Environment	Thermal	Dimethyl methylphosphonate (DMMP) undergoes degradation at above 700 °C, with TGA analysis (along with photo-ionisation for methyl and formaldehyde loss)	PO, methanol, formaldehyde	*Not reported in literature*	*Not reported in literature*	[85]
		Photolysis	Photodegradation of monocrotophos, under catalysis by TiO_2	Methylamine, formamide, acetic acid, formic acid	*Not reported in literature*	Half-life: 17-96 days	[87]
	Food	Thermal	*No study reported on food samples*				
		Photolysis	*No study reported on food samples*				
Mineral FRs	Environment	Thermal	Zinc borate in thermogravimetric analysis (TGA), degrades beginning at 290 °C	Cross linkage of B–O–Si structure between zinc borate with polydimethylsiloxane	*Not reported in literature*	*Not reported in literature*	[94]
		Photolysis	*Not reported in literature*				
	Food	Thermal	*No study reported on food samples*				
		Photolysis	*No study reported on food samples*				
Nitrogen-based FRs	Environment	Thermal	Melamine phosphate (MP) undergo progressive degradation, at 250–300 °C for formation of pyrophosphate, then at 300–330 °C for second stage	Melamine pyrophosphate (MDP) and melamine polyphosphate (MPP)	*Not reported in literature*	*Not reported in literature*	[98]
		Photolysis	Melamine mineralisation by Xe lamp, catalyzed by TiO_2	Ammeline and ammelide	*Not reported in literature*	*Not reported in literature*	[101]
	Food	Thermal	*No study reported on food samples*				
		Photolysis	*No study reported on food samples*				

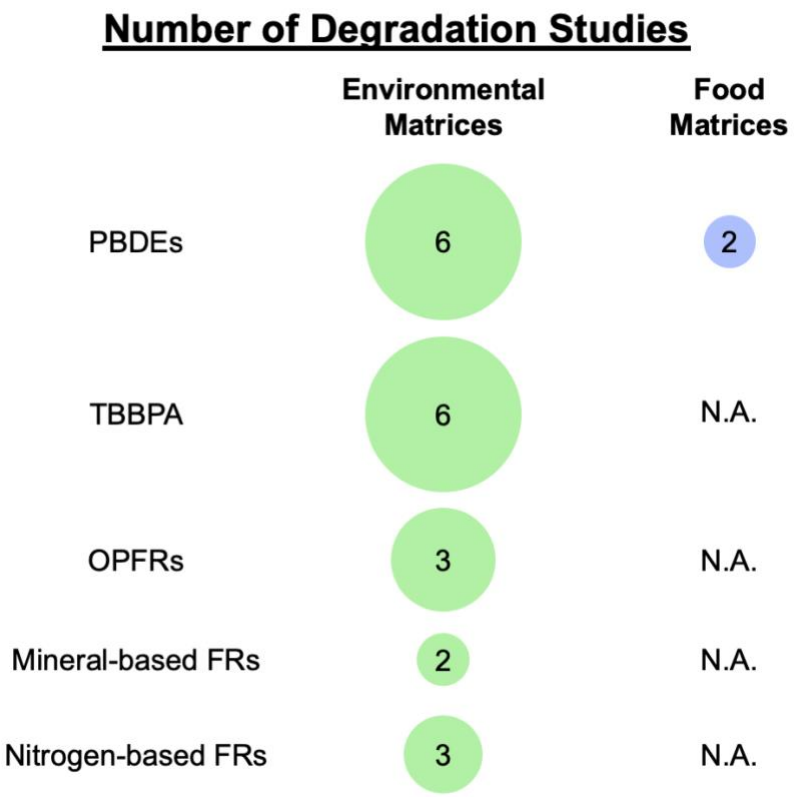


Figure 3.7 – Number of degradation studies of different FRs in the literature.

N.A.: not applicable.

3.3 **Discussion and recommendations**

Research to date on the degradation of FRs has mainly focused on processes in environmental matrices, and at high temperatures. More specifically, there is a reasonable understanding of abiotic degradation reactions (including thermal, photolytic and hydrolytic degradations) of BFRs in environmental models. In contrast, studies are limited to abiotic degradation of other FR classes, and insufficient information exists on degradation in the environment and food system models. As light and water could cause abiotic degradation, it is important to understand the different reaction mechanisms in the environmental contexts, in both short and long terms. Understanding the behaviour of these FRs could provide a better assessment of bioaccumulation or biomagnification in the food chain. Subsequently, these degradation products could also end up in the food humans consumed. In addition, most degradation information was determined to assess the quality of the chemical as flame retardants; therefore, information is missing as to whether these compounds undergo thermal transformations in food matrices, and at relatively lower cooking temperatures. In the context of food processing, some OPEs are also used in food packaging materials as plasticisers [106], it is also unknown if these FRs present would degrade if these materials are subjected to high-temperature treatment.

Regarding the limited information available from the literature, it was challenging to determine the association of the kinetics and mechanisms among FRs with environmental and food matrices. Nonetheless, the studies from environmental matrices could provide more understanding of the degradation in food matrices. For instance, the degradation of BDE-209 was studied in both the environmental [57] and food [61] matrices. Both studies pointed out various debromination products were formed during the degradation studies, with a few common products, such as BDE-

99 and BDE-183. Although the compounds react differently in the two matrices, there could be an association in the degradation mechanisms.

FRs used widely in the industry can leach into the natural environment and enter natural and agricultural food chains leading to human exposure. Therefore, from the perspective of risk assessment, it is crucial to know how these FR residues will behave during the food processing procedures, from the field to the cooking process. Currently, the literature focuses on the toxicity of the replacement FRs, while this is equally valuable information to evaluate the safety of these chemicals, understanding how these chemicals behave in the environment and food systems is important to assessing human exposures. The effect of cooking can also provide additional information on a more realistic level of these compounds being consumed through diet, for example, these FRs could be lost during cooking by the removal of oil or by degradation [48]. The estimation of daily intake of these chemicals should be based on cooked food, instead of raw food [107], as this can better indicate the risk of such chemicals by using sensible prediction of the consumption level. At present, no toxicity studies have been done specifically on the resulting degradation products that have been identified from these degradation studies, as well as the unknown degradation products. Yet, this is essential information for the novel FRs, as this could predict if the replacement was a safer alternative to the legacy FRs in the risk assessment process. Future studies should focus on how these FRs will degrade in food matrices, if these degradation products are less toxic than their parent compound, then this can ensure that the replacement is a better alternative for human health.

3.4 Acknowledgement

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Connecting Paragraph

Chapters 2 and 3 summarized the current knowledge of OPE levels found in food, and abiotic degradations of FRs in the environment and food, and identified the knowledge gap that the current risk assessment process does not consider the fate of these FRs in food. In addition, the source of FRs and OPEs is also essential for a better exposure assessment. In Chapter 4, the source of OPEs from the atmospheric air will be studied, by using honey samples collected in Quebec, Canada as a bio-indicator. This chapter will also present a non-targeted screening workflow developed for the identification of unknown NFRs and OPEs in the samples. Chapter 4 has been published in *Chemosphere*: Leung, G., Akiki, C., Bilamjian, S., Tian, L., Liu, L., & Bayen, S. (2023). Targeted and non-targeted screening of flame retardants in rural and urban honey. *Chemosphere, 341*, 139908. https://doi.org/10.1016/j.chemosphere.2023.139908.

Chapter 4. Targeted and Non-Targeted Screening of Flame Retardants in Rural and Urban Honey

Abstract

Flame retardants (FRs) are often added to commercial products to achieve flammability resistance, but they are not chemically bonded to the materials, so, they can be easily released into the environment during the production and disposal processes. When honeybees travel to collect nectar during the pollination process, they are prone to be contaminated by chemicals in the air. Therefore, honey contamination has been proposed as an indicator of the pollution status in a particular region. To date, the occurrence of flame retardants in urban honey has yet to be explored. In this study, a direct injection method was used, coupled with LC-QTOF-MS, to analyze honey samples. This method was applied to urban (n = 100) and rural (n = 100) honey samples from the Quebec province (Canada), and the levels of flame retardants in urban and rural honey samples were not significantly different. In the targeted approach, two of the target FRs, Tris(2-butoxyethyl) phosphate (TBOEP) and Triphenyl phosphate (TPHP), were detected and confirmed at an average trace concentration (<100 ng g^{-1}). Additionally, a non-targeted screening workflow with an in-house-built library was developed and validated to screen for flame retardants in honey. Tris(2-chloropropyl) phosphate (TCIPP) was identified in honey using the non-targeted screening workflow and confirmed using a pure analytical standard, but there are other compounds detected in the non-targeted analysis that have yet to be validated. This study was the first to report FR compounds based on a direct injection method, coupled with a non-targeted screening workflow, at a trace level in a honey matrix. It also showed that a non-targeted workflow was effective to detect and identify unknown compounds present in the honey sample; hence, this provided a novel angle for the occurrence of FRs in air, with honey as a bio-indicator.

Keywords: Honey, Non-targeted screening, Flame retardants, LC-MS

4.1 Introduction

With the ban on using legacy flame retardants (FRs), such as polybrominated diphenyl ethers (PBDEs), in the industry, it has become more popular for the industry to shift to novel FRs, such as organophosphate esters (OPEs), as a substitution. The global consumption of organophosphate flame retardants (OPFRs) went up 3.5 folds from 500,000 tons in 2004 to 680,000 tons in 2015, and it was estimated to account for 16% of the global market share in 2019 [1]. While some OPFRs may be found to be neurotoxic, especially to children [2], other classes of emerging FRs, such as novel brominated flame retardants (NBFRs) (e.g. bis(2,4,6-tribromophenoxy)ethane (BTBPE)) have been used in the industry. However, these have the same problem as OPEs as they possess toxic properties, and can be easily released into the environment by wastewater discharge, atmospheric disposition or runoffs [3]. In addition, hexabromocyclododecane (HBCDD), an aliphatic brominated FR, was also the most abundant FRs after PBDEs and tetrabromobisphenol A (TBBPA) in North America [4]. These FRs threaten human health not only because of their toxic properties, but also because most of them are lipophilic and can accumulate along the food chain.

Honey is a natural sweetener product produced by honeybees. During the pollination process, honeybees travel relatively long distances and are prone to be contaminated by chemicals in atmospheric air, and have them retained on their body surface or inhaled in their trachea. Honeybees may also be exposed to chemical residues from plant nectar, pollen or water. Therefore, these chemical residues found in honey can be used as a bioindicator for the pollution status in a particular region [5, 6]. According to recent studies, a range of OPEs has been detected in air samples in urban areas, such as Canada, Germany, and China. In particular, chlorinated-OPEs, such as tris(1-chloro-2-propyl) phosphate (TCIPP) and tris (2-chloroethyl) phosphate (TCEP) are

prevalent in the outdoor air samples. Other OPEs, including tri-n-butyl phosphate (TBP) and tris (2-butoxyethyl) phosphate (TBOEP), are also frequently found in outdoor air [7-9]. Since these FRs are hydrophobic in nature, they are likely to bind with dust, soil and sediment particles [10]. Therefore, it is very likely that these OPEs are one of the contaminants found in honey.

Currently, a range of chemicals has been detected from honey samples, including organochlorine pesticide [11], legacy FRs (PBDEs) [5] and plastic-related chemicals [12]. But for novel FRs, no relevant data is available, especially for honey samples from Canada. According to a study in Spain, OPEs were detected from foraging honeybees [13], meaning that these OPE contaminants in the atmospheric air can be attached to honeybees. Notably, a recent study on honey samples by von Eyken, Ramachandran and Bayen [12] detected TBOEP from a honey sample at 164.7 μg kg^{-1}. So, it was speculated that more types of OPEs and other FRs might be emitted into the air and are now contaminants in honey.

On the other hand, according to the current literature, these NBFRs have been detected in the aquatic biota, and accumulated along the food chain. More importantly, a study discovered that these NBFRs (specifically decabromodiphenyl ethane (DBDPE)) had undergone significant trophic magnification in Lake Winnipeg, Canada, meaning they have high trophic magnification potential in the aquatic environment [14]. Apart from this, these NBFRs have also been detected in different food samples [15]. Hence, this study can present a novel angle on the occurrence of flame retardants in urban air, and thus provide some insight into the pollution status of flame retardants.

The objectives of this study were to apply targeted and non-targeted approaches in the screening of flame retardants in honey samples obtained from Québec, with LC-MS, to compare FR levels from honey samples originating from different regions (rural and urban areas). In this

study, TBOEP, TBP, triphenyl phosphate (TPHP), and tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) were selected as the four target compounds, based on their frequent detection from food and the previous study on Québec honey samples [12, 16].

4.2 Material and Methods

4.2.1 Chemicals and reagents

The internal standard mix was composed of five labelled standards. Tributyl phosphate-d_{27} (TBP-d_{27}), tris(2-ethylhexyl) phosphate-d_{51} (TEHP-d_{51}), bis(2-ethylhexyl) phosphate-d_{34} (BEHP-d_{34}), diphenyl phosphate-d_{10} (DPHP-d_{10}) were purchased from Toronto Research Chemicals (Toronto, ON, Canada). Tetrabromobisphenol A-d_4 (TBBPA-d_4) was purchased from CDN Isotopes (Pointe-Claire, Canada). The *m*/*z* values of these internal standards are listed in Table S4.1.

Tris(2-butoxyethyl) phosphate (TBOEP), triphenyl phosphate (TPHP), tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), tributyl phosphate (TBP), bis(butoxyethyl) phosphate (BBOEP), diphenyl phosphate (DPHP), and 2-hydroxyethyl bis(2-butoxyethyl) phosphate (BTBOEP) standards were purchased from Toronto Research Chemicals (Toronto, ON, Canada). Tris(2-chloroisopropyl) phosphate (TCIPP) standard was purchased from Cambridge Isotope Laboratories (Saint-Laurent, Canada). The HPLC-grade solvents, including water, acetonitrile, methanol, and LC-MS grade formic acid were purchased from Fisher Scientific (Saint-Laurent, Canada). The LC-MS grade ammonium acetate was purchased from Sigma Aldrich. All glassware was baked at 325 °C for four hours prior to use.

4.2.2 Sample collection

The urban honey samples were supplied by two beekeeping companies based in Montreal. The two suppliers provided honey samples collected from different beehive locations in the Montreal region. These locations were categorized into regions based on their geographical areas, namely, Montreal-North, Montreal-East, Montreal-Centre-South, Montreal-Centre-West, Montreal-West and Laval. Approximately an equal number of samples were selected from each region (also based on availability). The samples selected were listed in Table S4.2.

The rural honey samples were purchased from local grocery stores, or online. These honey samples purchased were classified by the beehive location stated on the packaging labels or suppliers' websites. These locations were categorized by the administrative regions in Québec. Samples were selected based on these locations and availability, so that the samples would be originated from different regions. The samples selected were listed in Table S4.2.

4.2.3 Extraction by direct injection method

Approximately 0.2 g of honey was weighed in a polypropylene conical tube and 2 mL of a mixture of acetonitrile and water (1:1) was added. 20 µL of the spiking mix, as described in Section 4.2.1, was also added to each sample, in which each of the five internal standards was at 4 µg mL^{-1}. Samples were vortexed for about 2 minutes, or until the honey was dissolved entirely, and then filtered through a 0.22 µm PTFE filter (Agilent Technologies, Santa Clara, CA, USA) and polypropylene syringe. Before injection into the HPLC, the extract was further diluted with HPLC water to a final concentration corresponding to 1% of honey. These internal standards were not used for quantification in this study but were spiked to provide a reference for sensitivity and retention time, which is necessary for the future non-targeted data treatment [17]. One procedural

blank sample was per 10 samples in each batch; they followed the same extraction procedures without any honey samples.

4.2.4 Recovery test and matrix-matched calibration

To test for the recovery of the compounds of interest, rural and urban honey samples with the lowest signal of the target compounds, TBOEP and TPHP, were selected for a recovery test.4.2.3 The selected honey samples were spiked at three levels with their chemical standards in lieu of the internal standards mix following the same extraction procedure as described in Section 4.2.3. TBOEP was spiked at 10, 100, and 1000 ng mL^{-1} (corresponding to the concentrations of 1000, 10000, and 10000 ng g^{-1}), while TPHP was spiked at 0.5, 50 and 500 ng mL^{-1} (corresponding to the concentrations of 50, 5000, and 50000 ng g^{-1}). These samples were analyzed in triplicate for each compound.

To quantify the target compounds from the honey samples, a series of matrix-matched calibration curves were prepared. One honey sample from each of the four colours (honey colour definitions according to the guidelines by the Canadian Food Inspection Agency [18]), and with the lowest signal of the target compounds (TBOEP and TPHP) was selected. The selection was made in both rural and urban honey samples. The honey samples followed the same extraction procedure, as described in Section 4.2.3, where the target compounds were spiked at the final dilution step, so the final volume of each sample was 1 mL. The spiking range was from 0 to 10 ng mL^{-1} ($R^2 > 0.98$). A series of calibration curves were also prepared with methanol solvent as blanks ($R^2 > 0.98$).

4.2.5 Instrumental analysis

The samples were analyzed on a 1290 series LC system from Agilent Technologies (Santa Clara, CA, USA) equipped with a InfinityLab Poroshell 120 EC-C18 (3.0 × 100mm, 2.7 µm) column, fitted with InfinityLab Poroshell 120 EC-C18 (3.0 × 5mm, 2.7 µm) guard column from Agilent Technologies. The mobile phase for electrospray ionization positive mode consisted of water (solvent A) and methanol (solvent B), both with 0.1% formic acid. The mobile phase for electrospray ionization negative mode consisted of water (solvent A) and methanol (solvent B), both with 5 mM ammonium acetate. The flow rate was 0.3 mL min^{-1}. The mobile phase gradient was as follows: 0.5 min 5% B, from 0.5 to 8 min gradient to 100% B, from 8 to 12 min 100% B, from 12 to 12.1 min gradient to 5% B. The injection volume was 20 µL and the column temperature was set to 20 °C.

The LC system was coupled to a 6545 series Quadrupole-Time-of-Flight (Q-TOF) from Agilent Technologies equipped with a Dual AJS ESI ion source operating in both positive and negative modes. The drying gas temperature was 275 °C with a flow rate of 10 L min^{-1}, and the sheath gas temperature was 325°C with a flow rate of 12 L min^{-1}. The pressure of the nebulizer was 30 psi, the capillary voltage was 4000 V, the fragmentor voltage was 125 V, the skimmer voltage was 65 V, and the nozzle voltage was 250 V. All ions MS/MS data were collected as MS scans between *m/z* 70 to 1700 at a scan rate of 2 spectra/s. The first 2.5 minutes of elution was diverted to waste. Samples were kept at 4°C in the multi-sampler compartment.

Targeted MS/MS mode was also used to confirm the identity of the detected compounds, with the same analysis method described above. The spectra were recorded at three collision energies at 10, 20 and 40 V.

4.2.6 QA/QC

Six pooled quality control (QC) samples were prepared by pooling equal volumes of all extracted samples, and they were analyzed in the analysis queue. Samples in each batch were analyzed in a random order to ensure there was no trend in the results created by an instrumental drift [17]. A laboratory standard mix was also injected in the beginning, middle and end of the analysis queue to ensure the signals were consistent throughout the batch. Additionally, one of the honey samples was injected 5 times to check for the precision of the instrument.

4.2.7 Set-up of in-house screening library for flame retardants

To build the screening library of novel flame retardants, publications with the keyword 'novel flame retardants', and 'screening' was searched on the Web of Science. A total of 141 flame retardant compounds reported from the literature were compiled and categorized based on their chemical group, namely, hexabromocyclododecanes (HBCDDs), novel brominated flame retardants (NBFRs), and organophosphate esters (OPEs) [6, 16, 19-26]. Some compounds were reported in the literature and analyzed with gas-chromatography (GC), for instance, 2-ethylhexyl-2,3,4,5-tetrabromobenzoate [24]. These compounds may not be ionized well in the ESI instrument, but they were kept in the screening library for a more comprehensive screening of flame-retardant compounds in the honey samples.

Agilent PCDL Manager (v7.0) was used to organize the library by adding the chemical formula, mass and retention time (if chemical standards were available in the lab), and export as a '.cdb' file for screening in the subsequent data treatment. The compounds from this screening library were listed in Table S4.3.

4.2.8 Data treatment

MassHunter Profinder (v10.0) from Agilent Technologies was used for molecular feature extraction and peak alignments. The molecular feature extraction was done using the 'Batch Molecular Feature Extraction (Recursive, small molecules)', and the parameters are as follows: peak filter with height ≥300 counts, isotope model was common organic molecules, and the ions and adducts considered were H^+, Na^+, K^+, NH_4^+, CH_3COO^-, and H_2O. The RT tolerance was ±0.05 min, and the mass tolerance was ±10 ppm.

A suspect screening was also done by using the 'Targeted Molecular Feature Extraction' using an in-house flame-retardant screening library (Table S4.3). The mass tolerance of the library match was ±5 ppm, and the minimum total score to be considered was 70%.

Mass Profiler Professional from Agilent Technologies was used to generate PCA plots for the samples to assess the variability of the data. The number of principal components was 4, and component 1, 2 and 3 was picked for X-, Y- and Z-axis, respectively.

4.3 Results and discussion

4.3.1 QA/QC

Principal component analysis (PCA) plots were used to verify the positions of the QC samples, to ensure that the instrument run had no artifact and to monitor the quality of the run in a non-targeted analysis [12]. The PCA plots were based on different molecular feature extraction methods and ESI modes (Figure S4.1). For each PCA plot, the QC samples were shown to have a close clustering, except for ESI– in the targeted feature extraction. This demonstrated that the analysis was generally reproducible and could be used for further data treatments [12].

In addition, the precision and accuracy of the instrument were evaluated by the retention time (RT) and mass accuracy of the internal standards spiked respectively. The details can be found in Table S4.4. Briefly, the retention time precision for all five internal standards (TBP-d_{27}, TEHP-d_{51}, BEHP-d_{34}, DPHP-d_{10}, and TBBPA-d_4) has a relative standard deviation (RSD) below 0.1%. Moreover, one of the honey samples was injected five times, and the results showed that the RSD was 2.85% in terms of response. These results showed that there is a high precision of the LC run, and there is no significant time drift. The accuracy for the five internal standards was all within 15 ppm. The RSD% of the RT and mass accuracy for each internal standard was listed in Table S4.4.

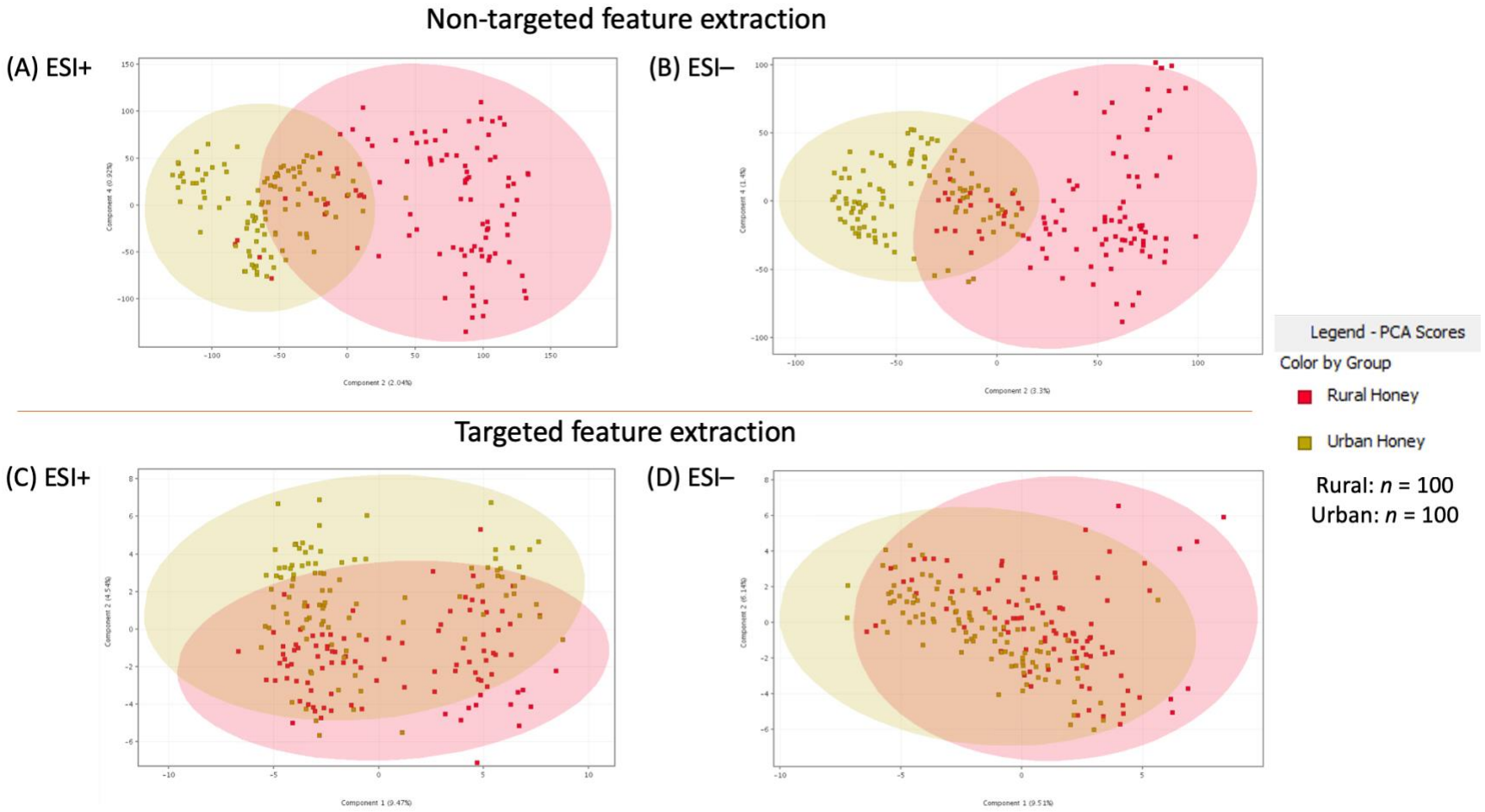

Figure 4.1 – PCA plots for different feature extraction algorithms and ESI modes. (A) plot for ESI+ in 'Batch recursive feature extraction (small molecules)', (B) plot for ESI– in 'Batch recursive feature extraction (small molecules)', (C) plot for ESI+ in 'Batch targeted feature extraction', (D) plot for ESI– in 'Batch targeted feature extraction'.

4.3.2 Comparison of rural and urban honey samples

With Agilent MassHunter Profinder (v10.0) ‘Batch Recursive Feature Extraction (small molecules)’, the compounds from the samples were extracted by a non-targeted method. The PCA plots of non-targeted extraction showed an apparent clustering of urban and rural honey samples, with a minor overlapping, meaning there is a distinctive difference in terms of its chemical composition (Figure 4.1 – PCA plots for different feature extraction algorithms and ESI modes. (A) plot for ESI+ in 'Batch recursive feature extraction (small molecules)', (B) plot for ESI– in 'Batch recursive feature extraction (small molecules)', (C) plot for ESI+ in ‘Batch targeted feature extraction’, (D) plot for ESI– in ‘Batch targeted feature extraction’.

A and Figure 4.1 – PCA plots for different feature extraction algorithms and ESI modes. (A) plot for ESI+ in 'Batch recursive feature extraction (small molecules)', (B) plot for ESI– in 'Batch recursive feature extraction (small molecules)', (C) plot for ESI+ in ‘Batch targeted feature extraction’, (D) plot for ESI– in ‘Batch targeted feature extraction’.

B).

Using the in-house-built novel flame-retardant database, the ‘targeted molecular feature extraction’ was also applied to the samples as a targeted feature extraction method. However, the clustering of urban and rural honey samples on the PCA plots was not as apparent. Although the honey samples originated from different areas, the flame-retardant profiles were not significantly different. The clustering of the sample groups in the non-targeted feature extraction method could be originated from other chemical contaminants, such as pesticides [11] and plastic-related chemicals [12].

4.3.3 Targeted MS/MS for TBOEP and TPHP and structural confirmation with SIRIUS

TBOEP, TBP, and TPHP showed a high detection frequency in the two honey sample groups (>90%), as well as the procedural blanks. At the same time, the detection frequency for TDCIPP was only ≥60% across the sample groups. Some of these flame retardants could be originated from the background signals, possibly from indoor dust, or it is ubiquitous in the environment [27]. Therefore, the samples were only further analyzed if their signal had to be higher than the $\bar{x}+3\sigma$ of procedural blanks. This value is also known as the limit of detection (LOD). The limit of quantification (LOQ), which is $\bar{x}+10\sigma$, was also calculated, to identify individual samples with a signal that is significantly higher than the procedural blanks.

For the samples with the detected flame retardants and signal above the detection limit, the samples were selected for a further MS/MS analysis to confirm the identity of the flame retardants. Using the targeted MS/MS mode, the fragmentation patterns were compared to their respective chemical standards. For each compound, the multiple reaction monitoring (MRM) precursor/product transitions were compared with the value obtained from the literature [28]. According to the results, TBOEP and TPHP, with their product-ion peaks at high signals, confirmed the presence of these flame retardants in the honey samples (Figure 4.2A and Figure 4.2B). While for TBP and TDCIPP, the product-ion peaks in the MS/MS spectrum of the sample had low signals, so it was concluded that TBP and TDCIPP were false identifications from the screening (Figure 4.2C and Figure 4.2D).

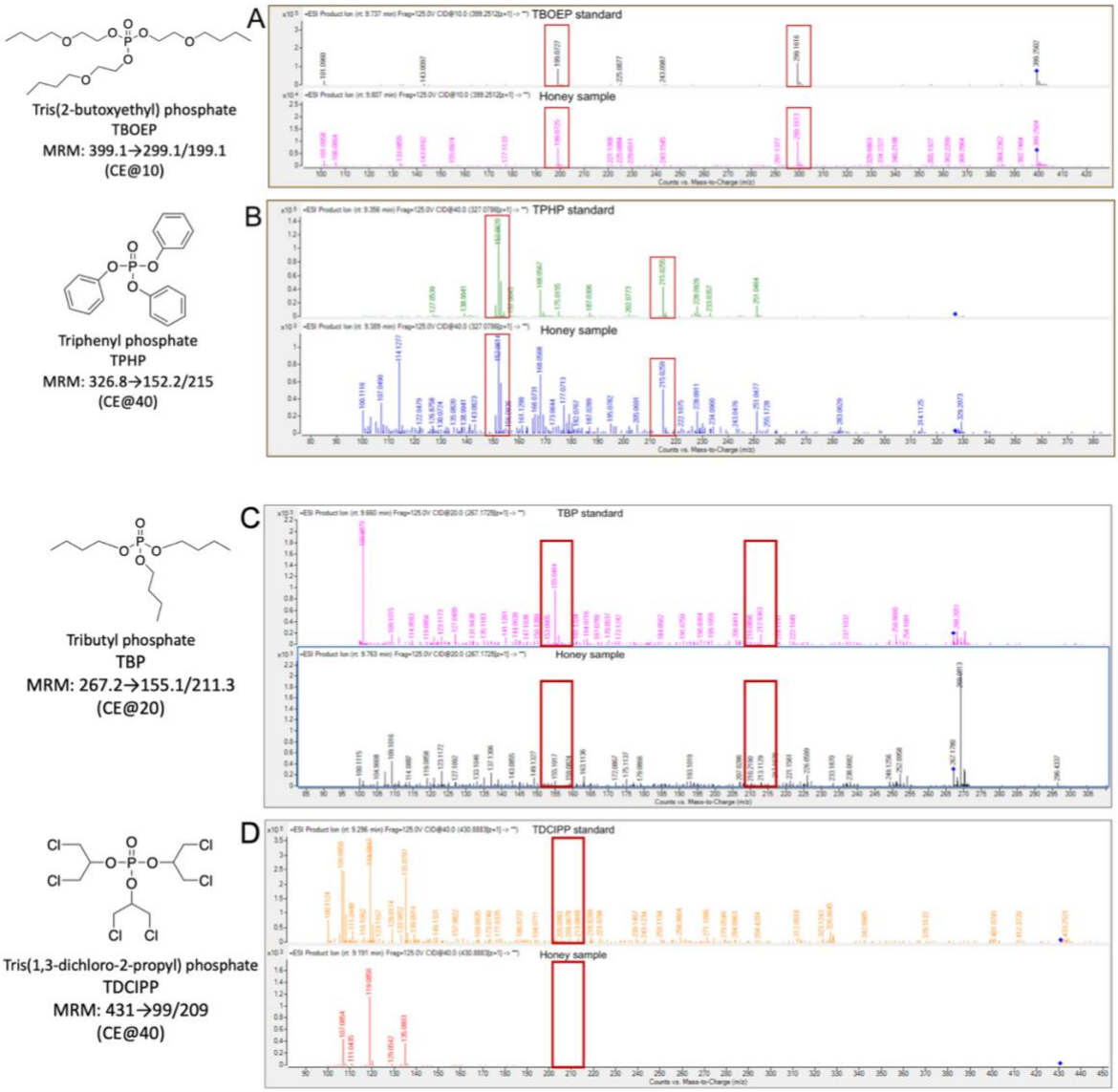

Figure 4.2 – Targeted MS/MS spectra for (A) TBOEP (B) TPHP (C) TBP (D) TDCIPP for the MRM transition patterns.

The software *SIRIUS* (Friedrich-Schiller-Universität Jena) was used to further confirm the detected compounds to analyze targeted MS/MS data generated. *SIRIUS* is an open-source software that provides rapid computational analysis for molecular structural identification, based

on online database searching [29]. Based on the results, *SIRIUS* was able to correctly identify the two compounds based on the fragmentation patterns on the targeted MS/MS spectra obtained in the honey matrix. Both compounds were on the training set of the machine learning process, and they were both the first candidate in the list, with a matching score above 75%. Therefore, this supported the matching of the MRM transition patterns from the targeted MS/MS spectra.

4.3.4 Limit of identification for TBOEP and TPHP

The limit of identification (LOI) for the two confirmed compounds, TBOEP and TPHP, were also investigated. This was done by spiking honey samples (with the lowest detected level) with the known concentration of the two compounds of interest. The detection and identification workflow was done using the same workflow as other honey samples, i.e. chemical feature extraction, targeted MS/MS analysis and identification by software *SIRIUS*.

The workflow was able to detect and identify the target compounds correctly. For TBOEP, the recovery ranged from 99.7% to 99.9%, for both rural and urban honey samples, at all three spiking levels, showing no significant loss of compounds resulting from the extraction method. Profinder could correctly detect the compounds with a high matching score (>96%) using the '*Targeted Feature Extraction'* mode at all three spiking levels. With *SIRIUS*, the compound could also be correctly identified, in terms of the structure, with a high matching score (>90%) (Figure 4.3A). For TPHP, a high recovery range was only obtained at high and medium levels (98.3-99.5%), and a low recovery resulted in low-level spiking (67%). Nonetheless, at all three levels, TPHP could be correctly detected by Profinder, with matching scores above 97%. *SIRIUS* could correctly identify TPHP with matching scores above 90% (Figure 4.3B). The results are summarized in Table S4.5 and the results showed that the LOIs are below the low level of spiking

for the two compounds. In other words, the LOI for TBOEP is below 1000 ng g^{-1}, while that of TPHP is below 50 ng g^{-1}.

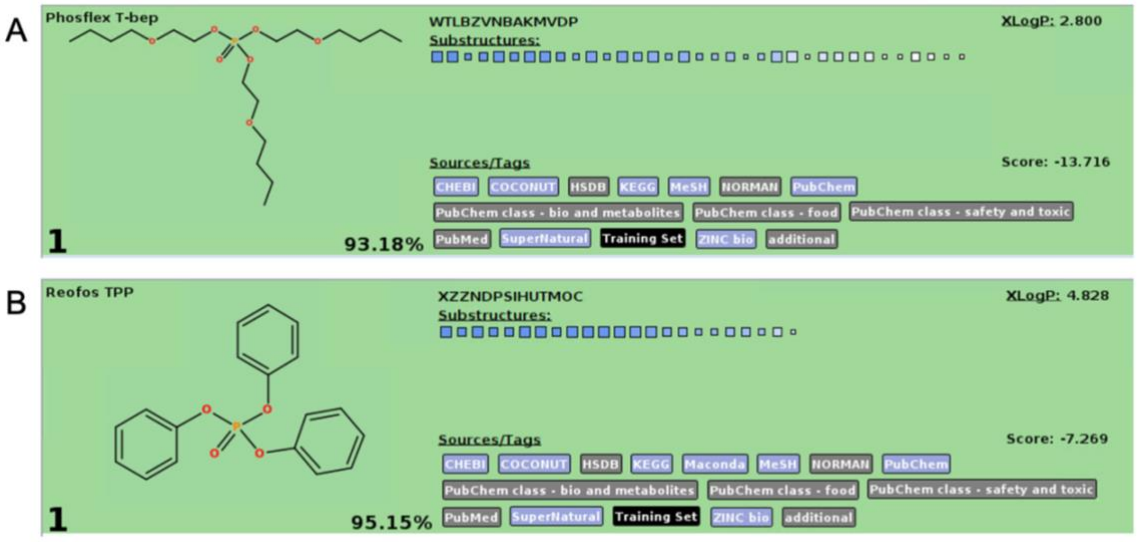

Figure 4.3 – Structural identification by SIRIUS of the spiked samples for (A) TBOEP and (B) TPHP.

4.3.5 Quantification by matrix-matched calibration

A series of calibration curves were prepared to understand the quantity of two detected and identified flame retardants. Knowing that honey may pose a matrix effect on the detected compounds, matrix-matched calibration curves were used to compare with the solvent-only calibration curves. Additionally, the effect on the honey colours and origin (rural and urban) was investigated by analyzing different calibration curves prepared based on these factors. For both TBOEP and TPHP, it was found that there were no significant differences among the slopes of calibration curves from different honey colours ($p > 0.05$), as well as their origins ($p > 0.05$). In terms of the matrix in honey and solvent, it was also found that there was no significant difference

($p > 0.05$) for the slopes. However, based on the matrix-effect calculations, only the concentrations of TPHP were within the acceptable range (82-117%). TBOEP showed a more significant matrix effect, especially in white honey (>120%), but also in rural golden and amber (>120%) and rural dark honey (<80%). Therefore, it was justified that the quantification was done using the matrix-matched calibration curve corresponding to the honey sample colour, in favour of correcting any possible matrix effects.

The quantification results performed based on the matrix-matched calibration was presented in Table 4.1. From the results, the average level of TBOEP was 76.3 ng g^{-1} in rural honey and 19.2 ng g^{-1} in urban honey. The average values were at the same magnitude as the previous study, which detected TBOEP at a level of 165 ng g^{-1} [12]. The highest detected level of TBOEP was at 1290 ng g^{-1}. Upon looking up the beehive location of this honey sample, it was found that it was produced within 30 km of Montreal Island. Since one of the hypotheses is that the flame-retardant pollution originated from anthropological activities, i.e. from the urban region, this could be a possible explanation for the higher detection of TBOEP in the honey sample. However, it is also worth noting that the other honey sample produced from the same region had a concentration of 100 ng g^{-1}. The average concentrations between rural and urban honey samples were not statistically different ($p > 0.05$). This showed that the hypothesis was not accurate.

Furthermore, the ubiquitous detection of the target compounds could also be due to the fact that these flame retardants could travel long distances in the air from the source of pollution. According to a screening assessment done by Environment and Climate Change Canada, the modelled half-life of TBOEP and TPHP (aryl organophosphates) are less than a day (gas phase). Still, there were found to be more persistent when bound to particulates, where TPHP and TBOEP can be persistent in the air for 5 and 137 days respectively [30]. Studies from the literature also

showed that these flame retardants had been detected in remote regions, such as the water [31] and air [32] in the Arctic region; such information showed the reason behind the frequent detections of these flame retardants far from the urban region.

Table 4.1 – Summary of the results of quantification of TBOEP and TPHP in honey samples, including minimum, maximum, and mean detection levels, standard deviations and detection frequencies.

	Min ($ng\ g^{-1}$)	Max ($ng\ g^{-1}$)	Mean ($ng\ g^{-1}$)	SD ($ng\ g^{-1}$)	Detection Frequency
TBOEP					
Rural Honey	2.77	1290	76.3	287	20/100
Urban Honey	3.74	35.7	19.2	9.69	13/100
TPHP					
Rural Honey	/	10.8	/	/	1/100
Urban Honey	/	/	/	/	0/100

4.3.6 Non-targeted screening of flame retardants

Besides using a targeted approach, a non-targeted screening method was applied to the data obtained from the high-resolution MS. These chemical features extracted from MassHunter Profinder (Agilent Technologies) were screened against the in-house built flame retardants database to detect possible flame retardants present in the honey samples. For each chemical entity identified, the software assigned a matching score from 0 to 100, based on the mass identified. The retention time was not used in the scoring algorithm, because only a small fraction of this

information was available, so this could lead to bias in the scoring algorithm. Among all the features extracted only the entities with a matching score over 80% were considered. Each entity detected was screened, for a neat Gaussian peak shape.

Apart from the four organophosphate esters flame retardants targets, some other compounds detected were also above the LOD level. According to Schymanski et. al. [33], the identification of compounds by high-resolution MS could be classified into levels of confidence. Four of the detected features were identified as OPEs based on the exact mass of interest, with a matching score on Profinder of >85%. Based on this putative identity, the samples with the identified features were analyzed by the targeted MS/MS mode on the LC-QTOF. The results were summarized in Table 4.2.

To illustrate that this workflow is valid, the samples incurred with the two detected target OPEs (TBOEP and TPHP) were also used for the MS/MS analysis. Both of them showed a matching score on *SIRIUS* above 75%. With the retention time and MS/MS fragments matched with their respective pure standards, the two compounds were concluded to be correct identifications in this workflow.

Feature 1 was tentatively identified as triethyl phosphate (TEP) according to the FR database, with a retention time of 5.43 minutes. *SIRIUS* was then used to search for possible molecular structures based on the library searching with the available MS/MS data. According to the software, TEP was correctly identified with a matching score of 46.7% (Figure S4.2A); however, its isomers, namely butyl ethyl hydrogen phosphate and butyl dimethyl phosphate, were also identified at 45% and 40.7%, respectively. A reference standard would be required to confirm the identity by the retention time. However, upon a closer inspection of the MS/MS spectrum available in the

molecular database, some of the fragment peaks were missing in the sample spectrum (Figure S4.2B), therefore, this could be concluded as a false identification.

Both Feature 2 and 3 were tentatively identified as metabolites of TBOEP, namely, 2-hydroxyethyl bis(2-butoxyethyl) phosphate (BTBOEP) and bis(2-butoxyethyl) phosphate (BBOEP), respectively. With the MS/MS data analyzed on *SIRIUS*, the structures of both features were correctly identified with a matching score of 60.5% (Figure S4.3A) and 47.0% (Figure S4.4A), respectively. The retention time between the sample and the reference standard also matched. Yet, when the MS/MS spectra of the sample and the reference standard were compared, there were missing peaks on the sample spectrum (Figure S4.3B, Figure S4.4B). Hence, both features were also concluded as false identifications.

Finally, feature 4 was tentatively identified as tris(2-chloroisopropyl) phosphate (TCIPP). According to the results from *SIRIUS*, the correct structure was identified with a matching score of 68.7%; however, from the list of probable structures, the isomer of TCIPP (tris(1-chloroisopropyl) phosphate) was also determined with a similar matching score at 75.3% (

Figure 4.4A). To confirm the identity, the MS/MS fragments were also compared to that of the reference standard, in which the spectrum of the sample showed a similar abundance distribution (

Figure 4.4B). Moreover, the retention time was compared against the reference standard, which matched at 8.8 minutes (

Figure 4.4C). Hence, the feature was confirmed to be TCIPP, with a level 1 identification confidence [33].

Based on the identification of TCIPP and TPHP from *SIRIUS*, despite the fact that both compounds showed a matching score in the medium range (68-76%), and the correct structure of

TCIPP was ranked number 9 in the list of candidates, this cannot be used to interpret as a false identification alone. While *SIRIUS* provided valuable information on the structure, these identifications showed that the *SIRIUS* can only attain a level 2 identification confidence, pure standards are still required for the highest level of identification confidence [33].

Table 4.2 – Identification outcome of four selected molecular features from suspect screening.

	TBOEP	TPHP	Feature 1	Feature 2	Feature 3	Feature 4
ESI mode	ESI+	ESI+	ESI+	ESI+	ESI+	ESI+
Matching score on Profinder	90.35	96.51	93.2	94.7	96.8	90.9
RT (min)	9.80	9.40	5.43	8.75	8.32	8.83
Chemical formula	$C_{18}H_{39}O_7P$	$C_{18}H_{15}O_4P$	$C_6H_{15}O_4P$	$C_{14}H_{31}O_7P$	$C_{12}H_{27}O_6P$	$C_9H_{18}Cl_3O_4P$
Putative identity based on molecular database	Tris(2-butoxyethyl) phosphate (TBOEP)	Triphenyl phosphate (TPHP)	Triethyl phosphate (TEP)	2-Hydroxyethyl bis(2-butoxyethyl) phosphate (BTBOEP)	Bis(2-butoxyethyl) phosphate (BBOEP)	Tris(2-chloroisopropyl) phosphate (TCIPP)
MS/MS fragments vs. literature or compound library (confidence level 2)	Matched	Matched	Not match	N.A.	N.A.	N.A.
Matching score from SIRIUS	87.5%	76.7%	46.7%	60.5%	47%	68.7%
RT comparison with pure standards	9.74	9.36	N.A.	8.74	8.40	8.81
MS/MS fragments vs. pure standards	Matched	Matched	N.A.	Not matched	Not matched	Matched
Samples vs. procedural blanks	Above LOD	Above LOD	Above LOD	Above LOD	Above LOD	Above LOD
Identification	Correct	Correct	False	False	False	Correct

N.A.: Not applicable

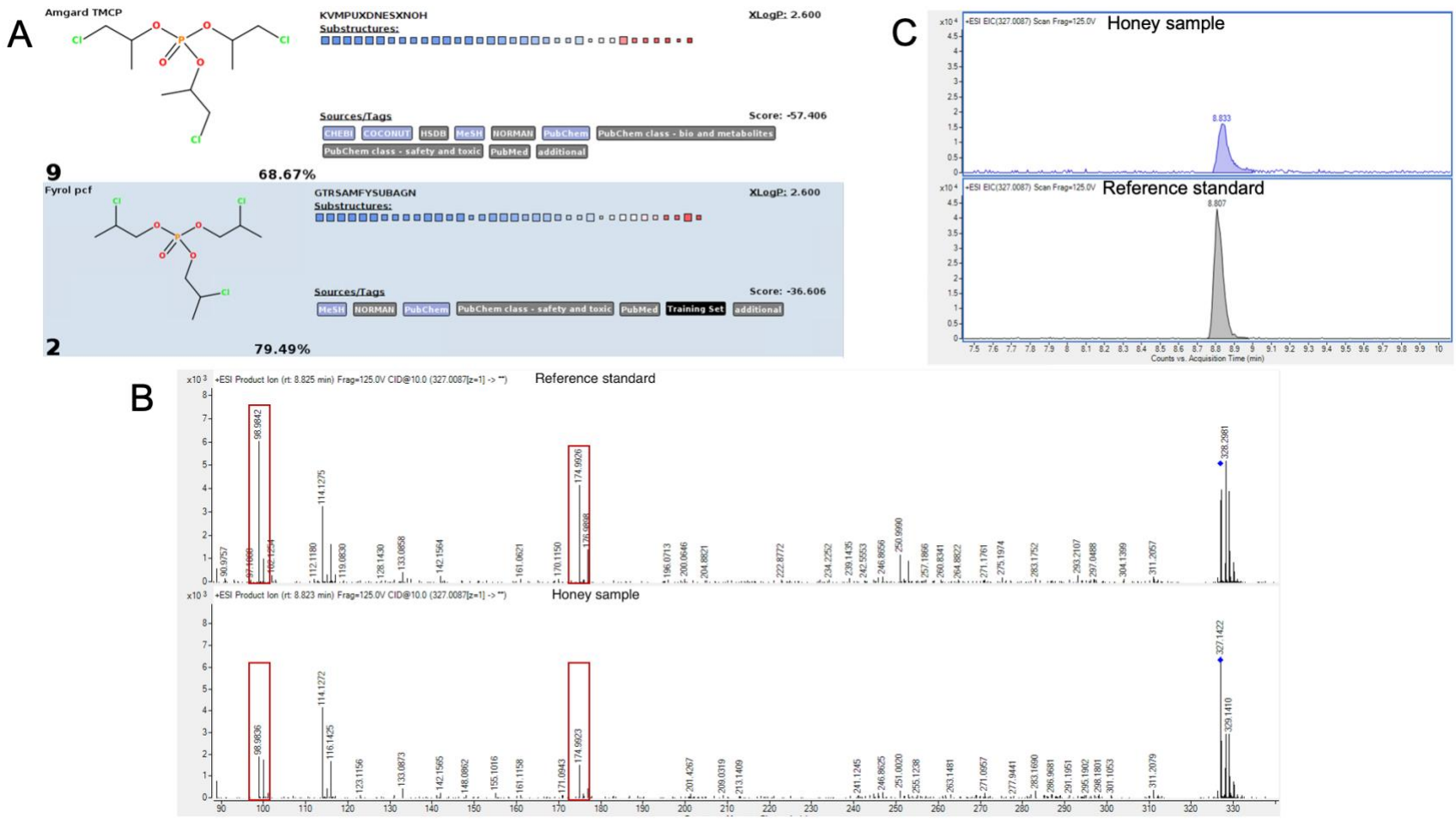

Figure 4.4 – (A) Structural identification of TCIPP with SIRIUS, with two candidates of the isomers of TCIPP. (B) Extracted ion chromatogram of TCIPP in honey sample and reference standard. (C) MS/MS spectrum of honey sample and reference standard at CE 10 V.

4.3.7 Non-targeted analysis for unique features in rural and urban honey

To examine the unique FR features present in the two groups of honey samples, a volcano plot was used to illustrate FR compounds that were statistically different in terms of the detected abundance. From the study, the compounds detected from the targeted feature extraction were filtered to remove compounds that were abundantly present in the blank samples (by 4 times). Among the 32 filtered compounds, 10 were common between the two sample groups, while 2 were unique to rural honey, and 20 were unique to urban honey samples (Figure S4.5). These compounds were used for a volcano plot to look for compounds with the most difference between the two groups in terms of abundance, as shown in Figure S4.6. On the left of the plot, compounds in blue (with negative values in the $\log_2$(fold change)) had a higher abundance in rural honey than that of urban honey samples, whereas compounds in red (with positive values in the $\log_2$(fold change)) had a higher abundance in urban honey than that of rural honey samples.

The plot showed that there were only two compounds that were statistically higher in rural honey samples (compounds listed in Table S4.6), which were 2-ethylhexyl diphenyl phosphate (EHDPP) and butyl diphenyl phosphate. In comparison, 12 compounds had a higher statistical abundance in the urban honey samples, but there are three groups of isomers (tri-cresyl phosphates, di-cresyl phosphates and isopropylphenyl diphenyl phosphates) as shown in Table S4.6. The other compounds were bis(methylphenyl) phenyl phosphate, N,N'-ethylenebis(3,4,5,6-tetrabromophthalimide) and tris(tribromoneopentyl) phosphate. Apart from the isomers, EHDPP was the only FR that has been reported from the literature that was detected from the food [16], aside from being FR, it is also used in the food industry as an adhesive, coating component and plasticizers in packaging materials, which was listed as an indirect food additive by the U.S. Food

and Drug Administration (FDA) [34]. The other compounds listed in Table S4.6 were first detected in food commodities in this current study.

While these unique features identified from the rural and urban honey samples have not been confirmed with the non-targeted workflow, this highlights that there are more compounds present in the honey samples. This also justifies the application of a non-targeted workflow for detecting and identifying chemical contaminants, as there could be unknown compounds present in the samples.

4.4 Conclusions

This was the first study to apply a direct injection method to study the occurrence of novel flame retardants in urban (n = 100) and rural (n = 100) honey samples collected from Quebec, Canada, with LC-QTOF. To summarize, the overall flame-retardant profile was compared between the two honey sample groups, and no significant difference was observed. Four target OPE compounds were detected, namely TBOEP, TBP, TDCIPP and TPHP. According to the MS/MS analysis, only TBOEP and TPHP were concluded as correct identifications, and this was verified by using *SIRIUS* to identify compounds with the provided MS/MS information. Based on this identification, the workflow was challenged by a spiking test to determine the LOI, in which the LOI of TBOEP was below 1000 ng g^{-1} and that of TPHP was below 50 ng g^{-1}.

In addition to the targeted approach, another novelty of this study was to apply a non-targeted approach to screen for the presence of other novel flame-retardant compounds, against an in-house built screening library. TCIPP was identified with a level 1 identification confidence. Since the samples were analyzed with a high-resolution MS, their accurate mass and isotopic distribution were also recorded, this could also be useful to screen for other groups of contaminants, with their respective screening library.

Overall, this study was the first study to demonstrate that the direct injection method coupled with LC-QTOF analysis was an effective method to detect and identify flame-retardant compounds at trace levels in a honey matrix (<100 ng g^{-1}), based on this non-targeted workflow. Since other chemical contaminants have been reported from honey samples, this non-targeted workflow could also be useful to screen for other families of contaminants in honey. This study also offered a novel angle to determine the occurrence of flame retardants in urban air, with honey as a bio-indicator.

For future studies, the sources of these flame-retardant contaminants from air samples could be investigated, more specifically, are these OPEs originated from the attachment to flowers or the honeybees from the air directly. Moreover, since honey is commonly used in cooking or hot beverage, the degradation of these OPEs detected under high temperatures could be studied to have a better understanding of the actual dietary intake of OPEs after cooking.

4.5 Acknowledgement

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Supplementary information

Table S4.1 – Supplier of internal standards used for extraction.

Compound	Type	m/z	Supplier
ESI+			
Tributyl phosphate-d27 (TBP-d27)	Surrogate standard	294.3414	Toronto Research Chemicals
Tris(2-ethylhexyl) phosphate-d51 (TEHP-d51)	Surrogate standard	486.6804	Toronto Research Chemicals
ESI–			
Bis(2-ethylhexyl) phosphate-d34 (BEHP-d34)	Surrogate standard	355.4329	Toronto Research Chemicals
Diphenyl phosphate-d10 (DPHP-d10)	Surrogate standard	259.0944	Toronto Research Chemicals
Tetrabromobiphsnol A-d4 (TBBPA-d4)	Surrogate standard	546.7709	CDN Isotopes

Table S4.2 – Original of urban and rural honey samples.

Origin of urban honey samples	Sub-total
Montreal-East	17
Montreal-Centre-South	21
Montreal-Centre-west	20
Montreal-West	18
Montreal-North	20
Laval	4
Total number of samples	100
Origin of rural honey samples	**Sub-total**
Saguenay-Lac-St-Jean	5
Capitale-Nationale	9
Estrie	8
Outaouais	11
Abitibi-Téminscamingue	3
Gaspésie-Îles-de-la-Madeleine	3
Chaudière-Appalaches	5
Lanaudière	8
Laurentides	3
Laurentides (Northwest of Montreal)	2
Laurentides (West of Montreal)	12
Montérégie (East of Montreal)	11
Montérégie (South of Montreal)	3
Montérégie (Southwest of Montreal)	4
Montérégie (West of Monteal)	3
Centre-du-Québec	10
Total number of samples	100

Table S4.3 – In-house-built flame retardant screening library, including the name, chemical formulae, mass, CAS number, and chemical group for each compound.

	Compound Name	Formula	Mass	CAS	Chemical Group
1.	αHBCDD α-Hexabromocyclododecane	C12H18Br6	635.65088	134237-50-6	HBCDD
2.	βHBCDD β-Hexabromocyclododecane	C12H18Br6	635.65088	134237-51-7	HBCDD
3.	γHBCDD γ-Hexabromocyclododecane	C12H18Br6	635.65088	134237-52-8	HBCDD
4.	1,1,2,2,3,3-Hexabromocyclododecane	C12H18Br6	635.65088	25637-99-4	HBCDD
5.	δHBCDD δ-Hexabromocyclododecane	C12H18Br6	635.65088	3194-55-6	HBCDD
6.	4-Bromophenol	C6H5BrO	171.95238	106-41-2	NBFR
7.	3-Bromostyrene	C8H7Br	181.97311	2039-86-3	NBFR
8.	2,4-Dibromophenol	C6H4Br2O	249.86289	615-58-7	NBFR
9.	2,6-Dibromophenol	C6H4Br2O	249.86289	608-33-3	NBFR
10.	2,4-Dibromostyrene	C8H6Br2	259.88363	24162-63-8	NBFR
11.	3,4-Dibromostyrene	C8H6Br2	259.88363	24162-64-9	NBFR
12.	3,5-Dibromostyrene	C8H6Br2	259.88363	120359-56-0	NBFR
13.	(2,2-Dibromovinyl)benzene	C8H6Br2	259.88363	31780-26-4	NBFR
14.	2,2-Bis(bromomethyl)propane-1,3-diol	C5H10Br2O2	259.90476	3296-90-0	NBFR
15.	3-Bromo-2,2-bis(bromomethyl)propanol	C5H9Br3O	321.82035	1522-92-5	NBFR
16.	2,4,6-Tribromophenol	C6H3Br3O	327.7734	118-79-6	NBFR

17.	Allyl 2,4,6-tribromophenyl ether	C9H7Br3O	367.8047	3278-89-5	NBFR
18.	Tetrabromophenol	C6H2Br4O	405.68392	14400-94-3	NBFR
19.	1-(2,3-Dibromopropyl)-3,5-diallyl-1,3,5- Triazine-2,4,6(1H,3H,5H)-trione	C12H15Br2N3O3	406.94802	57829-89-7	NBFR
20.	2,3,5,6-Tetrabromo-p-xylene	C8H6Br4	417.7203	23488-38-2	NBFR
21.	Tribromotrichlorocyclohexane	C6H6Br3Cl3	419.70852	30554-73-5	NBFR
22.	1,2-Dibromo-4-(1,2-dibromoethyl) cyclohexane	C8H12Br4	423.76725	3322-93-8	NBFR
23.	1,2,5,6-Tetrabromocyclooctane	C8H12Br4	423.76725	3194-57-8	NBFR
24.	2,3,4,5-Tetrabromobenzoic acid	C7H2Br4O2	433.67883	27581-13-1	NBFR
25.	2,3,4,5-Tetrabromo-6-chlorotoluene	C7H3Br4Cl	437.66568	39569-21-6	NBFR
26.	2,3,4,5-Tetrabromo-6-chlorotoluene	C7H3Br4Cl	437.66568	39569-21-6	NBFR
27.	2-Bromoallyl(2,4,6-tribromophenyl) ether	C9H6Br4O	445.71522	99717-56-3	NBFR
28.	Tetrabromophthalic anhydride	C8Br4O3	459.6581	632-79-1	NBFR
29.	Pentabromobenzene	C6HBr5	467.59951	608-90-2	NBFR
30.	Tetrabromobenzene-1,2-dicarboxylic acid	C8H2Br4O4	477.66866	13810-83-8	NBFR
31.	2,3,4,5,6-Pentabromotoluene	C7H3Br5	481.61516	87-83-2	NBFR
32.	Pentabromophenol	C6HBr5O	483.59443	608-71-9	NBFR
33.	2,3,4,5,6-Pentabromoethylbenzene	C8H5Br5	495.63081	85-22-3	NBFR
34.	1,2,3,4,5-Pentabromo-6-chlorocyclohexane	C6H6Br5Cl	507.60749	87-84-3	NBFR

35.	Allyl pentabromophenyl ether	C9H5Br5O	523.62573	3555-11-01	NBFR
36.	1,3,5-Tribromo-2-(2,3-dibromopropoxy)benzene	C9H7Br5O	525.64138	35109-60-5	NBFR
37.	Pentabromobenzoyl chloride	C7Br5ClO	529.55546	59646-51-4	NBFR
38.	7,8-Dibromo-1,2,3,4,11,11-hexachloro-1,4,4a,5,6,7,8,9,10,10a-decahydro-1,4-methanobenzocyclooctene	C13H12Br2Cl6	535.74369	51936-55-1	NBFR
39.	TBBPA Tetrabromobisphenol A	C15H12Br4O2	539.75708	79-94-7	NBFR
40.	Hexabromobenzene	C6Br6	545.51003	87-82-1	NBFR
41.	2-Ethylhexyl 2,3,4,5-Tetrabromobenzoate	C15H18Br4O2	545.80403	183658-27-7	NBFR
42.	(Pentabromophenyl)methyl acrylate	C10H5Br5O2	551.62064	59447-55-1	NBFR
43.	2,3,4,5,6,a-Hexabromotoluene	C7H2Br6	559.52568	38521-51-6	NBFR
44.	4,4'-Sulphonylbis(2,6-dibromophenol)	C12H6Br4O4S	561.67203	39635-79-5	NBFR
45.	Tetrabromobisphenol A bismethyl ether	C17H16Br4O2	567.78838	37853-61-5	NBFR
46.	Tetrabromobisphenol S Dimethyl Ether	C14H10Br4O4S	589.70333	70156-79-5	NBFR
47.	Hexabromocyclodecane	C10H14Br6	607.61958	25495-98-1	NBFR
48.	Tetrabromobisphenol A diallyl ether	C21H20Br4O2	619.81968	25327-89-3	NBFR
49.	2-(2-Hydroxyethoxy)ethyl 2-hydroxypropyl 3,4,5,6-tetrabromophthalate	C15H16Br4O7	623.76295	20566-35-2	NBFR

50.	4,4'-Isopropylidenebis(2,6-dibromophenyl) diacetate	C19H16Br4O4	623.77821	33798-02-6	NBFR
51.	4,4'-Isopropylidenebis[2-(2,6-dibromophenoxy)ethanol]	C19H20Br4O4	627.80951	4162-45-2	NBFR
52.	Tetrabromobisphenol A diacrylate	C21H16Br4O4	647.77821	55205-38-4	NBFR
53.	Tetrabromobisphenol A diglycidyl ether	C21H20Br4O4	651.80951	3072-84-2	NBFR
54.	Tebrabromobisphenol A bispropanoate	C21H20Br4O4	651.80951	37419-42-4	NBFR
55.	1,2-Bis(2,4,6-tribromophenoxy)ethane	C14H8Br6O2	681.56246	37853-59-1	NBFR
56.	1,2,3,4,7,7-Hexachloro-5-(tetrabromophenyl) bicyclo [2.2.1]hept-2-ene	C13H4Br4Cl6	685.51777	34571-16-9	NBFR
57.	Tris(2,3-dibromopropyl) phosphate	C9H15Br6O4P	691.58082	126-72-7	NBFR
58.	Bis(2-ethylhexyl) tetrabromophthalate	C24H34Br4O4	701.91906	26040-51-7	NBFR
59.	1,3,5-Tris (2,3-dibromopropyl)-1,3,5-triazine2,4,6(1H,3H,5H)-trione	C12H15Br6N3O3	722.62137	52434-90-9	NBFR
60.	(1-Methylethylidene)bis [(2,6-dibromo-4,1-phenylene)oxy-2,1-ethanediyl] diacrylate	C25H24Br4O6	735.83064	66710-97-2	NBFR
61.	3-(Tetrabromopentadecyl)-2,4,6- tribromophenol	C21H29Br7O	849.65021	168434-45-5	NBFR
62.	Octabromotrimethylphenyl indane	C18H12Br8	859.4406	1084889-51-9	NBFR
63.	2,2,4,5,6,7-Hexabromo-1-(2,3-dibromophenyl)-1,3,3-trimethylindane	C18H12Br8	859.4406	155613-93-7	NBFR

64.	2,2-Bis[3,5-dibromo-4-(2,3-dibromopropoxy)phenyl]propane	C21H20Br8O2	935.49303	21850-44-2	NBFR
65.	N,N'-Ethylenebis (3,4,5,6- tetrabromophthalimide)	C18H4Br8N2O4	943.36381	32588-76-4	NBFR
66.	Bis [3,5-dibromo-4-(2,3-dibromopropoxy) phenyl] sulphone	C18H14Br8O4S	957.40798	42757-55-1	NBFR
67.	1,1'-(Ethane-1,2-diyl)bis [pentabromobenzene]	C14H4Br10	961.21468	84852-53-9	NBFR
68.	Decabromodibenzyl ether	C14H4Br10O	977.20959	497107-13-8	NBFR
69.	1,1'-[Ethane-1,2-diylbisoxy]bis[pentabromobenzene]	C14H4Br10O2	993.20451	61262-53-1	NBFR
70.	Tris(tribromoneopentyl) phosphate	C15H24Br9O4P	1009.40626	19186-97-1	NBFR
71.	2,4,6-Tris(2,4,6-tribromophenoxy)-1,3,5-triazine	C21H6Br9N3O3	1058.30596	25713-60-4	NBFR
72.	Tetradecabromo-1,4-diphenoxybenzene	C18Br14O2	1352.84656	58965-66-5	NBFR
73.	Bis(pentabromobenzyl) tetrabromophthalate	C22H4Br14O4	1436.86769	82001-21-6	NBFR
74.	1,3,5-Triazine-2,4,6(1H,3H,5H)-trione,1,3-bis(2,3-dibromopropyl)-5-(2-propenyl)-	C12H15Br4N3O3	564.78469	75795-16-3	NBFR (bromide phthlate)
75.	Bis(pentabromobenzyl) terephthalate	C22H10Br10O4	1127.24129	90075-91-5	NBFR (bromide phthlate)
76.	Trimethyl phosphate	C3H9O4P	140.02385	512-56-1	OPE
77.	Triethyl phosphate	C6H15O4P	182.0708	78-40-0	OPE
78.	DBP Dibutyl phosphate	C8H19O4P	210.1021	107-66-4	OPE

79.	BCEP Bis(2-chloroethyl) phosphate	C4H9Cl2O4P	221.96155	3040-56-0	OPE
80.	TPrP Tripropyl phosphate	C9H21O4P	224.11775	513-08-6	OPE
81.	Triisopropyl phosphate	C9H21O4P	224.11775	513-02-0	OPE
82.	TCIPP Tris(1-chloro-2-propyl) phosphate	C9H18Cl3O4P	326.00083	13674-84-5	OPE
83.	BCIPP Bis-(1-chloro-2-propyl) phosphate	C6H13Cl2O4P	249.99285	789440-10-4	OPE
84.	DPHP Diphenyl phosphate	C12H11O4P	250.0395	838-85-7	OPE
85.	TBP Tributyl phosphate	C12H27O4P	266.1647	126-73-8	OPE
86.	TIBP Tri-iso-butyl phosphate	C12H27O4P	266.1647	126-71-6	OPE
87.	DoCP Dio-tolyl-phosphate	C14H15O4P	278.0708	35787-74-7	OPE
88.	DmCP Di-m-cresyl phosphate	C14H15O4P	278.0708	36400-46-1	OPE
89.	DpCP Di-p-cresyl phosphate	C14H15O4P	278.0708	843-24-3	OPE
90.	Monoisotridecyl phosphate	C13H29O4P	280.18035	50977-11-2	OPE
91.	TCEP Tris(2-chloroethyl) phosphate	C6H12Cl3O4P	283.95388	115-96-8	OPE
92.	Dibutyl phenyl phosphate	C14H23O4P	286.1334	2528-36-1	OPE
93.	o-ip-PPP o-Isopropylphenyl phenyl phosphate	C15H17O4P	292.08645		OPE
94.	m-ip-PPP m-Isopropylphenyl phenyl phosphate	C15H17O4P	292.08645		OPE
95.	p-ip-PPP p-Isopropylphenyl phenyl phosphate	C15H17O4P	292.08645	69415-02-7	OPE
96.	BBOEP Bis(2-butoxyethyl) phosphate	C12H27O6P	298.15453	14260-97-0	OPE
97.	Butyl diphenyl phosphate	C16H19O4P	306.1021	2752-95-6	OPE

98.	Tripentyl phosphate	C15H33O4P	308.21165	2528-38-3	OPE
99.	BDCIPP Bis(1,3-dichloro-2-propyl)phosphate	C6H11Cl4O4P	317.91491	72236-72-7	OPE
100.	BEHP Bis(2-ethylhexyl) phosphate	C16H35O4P	322.2273	298-07-7	OPE
101.	Tris(3-chloropropyl)phosphate	C9H18Cl3O4P	326.00083	1067-98-7	OPE
102.	Tris(2-chloropropyl) phosphate	C9H18Cl3O4P	326.00083	6145-73-9	OPE
103.	Tris(1-chloropropyl) phosphate	C9H18Cl3O4P	326.00083		OPE
104.	Tris(1-chloropropyl) phosphate	C9H18Cl3O4P	326.00083		OPE
105.	TPHP Triphenyl phosphate	C18H15O4P	326.0708	115-86-6	OPE
106.	Diphenyl p-tolyl phosphate	C19H17O4P	340.08645	78-31-9	OPE
107.	Cresyl diphenyl phosphate	C19H17O4P	340.08645	26444-49-5	OPE
108.	BTBOEP 2-Hydroxyethyl bis(2-butoxyethyl) phosphate	C14H31O7P	342.18074	1477494-86-2	OPE
109.	Bis(3,5,5-trimethylhexyl) hydrogen phosphate	C18H39O4P	350.2586	7153-98-2	OPE
110.	Bis(methylphenyl) phenyl phosphate	C20H19O4P	354.1021	26446-73-1	OPE
111.	(4-Tridecylphenyl) dihydrogen phosphate	C19H33O4P	356.21165		OPE
112.	2-Ethylhexyl diphenyl phosphate	C20H27O4P	362.1647	1241-94-7	OPE
113.	TOCP Tri-o-cresyl phosphate	C21H21O4P	368.11775	78-30-8	OPE
114.	TMCP Tri-m-cresyl phosphate	C21H21O4P	368.11775	563-04-2	OPE
115.	TPCP Tri-p-cresyl phosphate	C21H21O4P	368.11775	78-32-0	OPE

116.	2-ip-PDPP 2-Isopropylphenyl diphenyl phosphate	C21H21O4P	368.11775	64532-94-1	OPE
117.	4-ip-PDPP 4-Isopropylphenyl diphenyl phosphate	C21H21O4P	368.11775	55864-04-5	OPE
118.	3-ip-PDPP 3-Isopropylphenyl diphenyl phosphate	C21H21O4P	368.11775	69515-46-4	OPE
119.	tert-Butylphenyl Diphenyl Phosphate	C22H23O4P	382.1334	56803-37-3	OPE
120.	Isodecyl diphenyl phosphate	C22H31O4P	390.196	29761-21-5	OPE
121.	TBOEP Tris(2-butoxyethyl) phosphate	C18H39O7P	398.24334	78-51-3	OPE
122.	Tris(3,5-dimethylphenyl) phosphate	C24H27O4P	410.1647	25653-16-1	OPE
123.	Tris(3,4-dimethylphenyl) phosphate	C24H27O4P	410.1647	3862-11-01	OPE
124.	Trixylyl Phosphate	C24H27O4P	410.1647	25155-23-1	OPE
125.	TDCIPP Tris(1,3-dichloro-2-propyl)phosphate	C9H15Cl6O4P	427.88391	13674-87-8	OPE
126.	Tris(2,3-dichloropropyl) phosphate	C9H15Cl6O4P	427.88391	78-43-3	OPE
127.	Tris(1,3-dichloropropyl) phosphate	C9H15Cl6O4P	427.88391	40120-74-9	OPE
128.	TEHP Tris(2-ethylhexyl) phosphate	C24H51O4P	434.3525	78-42-2	OPE
129.	Tris(isopropylphenyl)phosphate	C27H33O4P	452.21165	64532-95-2	OPE
130.	Isopropylated triphenyl phosphate	C27H33O4P	452.21165	68937-41-7	OPE
131.	Bis[4-(2,2,4-trimethylpentan-3-yl)phenyl] hydrogen phosphate	C28H43O4P	474.2899		OPE
132.	Bis[4-(2,4,4-trimethylpentan-2-yl)phenyl] hydrogen phosphate	C28H43O4P	474.2899	1758-45-8	OPE

133.	Hexadecyl diphenyl phosphate	C28H43O4P	474.2899	56827-92-0	OPE
134.	Tris(4-tert-butylphenyl) phosphate	C30H39O4P	494.2586	78-33-1	OPE
135.	2,2-Bis(bromomethyl)-3-chloropropyl bis(2-chloro-1-(chloromethyl)ethyl) phosphate	C11H18Br2Cl5O4P	577.77521	66108-37-0	OPE
136.	Phosphoric acid, 2,2-bis(chloromethyl)-1,3-propanediyl tetrakis(2-chloroethyl) ester	C13H24Cl6O8P2	579.90776	38051-10-4	OPE
137.	Melamine	C3H6N6	126.06539	108-78-1	Others
138.	Tetrachlorophthalic anhydride	C8Cl4O3	283.86015	117-08-8	Others
139.	Chlorendic anhydride	C9H2Cl6O3	367.81351	115-27-5	Others
140.	Chlorendic acid	C9H4Cl6O4	385.82407	115-28-6	Others
141.	1,2,3,4,5-Pentabromo-6-(chloromethyl)benzene	C7H2Br5Cl	515.57619	58495-09-3	Others

Table S4.4 – Retention time, the precision of retention time and mass accuracy of internal standards.

Internal standards	Retention time (mins)	Range of retention time	RSD% of retention time	Range of mass error (ppm)	Average mass error (ppm)
Tributyl phosphate-d27	9.635	(9.628, 9.64)	0.02	(0.15, 4.6)	2.43
Tris(2-ethylhexyl) phosphate-d51	12.363	(12.35, 12.378)	0.04	(–3.4, 2.63)	0.14
Bis(2-ethylhexyl) phosphate-d34	9.755	(9.747, 9.765)	0.03	(9.06, 15.04)	12.5
Diphenyl phosphate-d10	7.38	(7.367, 3.395)	0.005	(–1.06, 11.48)	9.94
Tetrabromobisphenol A-d4	9.695	(9.686, 9.704)	0.03	(–3.68, 15.39)	13.1

Table S4.5 – Method detection limit for TBOEP and TPHP at three levels of concentrations.

Compounds	High level		Medium level		Low level	
	Detected	Identified	Detected	Identified	Detected	Identified
TBOEP	✓	✓	✓	✓	✓	✓
TPHP	✓	✓	✓	✓	✓	✓

Table S4.6 – List of compounds with statistically higher abundance in (1) rural, and (2) urban honey samples.

(1) Compounds with abundance statistically higher in rural honey samples	(2) Compounds with abundance statistically higher in urban honey samples
2-Ethylhexyl diphenyl phosphate (EHDPP)	Tri-m-cresyl phosphate (TMCP)
Butyl diphenyl phosphate	Tri-o-cresyl phosphate (TOCP)
	Tri-p-cresyl phosphate (TPCP)
	Di-m-cresyl phosphate (DmCP)
	Dio-tolyl-phosphate (DoCP)
	Di-p-cresyl phosphate (DpCP)
	2-Isopropylphenyl diphenyl phosphate (2-ip-PDPP)
	3-Isopropylphenyl diphenyl phosphate (3-ip-PDPP)
	4-Isopropylphenyl diphenyl phosphate (4-ip-PDPP)
	Bis(methylphenyl) phenyl phosphate
	N,N'-Ethylenebis (3,4,5,6-tetrabromophthalimide)
	Tris(tribromoneopentyl) phosphate

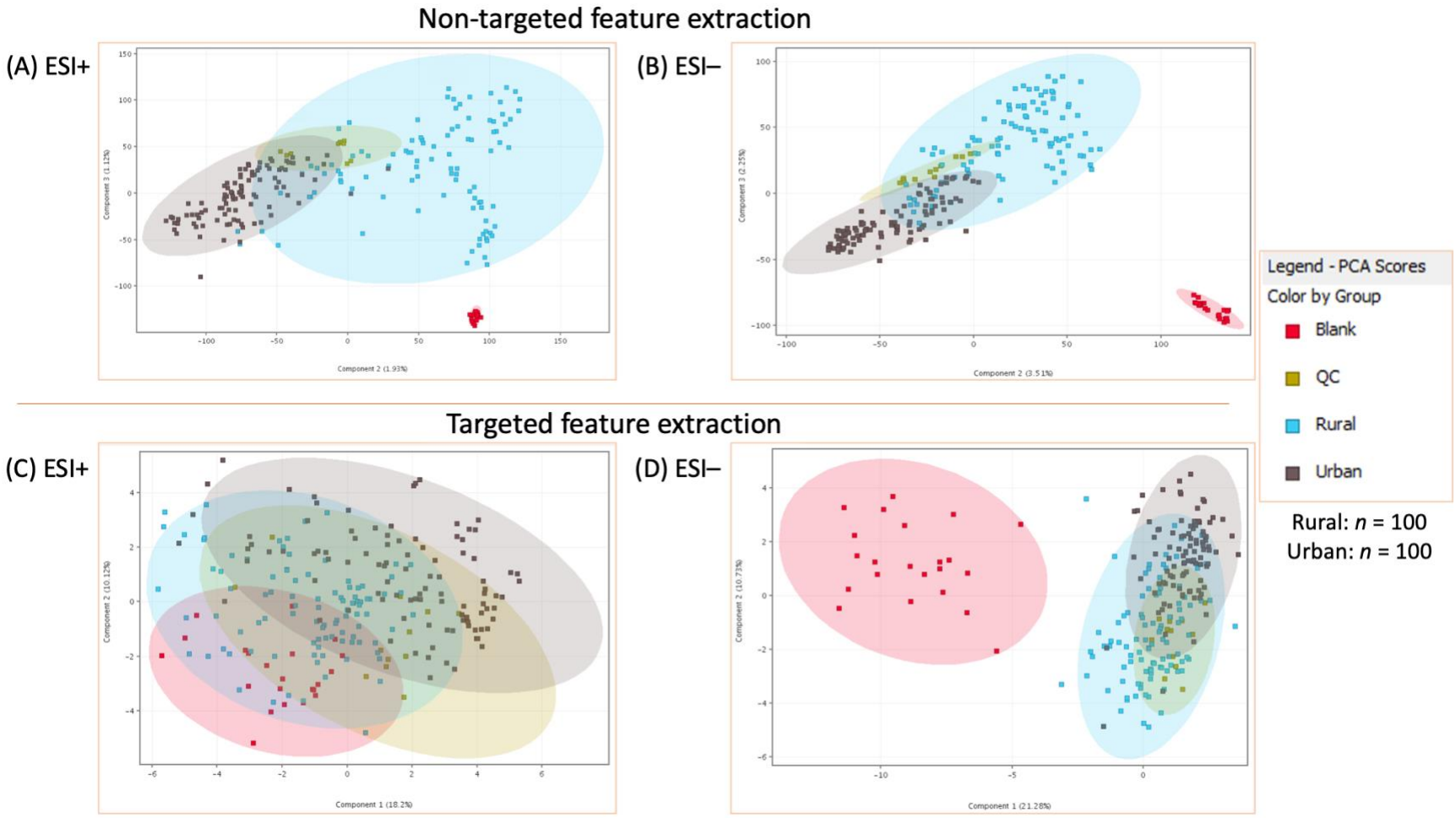

Figure S4.1 – PCA plots for different feature extraction algorithms and ESI modes.(A) plot for ESI+ in 'Batch recursive feature extraction (small molecules)', (B) plot for ESI– in 'Batch recursive feature extraction (small molecules)', (C) plot for ESI+ in 'Batch targeted feature extraction', (D) plot for ESI– in 'Batch targeted feature extraction'.

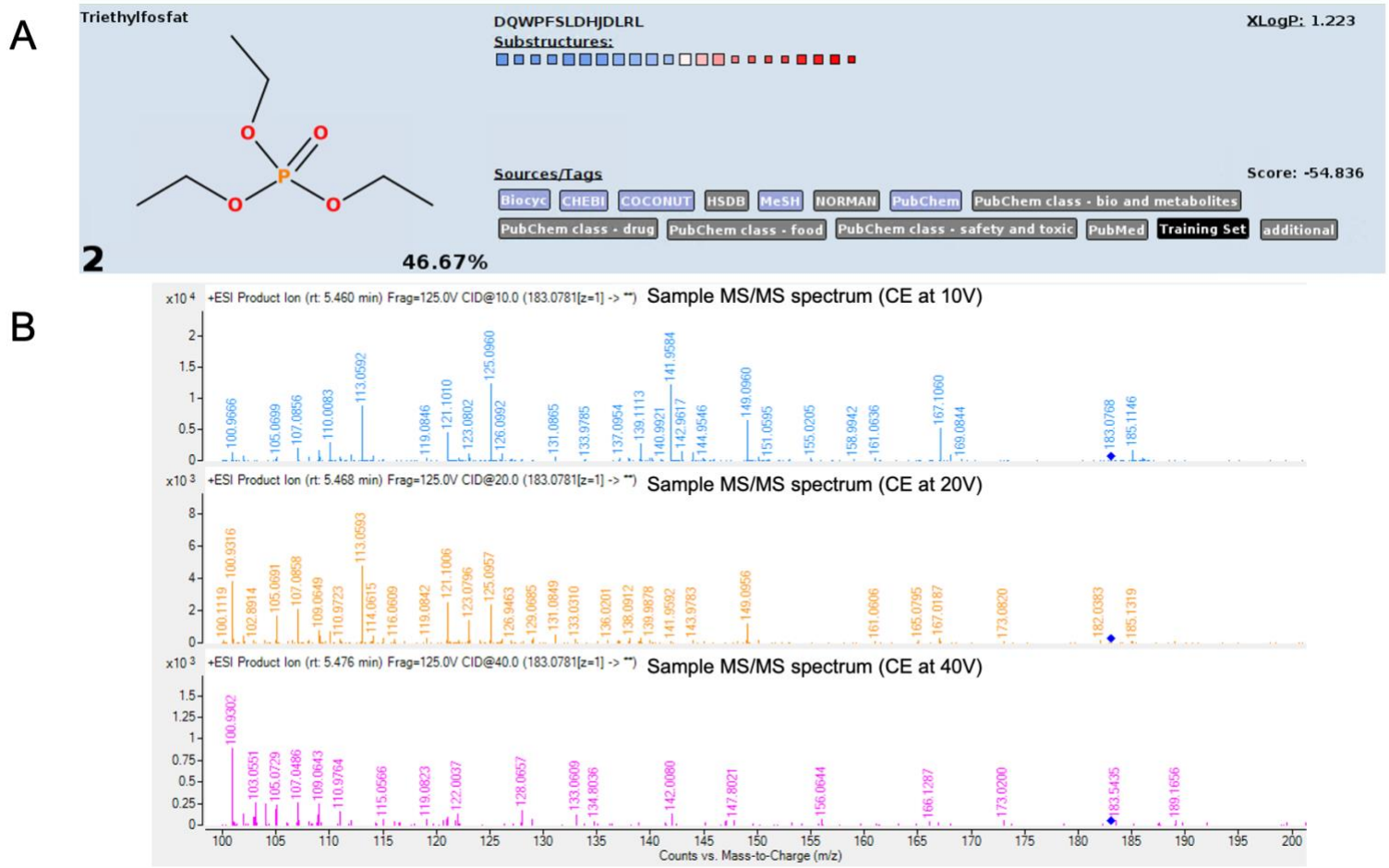

Figure S4.2 – Identification of triethyl phosphate (TEP). (A) Matching on SIRIUS. (B) MS/MS spectra of the honey sample at CE of 10, 20 and 40 V.

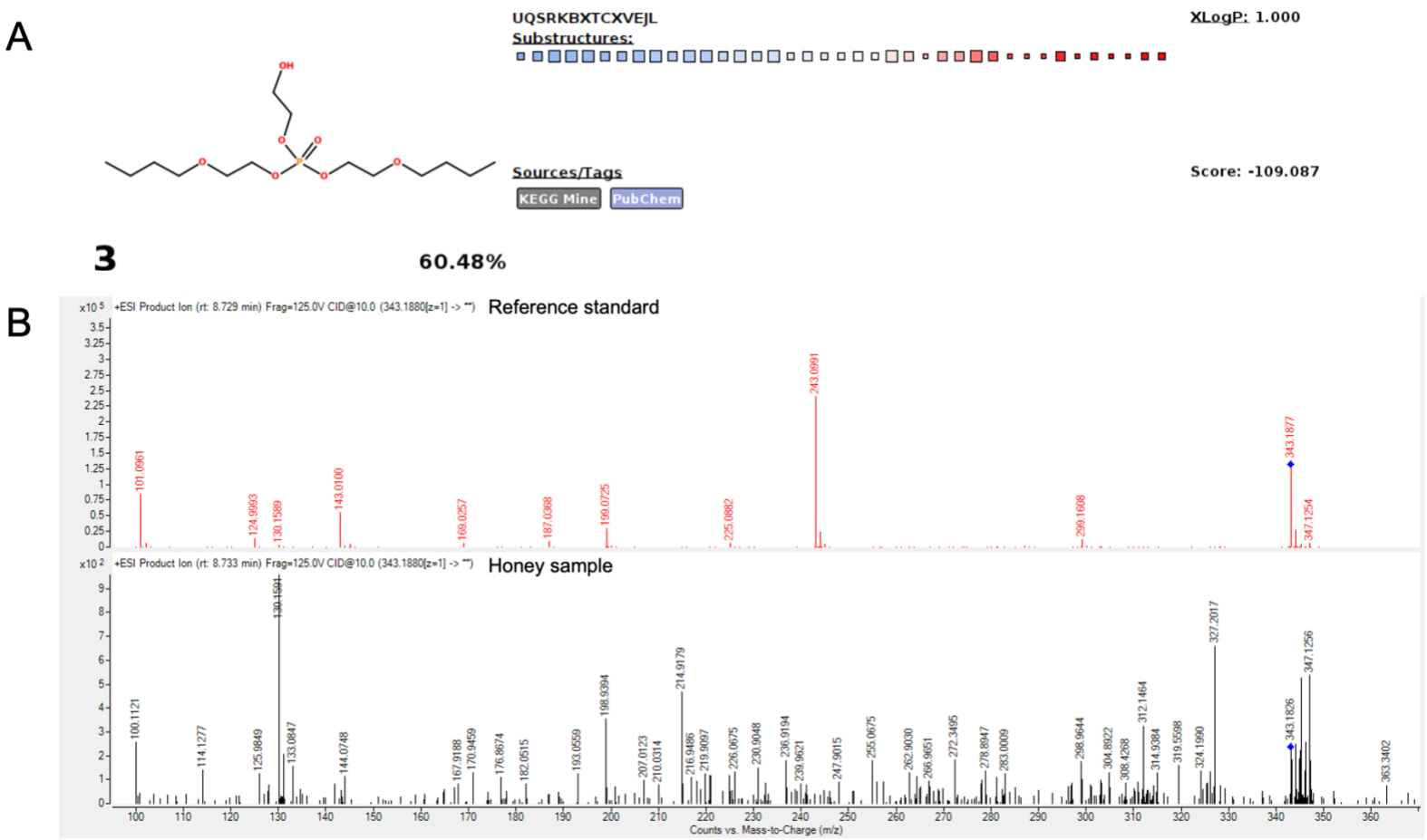

Figure S4.3 – Identification of 2-hydroxyethyl bis(2-butoxyethyl) phosphate (BTBOEP). (A) Matching on SIRIUS. (B) Comparison of MS/MS spectra of the honey sample and reference standard.

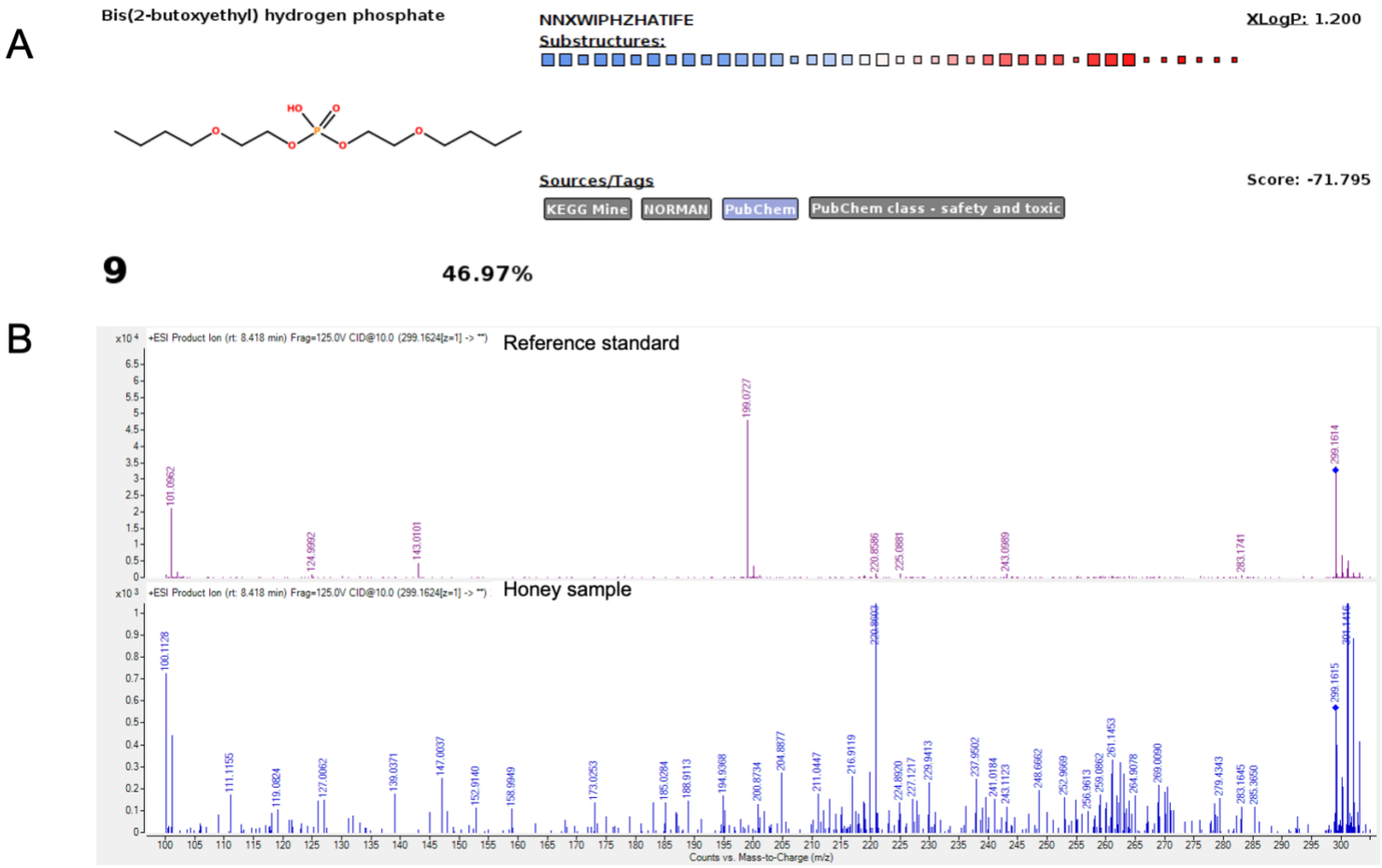

Figure S4.4 – Identification of bis(2-butoxyethyl) phosphate (BBOEP). (A) Matching on SIRIUS. (B) Comparison of MS/MS spectra of the honey sample and reference standard.

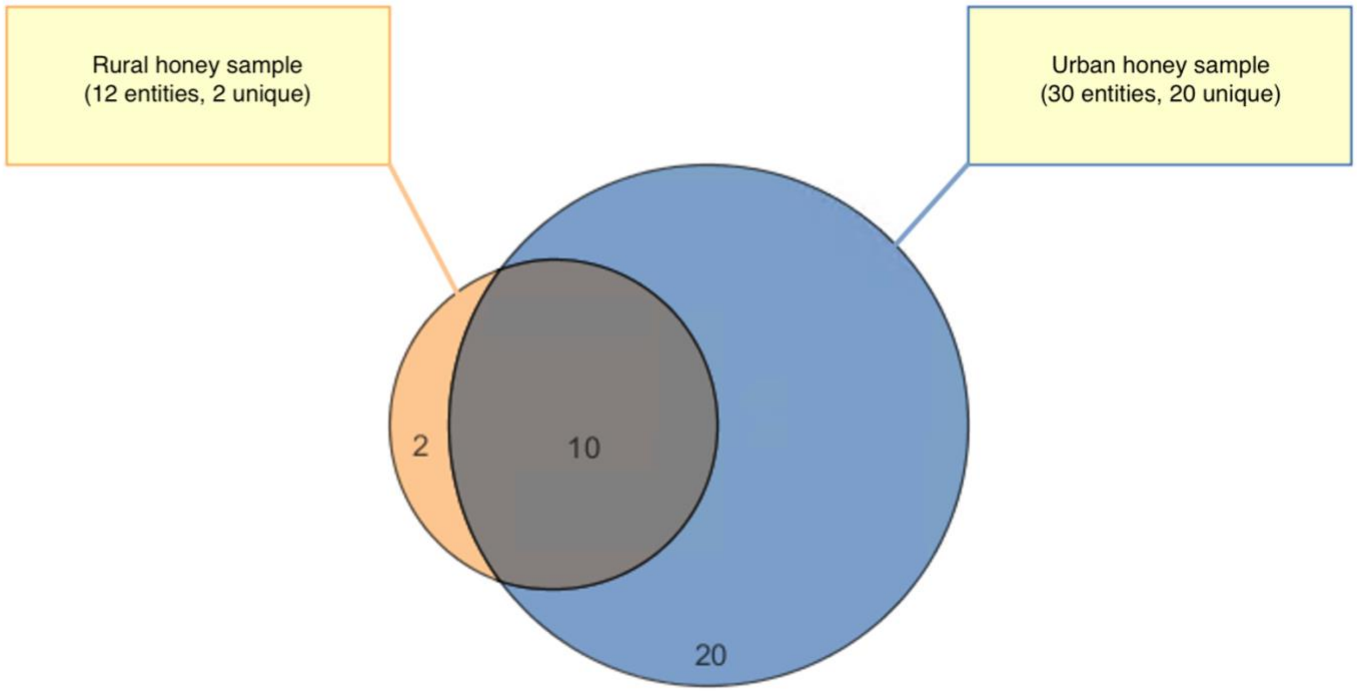

Figure S4.5 – Venn diagram showing entities unique to rural and urban honey samples after filtering.

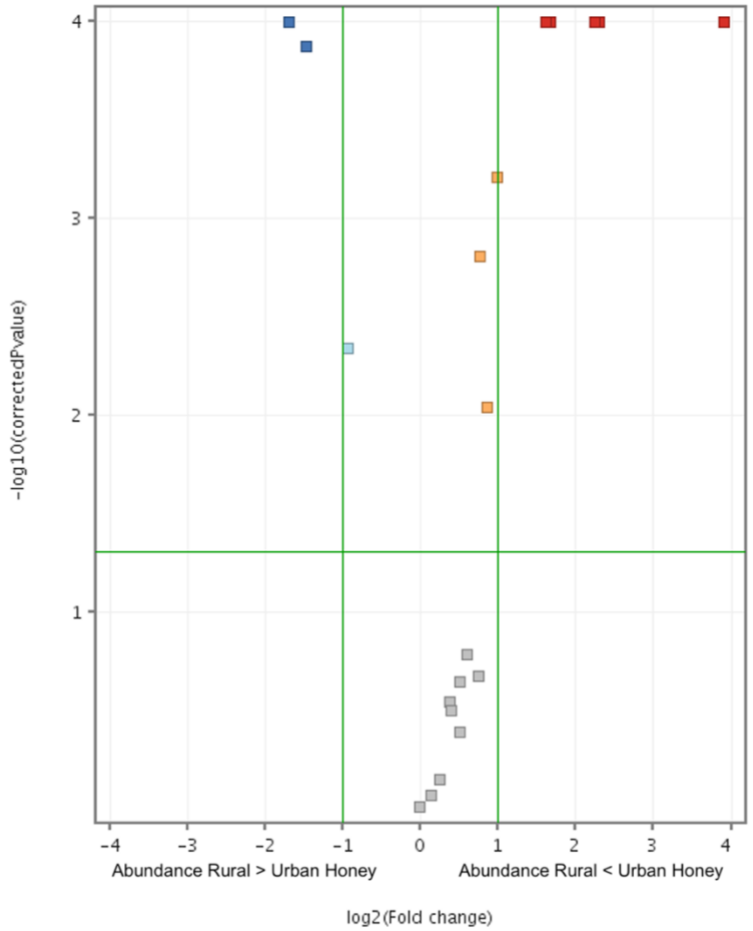

Figure S4.6 – Volcano plot comparing FR compounds that are statistically different from rural and urban honey samples.

Additional Supplementary Information

Table AS4.7 – Statistical analysis of matrix-matched calibration.

(A) TBOEP

Slope of matrix-matched calibration curves

Honey	Rural	Urban	Solvent
White	514060	396726	337206
Golden	390282	348073	315718
Amber	368024	340450	305296
Dark	167057	325529	299679

Two-way ANOVA

	Count	Sum	Average	Variance
Row 1	3	1247992	415997.333	8097872545
Row 2	3	1054073	351357.667	1398039300
Row 3	3	1013770	337923.333	988488529.3
Row 4	3	792265	264088.333	7228365361
Column 1	4	1439423	359855.75	20647595239
Column 2	4	1410778	352694.5	949341141.7
Column 3	4	1257899	314474.75	273806355.6

Source of Variation	*SS*	*df*	MS	*F*	*p*-value	F_{crit}
Rows	3.4949×10^{10}	3	1.165×10^{10}	2.27948991	0.1795311	4.757063
Columns	4761957270	2	2380978635	0.465890627	0.6485138	5.143251
Error	3.0664×10^{10}	6	5110595700			
Total	7.0374×10^{10}	11				

(B) TPHP

Slope of matrix-matched calibration curves

Honey	Rural	Urban	Solvent
White	966050	812604	849474
Golden	774176	749698	825314
Amber	728273	740690	774245
Dark	681149	741541	787796

Two-way ANOVA

	Count	Sum	Average	Variance
Row 1	3	2628128	876042.6667	6415839265
Row 2	3	2349188	783062.6667	1488674497
Row 3	3	2243208	747736	565590783
Row 4	3	2210486	736828.6667	2860050216
Column 1	4	3149648	787412	1.5625×10^{10}
Column 2	4	3044533	761133.25	1193928593
Column 3	4	3236829	809207.25	1187213034

Source of Variation	*SS*	*df*	MS	*F*	*p*-value	F_{crit}
Rows	3.5995×10^{10}	3	1199823624	3.99393387	0.0703021	4.75706266
Columns	4635620134	2	2317810067	0.77154508	0.5032756	5.14325285
Error	1.8025×10^{10}	6	3004114898			
Total	5.8655×10^{10}	11				

Connecting Paragraph

Chapter 4 concluded that there was contamination in a unique food matrix: honey. This case study provided a novel angle of the occurrence of FRs in atmospheric air and as a source of contaminants. It also developed a non-targeted screening workflow that was able to detect and identify unknown NFRs and OPEs present in the honey samples at trace levels. In Chapter 5, plastic food packaging materials as another source of FR contamination will be investigated, and the same workflow for non-targeted screening will be applied for samples. This chapter will also study the potential of OPEs migrating from thermal labels to food. This chapter will be submitted for publication in the *Journal of Agricultural and Food Chemistry*: Leung, G., Xu, Z., Liu, L., Goodyer, C. G., Hales, B. F., Bayen, S. An Investigation of Thermal Label and Other Plastic Food Packaging Materials as the Source of Exposure to Organophosphate Esters (OPEs) and Novel Flame Retardants in Food.

Chapter 5. An Investigation of Thermal Label and Other Plastic Food Packaging Materials as the Source of Exposure to Organophosphate Esters (OPEs) and Novel Flame Retardants in Food

Abstract

Some organophosphate esters (OPEs) are used as plasticizers in food packaging: they are often added as adhesives and are components in the polymers and coating on paper. In the current study, the objective was to analyze plastic food packaging materials for the occurrence of OPEs, and to investigate their migration from the packaging materials into food. In this study, a non-targeted screening workflow was applied to detect unknown flame retardants extracted from food packaging materials. A targeted study on TBOEP, TBP and TPHP in various plastic packaging materials used in fresh food, shown that the highest concentration was detected in thermal labels. The migration study demonstrated that these additives in food packaging could potentially lead to additional exposure to OPEs by humans via diet. This study, using both targeted and non-targeted approaches, provided a novel angle to assess the total flame-retardant exposure from food.

Keywords: plastic packaging materials, flame retardants, non-targeted screening, risk assessment, LC-MS

5.1 Introduction

Food packaging can provide useful information on the product, ingredients, and nutritional value. More importantly, it is essential to preserve the quality and ensure the safety of the food, by protecting food against physical, chemical, and microbiological hazards [1, 2]. Besides contaminants from the environment, these food packaging could also be a source of chemical contamination when they come in direct contact with food, this is also known as chemical migration [3]. Chemical migration refers to the phenomenon where a mass transfer of chemical compounds (migrants) occurs by molecular diffusion, from a region of high concentration to a low concentration. This process is usually caused by direct contact and interaction between the packaging material and the food [3, 4]. Plastic-related chemicals, such as plasticizers or monomers, have been reported in the literature that they can migrate from plastic food packaging to food [5-7]. This has led to food safety concerns as some of them are toxicants, such as bisphenol A being an endocrine disruptor [8], and an increased amount of studies done regarding the migration of these additives [9].

Organophosphate esters (OPEs) are a type of novel flame retardants (FRs), but they are also applied in food packaging materials [10]. For example, tris(2-butoxyethyl) phosphate (TBOEP), triphenyl phosphate (TPHP), and tris(2-ethylhexyl) phosphate (TEHP) are often added as plasticizers, adhesives, and components in coating and polymers [11, 12]. It has been suggested that OPEs involved in post-harvest food processing could be a source of contamination in food, more specifically, it was found that processed food or food in contact with plastic packaging has been found to have a higher OPE level [13]. Similar to plastic additives, these chemicals have been reported to be able to potentially migrate into food from the packaging materials, leading to additional exposure from the diet [14]. Exposure to OPEs is associated with endocrine disruption,

reproductive toxicity, and neurotoxicity [15]. Therefore, it is important to determine the OPE and FR profile from the packaging materials, in order to understand the magnitude and source of contamination in the food [10].

From the current studies, measurement of FR levels in food packaging materials have been conducted in China [16-19], Japan [11] and the United States [10]. The OPE levels were determined by extracting the flame retardants from food packaging materials with exhaustive methods. In terms of the sampling, most of the studies focused on processed food, or determined the occurrence without specifying the types of packaging material. The migration studies were also done with food simulant, instead of fresh food. Therefore, considering these knowledge gaps, a study on specific types of plastic packaging materials on fresh food is needed, to determine the identity and quantity of flame retardants that could be an additional source contaminating the food.

The objective of this study was to investigate the occurrence of OPEs and novel FRs in plastic food packaging materials, using both targeted and non-targeted analysis. The specific objectives were to study the occurrence and level of target OPEs from different types of plastic packaging materials from fresh food, and to apply a non-targeted screening to identify other unknown occurring OPEs and NFRs. Additionally, migration study was used to understand the migration potential of the identified OPEs in food simulant, and a model matrix, which is chicken meat.

5.2 Methodology

5.2.1 Chemicals and reagents

The native standards, tributyl phosphate (TBP), tris(2-butoxyethyl) phosphate (TBOEP), and triphenyl phosphate (TPHP) were purchased from Toronto Research Chemicals (Toronto, ON).

The internal standard mix was a mixture of seven compounds. Tributyl phosphate-d27 (TBP-d_{27}), tris(2-butoxyethyl) phosphate-d_{27} (TBOEP-d_{27}), triphenyl phosphate-d_{15} (TPHP-d_{15}), tris(1,3-dichloro-2-propyl) phosphate-d_{15} (TDCIPP-d_{15}), bis(2-ethylhexyl) phosphate-d_{34} (BEHP-d_{34}), diphenyl phosphate-d_{10} (DPHP-d_{10}), were purchased from Toronto Research Chemicals (Toronto, ON, Canada). The *m*/*z* values are listed in Table S5.1.

The HPLC-grade solvents, including water, methanol, reagent alcohol, and LC-MS grade formic acid were purchased from Fisher Scientific. The LC-MS grade ammonium acetate was purchased from Sigma Aldrich. All glassware was baked at 325 °C for four hours before use.

5.2.2 Sample collection

The packaging samples for the targeted and non-targeted screening were collected from six local grocery stores in Montréal, QC Canada from 2021 to 2022, as described in Xu et al. [7]. Briefly, five types of plastic packaging materials from fresh food were collected, including thermal labels, plastic wrapping films, absorbent pads, styrofoam trays and stickers. The samples were kept at –20 °C until sample preparation and analysis.

For the controlled migration study, the chicken breast samples, with the plastic wrapping film and thermal label on the packaging, were collected from three local grocery stores in Montréal, QC Canada.

5.2.3 Sample preparation and extraction

5.2.3.1 Direct extraction for targeted analysis and non-targeted screening

The sample preparation methods for direct extraction was described in Xu et al. [7]. Briefly, portions of the samples were extracted with 95% reagent alcohol as a food simulant in 250-mL amber jars. The volume used was determined according to the European Union Commission Regulation No 10/2011 [20], which was 6 dm^2 kg^{-1}. The extraction period was at 20 °C (± 1.2 °C) for ten days. The extract was filtered with 0.22 µm PTFE syringe filters and stored at –20 °C before analysis (Figure S5.1).

5.2.3.2 Thermal label migration study

The label migration study was described in Xu et al. [7]. A migration cell (MigraCell® (MC-60), FABES Forschungs-GmbH, Munich, Germany) was used, where labelled and non-labelled films were extracted with 95% reagent alcohol at 20 °C (±1.2 °C) for ten days (Figure S5.1). The extract was stored at –20 °C before analysis.

5.2.3.3 Controlled migration study

The migration study was done in three experimental settings, the original plastic wrapping film only, the original thermal label and wrapping film, and a spiked non-printed thermal label with wrapping film. The spiked label was prepared by adding 1 mL of the target native compounds at 1 µg mL^{-1} in methanol to the label evenly and was let air-dry.

The chicken breast samples collected were first homogenized to mix the portion that was in contact and without contact with the packaging materials, this would prevent false positive results if migration occurred before the experiment. Then, the meat samples were filled in a glass petri

dish (130 ± 0.01 g), and covered by the original plastic wrapping film. The same was prepared but the filled petri dish was covered with the original thermal label and wrapping film, and a spiked non-printed thermal label with plastic wrapping film. The samples were then wrapped in aluminum foil and stored at 4 °C for three days, which was the recommended maximum storage duration in the fridge for fresh poultry by Health Canada [21]. After three days, the samples were freeze-dried and ground to powder before analysis.

The extraction was done by a method developed by Tian et al. [5]. A mass of 0.5 (±0.005) g of freeze-dried samples were added to a centrifuge tube with 6 mL of methanol and spiked with the internal standard mix at 50 ng mL^{-1}. The tubes were mixed well by vortex for 2 minutes. Then, the tubes were sonicated for 30 minutes. Afterwards, the tubes were centrifuged at 3,170 *g* for 10 minutes at room temperature. The supernatant was collected and filtered by a 0.22 µm PTFE syringe filter (Figure S5.1). The extracted samples were stored at –20 °C before analysis.

5.2.4 Instrumental Analysis

The LC-QTOF analysis was performed using an Agilent Infinity II LC system from Agilent Technologies. The LC was performed using an InfinityLab Poroshell 120 EC- C_{18} (3.0 × 100 mm, 2.7 µm) column fitted with an InfinityLab Poroshell 120 EC-C_{18} (3.0 × 5 mm, 2.7 µm) guard column, from Agilent Technologies. The LC flow rate was 0.3 mL min^{-1}, and the column compartment was set at 30 °C. The QTOF system was from the 6545 series from Agilent Technologies, equipped with a Dual AJS ESI ion source operating in positive (ESI+) and negative ionization (ESI–) mode. The gas temperature was 150 °C, at a flow rate of 11 L min^{-1}. The nebulizer was set at 30 psi, and the sheath gas temperature was 375 °C. For compounds run in ESI+ mode, the mobile phase consisted of (A) 0.1% formic acid in HPLC- grade water and (B)

0.1% formic acid in HPLC-grade methanol. For compounds run in ESI– mode, the mobile phase consisted of (A) 5 mM of ammonium acetate in HPLC-grade water and (B): 5 mM ammonium acetate in HPLC-grade methanol.

The gradient programming used is described as follows: initial gradient 5% (B) hold for 0.50 minutes, increase (B) to 100% in 3.5 minutes then hold for 4 minutes, then decrease (B) back to 5% in 1 minutes. The column was equilibrated to starting conditions for 2 minutes before the next injection. The total run time for this method was 11 minutes.

Targeted MS/MS mode was also used to confirm the identity of the detected compounds, with the same analysis method described above. The spectra were recorded at three collision energies (CE) at 10, 20 and 40 V.

5.2.5 QA/QC

For the direct extraction and label migration studies, the limit of detection (LOD) and limit of quantification (LOQ) were determined by the 3σ and 10σ of the procedural blanks, respectively. For all the extractions, matrix-matched calibration curves were prepared to assess the influence of the matrix on the quantification of the target analytes. For the controlled migration study, six Quality Control samples were prepared by pooling equal volumes of all extracted samples, and were analyzed throughout the analysis queue.

To assess the instrument performance, the precision of the RT of the internal standards, and the mass accuracy were determined.

5.2.6 Set-up of in-house screening library for flame retardants

The flame-retardant screening library was set up as described by Leung et al. [22]. Briefly, a total of 141 flame-retardant compounds were compiled based on studies in literature, and were categorized based on their chemical group, namely, hexabromocyclododecanes (HBCDDs), novel brominated flame retardants (NBFRs), and organophosphate esters (OPEs). Agilent PCDL Manager (v7.0) was used to organize the library, including the chemical formulae and mass, and was exported as '.cdb' files for subsequent data treatment. The compounds from the screening library are listed in Table S5.2.

5.2.7 Data treatment

MassHunter Profinder (v10.0) from Agilent Technologies was used for the non-targeted screening. This was done by using 'Targeted Molecular Feature Extraction' with the in-house screening library for flame retardants. The mass tolerance of the library match was ±5 ppm, and the minimum total score to be considered was 80%. Each chemical feature identified was assigned a matching score from 0 to 100, based on the mass detected from the high-resolution MS. After the screening, samples with the putative entities were analyzed by the targeted MS/MS mode on LC-QTOF, and the data was analyzed on *SIRIUS* (version 5.5.1) [23] to confirm the identity and structures of these chemical features.

The statistical analyses were performed on IBM SPSS Statistics (version 29). Independent T-test was used to compare the level of OPEs concentration detected from different packaging materials, and migration study on the labelled and non-labelled films, as well as different sample groups in the controlled migration study. The difference was considered statistically different when the *p*-value was less than 0.05.

5.3 Results and Discussion

5.3.1 QA/QC

For the direct extraction and thermal label migration study, the LOD, LOQ, matrix effect and linearity of the three target OPEs are presented in Table S5.3. All three target OPEs had a linear range from 0.5 to 250 ng mL^{-1}, with $R^2 > 0.99$. The matrix effect between the alcohol extraction simulant and pure solvent (methanol) ranged from 96.6 to 99.4%, showing that there is no signal suppression of the compounds in reagent alcohol, therefore, the calibration was done in methanol solvent.

For the controlled migration study, the LOD, LOQ, linearity, recovery and matrix effect are presented in Table S5.3. The linearity was determined by using the response factors of the native compounds. Since there was a strong signal suppression from the chicken meat matrix (< 50%), a set of matrix-matched calibration curves was used, and the compounds were linear from 1 to 200 ng mL^{-1}, with the relative standard deviation (RSD%) of the response factors below 35.2%. The extraction efficiency was assessed by the recovery, which ranged from 111 ± 11.2 to 140 ± 13.1%. The precision and accuracy of the retention time and mass accuracy of the internal standards, respectively, are presented in Table S5.4. In summary, the retention time precisions of the six internal standards had RSD% values below 0.45%. The mass accuracy of the internal standards was within 1.6 ppm. This shows that there is no significant time and mass drift on the instrument in both ESI modes.

5.3.2 Direct extraction

5.3.2.1 Non-targeted screening of flame retardants in plastic packaging materials

A non-targeted screening was first applied to detect other flame-retardant compounds present in the food packaging samples from the direct extraction with the in-house flame-retardant screening library. The non-targeted screening workflow of flame retardants has been reported in a previous study [22].

Before applying the workflow for unknown compounds, TBOEP was selected to validate the method in the food simulant matrix. The samples with TBOEP were assigned a matching score of 97% in the Profinder software. In addition to the matching of retention time and MS/MS fragmentation patterns with its reference standard, the correct structure of TBOEP was also identified on *SIRIUS* with a matching score of 97.67%. This concludes that this workflow was able to identify the compounds from the samples correctly. The results and the features presented in the following are summarized in Table S5.5.

The other features detected were triethyl phosphate (TEP), 2-ethylhexyl diphenyl phosphate (EHDPP), and melamine. These features all had an assigned matching score of above 97% on Profinder, and were not detected in the procedural blank samples. For all three compounds, their signature fragments were present on their respective MS/MS spectrum, with reference to was the ones on *MassBank*, at 10, 20 and 40 V CE (Figure S2 and Figure S3). The retention time and the MS/MS spectrum of melamine was also compared with its reference standard, in which both matched (Figure S4). Also, *SIRIUS* was able to identify the correct structures of all three compound with a matching score above 80. Therefore, it was concluded that TEP and EHDPP achieved confidence level 2 of identification according to the level system proposed by Schymanski [24], while melamine achieved confidence level 1 of identification.

TEP was present in all five packaging materials analyzed, with a detection frequency of 44.3%. According to the Food and Drug Administration (FDA), TEP is applied as an adhesive and a component of coatings, and it has been also detected in food packaging samples from recent studies [10, 17, 25]. EHDPP was detected in two thermal label samples and one film sample, and the samples were collected from different stores. This also contradicted the frequent detection of EHDPP in food packaging materials from the literature [14, 17].

Melamine was detected in all five types of fresh food packaging materials (detection frequency = 65%); among the samples, the highest signals were observed from the thermal label samples collected from the same store. According to the FDA, melamine could be used as adhesives, and coating agents (as melamine-formaldehyde resins [26]). However, this was the first report of melamine in food packaging materials from North America, and it was previously reported in China from melamine-resin containers [27], Malaysia from melamine-ware products [28] and Taiwan from tableware [29].

From the results, the peak area of TEP and melamine showed a statistically higher level ($p < 0.05$) in thermal label samples compared to the wrapping film samples, showing that thermal label samples could be the source of unknown FRs, compared to the wrapping film. Also, a further experiment would be necessary to determine the specific migration limit of melamine from the packaging samples to the food, in order to understand the underlying risk associated with the use of this compound in food packaging materials.

5.3.2.2 Targeted analysis for specific OPEs

In this study, three OPEs were used as target analytes in the packaging materials, namely TBP, TBOEP, and TPHP. They were also identified from the non-targeted screening process.

According to the FDA, these compounds are classified as indirect food additives and are common components of coating, adhesive, and polyester resins in plastic packaging materials [30].

For the occurrence of these target OPEs in different plastic packaging materials, the levels were quantified with an external calibration method. Based on the detection frequency (Table 5.1), TPHP had the highest detection rate at 93.6% of the samples collected, followed by TBP (47.9%) and TBOEP (46.4%). Out of the five packaging materials, thermal labels had the highest detection frequency and all three OPEs were detected at above 80% of the samples. Regarding the concentrations, the average sums of the three OPEs detected from different types of packaging samples are presented in Figure 5.1. The highest average sum of target OPEs was found in thermal labels (63.9 ng cm^{-2}), followed by stickers (29.7 cm^{-2}), and their OPE levels were significantly higher than the other packaging materials ($p < 0.05$). This may suggest that thermal labels could be a main source of OPE contamination in food. This was the first study comparing the level of OPEs in different types of plastic packaging materials used in fresh food. Despite having the lowest detection frequency, TBOEP was the major source of OPEs in thermal labels and stickers, in terms of the concentrations detected relative to the other two OPEs, with an average of 56.2 and 24.8 ng cm^{-2}, respectively. Compared to other recent studies [10, 17, 18], TBOEP was also one of the major OPEs detected from plastic packaging materials, with concentrations at 2.53 ng mL^{-1} in unspecified plastic packaging materials [10], which are in the same magnitude of concentration detected in wrapping films (7.82 ± 10.4 ng mL^{-1}) and absorbent pads (2.88 ng mL^{-1}). While the other studies used exhaustive extraction methods, and it was not specified what types of plastic packaging materials were used for the analysis, the concentration could not be directly compared to the current study. Nonetheless, the high level of OPEs found in thermal labels and stickers suggests that the main application might be from the adhesive or the coating found on top of the

labels or stickers, but the specific use of these OPEs in each packaging material was not available. It could also be possible that there is contamination among the food packaging materials during usage if these OPEs could migrate, such as the adhesive side from the thermal label and sticker in contact with the wrapping film.

Table 5.1 – Detection frequency of target OPEs in different packaging materials.

	TBP			TBOEP			TPHP			Sum	
	Average Concentration (ng cm^{-2})	Range (ng cm^{-2})	Detection Frequency (%)	Average Concentration (ng cm^{-2})	Range (ng cm^{-2})	Detection Frequency (%)	Average Concentration (ng cm^{-2})	Range (ng cm^{-2})	Detection Frequency (%)	Average Concentration (ng cm^{-2})	Range (ng cm^{-2})
Thermal labels (n = 40)	3.65	0-35.3	80	56.2	0-749	80	1.24	0-2.70	95	61.1	1.93-755
Wrapping films (n = 39)	1.62	0-8.63	38.4	4.78	0-54.8	46.2	1.73	0-2.40	97.4	8.14	1.43-56.3
Stickers (n = 29)	1.52	0-10.1	37.9	24.8	0-233	48.3	3.36	0-21.7	89.7	29.7	0-234
Foam trays (n = 18)	0.520	0-3.27	16.7	0	0-0	0	1.45	0-1.67	94.4	1.97	0-4.94
Absorbent pads (n = 14)	3.03	0-21.0	42.9	0.274	0-3.84	7.14	1.54	0-3.53	85.7	4.84	0-21.0
Total (Average)	**22.5**	**0-35.3**	**47.9**	**2.18**	**0-749**	**46.4**	**1.87**	**0-21.7**	**93.6**	**26.6**	**0-755**

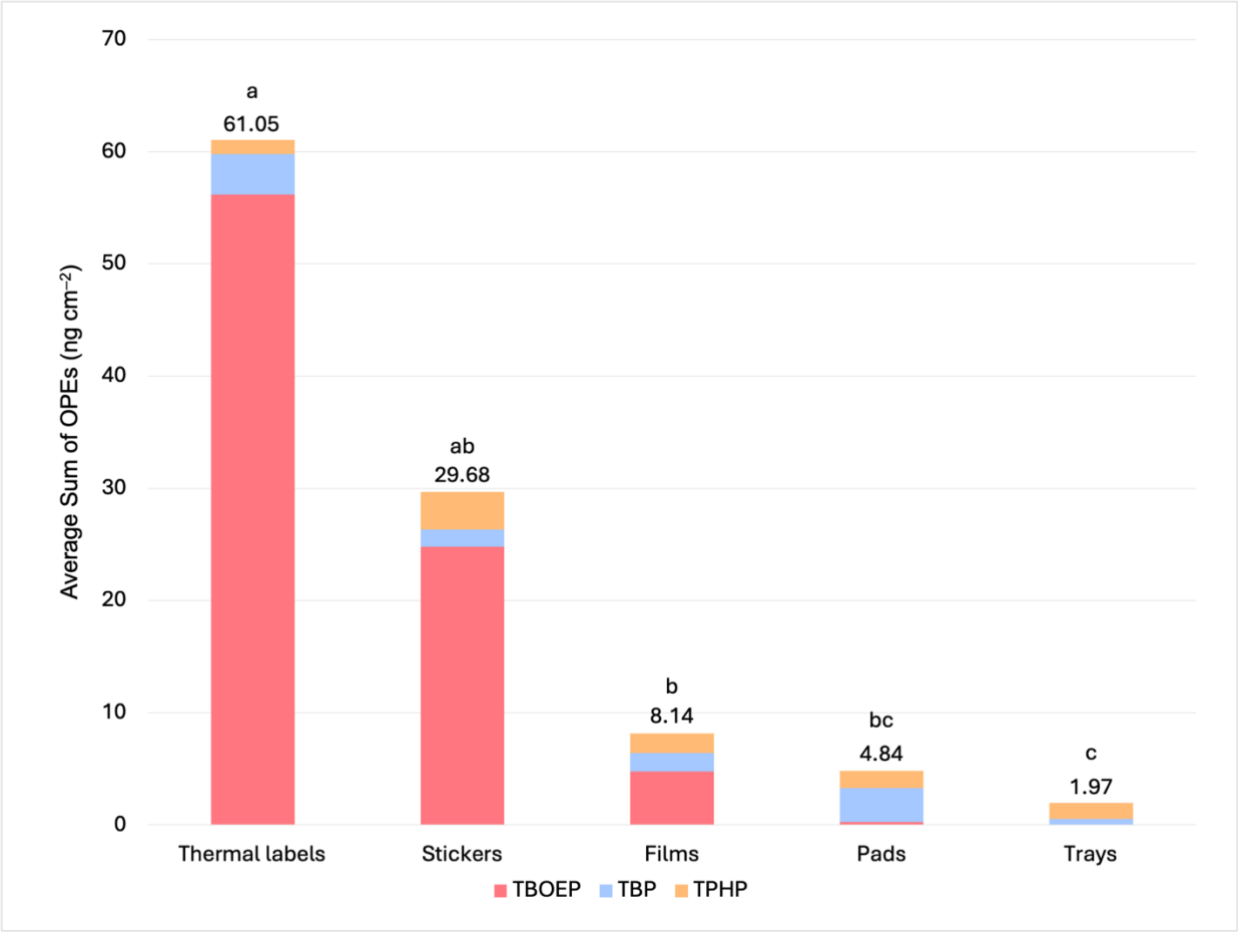

Figure 5.1 – Average sum of target OPEs from different packaging materials.

5.3.3 Thermal label migration study

Knowing that the thermal label was a main source of OPEs, a label migration study was also done to investigate the migration potential of these target OPEs from the thermal label to the food simulant. The results were presented in

Figure 5.2, where the concentration of the target OPEs from non-labelled films and labelled films were shown on the left and right sides of the chart, respectively. A similar trend was observed for TBOEP, where it showed the highest detection level at 43 ng cm^{-2} in labelled film, with a maximum level detected over 190 ng cm^{-2} in one of the samples. According to the statistical analysis, there was a significantly higher level of TBOEP detected in labelled than that in non-labelled films ($p < 0.05$), this demonstrated that TBOEP present from the thermal label could have a high potential of migration to the food through the wrapping film. However, the other two target OPEs, TBP and TPHP, showed no statistical significance between the two types of films ($p > 0.05$).

This trend was also consistent with another study done on samples collected in the United States, which compared the OPE levels detected in plastic packaged food and packaging materials [10]. From the results, it was also found that the TBOEP level was at a similar level between the food and the packaging materials, and not for TBP and TPHP, though there was no information on the type of plastic packaging materials used. Since the current study used the original thermal label in their intact form for the migration experiment, this further proves that there is a correlation between TBOEP level with the level present in plastic food packaging materials. In contrast, another recent study done in China showed a different trend [17], where the TBOEP level was found at a lower concentration in packaging materials than that in fresh food, and TPHP was found to be the predominant OPE found in packaging materials in terms of the concentration. The study also suggested that the difference in the OPEs found in the study done in the United States was

likely to be due to the different local usage of OPEs. As mentioned earlier, information regarding the exact application and volume applied to plastic packaging materials is not available in Canada. With the understanding of the migration potential of these OPEs, the information could be essential to understand the source of the detected OPEs from packaging materials and their behaviour in food.

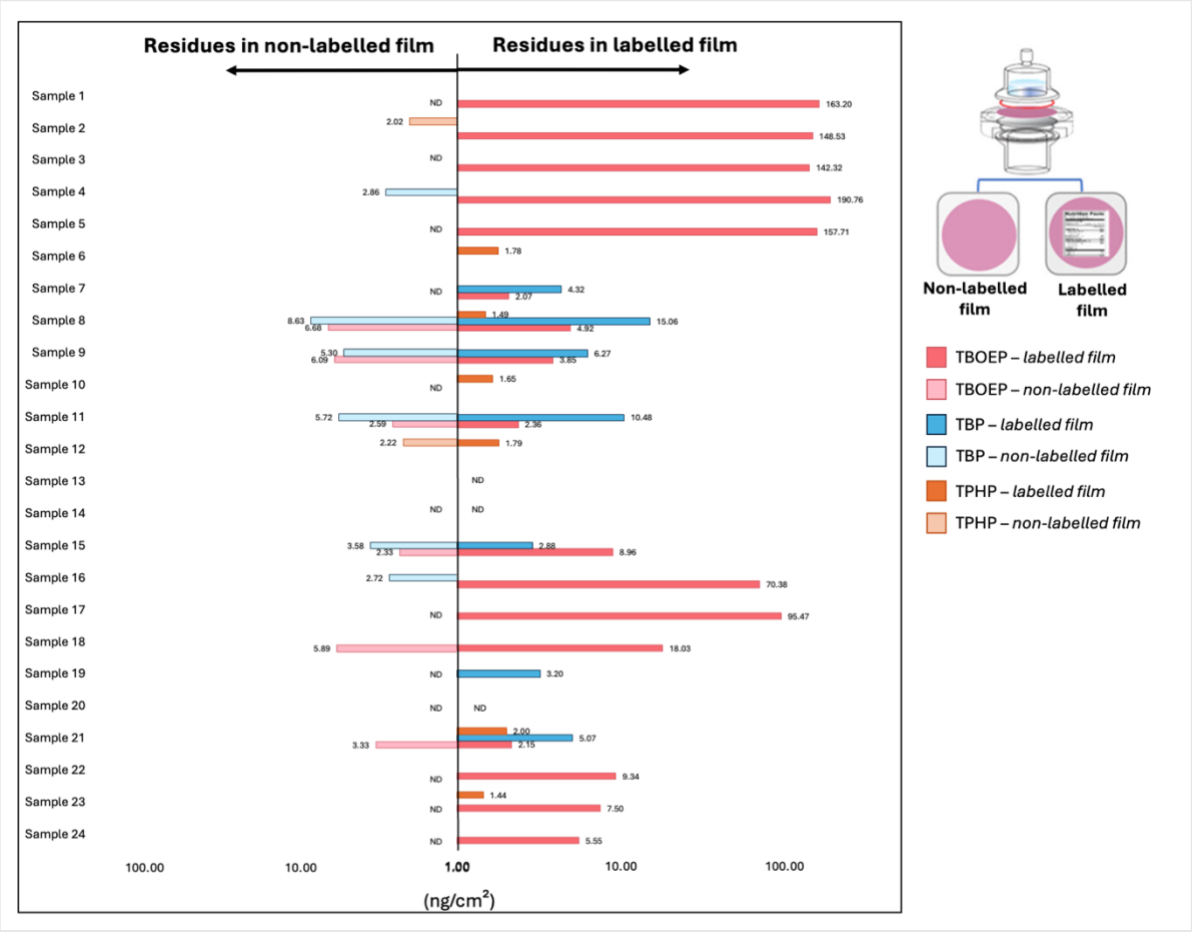

Figure 5.2 – Concentration of target OPEs from labelled and non-labelled films in the label migration study.

5.3.4 Controlled migration study of flame retardants in chicken

Based on the results of the migration cell study, a controlled migration study was conducted on chicken meat to understand the potential of the three target OPEs migrating from the thermal labels to the food. The results are shown in Table 5.2. For both stores A and B, no TBOEP residues were detected from the meat samples after incubation; while for Store C, TBOEP was detected from the original thermal and spiked label at the average of 19.9 and 14.4 ng g^{-1} wet weight (ww), respectively, which both levels were statistically higher ($p < 0.05$) than that without thermal labels. For TPHP, only a low level was detected in Store B, where 0.0725 ng g^{-1} ww was detected from meat samples after incubation, which was significantly ($p < 0.05$) lower than that without a label and with a spiked label. For TBP, it was detected in samples from all three stores, but 8.33 and 8.38 ng g^{-1} ww were detected from the original and spiked labels from Store C, and they were only found to have a significantly lower ($p < 0.05$) level from samples without the labels.

For TBOEP, it followed the same trend as the migration cell study where a significantly higher level was detected in the food simulant in contact with wrapping films with thermal labels than those without. The results here also indicated that TBOEP had a higher potential for migration from the thermal label to the food, hence, thermal labels could be an additional source of contamination of TBOEP. Nonetheless, the migration was below the specific migration limit of 50 ng g^{-1} ww, established by the Federal Office of Public Health of Switzerland [31]. For TPHP, the low migration potential in methanol (as a fatty food simulant) was also observed from a previous study, where the migration potential was less than 0.1 ng mL^{-1} [32]. In another study, the use of hexane as a fatty food simulant on plastic straws, cups and take-out boxes showed a migration concentration of over 15 ng g^{-1} for TPHP, suggesting that using a more lipophilic food matrix could increase the migration efficiency [18]. Currently, there is no study in the literature discussed

the migration of TBP to food. Since this is the first study in the literature reporting the migration of OPEs in food matrices, the study could be extended to other types of food matrices, more specifically for matrices in different lipophilicity, to understand the migration efficiency and behaviour of these OPEs.

Table 5.2 – The concentration of target OPEs in chicken meat samples from the controlled migration study.

		TBOEP			TPHP			TBP		
		No label	With label	With spiked label	No label	With label	With spiked label	No label	With label	With spiked label
Store A	Average (ng/g ww)	ND	ND	ND	ND	ND	ND	11.7	24.1	21.3
	SD							6.16	15.0	19.6
	Significance							/	$p > 0.05$	$p > 0.05$
Store B	Average (ng/g ww)	ND	ND	ND	0.365	0.0725	0.528	13.4	22.4	11.8
	SD				0.175	0.0794	0.248	8.42	12.9	2.99
	Significance				/	$p < 0.05$	$p > 0.05$	/	$p > 0.05$	$p > 0.05$
Store C	Average (ng/g ww)	6.83	19.9	14.4	ND	ND	ND	16.3	8.33	8.38
	SD	5.75	13.4	2.71				3.66	1.04	0.276
	Significance	/	$p < 0.05$	$p < 0.05$				/	$p < 0.05$	$p < 0.05$

ND: Non-detected

5.3.5 Research Implications

This was the first study to apply a non-targeted screening workflow to detect OPEs and novel flame retardants in plastic food packaging materials used in fresh food. Based on the targeted study, it was found that the thermal labels were the main source of OPEs, this could be due to the common usage of OPEs as adhesives and components in the coatings. From the target OPEs, it was also found that TBOEP had a high migration potential from the thermal label samples. The results from the controlled migration study showed that TBOEP could potentially migrate from the thermal label to the chicken meat matrix, but it was below the specific migration limit.

In this study, the results demonstrated that the OPEs present in food packaging samples could lead to additional contamination in food, and this was not accounted for in the current exposure assessment. Additionally, only a limited number of the OPEs and flame retardants in use currently in the industry had regulations, such as specific migration limits, to determine the safe consumption level via diet. Further studies are required to understand the migration behaviours of these OPEs from food packaging materials. For instance, the controlled migration study could be applied to other food matrices with different lipophilicity to understand the migration potential of OPEs in other food matrices. The conditions of storage, such as storage temperature, could also be investigated to understand the migration potential under different temperatures in food.

5.4 Acknowledgement

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Supplementary information

Table S5.1 – Supplier of internal standards used for extraction.

Compound	Type	*m/z*	Supplier
ESI+			
Tributyl phosphate-d27 (TBP-d27)	Surrogate standard	294.3414	Toronto Research Chemicals
Tributyl phosphate-d27 (TEHP-d51)	Surrogate standard	486.6804	Toronto Research Chemicals
Tris(2-butoxyethyl) phosphate-d27 (TBOEP-d27)	Surrogate standard	426.4201	Toronto Research Chemicals
Triphenyl phosphate-d15 (TPHP-d15)	Surrogate standard	342.1722	Toronto Research Chemicals
Tris(1,3-dichloro-2-propyl) phosphate-d15 (TDCIPP-d15)	Surrogate standard	445.9824	Toronto Research Chemicals
ESI–			
Bis(2-ethylhexyl) phosphate-d34 (BEHP-d34)	Surrogate standard	355.4329	Toronto Research Chemicals
Diphenyl phosphate-d10 (DPHP-d10)	Surrogate standard	259.0944	Toronto Research Chemicals

Table S5.2 – In-house-built flame retardant screening library, including the name, chemical formulae, mass, CAS number, and chemical group for each compound.

Please refer to *Table S4.3*.

Table S5.3 – The limit of detection (LOD), limit of quantification (LOQ), recovery, matrix effects and linearity of target OPEs.

Direct extraction and migration cell study					
	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	Recovery	Matrix effect (%)	Linearity (R^2)
TBOEP	0.0	0.0	N.A.	97.5	0.9973
TBP	1.41	4.69	N.A.	96.6	0.9995
TPHP	0.347	1.16	N.A.	99.4	0.9998
Controlled migration study					
	LOD (ng g^{-1})	LOQ (ng g^{-1})	Recovery (% ± SD)	Matrix effect (%)	Linearity (%RSD)
TBOEP	0.502	1.67	140 ± 13.1	0.383	29.0
TBP	2.99	9.97	111 ± 11.2	0.388	35.2
TPHP	0.0241	0.0803	115 ± 8.34	6.25	13.7

Table S5.4 – Retention time, the precision of retention time and mass accuracy of internal standards from the controlled migration study extraction.

Internal standards	ESI mode	*m/z*	Retention time (mins)	RSD% of retention time	Average mass error (ppm)
Tributyl phosphate-d27 (TBP-d27)	+	294.3414	6.09	0.111%	0.2
Tris(2-butoxyethyl) phosphate-d27 (TBOEP-d27)	+	426.4201	6.155	0.123%	0.7
Triphenyl phosphate-d15 (TPHP-d15)	+	342.1722	5.936	0.135%	0.9
Tris(1,3-dichloro-2-propyl) phosphate-d15 (TDCIPP-d15)	+	445.9824	5.903	0.171%	1.5
Bis(2-ethylhexyl) phosphate-d34 (BEHP-d34)	–	355.4329	6.054	0.182%	0.7
Diphenyl phosphate-d10 (DPHP-d10)	–	259.095	5.002	0.443%	1.2

Table S5.5 – Identification outcome of three selected molecular features from suspect screening.

	TBOEP	**Feature 1**	**Feature 2**	**Feature 3**
ESI mode	ESI+	ESI+	ESI+	ESI+
Matching score on Profinder	97	99	97	97
RT (min)	6.14	5.09	6.41	1.70
Chemical formula	$C_{18}H_{39}O_7P$	$C_6H_{15}O_4P$	$C_{20}H_{27}O_4P$	$C_3H_6N_6$
Putative identity based on molecular database	Tris(2-butoxyethyl) phosphate (TBOEP)	Triethyl phosphate (TEP)	2-Ethylhexyl diphenyl phosphate (EHDPP)	Melamine
MS/MS fragments vs. literature or compound library	Matched	Matched	Matched	Matched
Matching score from SIRIUS	97.7%	90.6%	83.9%	96.7%
RT comparison with pure standards	6.15	N.A.	N.A.	1.69
MS/MS fragments vs. reference standards	Matched	N.A.	N.A.	Matched
Samples vs. procedural blanks	Above LOD	Not detected in procedural blanks	Not detected in procedural blanks	Not detected in procedural blanks
Level of identification (Schymanski et al., 2014)	N.A.	Level 2	Level 2	Level 1

N.A.: Not applicable

Level of identification: Level of confidence of the identification based on a level system proposed by [1]

Table S5.6 – SPSS output of paired-sample t-test for comparison of sum OPE concentrations in different packaging materials.

	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		*t*	*df*	Significance	
				Lower	Upper			One-Sided *p*	Two-Sided *p*
Stickers – Trays	22.31249	53.49362	12.60857	–4.28926	48.91424	1.770	17	.047	.095
Stickers – Pads	23.83388	55.77166	14.90560	–8.36771	56.03548	1.599	13	.067	.134
Stickers – Labels	14.94390	64.21281	11.92402	–9.48135	39.36915	1.253	28	.110	.220
Stickers – Films	21.22477	62.01029	11.51502	–2.36268	44.81223	1.843	28	.038	.076
Trays – Pads	–2.96095	6.12834	1.63787	–6.49935	.57744	–1.808	13	.047	.094
Trays – Labels	–13.991	16.03021	3.77836	–21.96293	–6.01966	–3.703	17	<.001	.002
Trays – Films	–4.57011	8.04452	1.89611	–8.57055	–.56966	–2.410	17	.014	.028
Pads – Labels	–6.74752	10.37695	2.77336	–12.73900	–.75605	–2.433	13	.015	.030
Pads – Films	–2.81839	11.31473	3.02399	–9.35132	3.71454	–.932	13	.184	.368
Labels – Films	8.12930	20.44520	3.27385	1.50173	14.75686	2.483	38	.009	.018

Table S5.7 – SPSS output of paired-sample t-test for comparison of OPE concentrations in migration cell study.

	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		*t*	*df*	Significance	
				Lower	Upper			One-Sided *p*	Two-Sided *p*
TBOEP labelled film – TBOEP non-labelled film	41.91458	66.75575	13.62646	13.72610	70.10306	3.076	23	.003	.005
TBP labelled film – TBP non-labelled film	.76958	2.29749	.46897	–.20056	1.73973	1.641	23	.057	.114
TPHP labelled film – TPHP non-labelled film	.493	1.189	.343	–.263	1.248	1.435	11	.089	.179

Table S5.8 – SPSS output of paired-sample t-test for comparison of the area of detected FRs in extract.

	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		*t*	*df*	Significance	
				Lower	Upper			One-Sided *p*	Two-Sided *p*
TEP label – TEP film	747037.5263	1211836.267	278014.30662	162951.14203	1331123.9106	2.687	18	.008	.015
Melamine Film – Melamine Label	–95453.5556	186807.6470	44030.98466	–188350.8129	–2556.29821	–2.168	17	.022	.045

Table S5.9 – SPSS output of paired-sample t-test for comparison of concentration of target OPEs from chicken sample in contact without label, with original thermal label and spiked label, from Stores A, B and C.

		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		*t*	*df*	Significance	
					Lower	Upper			One-Sided *p*	Two-Sided *p*
TBOEP										
Pair 1	No Label – With Label	–15.31339	8.07316	4.66104	–35.36824	4.74146	–3.285	2	.041	.081
Pair 2	No Label – Spiked Label	–9.86209	3.37075	1.94610	–18.23550	–1.48868	–5.068	2	.018	.037
Pair 3	With Label – Spiked Label	5.45130	11.41564	6.59082	–22.90671	33.80932	.827	2	.248	.495
TBP										
Pair 1	Store A: No Label – With Label	–16.36889	13.94373	8.05042	–51.00704	18.26925	–2.033	2	.090	.179
Pair 2	Store A: No Label – Spiked Label	–13.55817	27.25784	15.73732	–81.27039	54.15406	–.862	2	.240	.480
Pair 3	Store A: With Label – Spiked Label	2.81072	31.77582	18.34578	–76.12478	81.74623	.153	2	.446	.892
Pair 4	Store B: No Label – With Label	–8.91773	11.15248	6.43889	–36.62204	18.78657	–1.385	2	.150	.300
Pair 5	Store B: No Label – Spiked Label	1.66427	6.20876	3.58463	–13.75915	17.08770	.464	2	.344	.688
Pair 6	Store B: With Label – Spiked Label	10.58201	13.39453	7.73333	–22.69185	43.85586	1.368	2	.152	.305

Pair 7	Store C: No Label – With Label	7.95489	4.62839	2.67221	–3.54268	19.45246	2.977	2	.048	.097
Pair 8	Store C: No Label – Spiked Label	7.90403	3.90633	2.25532	–1.79984	17.60791	3.505	2	.036	.073
Pair 9	Store C: With Label – Spiked Label	–.05086	.89031	.51402	–2.26251	2.16078	–.099	2	.465	.930
TPHP										
Pair 1	No Label – With Label	.31663	.15605	.09009	–.07102	.70427	3.514	2	.036	.072
Pair 2	No Label – Spiked Label	–.16255	.13773	.07952	–.50468	.17958	–2.044	2	.089	.178
Pair 3	With Label – Spiked Label	–.47918	.26400	.15242	–1.13499	.17663	–3.144	2	.044	.088

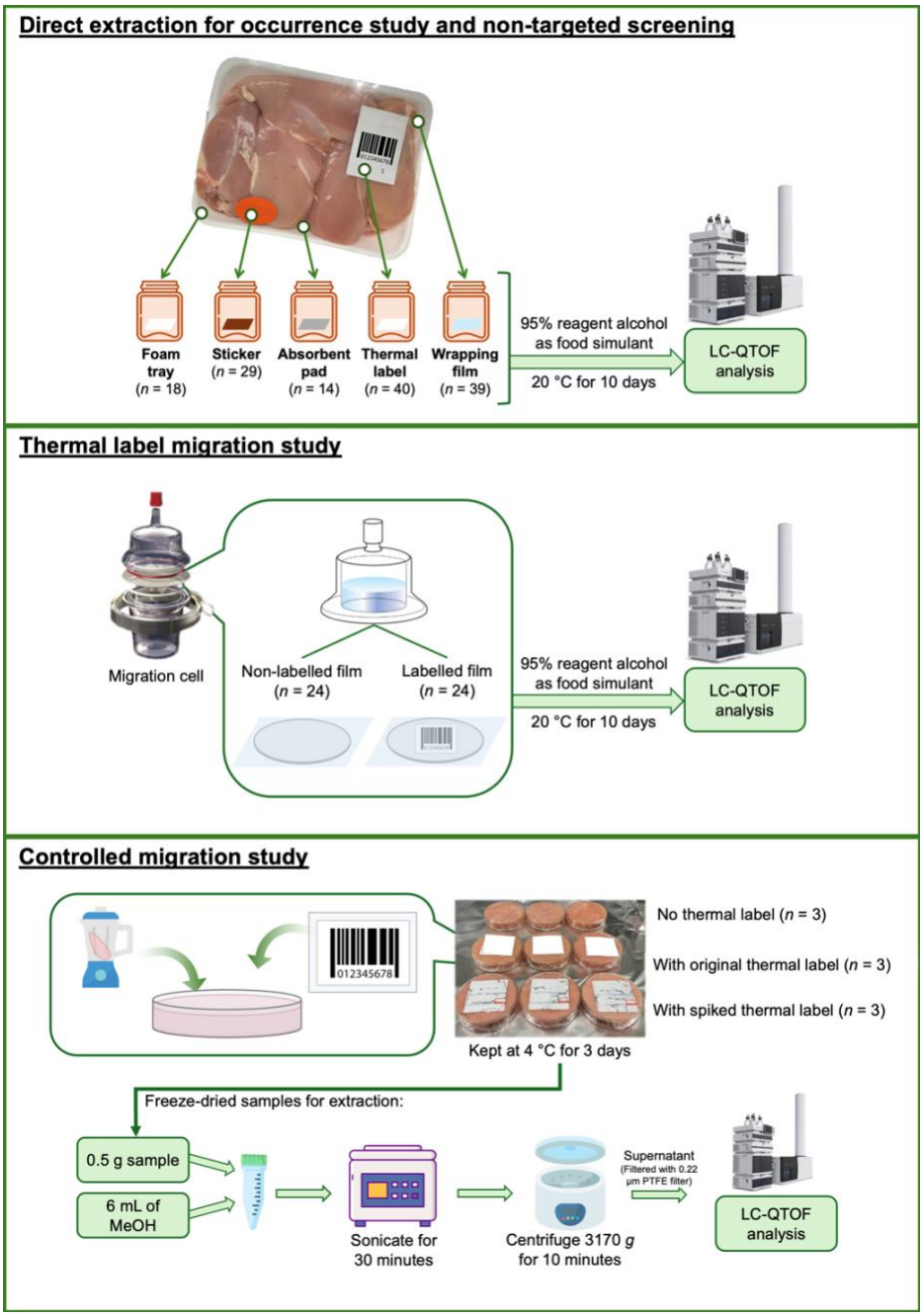

Figure S5.1 – Summary of the sample preparation and extraction for the direct extraction, thermal label migration study and controlled migration study.

Credits on the illustration: Jar, test tube and centrifuge icons made by Freepik from www.flaticon.com; bar-code product icon made by Ctrlastudio from www.flaticon.com; blender icon made by monkik from www.flaticon.com; science icon made by Paul J. from www.flaticon.com.

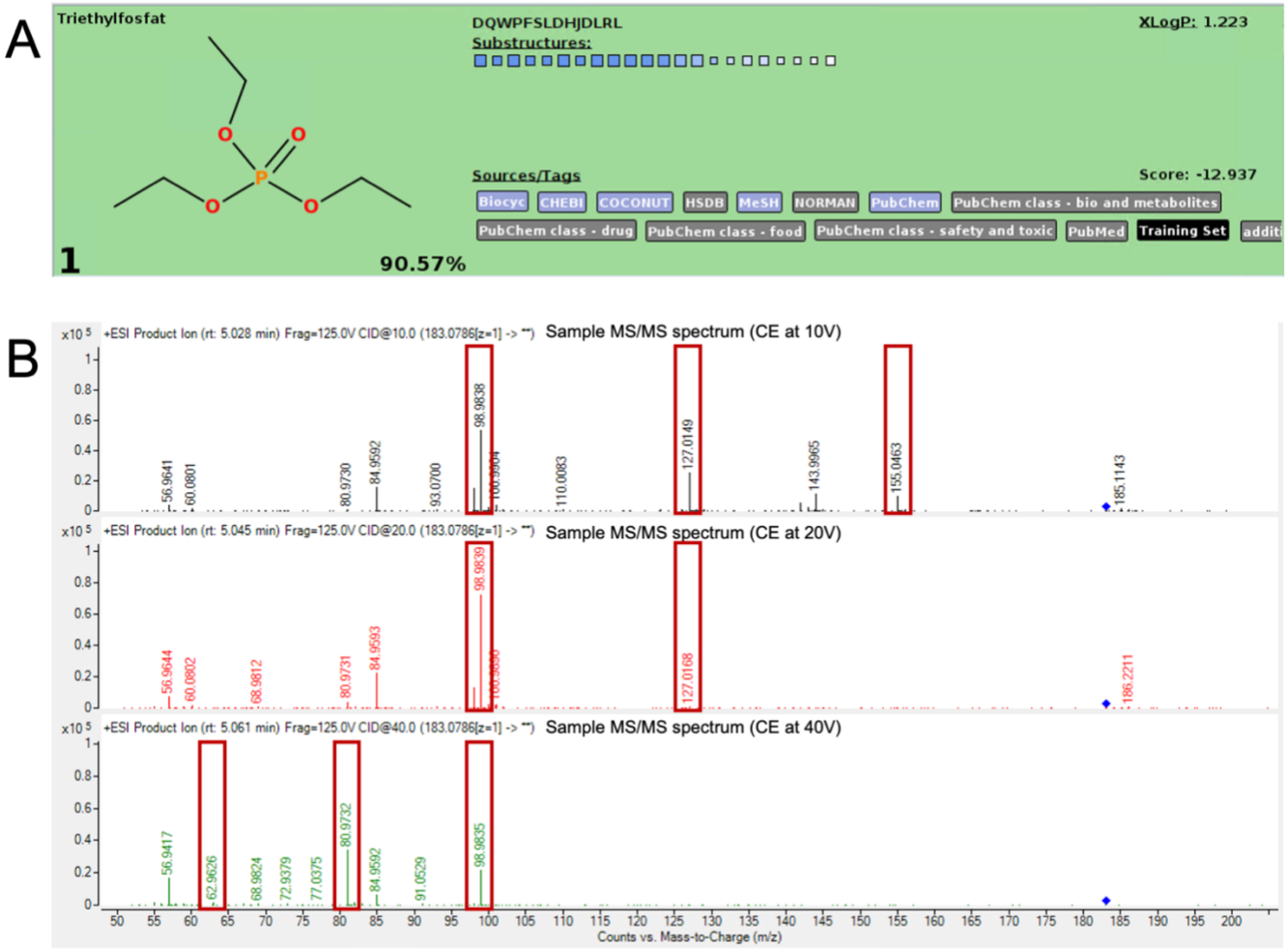

Figure S5.2 – Identification of triethyl phosphate (TEP). (A) Matching on SIRIUS. (B) MS/MS spectra of TEP in the packaging sample extract at CE of 10, 20 and 40 V.

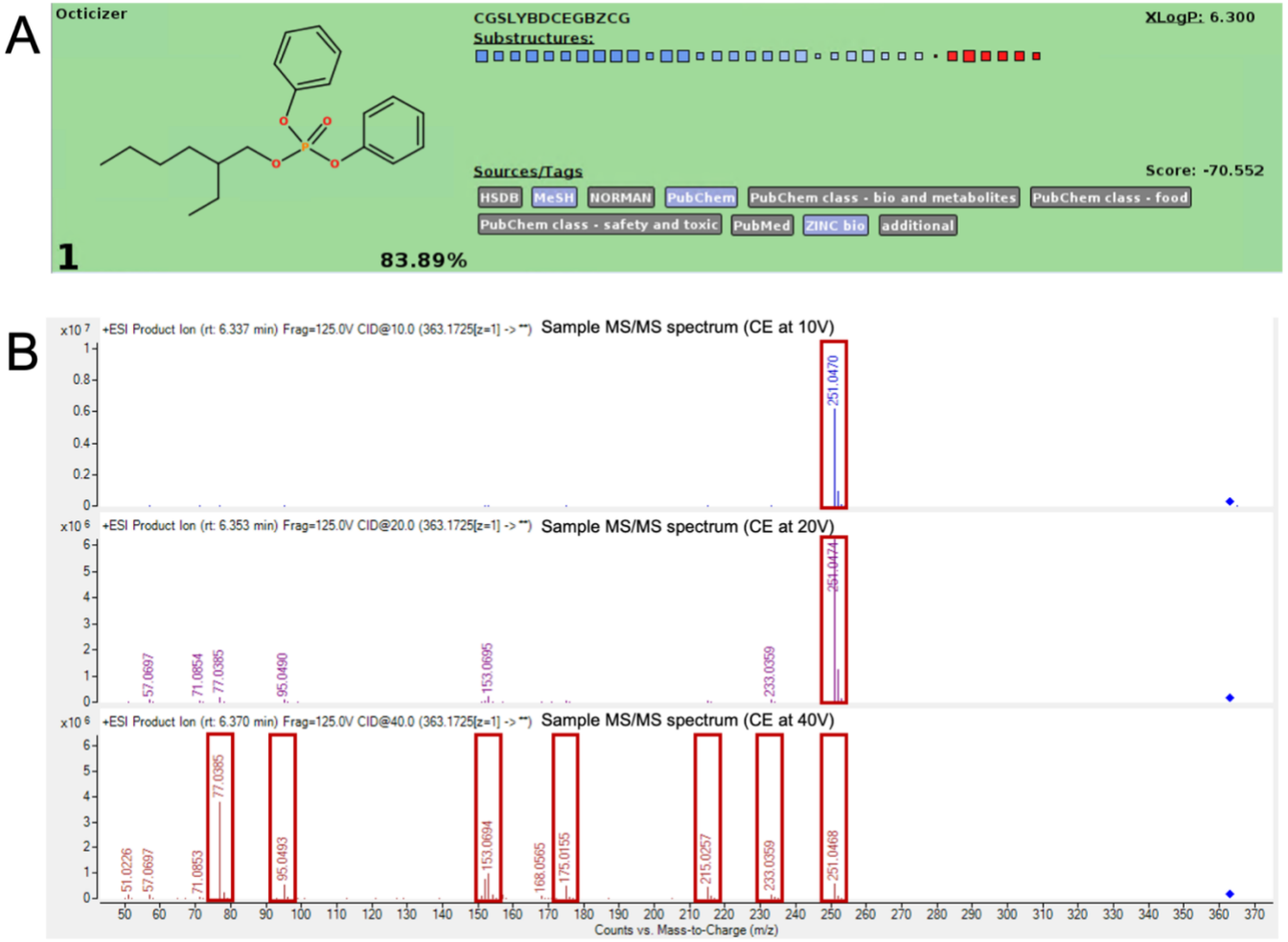

Figure S5.3 – Identification of 2-Ethylhexyl diphenyl phosphate (EHDPP). (A) MS/MS spectra of EHDPP in the packaging sample extract at CE of 10, 20 and 40 V.

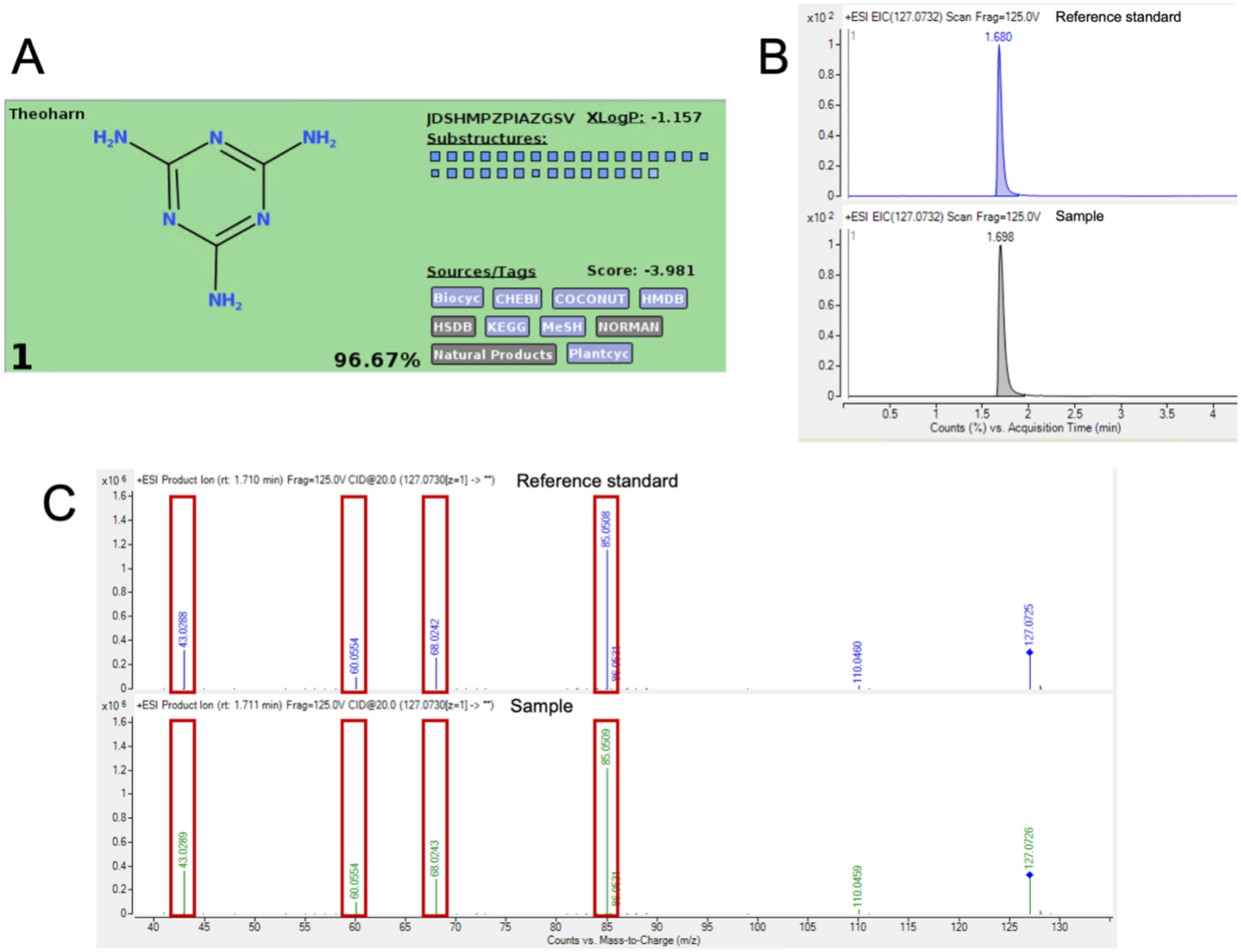

Figure S5.4 – Identification of melamine. (A) Structural identification of with SIRIUS. (B) Extracted ion chromatogram in sample and reference standard. (C) MS/MS spectrum of melamine in packaging sample extract and reference standard at CE 20 V.

Reference for supplementary information

[1] Schymanski, E. L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H. P., & Hollender, J. (2014). Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. *Environmental Science & Technology, 48*(4), 2097-2098. doi:10.1021/es5002105

Connecting Paragraph

Chapter 5 concluded that OPEs present in plastic food packaging materials, specifically thermal labels, could be a source of contamination in fresh food. This source is not accounted for in the current exposure assessment. After investigating other possible sources of FR contamination in food, the fate of FRs were investigated. Chapter 6 will introduce a study on the fate of OPEs in a model solution, which is water, and some selected food matrices under heating. The chapter described a trend of OPE degradation under typical processing conditions, which was also highlighted as a key knowledge gap in Chapter 3. This chapter will be submitted for publication in *Food Chemistry*: Leung, G., Liu, L., Bilamjian, S., Goodyer, C. G., Bayen, S. Non-Targeted Analysis of Thermal Degradation of Organophosphate Esters (OPEs) in Water and Food

Chapter 6. A Study on the Thermal Degradation of Organophosphate Esters (OPEs) in Water and Food with Application of Non-Targeted Analysis

Highlights:

- The first study to report the half-life of OPEs under heating in water and food
- Triester OPEs could degrade into their diester under heating by hydrolysis
- Different trend of degradation was observed across different matrices
- Other unknown degradation products were observed with non-targeted analysis

Abstract:

Organophosphate esters (OPEs) are flame retardants applied to commercial products, but they have been detected in food, due to contamination from the environment or food packaging. Currently, risk assessment only considers raw food samples, but most food humans consumed are cooked. Therefore, the objective of this study was to investigate the degradation of three selected OPEs in water and three food matrices, namely honey, chicken breast and salmon. The results showed that, in a model aqueous solution, triester OPEs could degrade significantly at 100 °C and form their diesters by hydrolysis, but not at 70 °C; triphenyl phosphate had the highest degradation rate, with a half-life of 29.6 minutes. However, the trend was different in the three food matrices, where most OPEs had a much slower rate of degradation. Non-targeted analysis was also applied to identify unknown degradation products found within the different heated matrices, showing that the degradation reactions were specific to each OPE as well as each food matrix involved.

Key Words: organophosphate esters, thermal degradation, LC-MS, non-targeted analysis, risk assessment, water and food matrices

6.1 Introduction

Organophosphate esters (OPEs) are commonly used as flame retardants and plasticizers in commercial products, or as adhesives and coating agents in food packaging materials [1]; two common examples include tris(2-butoxyethyl) phosphate (TBOEP) and triphenyl phosphate (TPHP). These OPEs are not chemically bonded to the polymers used, so they can be easily released into the environment [2]. Due to their lipophilicity, they can also accumulate in the environment and spread throughout the food chain [3].

Recent studies have reported the presence of OPEs in a variety of food types at concentrations ranging from 1 to 80 ng g^{-1} ww [4] and used the data to assess human exposure to OPEs from diet [5]. For example, TBOEP, TPHP and tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) were three of the most frequently detected OPEs from fresh chicken breast, fish and butter samples purchased in Montreal [6]. Currently, there are no maximum residue limits (MRLs) for OPEs in food in Canada [7], but characterizing and quantifying the OPEs and their metabolites (mOPEs) in food samples is essential for further risk assessment processes, to ensure consumer food safety. According to the current toxicity studies in the literature, OPEs pose adverse health effects on the human nervous and cognitive systems, affect adverse developmental behaviour in children, and are endocrine disruptors [8-11].

Currently, there are only a limited number of reports on the degradation reactions of OPEs; these studies have involved thermal and photo-degradation, hydrolysis and biotransformation [3, 12]. Most studies done on the degradation of flame-retardant compounds involved a much higher temperature than typical cooking conditions, and there is almost no information on the degradation of these flame-retardant compounds in food matrices [12]. In a previous study of Montreal foods, it was observed that thermal-processed food, such as bread, had a higher ratio of mOPEs (their

corresponding diesters) to OPEs, suggesting that thermal processing could lead to potential degradation of the triester parent OPEs [6]. However, in general, OPE studies have been limited to raw food samples [4], which is a simplified scenario as most of the food humans consume is cooked.

Aqueous models have been used to study the degradation products of antibiotics and antimicrobials with heating at cooking temperatures [13-16], but the toxicities and the exposure levels of these degradation products are often unknown. Some degradation products, for instance, the debrominated degradation products of polybrominated diphenyl ethers (PBDEs) showed higher toxicity than the parent compounds [17, 18]. Therefore, it is important to identify the unknown degradation products for better exposure assessment and toxicity testing.

The objectives of the present study were to study the degradation reactions of three target OPEs and their diester metabolites under heating in a model aqueous solution and three very different food matrices (honey, chicken breast, salmon). Endpoints included the effect of heating temperatures on degradation kinetics and the elucidation of possible novel degradation products by applying non-targeted analysis. The hypotheses were (i) these triester OPEs could potentially degrade into their corresponding diester OPEs or other products, and (ii) the degradation behaviour of these OPEs could be different among different food matrices.

6.2 Methodology

6.2.1 Chemicals and reagents

The OPE standards, namely tris(2-butoxyethyl) phosphate (TBOEP), bis(2-butoxyethyl) phosphate (BBOEP), tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), triphenyl phosphate (TPHP) and diphenyl phosphate (DPHP) were purchased from Toronto Research Chemicals (Toronto, ON, Canada). The internal standards tris(2-butoxyethyl) phosphate-d_{27} (TBOEP-d_{27}), bis(2-butoxyethyl) phosphate-d_8 (BBOEP-d_8), triphenyl phosphate-d_{15} (TPHP-d_{15}), diphenyl phosphate-d_{10} (DPHP-d_{10}), tris(1,3-dichloro-2-propyl) phosphate-d_{15} (TDCIPP-d_{15}), and bis(1,3-dichloro-2-propyl) phosphate-d_{10} (BDCIPP-d_{10}) were purchased from Toronto Research Chemicals (Toronto, ON, Canada). The *m*/*z* values are listed in Table S6.2.

The HPLC-grade solvents, including water, methanol, acetonitrile and LC-MS grade formic acid were purchased from Fisher Scientific. The LC-MS grade ammonium acetate was purchased from Sigma Aldrich. All glassware was baked at 325 °C for four hours before use.

6.2.2 Sample collection

The honey samples were purchased from local grocery stores or online in Montréal, QC Canada. Based on a previous study [19], honey samples with the lowest levels of TBOEP and TPHP, the closest pH values and the same colour class were selected for the degradation experiments, to limit the variability of results due to the difference in their characteristics. The chicken breast meat (referred to as ‘chicken’ in the following sections) and salmon samples were purchased from local grocery stores in Montréal, QC Canada.

6.2.3 Sample preparation for thermal degradation in water

TBOEP, TPHP, TDCIPP and their corresponding diesters (BBOEP, DPHP and BDCIPP, respectively), were chosen as targeted OPEs as they were frequently detected and were three of the OPEs with the highest detection level from the literature [4] and the previous study done on Montreal foodstuff. Each target OPE also represented a different side-chain group [16].

The stock standards for each of the six target compounds were first diluted to 1 mL of 100 and 1000 ng mL^{-1} in HPLC-grade water individually, in 2-mL glass amber vials in triplicate. The concentration in 100 ng mL^{-1} was used to simulate the approximate concentration detected in food samples according to the literature, and the concentration in 1000 ng mL^{-1} was used to detect possible degradation products at a low level. The pH of water HPLC-grade water was measured by a pH meter, and it was 7.8. Then, the vials were placed in a water bath at 100 °C (± 0.5 °C) for 0 (as control), 30, 60, 90, 120 and 240 minutes, with close monitoring of the temperature. After that, the vials were taken out of the water bath and cooled down in a cool-water bath immediately. The same procedures were done for heating at 70 °C (± 0.5 °C). The temperature 100 °C was used to simulate cooking temperature, for which 100 °C is the highest in an aqueous model; while for 70 °C, it was used to simulate the temperature for hot beverages.

6.2.4 Sample preparation and extraction for thermal degradation in food

For honey samples, 0.2 (± 0.01) grams of honey samples were weighed in 40-mL amber vials, and 4 mL of HPLC-grade water was added to each vial and mixed well by vortex. This produced a honey solution at 5% concentration, which is about the same concentration of honey used as a sweetener in beverages. Then, a mix of the six target OPEs at 1000 ng mL^{-1} was spiked to the samples and left overnight to equilibrate. One mL of the honey solution was transferred to

glass amber vials in triplicate. Afterwards, the vials were placed in a water bath at 100 °C (± 0.5 °C) for 0 (as control), 60 and 240 minutes. After heating, the vials were taken out of the water bath and cooled down in a cool-water bath immediately. The same procedures were done for heating at 70 °C (± 0.5 °C). The extraction by a direct injection method was done based on the previous studies [13, 19, 20]. Briefly, 1 mL of acetonitrile was mixed with the 1 mL heated honey solution in a 15-mL centrifuge tube, and an internal spiking mix (with final concentration at 50 ng mL^{-1}) was added. The extract was filtered through 0.22 µm PTFE filters (Agilent Technologies, Santa Clara, CA, USA). Finally, the extract was further diluted to 1% honey. The extract was kept at –20 °C until the analysis on the LC-QTOF.

For the chicken breast and salmon matrices, the samples were first homogenized in a blender. 5 (± 0.05) grams of the sample was added to 40-mL amber vials. Then, a mix of the six target OPEs at 1000 ng mL^{-1} was spiked to the samples, followed by careful mixing of the spiking solution and the sample by spatulas, and left overnight to equilibrate. Afterwards, the vials were placed in a water bath at 100 °C (± 0.5 °C) for 0 (as control), 60 and 240 minutes. After heating, the vials were taken out of the water bath and cooled down in a cool-water bath immediately, and the cooked sample and the juice were mixed carefully with a spatula to form a homogenous matrix before the extraction. The extraction was done based on a previous study [21]. Briefly, 15 mL of methanol and an internal spiking mix (with the final concentration at 50 ng mL^{-1}) were added to each vial. The vials were mixed well by vortex, followed by sonication for 30 minutes. The pellets in the vials were let to settle down for 30 minutes before the supernatant was collected. The extract was kept in the freezer at –20 °C. Before the analysis on the LC-QTOF, the extracts were centrifuged to remove the lipid precipitates at 4500 rpm for 10 minutes at room temperature, and filtered through 0.22 µm PTFE filters (Agilent Technologies).

To understand the effect of heated chicken and salmon matrices on the degradation of OPEs, a re-heating experiment was carried out. This was because spiking these compounds in raw and cooked meat matrices may influence the efficiency of extraction, due to changes in the food matrices after heating. Non-spiked matrixes after heating for 240 minutes at 100 °C were spiked with the same OPE standard mix, and carefully mixed with a spatula to ensure that the heated sample and juice formed a homogenous matrix. After equilibrating overnight, the samples were heated again for 240 minutes at 100 °C. The extraction procedure afterwards was the same.

6.2.5 Instrumental Analysis

The LC-QTOF analysis was performed using an Agilent Infinity II LC system from Agilent Technologies. The LC was performed using an InfinityLab Poroshell 120 EC-C_{18} (3.0 × 100 mm, 2.7 μm) column fitted with an InfinityLab Poroshell 120 EC-C_{18} (3.0 × 5 mm, 2.7 μm) guard column, from Agilent Technologies. The Q-TOF system was from the 6545 series from Agilent Technologies, equipped with a Dual AJS ESI ion source operating in positive (ESI+) and negative ionization (ESI–) modes. All samples were analyzed in both ESI modes. For the ESI+ mode, the mobile phase consisted of (A) 0.1% formic acid in HPLC-grade water and (B) 0.1% formic acid in HPLC-grade methanol. For ESI– mode, the mobile phase consisted of (A) 5mM ammonium acetate in HPLC-grade water and (B) 5mM ammonium acetate in HPLC-grade methanol. The LC gradients are presented in Supplementary Text 6.1.

6.2.6 Method validation

The limit of detection (LOD) and limit of quantification (LOQ) were determined by the 3σ and 10σ of the procedural blanks. For the degradation experiment in the model solution, an

external calibration method was used to reduce the interference from labelled standards. For the degradation experiment in food matrices, matrix-matched calibration curves were prepared to assess the influence of the matrix on the linearity. For each experiment, 6 quality control samples were prepared by pooling equal volumes of all extracted samples, and they were analyzed in the analysis queue. To assess the instrument performance, the precision of the RT of the internal standards, and the mass accuracy were determined.

6.2.7 Data treatment and statistical analysis

MassHunter Qualitative Analysis (v10.0) from Agilent Technologies was used to extract peak information and targeted MS/MS data. MassHunter Quantitative Analysis for Q-TOF (v10.0) from Agilent Technologies was used to integrate the peak area and quantify the concentration of OPEs from the samples.

For the kinetics study, a first-order degradation model was applied for the OPEs, where a plot for the natural logarithm of the measured concentration over time was plotted by the following equation [14, 15]:

$$\ln C = \ln C_0 - kt \quad \text{(Equation 6.1)}$$

where C was the concentration at time point t, C_0 was the initial concentration, and k was the rate constant. The half-life of the OPEs was calculated by:

$$t_{\frac{1}{2}} = \frac{\ln 2}{k} \quad \text{(Equation 6.2)}$$

For the non-targeted analysis, MassHunter Profinder (v10.0) from Agilent Technologies was first used for molecular feature extraction and peak alignments. Then, Mass Profiler Professional from Agilent Technologies was used to analyze the data extracted and for non-targeted analysis

for possible degradation products of the target OPEs. This includes fold-change and volcano plot analyses to determine features with the most significant increase in abundance after heating, followed chemical formula assignment of the putative degradation products. The details of the parameters used are described in Supplementary Text 6.2. Afterwards, these putative chemical features of degradation products were analyzed by the targeted MS/MS mode on LC-QTOF, and the data was analyzed on *SIRIUS* (version 5.5.1) [21] to confirm the identity and structures of these chemical features. The overall non-targeted analysis workflow can be found in Figure S6.1.

The statistical analyses were performed on IBM SPSS Statistics (version 29). Paired-sample T-test was used to compare the concentrations of OPEs detected from different heating durations. The difference was considered statistically different when the *p*-value was less than 0.05.

6.3 Results and Discussion

6.3.1 QA/QC

PCA plots were used to verify the position of the QC samples to monitor the quality of the instrument run before further analysis [13]. The chemical features extracted from molecular feature extraction on Profinder (v10.0) were plotted, based on the experiment in different ESI modes (Figure S6.2 to Figure S6.10). From each PCA plot, the QC samples showed a clear clustering, demonstrating that the analysis was reproducible and that the data could be used for non-targeted analysis [13].

Table S6.3 contains the LOD, LOQ and linearity data from the direct extraction method of the three target OPEs. The concentrations below LOD were denoted as non-detected (ND). For the degradation studies in the model aqueous solution, all six target OPEs had a linear range from 10 to 200 ng mL^{-1}, with $R^2 > 0.97$. For the degradation experiments in the food matrices, the linearity was determined by using the response factors of the native compounds. Since there was a strong signal suppression from the chicken and salmon matrices (matrix effect < 50%), a set of matrix-matched calibration curves was used, and the compounds were linear from 1 to 200 ng mL^{-1}, with the %RSD below 38.8%. The extraction efficiency for the food matrices was assessed by the recovery, in which the values were in the acceptable range [23], from 81.6 ± 3.63 to 125 ± 17.0%; except TDCIPP in chicken matrix, which was at 152 ± 83.6 %.

For the degradation experiment in food matrices, the precision and accuracy of the retention times and mass accuracies of the internal standards, respectively, are presented in Table S6.4. In summary, the retention time precisions of the six internal standards had a relative standard deviation (RSD) below 1.1%. The mass accuracy of the internal standards was within 3.8 ppm. This shows that there is no significant time and mass drift on the instrument in both ESI modes.

6.3.2 Degradation and the kinetics of target OPEs in the model solution

Degradation of the target OPEs was observed on the total ion chromatograms (TIC) and extraction ion chromatograms (EIC). For example, in Figure S6.11, the TIC and EIC of TPHP before and after heating at 100 °C, detected the TPHP peak at 3.7 minutes. A comparison with the TIC of the blank sample, where there was no peak at 3.7 minutes, showed that TPHP did not originate from the background signal or contamination from the solvent. The peak for TPHP after heating showed a smaller peak area on the EIC, and additional peaks appeared on the TIC, meaning new compounds were formed and detected during the heating process. The results for the model solution, at 70 °C and 100 °C, are presented in Figure 6.1 and S6.12 and Tables S6.5A and B. Degradation was concluded to have occurred when the precision of the concentration measured was below the degradation percentage, and the concentration between the heated and non-heated samples had a statistical difference ($p < 0.05$). All three target triester OPEs degraded at 100 °C but not at 70 °C. TPHP showed the highest degradation percentage, with over 99% after 240 minutes of heating; TBOEP showed a slower degradation trend after 90 minutes, where the concentration did not show any significant change afterwards. Figure S6.13 and Table 6.1 provided the rate constant (k) and half-life values of the target OPEs that showed degradation.

To the best of our knowledge, the current study is the first to report the degradation of OPEs at a high temperature. However, a similar trend was observed in a study on OPE hydrolysis over 35 days at 20 °C, by Su [16]. Their results showed that aryl-OPEs (TPHP in the present study) demonstrated the lowest stability at a basic pH, followed by chlorinated-OPEs (TDCIPP in this study). TBOEP was also found to be stable during the experimental period and no significant depletion was observed. The study also summarized the pKa values of the resulting alcohol (the

leaving group in the hydrolysis reaction), and the observed trend from this study is also consistent with the literature stating that the rate of the hydrolysis reaction generally increases with a lower p*K*a value [16, 24], TPHP > TDCIPP > TBOEP. The *k* of TPHP degradation at 20 °C was 4.29 × 10^{-6} and 6.29 × 10^{-6} $mins^{-1}$ at pH 7 and 9 respectively [16], which was over 3,500 times lower than that at 100 °C. Diesters are potential degradation products of triester OPEs by hydrolysis [16], and they have been detected in drinking and surface water [25]. Results from the present study showed that, at a cooking temperature setting (above 100 °C), the formation of these potential hydrolysis degradation products occurred at a much higher rate As a result, many of the OPEs humans are exposed to through cooked or heated food would be mostly diesters or other degradation products instead of the parent triesters OPEs, with the exception of those stable under high temperatures, such as TBOEP.

Of the three diester OPEs investigated, only BDCIPP showed a significant ($p < 0.05$) decrease in concentration after heating for 120 minutes at 100 °C. This finding was different from Su et al where no degradation of BDCIPP was detected at the experimental setting at 20 °C for 35 days [16]. Currently, there are no studies done on the degradation of diester OPEs, so the reaction involved remains unknown.

Based on the literature, the major degradation products of the triester OPEs are their corresponding diesters, and these were confirmed using reference standards (Section 6.3.4). Quantification of the diester OPEs was carried out in the triester samples heated at 100 °C, and the results are presented in Figure 6.2. All three of the diesters of the target OPEs had their concentrations increased significantly ($p < 0.05$). Notably, the formation of DPHP exceeded 300 ng mL^{-1} after heating for 240 minutes, while the formation of both BBOEP and BDCIPP was below 6 ng mL^{-1}. Assuming that diesters are the major degradation products of the triester OPEs,

this trend is consistent with the rate constant determined where TPHP had the highest rate of degradation. Comparing BBOEP and BDCIPP formation at a similar level, since it was found that BDCIPP degraded at 100 °C, it could be hypothesized that the BDCIPP formed continued to degrade and formed other unknown degradation products. For all three OPEs, no monoester was detected in the sample. This stability trend was also reported in the literature, where it has been proposed that the reactivity of hydrolysis of OPEs follows the trend: "triester >> monoester > diester" [24]. Therefore, it was expected that BBOEP and DPHP would not degrade significantly in the present study.

In terms of the toxicity of the diester OPEs compared to the triester parent compound, Liu et al. [26] found that the lipid-normalized bioaccumulation factor for DPHP was higher than that of TPHP by more than 10-fold, suggesting that the diester product could potentially be more bioaccumulative. The same study also reported that DPHP showed a lower LD_{50} level for rats through oral exposure, meaning that these diester degradation products could potentially be more toxic than the parent compound [26]. While there are a few studies in the literature focusing on the toxicities of OPE diesters [27], these degradation products are still not the main concern in the current risk assessment process for OPEs.

Table 6.1 – Summary of rate constants (k), half-lives ($t_{1/2}$) and R^2 of the fitted linear curves for the degradation of target OPEs in the model aqueous solution at 100 and 70 °C.

	100 °C				70 °C			
OPEs	Degradation	k (min^{-1})	$t_{1/2}$ (mins)	R^2	Degradation	k (min^{-1})	$t_{1/2}$ (mins)	R^2
TBOEP	✓	0.0005	1390	0.0334	✗	N.A.	N.A.	N.A.
BBOEP	✗	N.A.	N.A.	N.A.	✗	N.A.	N.A.	N.A.
TPHP	✓	0.0234	29.6	0.9914	✗	N.A.	N.A.	N.A.
DPHP	✗	N.A.	N.A.	N.A.	✗	N.A.	N.A.	N.A.
TDCIPP	✓	0.0033	210	0.9897	✗	N.A.	N.A.	N.A.
BDCIPP	✓	0.0041	169	0.7139	✗	N.A.	N.A.	N.A.

N.A.: non-applicable

✓– The degradation observed was statistically significant ($p < 0.05$) and the precision of the concentration was below the degradation %.

✗ – The degradation observed was not statistically significant, $p > 0.05$.

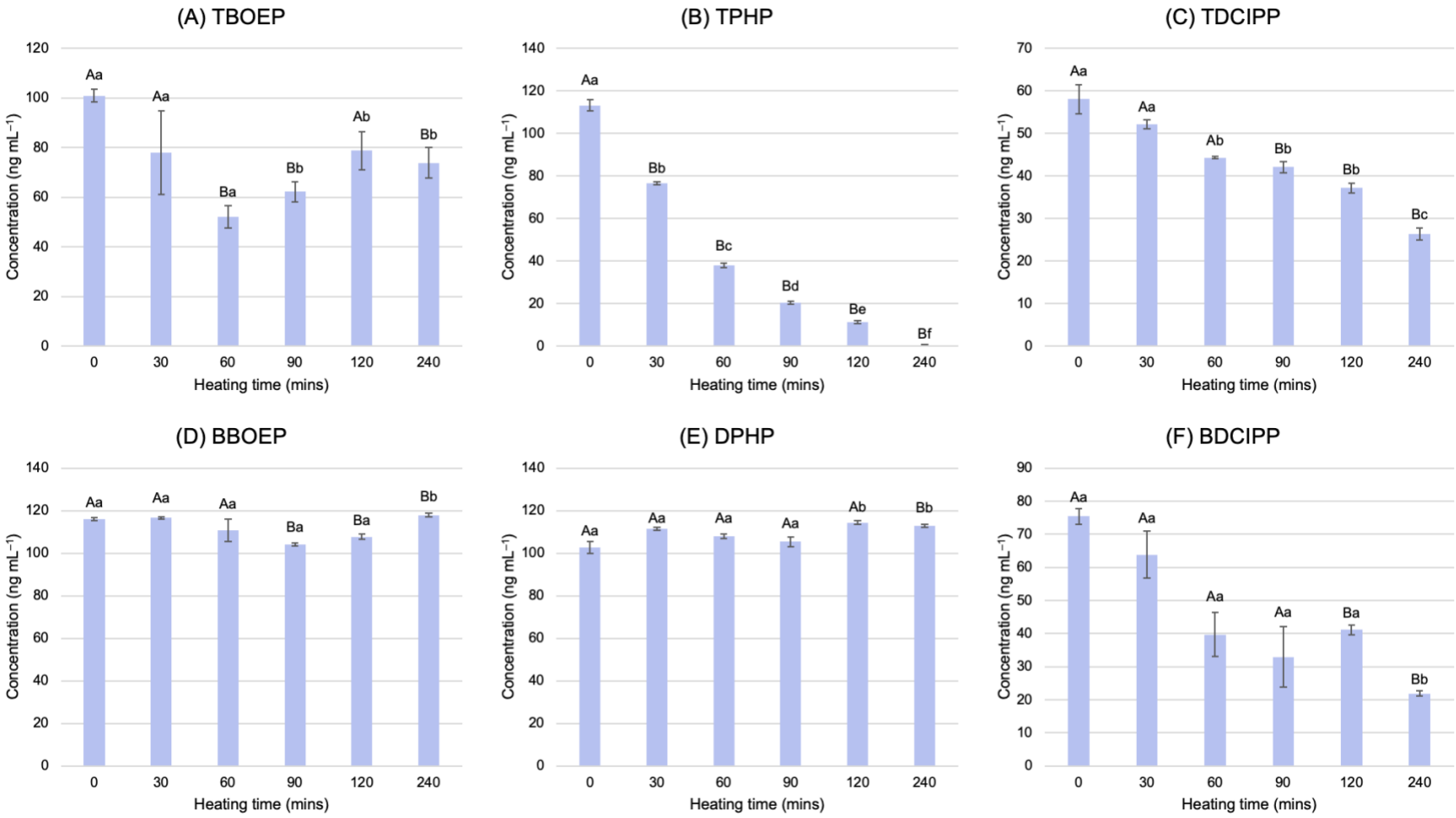

Figure 6.1 – The concentration of target OPEs heating at 100 °C over 240 minutes. (A) TBOEP, (B) TPHP, (C) TDCIPP, (D) BBOEP, (E), DPHP and (F) BDCIPP. Statistical significance between time t and 0 was denoted by uppercase letters; statistical significance between t and its previous time point was denoted by lowercase letters. (n = 3, error bars represent the SD).

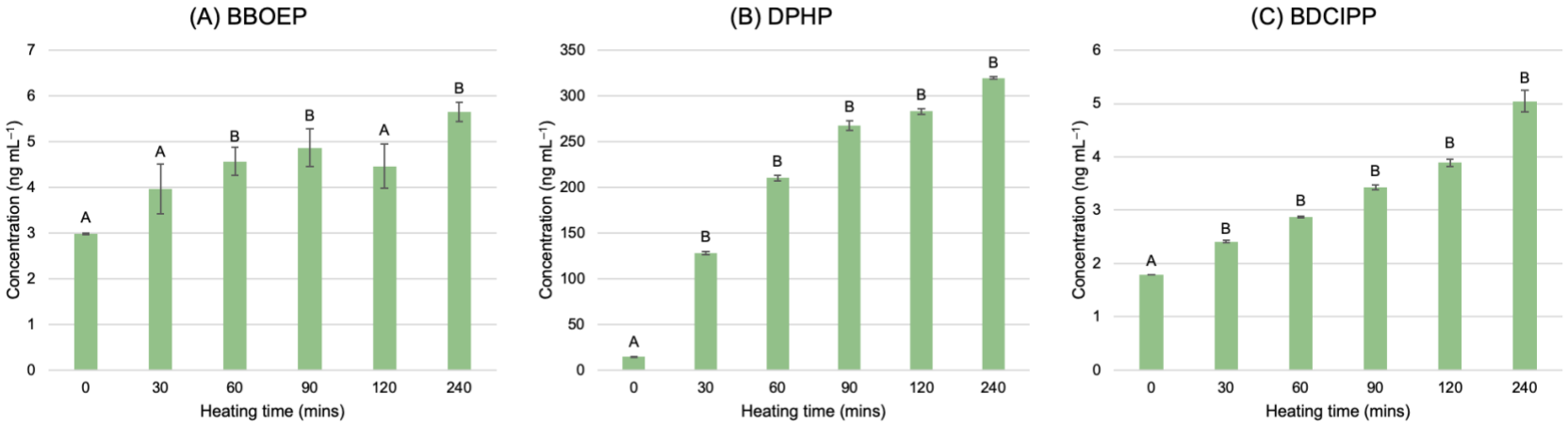

Figure 6.2 – Quantification of diester OPEs of triester degradation samples in the model aqueous solution at 100 °C. (A) BBOEP in TBOEP sample, (B) DPHP in TPHP sample, (C), BDCIPP in TDCIPP samples. (n = 3, error bars represent the SD).

6.3.3 Degradation and the kinetics of target OPEs in different food matrices

The thermal degradation of OPEs was also investigated in three different food matrices (Figure 6.3, Table S6.5C). At 100 °C, both TBOEP and TPHP showed a significant decrease ($p < 0.05$) in concentration (–20.3 ± 6.67% and –18.9 ± 2.94%, respectively) after heating, but no degradation was observed for TDCIPP ($p > 0.05$). For the diesters, the concentration of DPHP significantly increased after heating for 240 minutes ($p < 0.01$); BDCIPP showed degradation, with over a 75% decrease in concentration ($p < 0.001$), while no degradation was observed for BBOEP ($p > 0.05$). At 70 °C (Figure S6.13, Table S6.5D), only TBOEP and TPHP showed a significant decrease ($p < 0.01$). Surprisingly, BBOEP also had a significant decrease ($p < 0.05$) in the concentration of 21.5 ± 4.51% after heating for 240 minutes. Knowing that these OPEs did not degrade under 70 °C in the model aqueous solution (Figure S6.12), other degradation reactions must have occurred with the ingredients in honey that did not involve a high temperature of 100 °C.

In terms of the degradation kinetics in the honey matrix (Table 6.2), TBOEP had the same *k* value as the model aqueous solution, suggesting that the rate of TBOEP degradation could be unaffected by the honey matrix. The *k* of TPHP was reduced by over 30-fold to 0.0006 $mins^{-1}$ in the honey matrix. In a previous study, it was found that the rate of hydrolysis is pH-dependent; more specifically, the half-lives of TPHP hydrolysis was 28 days at pH 5, compared to 19 days at pH 7 [28]. A possible explanation was that the pH of the honey sample chosen was around 4.1, so the degradation rate of TPHP was reduced in the honey matrix under a lower pH, compared with the model solution. In contrast, the *k* of BDCIPP increased in the honey matrix, suggesting that degradation of the diester could involve other reactions than hydrolysis, or BDCIPP could react to a component in honey. This is the first report of BDCIPP being degraded in an abiotic matrix.

The degradation of OPEs under the chicken and salmon matrix was also studied at 100 °C (Figure 6.3, Table 6.2). In both matrices, TBOEP and TPHP showed a significant decrease in concentration ($p < 0.05$) after heating for 240 minutes. However, in the chicken matrix, the degradation percentages for the two compounds were 56.7 ± 4.12% and 45.7 ± 2.99%, respectively, more than two times that in the salmon matrix. In terms of the degradation rate, TBOEP and TPHP degradation in the chicken matrix was more than three times higher than in the salmon matrix. Comparing the two matrices: chicken breasts have an average moisture content of 76%, while that of salmon is 74%, chicken breasts have an average fat content of 1.05%, while that of salmon is 5.70% [6] and the pH of chicken breasts is 5.0 to 6.0 [29] compared to salmon at 6.1 to 6.3 [30]. Since the moisture content and pH of the two matrices are comparable, it could be hypothesized that, considering both TBOEP and TPHP are lipophilic compounds, they can partition in the lipid of the salmon matrix, similar to food preservatives found to partition in the fish oil-water system [31]. Partitioning in the lipids could offer a protective effect on the OPEs from the hydrolysis reaction, while other reactions within the matrix may still occur. An effect of lipids on the hydrolysis rate could also be responsible for the DPHP formation in the two matrices: the DPHP concentration increased significantly (119 ± 13.1%; $p < 0.01$) in the chicken matrix, compared to an insignificant change ($p > 0.05$) in the salmon matrix.

For BDCIPP in the chicken and salmon matrices, a significant decrease in concentration ($p < 0.01$) was observed, with degradation percentages at 59.8 ± 6.50% and 65.4 ± 4.80%, respectively. It was also observed that the degradation percentages of BDCIPP in these two matrices, as well as the values of the rate constant k, were close to that in the model aqueous solution. These results further suggest that the degradation of BDCIPP involves other reactions, and could be independent of matrix moisture content, fat content and pH.

Other unexpected trends included that the rate constant *k* for the degradation of TBOEP in chicken was higher than that in the model solution; this could be due to other interactions of TBOEP with the chicken matrix. Also, for BBOEP, it was found that there was a 5.82 ± 0.575% increase ($p < 0.01$) in concentration after heating in the salmon matrix, which was not observed in other food matrices studied. This could be due to a specific interaction of BBOEP with a component in the salmon matrix.

In the literature, different types of interactions between nutrients and food matrices have been described, including the presence of physical barriers or binding of the chemicals with matrix components [32]. In contrast, the interactions between contaminants and the food matrices have not been well-studied, leaving the field open to hypotheses to be tested. For instance, the protective effect of the lipid in salmon on the OPE degradation by partition could be investigated by determining the experimental partition coefficient between the lipids and water, which was previously done for studies on the release of aromatic compounds in food [33]. Based on the type of lipids found in salmon, and the lipophilicity of OPEs, the partition of other OPEs could be predicted as well. It should also be noted that, although it has been suggested that the occurrence of OPEs is not associated with the lipid content in the food, the degradation of OPEs within the matrices could have a different trend, based on the current study. This could be due to the interaction of OPEs with the lipids as suggested, or other micronutrients, such as pyridoxine phosphate formation with phosphate with vitamin B_6 [34]. Another example could be the formation of hydroxymethylfurfural from glucose or fructose in honey [35], it is unknown whether OPEs are involved in the phosphorylation of glucose or fructose [36]. To model specific OPE interactions with the food matrices, its chemical and structural properties will need to be characterized, and correlated to chemical reactivity [37]. This approach would provide information

on the effect of catalysis, diffusivity and temperature on the food matrices, as these could alter the food matrices during cooking and thermal processing [37]. This modelling technique could also be applied to other food matrices to better understand the types and modes of interactions for contaminants in food.

Table 6.2 – Summary of rate constant (k), half-life and R^2 of the fitted linear curves for the degradation of target OPEs at 100 °C in different food matrices.

	Honey matrix				Chicken matrix				Salmon matrix			
OPEs	Degradation	k (min^{-1})	$t_{1/2}$ (mins)	R^2	Degradation	k (min^{-1})	$t_{1/2}$ (mins)	R^2	Degradation	k (min^{-1})	$t_{1/2}$ (mins)	R^2
TBOEP	✓	0.0005	1380	0.0972	✓	0.0023	301	0.2319	✓	0.0006	1160	0.9794
BBOEP	✗	N.A.	N.A.	N.A.	✗	N.A.	N.A.	N.A.	✓	-0.0005	-1390	0.2231
TPHP	✓	0.0006	1160	0.2504	✓	0.0025	277	0.9992	✓	0.0007	990	0.8079
DPHP	✗	N.A.	N.A.	N.A.	✓	-0.0033	-210	1	✗	N.A.	N.A.	N.A.
TDCIPP	✗	N.A.	N.A.	N.A.	✗	N.A.	N.A.	N.A.	✗	N.A.	N.A.	N.A.
BDCIPP	✓	0.0055	126	0.9282	✓	0.0039	178	0.9957	✓	0.0043	161	0.9907

N.A.: Non-applicable

✓– The degradation observed was statistically significant ($p < 0.05$) and the precision of the concentration was below the degradation %.

✗ – The degradation observed was not statistically significant, $p > 0.05$.

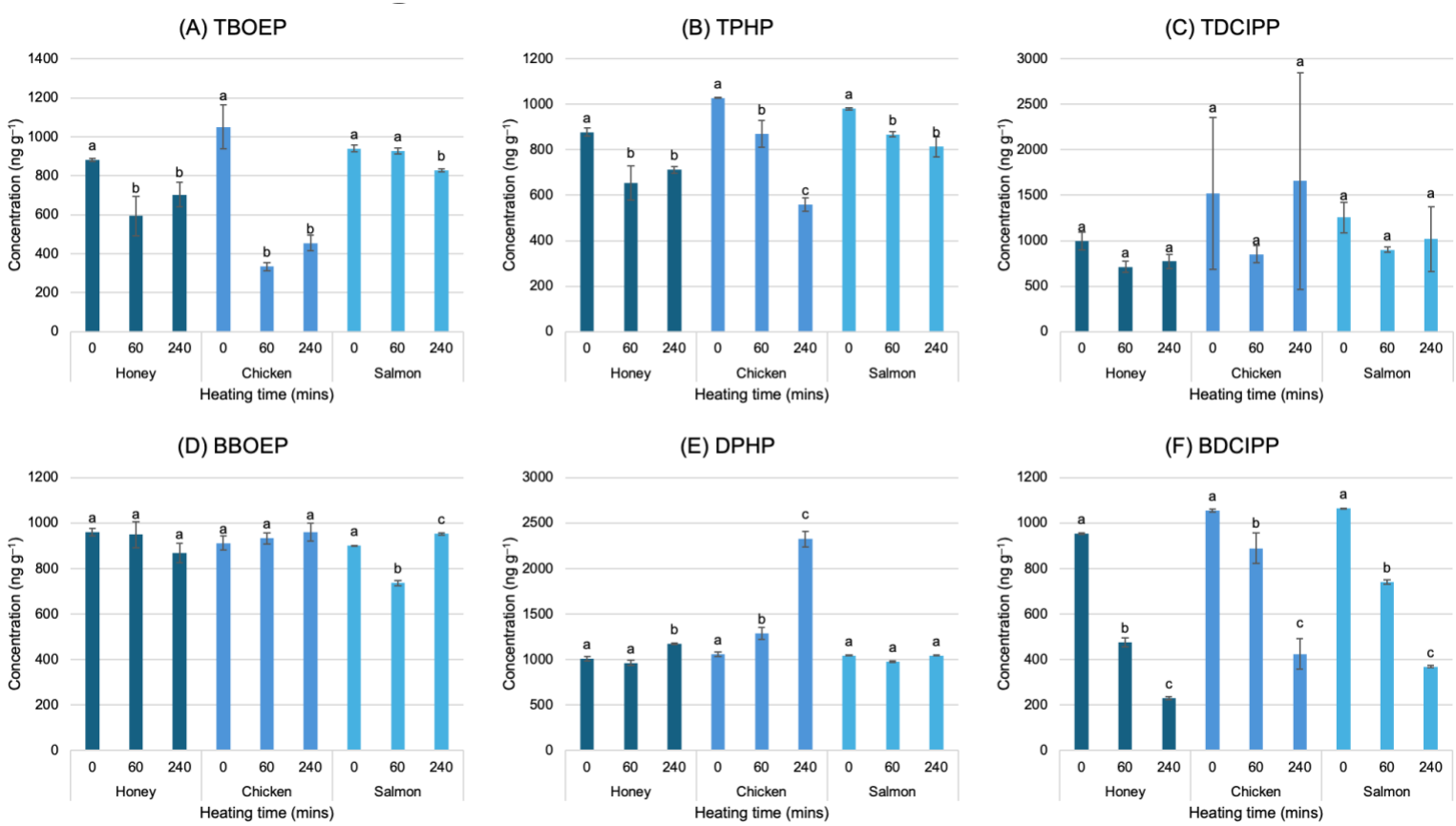

Figure 6.3 – The concentration of target OPEs heating at 100 °C in different food matrices at 60 and 240 minutes. (A) TBOEP, (B) TPHP, (C) TDCIPP, (D) BBOEP, (E), DPHP and (F) BDCIPP. Statistical significance was denoted by the letters on the bars (n = 3, error bars represent the SD).

6.3.4 Non-targeted analysis of thermal degradation products in the model solution

Based on the results in the previous section, non-targeted analysis was applied to four of the six target OPEs which showed a degradation trend in the model aqueous solution, i.e. TBOEP, TPHP, TDCIPP and BDCIPP at 100 °C. Before analyzing the features on the targeted MS/MS mode on the LC-QTOF, these features were examined individually to ensure that the chemical formula generated had a matching score of 80, and that they were not detected in the blank samples. The chemical formulae of the possible degradation products of the four OPEs were summarized in Table S6.7. The structure of four confirmed degradation products, as well as proposed structures for four other degradation products are presented in Figure 6.4.

For TBOEP, all the features remaining after filtering were from the ESI+ mode. BBOEP's structure and identity were confirmed with the analysis on *SIRIUS* and the matching of retention time of the reference standard (Figure S6.14). Another degradation product, TBOEP-DP2, was predicted as bis(2-butoxyethyl) 2-hydroxyethyl phosphate (BTBOEP) by *SIRIUS*. By comparing the retention time and fragmentation patterns on the MS/MS spectra with the reference standard, this identity was also confirmed (Figure S6.15); this was the only degradation product that achieved a level 1 confidence level of identification [38]. BTBOEP has also been detected as a degradation product of TBOEP under anaerobic conditions in bacterial enrichment culture [39] and an aquatic media by electrochemical oxidation [40]. Another degradation product, TBOEP-DP3, bis(2-butoxybutyl) hydrogen phosphate, was an alkylated product of BBOEP. TBOEP-DP4 (*m*/*z* 363.1536, $C_{12}H_{26}O_{12}$) had the highest peak height among the other degradation products and had two possible structures according to *SIRIUS*, with a similar matching score: both were hydroxylated alkane chains, which could be hydroxylated products from the side chains cleaved from the parent TBOEP compound. However, the mechanisms for the formation of these

degradation compounds are still unknown. Other degradation products of TBOEP reported in the literature were not found in the current study.

For TPHP, no structures could be proposed by *SIRIUS* based on the chemical formulae and MS/MS of the putative degradation products, except DPHP (Figure S6.16). DPHP was also reported as a degradation product under a bioelectrochemical system, while some biotransformation products were also identified in an aquatic setting, yet none of these biotransformation products matched with the degradation products found in the present study [41, 42]. This could mean that the degradation reactions could involve a more complicated mechanism than the transformation involved in a biotic setting, such as carboxylation or hydroxylation.

After filtering, none of the degradation products of TDCIPP, except BDCIPP, had a chemical formula with a matching score of 80. The identity of BDCIPP was also confirmed by reference standards (Figure S6.17). For the degradation products of BDCIPP, all the degradation products reported had a higher mass than the parent BDCIPP, in particular, BDCIPP-DP4 (*m*/*z* 691.5983, $C_{40}H_{83}O_6P$) had three suggested structures from *SIRIUS*, which all have long side chains. Although the mechanism is unknown, considering some of the suggested chemical formulae of degradation products from different OPEs had more than one phosphorus atom, this may imply that the OPEs could also react with each other to form more complex products during the heating process.

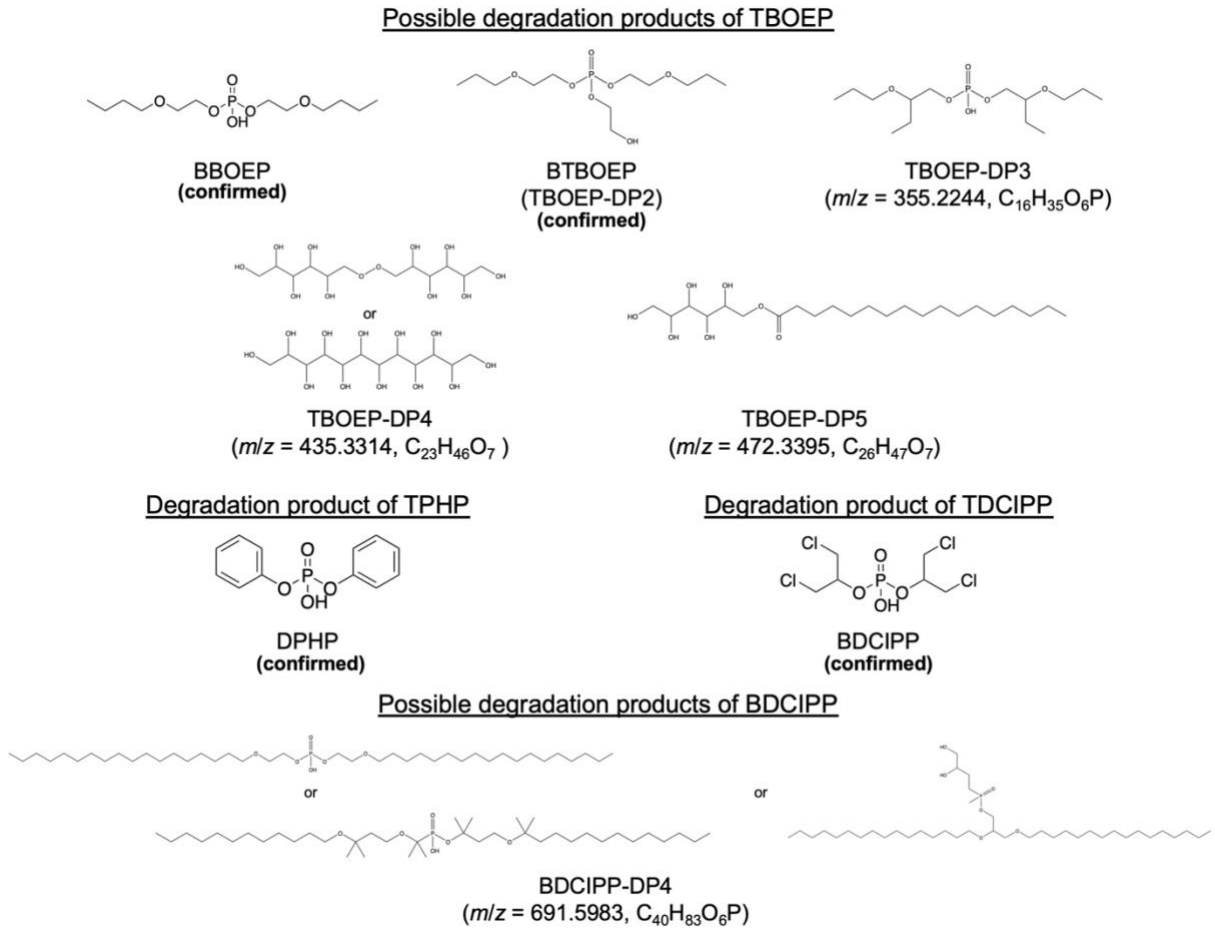

Figure 6.4 – Possible and confirmed degradation products of TBOEP, TPHP, TDCIPP and BDCIPP.

6.3.5 Non-targeted analysis of thermal degradation products in food matrices

Similarly to the different trends of the degradation between the model solution and the food matrices, different degradation products were observed as well. Among the three food matrices, different degradation products were observed, which confirmed the second hypothesis that these OPEs could interact with food matrices in a unique manner, and produce different degradation products. These possible degradation products were not detected in the blank, non-spiked and non-heated samples. The results are summarized in Table S6.8, and the possible structures are presented in

Figure 6.5. None of these degradation products have been reported in the literature, and no information regarding their occurrence and relationship with the matrices was available.

From the list of degradation products, new phosphate compounds have been formed, including H100-DP5 (*m*/*z* 233.0427, $C_5H_{13}O_8P$), H70-DP2 (*m*/*z* 298.9405, $C_6H_{12}Cl_3O_5P$), C100-DP1 (*m*/*z* 170.9614, $C_3H_6ClO_4P$), C100-DP8 (*m*/*z* 390.9437, $C_9H_{17}Cl_4O_6P$), and S100-DP1 (*m*/*z* 280.9746, $C_6H_{13}Cl_2O_6P$). This indicated that novel degradation products can be formed from the interaction of OPEs with food matrices. Some of these degradation products resembled the side chain of the parent OPE. For example, H100-DP4 (*m*/*z* 231.0843, $C_{10}H_{14}O_6$) has a similar structure to the TBOEP side chain; it could be hypothesized that the leaving group cleaved could further react with other OPEs or the food matrix.

The results, despite the lack of confirmation of the proposed structures and chemical formulae, could aid in the prediction of the reactions of OPEs under thermal processing in future studies. Additionally, these data demonstrate that the non-targeted workflow was effective in determining unknown compounds found in the complex matrices.

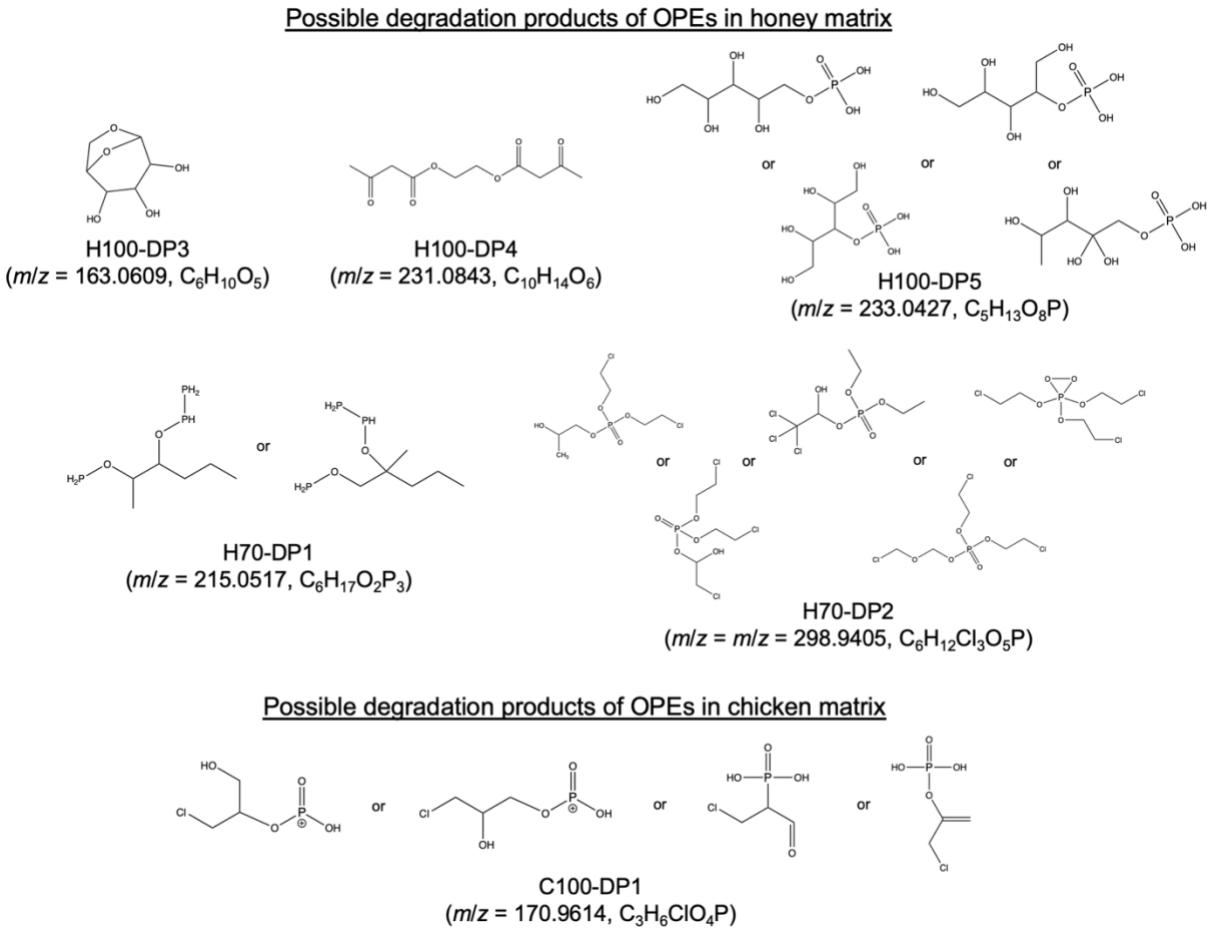

Figure 6.5 – Possible structures of the OPEs degradation products in honey and chicken matrices.

6.3.6 Effect of heated chicken and salmon matrices on the degradation of OPEs

Heating of meat matrices, including chicken and salmon samples in this study, could lead to shrinkage of the matrix and solubilization of the collagen into gelatin [32]. Also, it was suggested that spiked samples prepared by adding analytes to the matrix could have high variability, in contrast to using incurred samples [43]. In terms of OPE degradation, it could be interpreted that the degradation observed was the OPEs being trapped in the cooked matrices during extraction. Therefore, the degradation percentages were compared between spiked non-heated and heated chicken and salmon samples, to understand the effect of heated matrices on degradation. The results are presented in Figure 6.6 and Table S6.9.

For BBOEP, TPHP, TDCIPP and BDCIPP, no significant differences ($p < 0.05$) were found between the degradation percentages in both matrices for the spiked non-heated and heated samples, signifying that these OPEs were not trapped in the matrix, and hence, not extracted from the samples. In contrast, TBOEP had significantly lower ($p < 0.01$) degradation percentages for the spiked heated matrices for both chicken and salmon matrices. In the chicken matrix, the concentration before re-heating in the heated sample was less than half of that in the non-heated sample, but the concentration after heating and re-heating was at a similar level. This could be due to a specific and unknown interaction between TBOEP and cooked chicken matrix, something that needs further investigation. DPHP in the salmon matrix had a significant difference ($p < 0.05$) in the degradation percentage too, but this was due to a significant increase in its concentration. Based on the findings from previous sections, that DPHP was formed by the hydrolysis of TPHP, it could be hypothesized that a prolonged heating period could generate more DPHP.

Overall, the detectability of most OPEs was not hindered by the conformational change of the matrix from heating, which was unlike the trend previously suggested [44]. It should also be

considered that the current study homogenized the samples before and after heating, so the reactions and rates would be homogenous in the food matrix [37]. Using incurred samples could be a more accurate representation of how these OPEs could behave in the food matrix, and how they interact with the matrix under thermal processing. However, the extraction method would need to be optimized to effectively extract incurred contaminants in the food matrix.

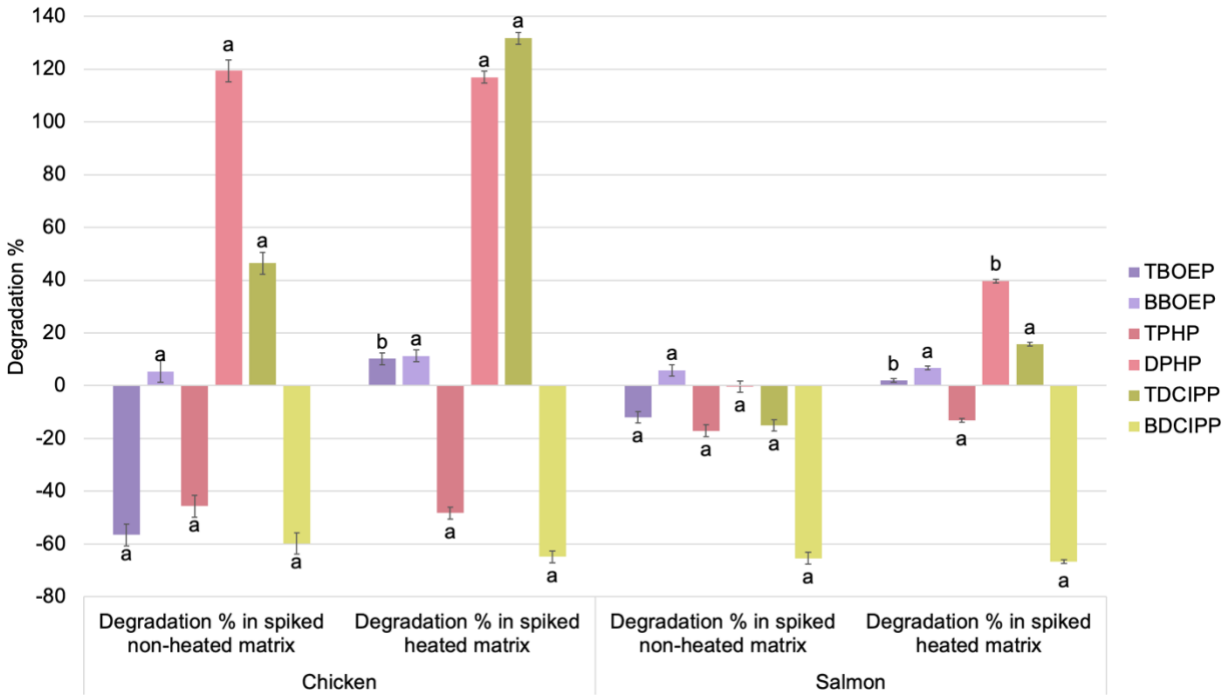

Figure 6.6 – The degradation of OPEs in non-heated and heated chicken and salmon matrices (n = 3, error bars represent the SD).

6.4 Conclusion

To summarize, OPEs can degrade under thermal processing at 100 °C, in both the model aqueous solution and the three examples of food matrices tested in the present study. This study confirmed that diesters of the triester OPEs could be formed by hydrolysis, and that the degradation in food matrices may involve more complex mechanisms as several alternative degradation products were also formed. This was the first study to report degradation of diester OPEs, showing that while diester OPEs are more stable, some may degrade under heating, such as BDCIPP. This was also the first study to investigate the half-life of OPEs under thermal processing in food matrices, which is in the range of less than an hour to a day, relative to the environment matrices that could be days to months, demonstrating that these OPEs are much less stable in food during heating.

In terms of risk assessment, the current study applied an extreme condition, i.e. heating the samples for up to four hours and spiking at a high level, to assess the worst possible outcome, in terms of the degradation of OPEs and the degradation products produced. Knowing that the level for all three target triester OPEs decreased significantly in the heating process, it could be incorrectly inferred that cooking is a mitigation of OPE exposure. However, in the current hazard characterization process, the toxicities of the diester OPEs are not the focus, and the new unknown compounds produced have also not been considered. Based on the current results, the three target triester OPEs with different side-chain chemistry, had distinctive degradation rates in different matrices and could generate a wide range of unknown compounds. While the trend of degradation reactions could be generalized, it was also observed that the degradation reactions are compound- and matrix-dependent. Therefore, it is essential to understand that these the experimental findings should not be extrapolated directly to other food matrices, specific experiments are required to test

for possible interactions between the OPEs and food matrices. The results studying these degradation reactions of OPEs could facilitate a more accurate estimation of the exposure via diet, and provide insights to predict how other OPEs, currently being detected in occurrence studies, would react in the matrices.

6.5 Acknowledgement

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Supplementary Information

Supplementary Text 6.1 – LC analysis gradients

For the model solution, the mobile phase gradient was as follows: 1.0 minute 70% B, from 1.0 to 3.0 minute gradient to 80% B, from 3.0 to 5.0 minute 100% B and hold for 4 minutes, from 9.0 to 9.01 min gradient to 70% B, from 9.1 to 12.0 minute return to 70% B. The total run time for this method was 12.00 minutes.

For the honey solution, the mobile phase gradient was as follows: 0.5 minute 5% B, from 0.5 to 8 minute gradient to 100% B, from 8 to 12 minute 100% B, and from 12 to 12.1 minute gradient to 5% B. The first 2.5 minutes of elution was diverted to waste. The total run time for this method was 15.00 minutes.

For chicken and salmon matrices, the mobile phase gradient was as follows: 0.5 minute 5% B, from 0.5 to 4.0 minute gradient to 100% B and hold for 4 minutes, from 8.0 to 8.01 min gradient to 5% B, from 8.01 to 9.0 minute hold at 5% B. A post-run of 2 minutes was included. The total run time for this method was 11.00 minutes.

Supplementary Text 6.2 – Data analysis workflow for non-targeted analysis

For the non-targeted analysis, MassHunter Profinder (v10.0) from Agilent Technologies was used for molecular feature extraction and peak alignments. The molecular feature extraction was done using the 'Batch Molecular Feature Extraction (Recursive, small molecules)', and the parameters are as follows: peak filter with height ≥300 counts, isotope model was common organic molecules, and the ions and adducts considered were H^+, Na^+, K^+, NH_4^+, CH_3COO^-, and H_2O. The RT tolerance was ±0.05 min, and the mass tolerance was ±10 ppm. The molecular feature extraction score was set at ≥ 70.

Mass Profiler Professional from Agilent Technologies was used to analyze the data extracted. First, the data normalization and baselining options were set to none. Next, the entities were filtered by frequency to be present in at least 80% of samples in at least one condition. Then, a principal component analysis (PCA) plot was used to assess the variability of the data. The number of principal components was 4, and components 1, 2 and 3 were picked for the X-, Y- and Z-axis, respectively. Mass Profiler Professional was also used for non-targeted analysis for possible degradation products of the target OPEs, by fold-change and volcano plot analyses. The fold-change cut-off at 2.0 and the abundance difference cut-off at 10,000, to remove features found in the blank samples. Then, the volcano plot analysis was done with a moderated T-test, with Benjamini-Hochberg multiple testing correction, and the correct p-value cut-off was 0.05, to determine features that had a significantly higher abundance in the heated sample. The chemical formulae were assigned by IDBrowser, a matching score was assigned from 0 to 100 based on the mass accuracy, 100 being the highest.

Table S6.1 – Name, chemical formula, molecular weight, m/z and log K_{ow} of the six target OPEs.

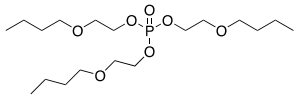
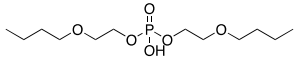
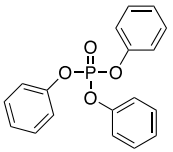
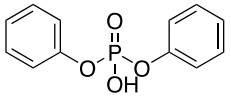
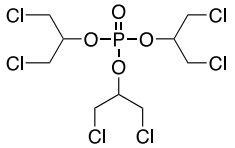
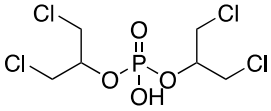
Acronym	Name	CAS#	Chemical formula	Molecular weight	ESI Mode	*m/z*	log K_{ow}	Molecular structure
TBOEP	Tris(2-butoxyethyl) phosphate	78-51-3	$C_{18}H_{39}O_7P$	398.24	+	399.2512	3.75	
BBOEP	Bis(2-butoxyethyl) phosphate	14260-97-0	$C_{12}H_{27}O_6P$	298.31	+	299.1624	2.22	
TPHP	Triphenyl phosphate	115-86-6	$C_{18}H_{15}O_4P$	326.3	+	327.0786	4.59	
DPHP	Diphenyl phosphate	838-85-7	$C_{12}H_{11}O_4P$	250.19	–	249.0317	2.88	
TDCIPP	Tris(1,3-dichloro-2-propyl) phosphate	13674-87-8	$C_9H_{15}Cl_6O_4P$	430.9	+	428.8917	3.65	
BDCIPP	Bis(1,3-dichloro-2-propyl) phosphate	72236-72-7	$C_6H_{11}Cl_4O_4P$	319.94	–	316.9071	1.61	

Table S6.2 – Supplier of internal standards used for extraction.

Compound	Type	*m*/*z*	Supplier
ESI+			
Tris(2-butoxyethyl) phosphate-d27 (TBOEP-d27)	Surrogate standard	426.4201	Toronto Research Chemicals
Triphenyl phosphate-d15 (TPHP-d15)	Surrogate standard	342.1722	Toronto Research Chemicals
Tris(1,3-dichloro-2-propyl) phosphate-d15 (TDCIPP-d15)	Surrogate standard	445.9824	Toronto Research Chemicals
Bis(2-butoxyethyl) phosphate-d8 (BBOEP-d8)	Surrogate standard	307.212	Toronto Research Chemicals
ESI–			
Bis(1,3-dichloro-2-propyl) phosphate-d10 (BDCIPP-d10)	Surrogate standard	316.9076	Toronto Research Chemicals
Diphenyl phosphate-d10 (DPHP-d10)	Surrogate standard	259.0944	Toronto Research Chemicals

Table S6.3 – The limit of detection (LOD), limit of quantification (LOQ), recovery, matrix effects and linearity of target OPEs, in (A) the model solution, (B) the honey matrix, (C) the chicken matrix and (D) the salmon matrix.

(A) Degradation experiment in model solution					
Compounds	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	Recovery	Matrix effect (%)	Linearity (R^2)
TBOEP	1.34	4.47	N.A.	N.A.	0.978
BBOEP	0.326	1.09	N.A.	N.A.	0.999
TPHP	0.0667	0.222	N.A.	N.A.	0.972
DPHP	0.380	1.27	N.A.	N.A.	0.992
TDCIPP	0.00	0.00	N.A.	N.A.	0.999
BDCIPP	0.00	0.00	N.A.	N.A.	0.999

(B) Degradation experiment in honey matrix																
Compounds	LOD (ng g^{-1})	LOQ (ng g^{-1})	Recovery (% ± SD)		Matrix effect (%)						Linearity (%RSD)					
			70 °C	100 °C	70 °C, 0 mins	70 °C, 60 mins	70 °C, 240 mins	100 °C, 0 mins	100 °C, 60 mins	100 °C, 240 mins	70 °C, 0 mins	70 °C, 60 mins	70 °C, 240 mins	100 °C, 0 mins	100 °C, 60 mins	100 °C, 240 mins
TBOEP	4.43	14.8	85.1 ± 0.972	88.0 ± 6.22	86.1	82.5	79.3	76.7	74.3	74.6	17.1	18.1	17.9	17.8	17.2	17.8
BBOEP	2.01	6.70	103 ± 1.79	95.2 ± 1.66	89.0	85.1	83.4	78.5	79.5	76.9	30.0	26.4	20.4	14.3	13.2	25.7
TPHP	0.483	1.61	88.1 ± 0.169	87.8 ± 1.83	85.5	80.4	74.4	73.6	71.4	69.3	26.0	29.1	24.0	26.9	26.7	16.4
DPHP	0.0617	0.206	100 ± 1.60	101 ± 2.26	89.7	92.7	94.6	92.7	94.6	94.5	25.0	26.5	26.3	21.9	24.6	22.6
TDCIPP	4.79	16.0	81.6 ± 3.63	99.4 ± 9.80	79.6	75.5	74.4	66.4	73.7	65.7	30.1	30.9	32.2	24.3	22.9	30.5
BDCIPP	0.0283	0.0943	97.4 ± 3.53	95.4 ± 0.387	88.7	95.8	94.4	92.5	101	99.7	24.0	23.5	25.0	23.7	24.8	27.2

(C) Degradation experiment in chicken matrix											
Compounds	LOD ($ng\ g^{-1}$)	LOQ ($ng\ g^{-1}$)	Recovery (% ± SD)	Matrix effect (%)				Linearity (%RSD)			
			100 °C	100 °C, 0 mins	100 °C, 60 mins	100 °C, 240 mins	100 °C, 240+240 mins	100 °C, 0 mins	100 °C, 60 mins	100 °C, 240 mins	100 °C, 240+240 mins
TBOEP	0.187	0.622	102 ± 11.5	32.7	42.7	35.5	25.3	25.8	25.2	26.1	21.6
BBOEP	2.00	6.68	91.0 ± 2.93	58.4	62.4	56.7	42.1	23.9	21.4	28.6	28.0
TPHP	0.107	0.356	103 ± 0.270	28.1	38.3	30.1	18.1	28.8	27.2	30.3	37.7
DPHP	0.182	0.606	106 ± 2.32	130	140	130	107	31.2	30.2	37.1	25.9
TDCIPP	2.51	8.38	152 ± 83.6	16.8	25.8	19.6	10.0	32.2	38.8	26.4	37.2
BDCIPP	0.107	0.356	106 ± 0.643	94.9	101	97.1	82.8	29.3	24.8	27.5	19.0

(D) Degradation experiment in salmon matrix											
Compounds	LOD ($ng\ g^{-1}$)	LOQ ($ng\ g^{-1}$)	Recovery (% ± SD)	Matrix effect (%)				Linearity (%RSD)			
			100 °C	100 °C, 0 mins	100 °C, 60 mins	100 °C, 240 mins	100 °C, 240+240 mins	100 °C, 0 mins	100 °C, 60 mins	100 °C, 240 mins	100 °C, 240+240 mins
TBOEP	0.691	2.30	94.0 ± 1.79	16.5	20.7	12.4	15.0	18.6	20.4	21.2	26.8
BBOEP	1.31	4.38	89.6 ± 0.906	70.3	64.9	60.0	53.9	26.1	18.2	27.1	22.4
TPHP	0.0548	0.183	98.1 ± 0.563	5.47	8.60	5.03	7.52	30.8	30.3	23.6	28.2
DPHP	0.0767	0.256	105 ± 0.588	118	123	121	118	18.1	21.2	27.5	28.4
TDCIPP	3.71	12.3	125 ± 17.0	7.00	9.99	6.11	8.22	17.2	15.0	26.1	12.0
BDCIPP	0.0552	0.184	106 ± 1.54	106	110	104	92.4	22.9	18.6	20.3	23.0

Table S6.4 – Retention time, the precision of retention time and mass accuracy of internal standards from the controlled migration study extraction.

			Honey matrix			Chicken matrix			Salmon matrix		
Internal standards	ESI mode	*m/z*	Retention time (mins)	RSD% of retention time (%)	Average mass error (ppm)	Retention time (mins)	RSD% of retention time (%)	Average mass error (ppm)	Retention time (mins)	RSD% of retention time (%)	Average mass error (ppm)
TBOEP-d27	+	426.4201	9.71	0.188	1.96	6.18	0.0947	3.76	6.18	0.666	2.37
BBOEP-d8	+	307.212	8.37	0.376	0.803	5.54	0.767	3.41	5.58	1.08	1.46
TPHP-d15	+	342.1722	9.30	0.0954	1.69	5.96	0.217	2.24	5.95	0.673	1.57
DPHP-d10	–	259.095	7.38	0.162	0.983	5.02	0.448	2.35	5.01	0.0997	0.619
TDCIPP-d15	+	445.9824	9.28	0.110	0.993	5.91	0.280	0.0703	5.91	0.716	0.0862
BDCIPP-d10	–	326.9704	8.04	0.257	0.108	5.23	0.591	0.204	5.30	0.150	0.794

Table S6.5 – Summary of precision, degradation percentage and p-values of OPE degradation in (A) the model solution at 100 °C, (B) the model solution at 70°C, (C) the honey solution at 100 °C, (D) the honey solution at 70 °C, (E) the chicken matrix at 100 °C and (F) the salmon matrix at 100 °C.

(A) Degradation in model solution at 100 °C																			
	30 minutes				60 minutes				90 minutes				120 minutes				240 minutes			
OPEs	Precision (%)	Degradation (% ± SD)	*p*-value	Degradation observed	Precision	Degradation (% ± SD)	*p*-value	Degradation observed	Precision	Degradation (% ± SD)	*p*-value	Degradation observed	Precision	Degradation (% ± SD)	*p*-value	Degradation observed	Precision	Degradation (% ± SD)	*p*-value	Degradation observed
TBOEP	37.2	–22.7 ± 25.0	> 0.05	✗	14.9	–48.3 ± 8.21	< 0.05	✓	11.3	–38.4 ± 8.34	< 0.05	✓	16.6	–22.0 ± 16.2	> 0.05	✗	14.3	–26.8 ± 7.24	< 0.05	✓
BBOEP	0.797	0.453 ± 1.05	> 0.05	✗	8.34	–4.54 ± 7.91	> 0.05	✗	1.20	–10.4 ± 0.481	< 0.001	✓	1.92	–7.22 ± 2.28	< 0.05	✓	1.22	–1.62 ± 0.529	< 0.05	✓
TPHP	1.43	–32.4 ± 2.66	< 0.01	✓	5.07	–66.5 ± 0.539	< 0.001	✓	5.99	–81.9 ± 1.75	< 0.01	✓	9.79	–90.1 ± 0.645	< 0.001	✓	56.6	–99.6 ± 0.237	< 0.001	✓
DPHP	1.29	8.64 ± 6.53	> 0.05	✗	1.67	5.09 ± 3.40	> 0.05	✗	3.94	2.65 ±3.90	> 0.05	✗	1.29	11.5 ± 5.96	> 0.05	✗	1.16	9.90 ± 4.41	< 0.05	✓
TDCIPP	3.49	–10.2 ± 6.61	> 0.05	✗	0.794	–23.7 ± 8.49	> 0.05	✗	5.26	–27.6 ± 7.51	< 0.05	✓	5.23	–36.0 ± 6.11	< 0.05	✓	9.37	–54.8 ± 9.19	< 0.05	✓
BDCIPP	19.4	–15.3 ± 15.5	> 0.05	✗	29.0	–47.2 ± 17.7	> 0.05	✗	47.7	–56.3 ± 22.7	> 0.05	✗	6.53	–45.5 ± 3.36	< 0.01	✓	5.74	–71.0 ± 3.31	< 0.01	✓

(B) Degradation in model solution at 70 °C																			
	30 minutes				60 minutes				90 minutes				120 minutes				240 minutes			
OPEs	Precision (%)	Degradation (% ± SD)	*p*-value	Degradation observed	Precision	Degradation (% ± SD)	*p*-value	Degradation observed	Precision	Degradation (% ± SD)	*p*-value	Degradation observed	Precision	Degradation (% ± SD)	*p*-value	Degradation observed	Precision	Degradation (% ± SD)	*p*-value	Degradation observed
TBOEP	29.8	42.3 ± 56.1	> 0.05	✗	24.2	0.917 ± 33.4	> 0.05	✗	14.1	14.3 ± 6.19	> 0.05	✗	11.3	16.9 ± 8.06	> 0.05	✗	20.2	93.8 ± 55.4	> 0.05	✗
BBOEP	0.699	– 0.381 ± 1.10	> 0.05	✗	27.4	–11.6 ± 23.7	> 0.05	✗	1.53	7.28 ± 0.635	< 0.01	✓	0.592	8.11 ± 2.17	< 0.05	✓	14.0	–3.61 ± 13.1	> 0.05	✗
TPHP	5.11	11.7 ± 19.3	> 0.05	✗	5.23	9.21 ± 22.2	> 0.05	✗	0.630	8.04 ± 16.8	> 0.05	✗	4.21	4.16 ± 20.1	> 0.05	✗	13.2	–15.3 ± 12.9	> 0.05	✗
DPHP	2.29	2.12 ± 8.11	> 0.05	✗	1.13	– 0.487 ± 7.06	> 0.05	✗	0.343	0.746 ± 6.93	> 0.05	✗	1.32	–1.47 ± 5.26	> 0.05	✗	1.17	–1.67 ± 7.42	> 0.05	✗
TDCIPP	1.71	–2.30 ± 5.93	> 0.05	✗	13.9	2.71 ± 10.7	> 0.05	✗	8.17	8.63 ± 2.68	< 0.05	✓	3.25	10.7 ± 9.77	> 0.05	✗	16.2	37.5 ± 13.7	> 0.05	✗
BDCIPP	24.9	4.64	> 0.05	✗	1.83	–23.9	> 0.05	✗	25.6	–45.1	> 0.05	✗	54.0	–32.1	> 0.05	✗	8.86	–5.47	> 0.05	✗

(C) Degradation in honey solution at 100 °C								
	60 minutes				240 minutes			
OPEs	Precision (%)	Degradation (% ± SD)	*p*-value	Degradation observed	Precision	Degradation (% ± SD)	*p*-value	Degradation observed
TBOEP	16.8	–32.7 ±11.1	< 0.05	✓	10.9	–20.3 ± 6.67	< 0.05	✓
BBOEP	5.92	–1.06 ± 6.52	> 0.05	✗	3.34	–9.41 ± 5.71	> 0.05	✗
TPHP	11.4	–25.6 ± 8.92	< 0.05	✓	1.94	–18.9 ± 2.94	< 0.01	✓
DPHP	2.73	–4.54 ± 4.54	> 0.05	✗	2.18	15.9 ± 3.32	< 0.01	✓
TDCIPP	9.04	–28.5 ± 11.4	> 0.05	✗	10.2	–22.6 ± 9.79	> 0.05	✗
BDCIPP	4.16	–50.1 ± 2.13	< 0.001	✓	2.88	–75.7 ± 0.796	< 0.001	✓
(D) Degradation in honey solution at 70 °C								
	60 minutes				240 minutes			
OPEs	Precision (%)	Degradation (% ± SD)	*p*-value	Degradation observed	Precision	Degradation (% ± SD)	*p*-value	Degradation observed
TBOEP	2.52	–13.5 ± 2.74	< 0.05	✓	3.59	–15.5 ± 2.38	< 0.01	✓
BBOEP	2.50	–4.86 ± 3.90	> 0.05	✗	5.76	–21.5 ± 4.51	< 0.05	✓
TPHP	0.451	–13.4 ± 0..318	< 0.05	✓	2.34	–12.4 ± 2.22	< 0.001	✓
DPHP	1.04	–0.615 ± 1.42	> 0.05	✗	1.06	–1.75 ± 2.34	> 0.05	✗
TDCIPP	6.16	–1.96 ± 3.95	> 0.05	✗	9.18	–5.84 ± 11.9	> 0.05	✗
BDCIPP	1.78	–4.45 ± 1.88	> 0.05	✗	0.574	–6.32 ± 2.87	> 0.05	✗

(E) Degradation in chicken meat at 100 °C								
	60 minutes				240 minutes			
OPEs	Precision (%)	Degradation (% ± SD)	*p*-value	Degradation observed	Precision	Degradation (% ± SD)	*p*-value	Degradation observed
TBOEP	6.64	–68.23 ± 5.55	< 0.01	✓	8.54	–56.7 ± 4.12	< 0.01	✓
BBOEP	2.58	2.31 ± 3.52	> 0.05	✗	0.218	5.43 ± 2.46	> 0.05	✗
TPHP	6.71	–15.4 ± 5.77	< 0.01	✓	5.29	–45.7 ± 2.99	< 0.001	✓
DPHP	5.20	21.5 ± 4.92	< 0.01	✓	3.72	119 ± 13.1	< 0.01	✓
TDCIPP	11.2	–44.0 ± 33.3	> 0.05	✗	71.8	9.03 ± 145	> 0.05	✗
BDCIPP	7.46	–15.7 ± 6.21	< 0.05	✓	16.0	–59.8 ± 6.50	< 0.01	✓
(F) Degradation in salmon meat at 100 °C								
	60 minutes				240 minutes			
OPEs	Precision (%)	Degradation (% ± SD)	*p*-value	Degradation observed	Precision	Degradation (% ± SD)	*p*-value	Degradation observed
TBOEP	1.78	–1.37 ± 3.51	> 0.05	✗	1.07	–12.0 ± 2.18	< 0.01	✓
BBOEP	1.48	–18.1 ± 2.40	< 0.01	✓	0.458	5.82 ± 0.575	< 0.01	✓
TPHP	1.34	–11.5 ± 1.64	< 0.01	✓	5.52	–17.0 ± 4.58	< 0.05	✓
DPHP	1.11	–6.58 ± 2.98	> 0.05	✗	0.418	–0.304 ± 0.714	> 0.05	✗
TDCIPP	3.05	–28.2 ± 11.8	> 0.05	✗	34.9	–18.7 ± 42.5	> 0.05	✗
BDCIPP	1.47	–30.3 ± 2.26	< 0.01	✓	1.18	–65.4 ± 4.80	< 0.01	✓

Notations:

✓– The degradation observed was statistically significant ($p < 0.05$) and the precision of the concentration was below the degradation %.

✗ – The degradation observed was not statistically significant, $p > 0.05$.

Note: The 'Degradation' column represented the % change in concentrations in comparison with the non-heated sample. A negative % change represented a potential degradation, if the this change was statistically significant, and the precision of the concentration was also below this degradation %; a positive % change meant that there was an increase in concentration after heating.

Table S6.6 – Summary of rate constant (k), half-life and R2 of the fitted linear curves for the degradation of target OPEs at 70 °C in the honey matrix.

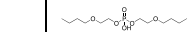
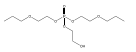
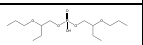
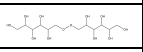
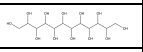
OPEs	Degradation	k (min^{-1})	$t_{1/2}$ (mins)	R^2
TBOEP	✓	0.0006	1160	0.6123
BBOEP	✓	0.001	693	0.9568
TPHP	✓	0.0004	1730	0.4077
DPHP	✗	N.A.	N.A.	N.A.
TDCIPP	✗	N.A.	N.A.	N.A.
BDCIPP	✗	N.A.	N.A.	N.A.

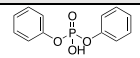
Notations:

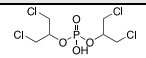
✓– The degradation observed was statistically significant ($p < 0.05$) and the precision of the concentration was below the degradation %.

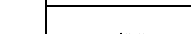
✗ – The degradation observed was not statistically significant, $p > 0.05$.

Table S6.7 – Summary of possible degradation products in the model solution, after heating for 240 minutes at 100 °C, of (A) TBOEP, (B) TPHP, (C) TDCIPP and (D) BDCIPP.

(A) TBOEP										
Degradation product (DP)	*m/z*	R_t (mins)	ESI mode	Chemical formula	Formula matching score	Mass accuracy (ppm)	Mass difference with parent OPE	Detection in blank	Proposed structure by *SIRIUS*	Structure matching score (%)
BBOEP*	299.1629	1.79	+	$C_{12}H_{27}O_6P$	95.56	3.68	100.0883	No		80.0
TBOEP-DP1	219.1955	3.62	+	$C_{12}H_{26}O_3$	99.73	0.05	–180.0557	No	No	N.A.
TBOEP-DP2* (BTBOEP)	343.1895	2.29	+	$C_{14}H_{31}O_7P$	93.43	4.47	–56.0617	No		83.00
TBOEP-DP3	355.2244	4.63	+	$C_{16}H_{35}O_6P$	99.75	–0.01	–44.0268	No		66.37
TBOEP-DP4	363.1536	3.63	+	$C_{12}H_{26}O_{12}$	89.68	5.08	–36.0976	No		52.56
										51.28
TBOEP-DP5	435.3314	2.17	+	$C_{23}H_{46}O_7$	98.99	0.45	+36.0802	No		40.59
TBOEP-DP6	472.3395	4.79	+	$C_{26}H_{47}O_7$	99.18	–0.07	+73.0883	No	No	N.A.
TBOEP-DP7	623.3315	5.24	+	$C_{26}H_{56}O_{12}P_2$	99.26	–0.62	+224.0803	No	No	N.A.

(B) TPHP										
Degradation product (DP)	*m*/*z*	R_t (mins)	ESI mode	Chemical formula	Formula matching score	Mass accuracy (ppm)	Mass difference with parent OPE	Detection in blank	Proposed structure by *SIRIUS*	Structure matching score (%)
DPHP*	249.0326	1.17	–	$C_{12}H_{11}O_4P$	98.89	1.50	–77.046	Yes		90.65
TPHP-DP1	145.0023	1.27	+	$C_6H_9O_{11}P$	92.32	2.55	–181.0763	No	No	N.A.
TPHP-DP2	153.0708	1.27	+	$C_{12}H_{10}O$	93.76	2.54	–173.0078	No	No	N.A.
TPHP-DP3	165.5252	1.27	+	$C_{12}H_9O_{11}$	95.87	3.03	–160.5534	No	No	N.A.
TPHP-DP4	358.1207	3.71	+	$C_{23}H_{17}O_4$	97.05	2.39	+32.0421	No	No	N.A.
TPHP-DP5	525.0503	1.27	+	$C_{25}H_{20}O_{10}P_2$	99.21	0.53	+198.9717	No	No	N.A.

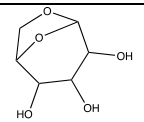
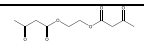
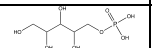
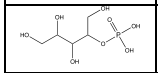
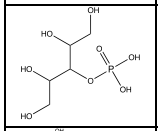
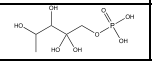
(C) TDCIPP										
Degradation product (DP)	*m*/*z*	R_t (mins)	ESI mode	Chemical formula	Formula matching score	Mass accuracy (ppm)	Mass difference with parent OPE	Detection in blank	Proposed structure by *SIRIUS*	Structure matching score (%)
BDCIPP*	316.9074	1.40	–	$C_6H_{11}Cl_4O_4P$	97.62	–1.13	–109.9669	No		56.79

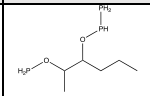
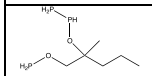
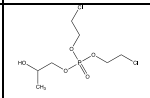
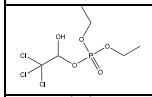
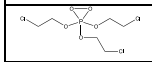
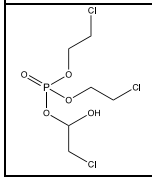
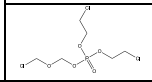
(D) BDCIPP										
Degradation product (DP)	*m/z*	R_t (mins)	ESI mode	Chemical formula	Formula matching score	Mass accuracy (ppm)	Mass difference with parent OPE	Detection in blank	Proposed structure by *SIRIUS*	Structure matching score (%)
BDCIPP-DP1	349.1153	0.93	+	$C_{15}H_{28}OP_4$	94.09	–2.77	+30.1903	No	No	N.A.
BDCIPP-DP2	667.5631	6.01	+	$C_{41}H_{81}P_3$	95.89	–1.21	+348.6381	No	No	N.A.
BDCIPP-DP3	677.5840	6.10	+	$C_{39}H_{81}O_6$ P	98.34	–0.96	+358.6590	No	No	N.A.
BDCIPP-DP4	691.5983	6.15	+	$C_{40}H_{83}O_6P$	92.14	–2.37	+372.5983	No		37.98
										38.26
										39.35

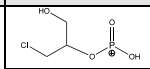
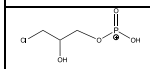
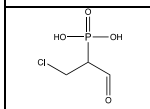
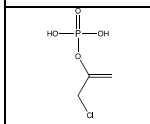
N.A.: Non-applicable

*: Confirmed with reference standards

Table S6.8 – Summary of possible degradation products of OPEs in the food matrices after heating for 240 minutes in (A) honey at 100 °C, (B) honey at 70 °C, (C) chicken at 100 °C and (D) salmon at 100 °C.

(A) Honey at 100 °C										
Degradation product (DP)	*m/z*	R_t (mins)	ESI mode	Chemical formula	Formula matching score	Mass accuracy (ppm)	Detection in blank	Detection in non-spiked sample	Proposed structure by *SIRIUS*	Structure matching score (%)
H100-DP1	85.0289	2.79	+	$C_4H_4O_2$	99.6	0.92	No	No	No	N.A.
H100-DP2	157.0866	5.05	–	$C_8H_{14}O_3$	87.04	–0.35	No	No	No	N.A.
H100-DP3	163.0609	2.78	+	$C_6H_{10}O_5$	99.35	2.06	No	No		81.69
H100-DP4	231.0843	3.59	+	$C_{10}H_{14}O_6$	82.64	–6.95	No	No		22.50
H100-DP5	233.0427	2.79	+	$C_5H_{13}O_8P$	83.49	1.32	No	No		78.86
										71.43
										72.95
										69.53

(B) Honey at 70 °C										
Degradation product (DP)	*m/z*	R_t (mins)	ESI mode	Chemical formula	Formula matching score	Mass accuracy (ppm)	Detection in blank	Detection in non-spiked sample	Proposed structure by *SIRIUS*	Structure matching score (%)
H70-DP1	215.0517	4.1	+	$C_6H_{17}O_2P_3$	82.2	2.75	No	No		17.72
										17.05
H70-DP2	298.9405	6.76	–	$C_6H_{12}Cl_3O_5P$	98.91	0.03	No	No		48.94
										50.55
										43.37
										50.55
										48.31

(C) Chicken at 100 °C										
Degradation product (DP)	*m/z*	R_t (mins)	ESI mode	Chemical formula	Formula matching score	Mass accuracy (ppm)	Detection in blank	Detection in non-spiked sample	Proposed structure by *SIRIUS*	Structure matching score (%)
C100-DP1	170.9614	4.71	–	$C_3H_6ClO_4P$	99.4	–0.04	No	No		55.56
										60.20
										36.79
										45.36
C100-DP2	198.1132	3.13	+	No formula	N.A.	N.A.	No	No	N.A.	N.A.
C100-DP3	199.1082	4.84	–	No formula	N.A.	N.A.	No	No	N.A.	N.A.
C100-DP4	229.0546	3.61	–	No formula	N.A.	N.A.	No	No	N.A.	N.A.
C100-DP5	298.9412	4.74	–	No formula	N.A.	N.A.	No	No	N.A.	N.A.
C100-DP6	299.1908	2.69	+	No formula	N.A.	N.A.	No	No	N.A.	N.A.
C100-DP7	302.9354	4.74	–	No formula	N.A.	N.A.	No	No	N.A.	N.A.
C100-DP8	390.9437	5.22	–	$C_9H_{17}Cl_4O_6P$	99.31	–0.58	No	No	No	N.A.

(D) Salmon at 100 °C										
Degradation product (DP)	*m/z*	R_t (mins)	ESI mode	Chemical formula	Formula matching score	Mass accuracy (ppm)	Detection in blank	Detection in non-spiked sample	Proposed structure by *SIRIUS*	Structure matching score (%)
S100-DP1	280.9746	3.6	–	$C_6H_{13}Cl_2O_6P$	99.14	–0.5	No	No	No	N.A.

N.A.: Non-applicable

Table S6.9 – The results of the reheating experiment on the effect of cooked (A) chicken and (B) salmon matrices on the degradation of OPEs. (n = 3 ± SD).

(A) Chicken heated at 100 °C							
OPEs	Spiked on non-heated matrix			Spiked on heated matrix (for 240 minutes)			Statistical significance
	Concentration without heating (ng g^{-1} ± SD)	Concentration with heating for 240 minutes (ng g^{-1} ± SD)	Degradation (% ± SD)	Concentration without reheating (ng g^{-1})	Concentration with reheating for an additional 240 minutes (ng g^{-1})	Degradation (% ± SD)	
TBOEP	1051 ± 114	455 ± 38.9	–56.7 ± 4.12	408 ± 22.6	450 ± 19.3	–10.1 ± 2.25	***p* < 0.001**
BBOEP	911 ± 30.5	961 ± 39.5	5.43 ± 2.46	824 ± 20.2	916 ± 86.0	–11.1 ± 12.4	$p > 0.05$
TPHP	1028 ± 2.70	558 ± 29.6	–45.7 ± 2.99	1103 ± 18.6	571 ± 12.3	–48.3 ± 0.265	$p > 0.05$
DPHP	1060 ± 23.2	2323 ± 86.4	119 ± 13.1	1113 ± 49.1	2410 ± 34.4	+116 ± 11.9	$p > 0.05$
TDCIPP	1519 ± 834	1656 ± 1190	9.03 ± 145	1188 ± 459	2258 ± 1490	90 ± 190	$p > 0.05$
BDCIPP	1054 ± 6.45	424 ± 68.0	–59.8 ± 6.50	1078 ± 8.15	379 ± 24.3	–64.8 ± 2.36	$p > 0.05$

(B) Salmon heated at 100 °C							
OPEs	Spiked on non-heated matrix			Spiked on heated matrix (for 240 minutes)			Statistical significance
	Concentration without heating (ng g^{-1} ± SD)	Concentration with heating for 240 minutes (ng g^{-1} ± SD)	Degradation (% ± SD)	Concentration without reheating (ng g^{-1})	Concentration with reheating for an additional 240 minutes (ng g^{-1})	Degradation (% ± SD)	
TBOEP	939 ± 17.9	827 ± 8.86	–12.0 ± 2.18	958 ± 26.1	977 ± 28.0	1.97 ± 0.717	***p* < 0.01**
BBOEP	899 ± 7.47	952 ± 11.7	5.82 ± 0.575	713 ± 47.4	762 ± 88.7	6.75 ± 8.88	$p > 0.05$
TPHP	980 ± 5.63	813 ± 44.9	–17.0 ± 4.58	1012 ± 43.8	879 ± 18.4	–13.2 ± 2.46	$p > 0.05$
DPHP	1045 ± 5.89	1042 ± 13.1	–0.304 ± 0.714	810 ± 32.4	1132 ± 33.2	39.6 ± 6.26	***p* < 0.05**
TDCIPP	1255 ± 1190	1020 ± 356	–18.7 ± 42.5	1057 ± 194	1168 ± 211	10.5 ± 43.0	$p > 0.05$
BDCIPP	1063 ± 15.4	368 ± 50.5	–65.4 ± 4.80	1093 ± 17.5	364 ± 10.3	–66.7 ± 0.821	$p > 0.05$

Note: The ‘Degradation’ column represented the % change in concentrations in comparison with the non-heated sample. A negative % change represented a decrease in concentration after heating; a positive % change meant that there was an increase in concentration after heating.

Table S6.10 – SPSS output of paired-sample t-test for comparison of concentrations of OPEs in the model aqueous solution heated in different durations.

		Paired Differences					*t*	df	Significance	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				One–Sided *p*	Two–Sided *p*
					Lower	Upper				
Pair 1	TBOEP 100C 0M – TBOEP 100C 30M	22.9338	24.8856	14.3677	–38.8854	84.7530	1.60	2	.13	.25
Pair 2	TBOEP 100C 0M – TBOEP 100C 60M	48.8102	8.8973	5.1369	26.7079	70.9124	9.50	2	.01	.01
Pair 3	TBOEP 100C 0M – TBOEP 100C 90M	38.7601	9.2924	5.3650	15.6764	61.8438	7.22	2	.01	.02
Pair 4	TBOEP 100C 0M – TBOEP 100C 120M	22.2046	17.3265	10.0035	–20.8369	65.2460	2.22	2	.08	.16
Pair 5	TBOEP 100C 0M – TBOEP 100C 240M	27.0817	6.1919	3.5749	11.7002	42.4631	7.58	2	.01	.02
Pair 6	TBOEP 100C 30M – TBOEP 100C 60M	25.8764	26.9551	15.5625	–41.0837	92.8365	1.66	2	.12	.24
Pair 7	TBOEP 100C 30M – TBOEP 100C 90M	15.8263	29.1041	16.8033	–56.4722	88.1249	.94	2	.22	.45
Pair 8	TBOEP 100C 30M – TBOEP 100C 120M	–.7292	39.6811	22.9099	–99.3026	97.8441	–.03	2	.49	.98
Pair 9	TBOEP 100C 30M – TBOEP 100C 240M	4.1479	20.7397	11.9741	–47.3725	55.6682	.35	2	.38	.76
Pair 10	TBOEP 100C 60M – TBOEP 100C 90M	–10.0501	2.2764	1.3143	–15.7049	–4.3953	–7.65	2	.01	.02
Pair 11	TBOEP 100C 60M – TBOEP 100C 120M	–26.6056	12.8215	7.4025	–58.4560	5.2448	–3.59	2	.03	.07

Pair 12	TBOEP 100C 60M – TBOEP 100C 240M	–21.7285	14.0301	8.1003	–56.5813	13.1243	–2.68	2	.06	.12
Pair 13	TBOEP 100C 90M – TBOEP 100C 120M	–16.5555	10.6010	6.1205	–42.8898	9.7787	–2.70	2	.06	.11
Pair 14	TBOEP 100C 90M – TBOEP 100C 240M	–11.6784	14.9857	8.6520	–48.9050	25.5482	–1.35	2	.15	.31
Pair 15	TBOEP 100C 120M – TBOEP 100C 240M	4.8771	23.5160	13.5770	–53.5399	63.2941	.36	2	.38	.75
Pair 16	BBOEP 100C 0M – BBOEP 100C 30M	–.5262	1.2181	.7033	–3.5522	2.4997	–.75	2	.27	.53
Pair 17	BBOEP 100C 0M – BBOEP 100C 60M	5.2731	9.1279	5.2700	–17.4020	27.9481	1.00	2	.21	.42
Pair 18	BBOEP 100C 0M – BBOEP 100C 90M	12.0818	.5487	.3168	10.7187	13.4449	38.14	2	<.001	<.001
Pair 19	BBOEP 100C 0M – BBOEP 100C 120M	8.3823	2.6871	1.5514	1.7072	15.0573	5.40	2	.02	.03
Pair 20	BBOEP 100C 0M – BBOEP 100C 240M	–1.8807	.6156	.3554	–3.4100	–.3515	–5.29	2	.02	.03
Pair 21	BBOEP 100C 30M – BBOEP 100C 60M	5.7993	8.3254	4.8067	–14.8820	26.4807	1.21	2	.18	.35
Pair 22	BBOEP 100C 30M – BBOEP 100C 90M	12.6081	1.6481	.9516	8.5139	16.7023	13.25	2	.00	.01
Pair 23	BBOEP 100C 30M – BBOEP 100C 120M	8.9085	2.9917	1.7272	1.4768	16.3402	5.16	2	.02	.04
Pair 24	BBOEP 100C 30M – BBOEP 100C 240M	–1.3545	1.0566	.6100	–3.9792	1.2702	–2.22	2	.08	.16
Pair 25	BBOEP 100C 60M – BBOEP 100C 90M	6.8087	9.6767	5.5868	–17.2294	30.8469	1.22	2	.17	.35
Pair 26	BBOEP 100C 60M – BBOEP 100C 120M	3.1092	11.2691	6.5062	–24.8847	31.1031	.48	2	.34	.68

Pair 27	BBOEP 100C 60M – BBOEP 100C 240M	–7.1538	8.5328	4.9264	–28.3505	14.0428	–1.45	2	.14	.28
Pair 28	BBOEP 100C 90M – BBOEP 100C 120M	–3.6996	2.3088	1.3330	–9.4350	2.0359	–2.78	2	.05	.11
Pair 29	BBOEP 100C 90M – BBOEP 100C 240M	–13.9626	1.1553	.6670	–16.8324	–11.0927	–20.93	2	.00	.00
Pair 30	BBOEP 100C 120M – BBOEP 100C 240M	–10.2630	3.2448	1.8734	–18.3235	–2.2025	–5.48	2	.02	.03
Pair 31	TPHP 100C 0M – TPHP 100C 30M	36.6973	4.4484	2.5683	25.6468	47.7479	14.29	2	.00	.00
Pair 32	TPHP 100C 0M – TPHP 100C 60M	75.2093	2.9000	1.6743	68.0052	82.4134	44.92	2	<.001	<.001
Pair 33	TPHP 100C 0M – TPHP 100C 90M	92.7283	5.6418	3.2573	78.7132	106.7435	28.47	2	<.001	.00
Pair 34	TPHP 100C 0M – TPHP 100C 120M	101.9726	3.7598	2.1707	92.6327	111.3125	46.98	2	<.001	<.001
Pair 35	TPHP 100C 0M – TPHP 100C 240M	112.6580	4.4481	2.5681	101.6083	123.7077	43.87	2	<.001	<.001
Pair 36	TPHP 100C 30M – TPHP 100C 60M	38.5120	1.5567	.8987	34.6450	42.3789	42.85	2	<.001	<.001
Pair 37	TPHP 100C 30M – TPHP 100C 90M	56.0310	2.2569	1.3030	50.4245	61.6375	43.00	2	<.001	<.001
Pair 38	TPHP 100C 30M – TPHP 100C 120M	65.2753	.7952	.4591	63.3000	67.2506	142.19	2	<.001	<.001
Pair 39	TPHP 100C 30M – TPHP 100C 240M	75.9607	1.1854	.6844	73.0160	78.9054	110.99	2	<.001	<.001
Pair 40	TPHP 100C 60M – TPHP 100C 90M	17.5190	3.0647	1.7694	9.9059	25.1322	9.90	2	.01	.01
Pair 41	TPHP 100C 60M – TPHP 100C 120M	26.7633	.8772	.5064	24.5843	28.9423	52.85	2	<.001	<.001

Pair 42	TPHP 100C 60M – TPHP 100C 240M	37.4487	1.7417	1.0056	33.1220	41.7754	37.24	2	<.001	<.001
Pair 43	TPHP 100C 90M – TPHP 100C 120M	9.2443	2.2985	1.3271	3.5344	14.9542	6.97	2	.01	.02
Pair 44	TPHP 100C 90M – TPHP 100C 240M	19.9297	1.3307	.7683	16.6239	23.2354	25.94	2	<.001	.00
Pair 45	TPHP 100C 120M – TPHP 100C 240M	10.6854	.9778	.5645	8.2564	13.1144	18.93	2	.00	.00
Pair 46	DPHP 100C 0M – DPHP 100C 30M	–8.8688	6.1646	3.5592	–24.1826	6.4450	–2.49	2	.07	.13
Pair 47	DPHP 100C 0M – DPHP 100C 60M	–5.2268	3.1586	1.8236	–13.0733	2.6197	–2.87	2	.05	.10
Pair 48	DPHP 100C 0M – DPHP 100C 90M	–2.7208	4.0242	2.3234	–12.7176	7.2759	–1.17	2	.18	.36
Pair 49	DPHP 100C 0M – DPHP 100C 120M	–11.7924	5.5164	3.1849	–25.4959	1.9112	–3.70	2	.03	.07
Pair 50	DPHP 100C 0M – DPHP 100C 240M	–10.1593	4.0123	2.3165	–20.1263	–.1922	–4.39	2	.02	.05
Pair 51	DPHP 100C 30M – DPHP 100C 60M	3.6420	3.2285	1.8640	–4.3780	11.6620	1.95	2	.09	.19
Pair 52	DPHP 100C 30M – DPHP 100C 90M	6.1480	4.8258	2.7862	–5.8400	18.1360	2.21	2	.08	.16
Pair 53	DPHP 100C 30M – DPHP 100C 120M	–2.9235	1.2396	.7157	–6.0028	.1558	–4.08	2	.03	.06
Pair 54	DPHP 100C 30M – DPHP 100C 240M	–1.2904	2.3141	1.3360	–7.0389	4.4581	–.97	2	.22	.44
Pair 55	DPHP 100C 60M – DPHP 100C 90M	2.5060	4.0198	2.3208	–7.4797	12.4916	1.08	2	.20	.39
Pair 56	DPHP 100C 60M – DPHP 100C 120M	–6.5655	3.0025	1.7335	–14.0243	.8932	–3.79	2	.03	.06

Pair 57	DPHP 100C 60M – DPHP 100C 240M	–4.9324	1.7691	1.0214	–9.3272	–.5377	–4.83	2	.02	.04
Pair 58	DPHP 100C 90M – DPHP 100C 120M	–9.0715	3.6268	2.0939	–18.0810	–.0620	–4.33	2	.02	.05
Pair 59	DPHP 100C 90M – DPHP 100C 240M	–7.4384	2.8581	1.6501	–14.5382	–.3386	–4.51	2	.02	.05
Pair 60	DPHP 100C 120M – DPHP 100C 240M	1.6331	1.5049	.8689	–2.1054	5.3716	1.88	2	.10	.20
Pair 61	TDCIPP 100C 0M – TDCIPP 100C 30M	5.9098	4.3689	2.5224	–4.9431	16.7627	2.34	2	.07	.14
Pair 62	TDCIPP 100C 0M – TDCIPP 100C 60M	13.7619	6.1078	3.5263	–1.4107	28.9345	3.90	2	.03	.06
Pair 63	TDCIPP 100C 0M – TDCIPP 100C 90M	16.0079	5.5439	3.2008	2.2360	29.7799	5.00	2	.02	.04
Pair 64	TDCIPP 100C 0M – TDCIPP 100C 120M	20.8909	5.5606	3.2104	7.0776	34.7042	6.51	2	.01	.02
Pair 65	TDCIPP 100C 0M – TDCIPP 100C 240M	31.7995	8.3606	4.8270	11.0307	52.5682	6.59	2	.01	.02
Pair 66	TDCIPP 100C 30M – TDCIPP 100C 60M	7.8521	2.1391	1.2350	2.5381	13.1660	6.36	2	.01	.02
Pair 67	TDCIPP 100C 30M – TDCIPP 100C 90M	10.0981	3.0429	1.7568	2.5392	17.6571	5.75	2	.01	.03
Pair 68	TDCIPP 100C 30M – TDCIPP 100C 120M	14.9811	1.3801	.7968	11.5528	18.4094	18.80	2	.00	.00
Pair 69	TDCIPP 100C 30M – TDCIPP 100C 240M	25.8896	4.1576	2.4004	15.5615	36.2178	10.79	2	.00	.01
Pair 70	TDCIPP 100C 60M – TDCIPP 100C 90M	2.2460	2.0293	1.1716	–2.7950	7.2871	1.92	2	.10	.20
Pair 71	TDCIPP 100C 60M – TDCIPP 100C 120M	7.1290	2.2854	1.3195	1.4516	12.8064	5.40	2	.02	.03

Pair 72	TDCIPP 100C 60M – TDCIPP 100C 240M	18.0376	2.2799	1.3163	12.3740	23.7011	13.70	2	.00	.01
Pair 73	TDCIPP 100C 90M – TDCIPP 100C 120M	4.8830	3.9102	2.2576	–4.8306	14.5965	2.16	2	.08	.16
Pair 74	TDCIPP 100C 90M – TDCIPP 100C 240M	15.7915	3.8395	2.2167	6.2537	25.3293	7.12	2	.01	.02
Pair 75	TDCIPP 100C 120M – TDCIPP 100C 240M	10.9086	3.6027	2.0800	1.9590	19.8581	5.24	2	.02	.03
Pair 76	BDCIPP 100C 0M – BDCIPP 100C 30M	11.5339	12.1056	6.9892	–18.5380	41.6059	1.65	2	.12	.24
Pair 77	BDCIPP 100C 0M – BDCIPP 100C 60M	35.7524	14.9014	8.6033	–1.2646	72.7694	4.16	2	.03	.05
Pair 78	BDCIPP 100C 0M – BDCIPP 100C 90M	42.4701	18.6871	10.7890	–3.9513	88.8914	3.94	2	.03	.06
Pair 79	BDCIPP 100C 0M – BDCIPP 100C 120M	34.2980	3.7342	2.1559	25.0218	43.5742	15.91	2	.00	.00
Pair 80	BDCIPP 100C 0M – BDCIPP 100C 240M	53.5394	5.3637	3.0967	40.2153	66.8634	17.29	2	.00	.00
Pair 81	BDCIPP 100C 30M – BDCIPP 100C 60M	24.2185	22.7464	13.1326	–32.2867	80.7237	1.84	2	.10	.21
Pair 82	BDCIPP 100C 30M – BDCIPP 100C 90M	30.9361	13.0456	7.5319	–1.4709	63.3431	4.11	2	.03	.05
Pair 83	BDCIPP 100C 30M – BDCIPP 100C 120M	22.7640	9.8006	5.6584	–1.5821	47.1102	4.02	2	.03	.06
Pair 84	BDCIPP 100C 30M – BDCIPP 100C 240M	42.0054	13.0808	7.5522	9.5108	74.5000	5.56	2	.02	.03
Pair 85	BDCIPP 100C 60M – BDCIPP 100C 90M	6.7177	19.6021	11.3173	–41.9768	55.4121	.59	2	.31	.61
Pair 86	BDCIPP 100C 60M – BDCIPP 100C 120M	–1.4544	14.0569	8.1157	–36.3737	33.4648	–.18	2	.44	.87

Pair 87	BDCIPP 100C 60M – BDCIPP 100C 240M	17.7870	10.3638	5.9836	–7.9583	43.5322	2.97	2	.05	.10
Pair 88	BDCIPP 100C 90M – BDCIPP 100C 120M	–8.1721	14.9532	8.6332	–45.3178	28.9737	–.95	2	.22	.44
Pair 89	BDCIPP 100C 90M – BDCIPP 100C 240M	11.0693	15.3028	8.8351	–26.9449	49.0835	1.25	2	.17	.34
Pair 90	BDCIPP 100C 120M – BDCIPP 100C 240M	19.2414	3.7020	2.1374	10.0451	28.4377	9.00	2	.01	.01
Pair 91	TBOEP 70C 0M – TBOEP 70C 30M	–30.9270	37.3035	21.5372	–123.5941	61.7401	–1.44	2	.14	.29
Pair 92	TBOEP 70C 0M – TBOEP 70C 60M	–.6697	23.3256	13.4670	–58.6138	57.2743	–.05	2	.48	.96
Pair 93	TBOEP 70C 0M – TBOEP 70C 90M	–10.4303	5.2380	3.0242	–23.4422	2.5817	–3.45	2	.04	.07
Pair 94	TBOEP 70C 0M – TBOEP 70C 120M	–12.3226	5.8574	3.3817	–26.8731	2.2279	–3.64	2	.03	.07
Pair 95	TBOEP 70C 0M – TBOEP 70C 240M	–68.5333	33.5064	19.3449	–151.7679	14.7012	–3.54	2	.04	.07
Pair 96	TBOEP 70C 30M – TBOEP 70C 60M	30.2573	15.2673	8.8146	–7.6688	68.1834	3.43	2	.04	.08
Pair 97	TBOEP 70C 30M – TBOEP 70C 90M	20.4967	42.5359	24.5581	–85.1683	126.1618	.83	2	.25	.49
Pair 98	TBOEP 70C 30M – TBOEP 70C 120M	18.6044	40.4570	23.3579	–81.8963	119.1051	.80	2	.25	.51
Pair 99	TBOEP 70C 30M – TBOEP 70C 240M	–37.6063	12.6777	7.3195	–69.0995	–6.1132	–5.14	2	.02	.04
Pair 100	TBOEP 70C 60M – TBOEP 70C 90M	–9.7605	28.5095	16.4600	–80.5822	61.0611	–.59	2	.31	.61
Pair 101	TBOEP 70C 60M – TBOEP 70C 120M	–11.6529	27.5130	15.8846	–79.9989	56.6931	–.73	2	.27	.54

Pair 102	TBOEP 70C 60M – TBOEP 70C 240M	–67.8636	10.8594	6.2697	–94.8400	–40.8873	–10.82	2	.00	.01
Pair 103	TBOEP 70C 90M – TBOEP 70C 120M	–1.8923	5.4323	3.1363	–15.3869	11.6022	–.60	2	.30	.61
Pair 104	TBOEP 70C 90M – TBOEP 70C 240M	–58.1031	38.5491	22.2563	–153.8643	37.6582	–2.61	2	.06	.12
Pair 105	TBOEP 70C 120M – TBOEP 70C 240M	–56.2107	38.0949	21.9941	–150.8438	38.4223	–2.56	2	.06	.13
Pair 106	BBOEP 70C 0M – BBOEP 70C 30M	.4601	1.3429	.7753	–2.8757	3.7959	.59	2	.31	.61
Pair 107	BBOEP 70C 0M – BBOEP 70C 60M	13.9848	28.3289	16.3557	–56.3879	84.3576	.86	2	.24	.48
Pair 108	BBOEP 70C 0M – BBOEP 70C 90M	–8.7954	.7497	.4328	–10.6577	–6.9331	–20.32	2	.00	.00
Pair 109	BBOEP 70C 0M – BBOEP 70C 120M	–9.8017	2.4937	1.4397	–15.9963	–3.6070	–6.81	2	.01	.02
Pair 110	BBOEP 70C 0M – BBOEP 70C 240M	4.3648	15.7798	9.1105	–34.8344	43.5641	.48	2	.34	.68
Pair 111	BBOEP 70C 30M – BBOEP 70C 60M	13.5247	28.4406	16.4202	–57.1256	84.1750	.82	2	.25	.50
Pair 112	BBOEP 70C 30M – BBOEP 70C 90M	–9.2555	1.2288	.7095	–12.3081	–6.2029	–13.05	2	.00	.01
Pair 113	BBOEP 70C 30M – BBOEP 70C 120M	–10.2618	1.6150	.9324	–14.2737	–6.2498	–11.01	2	.00	.01
Pair 114	BBOEP 70C 30M – BBOEP 70C 240M	3.9047	16.6642	9.6211	–37.4914	45.3008	.41	2	.36	.72
Pair 115	BBOEP 70C 60M – BBOEP 70C 90M	–22.7802	27.6727	15.9769	–91.5231	45.9626	–1.43	2	.15	.29
Pair 116	BBOEP 70C 60M – BBOEP 70C 120M	–23.7865	29.9766	17.3070	–98.2524	50.6794	–1.37	2	.15	.30

Pair 117	BBOEP 70C 60M – BBOEP 70C 240M	–9.6200	41.3220	23.8573	–112.2696	93.0295	–.40	2	.36	.73
Pair 118	BBOEP 70C 90M – BBOEP 70C 120M	–1.0063	2.7367	1.5800	–7.8047	5.7921	–.64	2	.29	.59
Pair 119	BBOEP 70C 90M – BBOEP 70C 240M	13.1602	16.5075	9.5306	–27.8466	54.1670	1.38	2	.15	.30
Pair 120	BBOEP 70C 120M – BBOEP 70C 240M	14.1665	16.1172	9.3053	–25.8709	54.2039	1.52	2	.13	.27
Pair 121	TPHP 70C 0M – TPHP 70C 30M	–17.6696	24.3925	14.0830	–78.2640	42.9247	–1.25	2	.17	.34
Pair 122	TPHP 70C 0M – TPHP 70C 60M	–13.9568	29.9667	17.3013	–88.3982	60.4846	–.81	2	.25	.50
Pair 123	TPHP 70C 0M – TPHP 70C 90M	–12.1873	22.3335	12.8942	–67.6668	43.2922	–.95	2	.22	.44
Pair 124	TPHP 70C 0M – TPHP 70C 120M	–6.3052	27.9416	16.1321	–75.7159	63.1055	–.39	2	.37	.73
Pair 125	TPHP 70C 0M – TPHP 70C 240M	23.1825	23.1799	13.3829	–34.3996	80.7647	1.73	2	.11	.23
Pair 126	TPHP 70C 30M – TPHP 70C 60M	3.7128	11.4652	6.6194	–24.7683	32.1939	.56	2	.32	.63
Pair 127	TPHP 70C 30M – TPHP 70C 90M	5.4824	8.6440	4.9906	–15.9906	26.9553	1.10	2	.19	.39
Pair 128	TPHP 70C 30M – TPHP 70C 120M	11.3645	10.1793	5.8770	–13.9224	36.6513	1.93	2	.10	.19
Pair 129	TPHP 70C 30M – TPHP 70C 240M	40.8522	25.5475	14.7499	–22.6114	104.3157	2.77	2	.05	.11
Pair 130	TPHP 70C 60M – TPHP 70C 90M	1.7696	7.6386	4.4101	–17.2057	20.7448	.40	2	.36	.73
Pair 131	TPHP 70C 60M – TPHP 70C 120M	7.6516	2.0307	1.1724	2.6070	12.6963	6.53	2	.01	.02

Pair 132	TPHP 70C 60M – TPHP 70C 240M	37.1394	20.6976	11.9498	–14.2763	88.5551	3.11	2	.04	.09
Pair 133	TPHP 70C 90M – TPHP 70C 120M	5.8821	5.6100	3.2389	–8.0539	19.8181	1.82	2	.11	.21
Pair 134	TPHP 70C 90M – TPHP 70C 240M	35.3698	17.1348	9.8928	–7.1954	77.9350	3.58	2	.04	.07
Pair 135	TPHP 70C 120M – TPHP 70C 240M	29.4877	19.5917	11.3113	–19.1808	78.1562	2.61	2	.06	.12
Pair 136	DPHP 70C 0M – DPHP 70C 30M	–2.2350	8.2394	4.7570	–22.7029	18.2328	–.47	2	.34	.68
Pair 137	DPHP 70C 0M – DPHP 70C 60M	.5132	7.2833	4.2050	–17.5796	18.6060	.12	2	.46	.91
Pair 138	DPHP 70C 0M – DPHP 70C 90M	–.7858	7.0065	4.0452	–18.1910	16.6194	–.19	2	.43	.86
Pair 139	DPHP 70C 0M – DPHP 70C 120M	1.5499	5.4382	3.1397	–11.9593	15.0590	.49	2	.34	.67
Pair 140	DPHP 70C 0M – DPHP 70C 240M	1.7557	7.6453	4.4140	–17.2361	20.7476	.40	2	.36	.73
Pair 141	DPHP 70C 30M – DPHP 70C 60M	2.7483	1.2819	.7401	–.4361	5.9327	3.71	2	.03	.07
Pair 142	DPHP 70C 30M – DPHP 70C 90M	1.4492	2.3356	1.3484	–4.3527	7.2511	1.07	2	.20	.39
Pair 143	DPHP 70C 30M – DPHP 70C 120M	3.7849	3.6876	2.1291	–5.3757	12.9455	1.78	2	.11	.22
Pair 144	DPHP 70C 30M – DPHP 70C 240M	3.9908	2.8357	1.6372	–3.0536	11.0351	2.44	2	.07	.14
Pair 145	DPHP 70C 60M – DPHP 70C 90M	–1.2991	1.1164	.6445	–4.0723	1.4742	–2.02	2	.09	.18
Pair 146	DPHP 70C 60M – DPHP 70C 120M	1.0366	2.4220	1.3984	–4.9800	7.0532	.74	2	.27	.54

Pair 147	DPHP 70C 60M – DPHP 70C 240M	1.2425	1.8293	1.0561	–3.3017	5.7867	1.18	2	.18	.36
Pair 148	DPHP 70C 90M – DPHP 70C 120M	2.3357	1.6818	.9710	–1.8422	6.5135	2.41	2	.07	.14
Pair 149	DPHP 70C 90M – DPHP 70C 240M	2.5415	.9139	.5276	.2713	4.8118	4.82	2	.02	.04
Pair 150	DPHP 70C 120M – DPHP 70C 240M	.2059	2.2079	1.2747	–5.2787	5.6905	.16	2	.44	.89
Pair 151	TDCIPP 70C 0M – TDCIPP 70C 30M	1.4787	3.8166	2.2035	–8.0022	10.9596	.67	2	.29	.57
Pair 152	TDCIPP 70C 0M – TDCIPP 70C 60M	–1.7451	6.7466	3.8951	–18.5045	15.0144	–.45	2	.35	.70
Pair 153	TDCIPP 70C 0M – TDCIPP 70C 90M	–5.5583	1.8962	1.0948	–10.2687	–.8479	–5.08	2	.02	.04
Pair 154	TDCIPP 70C 0M – TDCIPP 70C 120M	–6.8867	5.9460	3.4329	–21.6573	7.8839	–2.01	2	.09	.18
Pair 155	TDCIPP 70C 0M – TDCIPP 70C 240M	–24.1630	10.4908	6.0569	–50.2237	1.8976	–3.99	2	.03	.06
Pair 156	TDCIPP 70C 30M – TDCIPP 70C 60M	–3.2238	9.5183	5.4954	–26.8687	20.4211	–.59	2	.31	.62
Pair 157	TDCIPP 70C 30M – TDCIPP 70C 90M	–7.0370	5.5144	3.1838	–20.7356	6.6616	–2.21	2	.08	.16
Pair 158	TDCIPP 70C 30M – TDCIPP 70C 120M	–8.3654	3.3841	1.9538	–16.7719	.0411	–4.28	2	.03	.05
Pair 159	TDCIPP 70C 30M – TDCIPP 70C 240M	–25.6418	13.5777	7.8391	–59.3708	8.0872	–3.27	2	.04	.08
Pair 160	TDCIPP 70C 60M – TDCIPP 70C 90M	–3.8133	5.0298	2.9040	–16.3081	8.6816	–1.31	2	.16	.32
Pair 161	TDCIPP 70C 60M – TDCIPP 70C 120M	–5.1416	9.3205	5.3812	–28.2951	18.0118	–.96	2	.22	.44

Pair 162	TDCIPP 70C 60M – TDCIPP 70C 240M	–22.4180	12.9981	7.5045	–54.7071	9.8711	–2.99	2	.05	.10
Pair 163	TDCIPP 70C 90M – TDCIPP 70C 120M	–1.3284	6.9817	4.0309	–18.6720	16.0153	–.33	2	.39	.77
Pair 164	TDCIPP 70C 90M – TDCIPP 70C 240M	–18.6047	10.1324	5.8499	–43.7749	6.5655	–3.18	2	.04	.09
Pair 165	TDCIPP 70C 120M – TDCIPP 70C 240M	–17.2763	16.3823	9.4583	–57.9723	23.4196	–1.83	2	.10	.21
Pair 166	BDCIPP 70C 0M – BDCIPP 70C 30M	–3.2448	33.1834	19.1585	–85.6770	79.1874	–.17	2	.44	.88
Pair 167	BDCIPP 70C 0M – BDCIPP 70C 60M	16.6778	21.9128	12.6513	–37.7565	71.1122	1.32	2	.16	.32
Pair 168	BDCIPP 70C 0M – BDCIPP 70C 90M	31.4580	27.1659	15.6843	–36.0259	98.9419	2.01	2	.09	.18
Pair 169	BDCIPP 70C 0M – BDCIPP 70C 120M	22.3945	39.4443	22.7732	–75.5905	120.3795	.98	2	.21	.43
Pair 170	BDCIPP 70C 0M – BDCIPP 70C 240M	–3.8190	26.5880	15.3506	–69.8673	62.2293	–.25	2	.41	.83
Pair 171	BDCIPP 70C 30M – BDCIPP 70C 60M	19.9226	18.8039	10.8564	–26.7889	66.6341	1.84	2	.10	.21
Pair 172	BDCIPP 70C 30M – BDCIPP 70C 90M	34.7028	25.7124	14.8451	–29.1704	98.5760	2.34	2	.07	.14
Pair 173	BDCIPP 70C 30M – BDCIPP 70C 120M	25.6393	40.2129	23.2169	–74.2551	125.5337	1.10	2	.19	.38
Pair 174	BDCIPP 70C 30M – BDCIPP 70C 240M	–.5742	21.2430	12.2647	–53.3448	52.1964	–.05	2	.48	.97
Pair 175	BDCIPP 70C 60M – BDCIPP 70C 90M	14.7802	8.8516	5.1105	–7.2085	36.7689	2.89	2	.05	.10
Pair 176	BDCIPP 70C 60M – BDCIPP 70C 120M	5.7167	24.6162	14.2122	–55.4334	66.8667	.40	2	.36	.73

Pair 177	BDCIPP 70C 60M – BDCIPP 70C 240M	–20.4968	5.6021	3.2344	–34.4132	–6.5804	–6.34	2	.01	.02
Pair 178	BDCIPP 70C 90M – BDCIPP 70C 120M	–9.0635	15.7710	9.1054	–48.2408	30.1138	–1.00	2	.21	.42
Pair 179	BDCIPP 70C 90M – BDCIPP 70C 240M	–35.2770	4.4694	2.5804	–46.3797	–24.1743	–13.67	2	.00	.01
Pair 180	BDCIPP 70C 120M – BDCIPP 70C 240M	–26.2135	19.7841	11.4224	–75.3600	22.9331	–2.29	2	.07	.15

Notations:

100C: Heated at 100 °C

70C: Heated at 70 °C

0M: Heated for 0 minutes (non-heated)

30M: Heated for 30 minutes

60M: Heated for 60 minutes

90M: Heated for 90 minutes

120M: Heated for 120 minutes

240M: Heated for 240 minutes

Table S6.11 – SPSS output of paired-sample t-test for comparison of concentrations of OPEs in different food matrices heated at different durations.

		Paired Differences					*t*	df	Significance	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				One–Sided *p*	Two–Sided *p*
					Lower	Upper				
Pair 1	TBOEP Honey 100C 0H – TBOEP Honey 100C 1H	288.1881	97.0501	56.0319	47.1022	529.2740	5.14	2	.02	.04
Pair 2	TBOEP Honey 100C 0H – TBOEP Honey 100C 4H	178.6794	58.1567	33.5768	34.2103	323.1486	5.32	2	.02	.03
Pair 3	TBOEP Honey 100C 1H – TBOEP Honey 100C 4H	–109.5087	46.1922	26.6691	–224.2564	5.2391	–4.11	2	.03	.05
Pair 4	TBOEP Chicken 100C 0H – TBOEP Chicken 100C 1H	717.2837	135.3965	78.1712	380.9401	1053.6273	9.18	2	.01	.01
Pair 5	TBOEP Chicken 100C 0H – TBOEP Chicken 100C 4H	596.0426	101.1957	58.4253	344.6587	847.4266	10.20	2	.00	.01
Pair 6	TBOEP Chicken 100C 1H – TBOEP Chicken 100C 4H	–121.2411	57.6470	33.2825	–264.4443	21.9621	–3.64	2	.03	.07
Pair 7	TBOEP Salmon 100C 0H – TBOEP Salmon 100C 1H	12.8280	33.1760	19.1542	–69.5858	95.2419	.67	2	.29	.57
Pair 8	TBOEP Salmon 100C 0H – TBOEP Salmon 100C 4H	112.4589	22.6075	13.0524	56.2988	168.6190	8.62	2	.01	.01
Pair 9	TBOEP Salmon 100C 1H – TBOEP Salmon 100C 4H	99.6309	18.6450	10.7647	53.3141	145.9477	9.26	2	.01	.01
Pair 10	BBOEP Honey 100C 0H – BBOEP Honey 100C 1H	10.1650	62.0491	35.8241	–143.9736	164.3036	.28	2	.40	.80
Pair 11	BBOEP Honey 100C 0H – BBOEP Honey 100C 4H	90.2491	56.8037	32.7956	–50.8591	231.3573	2.75	2	.06	.11

Pair 12	BBOEP Honey 100C 1H – BBOEP Honey 100C 4H	80.0841	77.4654	44.7247	–112.3507	272.5189	1.79	2	.11	.22
Pair 13	BBOEP Chicken 100C 0H – BBOEP Chicken 100C 1H	–21.0119	32.1910	18.5855	–100.9787	58.9549	–1.13	2	.19	.38
Pair 14	BBOEP Chicken 100C 0H – BBOEP Chicken 100C 4H	–49.5313	22.4919	12.9857	–105.4043	6.3417	–3.81	2	.03	.06
Pair 15	BBOEP Chicken 100C 1H – BBOEP Chicken 100C 4H	–28.5194	51.5290	29.7503	–156.5246	99.4857	–.96	2	.22	.44
Pair 16	BBOEP Salmon 100C 0H – BBOEP Salmon 100C 1H	163.1863	20.2056	11.6657	112.9927	213.3798	13.99	2	.00	.01
Pair 17	BBOEP Salmon 100C 0H – BBOEP Salmon 100C 4H	–52.3853	5.4540	3.1489	–65.9338	–38.8367	–16.64	2	.00	.00
Pair 18	BBOEP Salmon 100C 1H – BBOEP Salmon 100C 4H	–215.5715	18.6523	10.7689	–261.9064	–169.2367	–20.02	2	.00	.00
Pair 19	TPHP Honey 100C 0H – TPHP Honey 100C 1H	224.5545	81.2821	46.9282	22.6386	426.4704	4.79	2	.02	.04
Pair 20	TPHP Honey 100C 0H – TPHP Honey 100C 4H	166.3104	28.7036	16.5720	95.0067	237.6141	10.04	2	.00	.01
Pair 21	TPHP Honey 100C 1H – TPHP Honey 100C 4H	–58.2441	84.0949	48.5522	–267.1474	150.6593	–1.20	2	.18	.35
Pair 22	TPHP Chicken 100C 0H – TPHP Chicken 100C 1H	158.1807	59.6113	34.4166	10.0979	306.2635	4.60	2	.02	.04
Pair 23	TPHP Chicken 100C 0H – TPHP Chicken 100C 4H	469.7338	31.7158	18.3112	390.9473	548.5204	25.65	2	<.001	.00
Pair 24	TPHP Chicken 100C 1H – TPHP Chicken 100C 4H	311.5531	34.6155	19.9853	225.5634	397.5429	15.59	2	.00	.00
Pair 25	TPHP Salmon 100C 0H – TPHP Salmon 100C 1H	112.8158	16.6318	9.6024	71.5002	154.1315	11.75	2	.00	.01
Pair 26	TPHP Salmon 100C 0H – TPHP Salmon 100C 4H	167.0935	44.9891	25.9745	55.3344	278.8526	6.43	2	.01	.02

Pair 27	TPHP Salmon 100C 1H – TPHP Salmon 100C 4H	54.2777	52.3694	30.2355	–75.8151	184.3704	1.80	2	.11	.21
Pair 28	DPHP Honey 100C 0H – DPHP Honey 100C 1H	45.8360	47.3014	27.3095	–71.6671	163.3391	1.68	2	.12	.24
Pair 29	DPHP Honey 100C 0H – DPHP Honey 100C 4H	–160.3965	30.3790	17.5393	–235.8620	–84.9309	–9.14	2	.01	.01
Pair 30	DPHP Honey 100C 1H – DPHP Honey 100C 4H	–206.2325	19.7481	11.4016	–255.2896	–157.1754	–18.09	2	.00	.00
Pair 31	DPHP Chicken 100C 0H – DPHP Chicken 100C 1H	–227.7208	54.4470	31.4350	–362.9747	–92.4669	–7.24	2	.01	.02
Pair 32	DPHP Chicken 100C 0H – DPHP Chicken 100C 4H	–1263.5463	109.6027	63.2791	–1535.8144	–991.2781	–19.97	2	.00	.00
Pair 33	DPHP Chicken 100C 1H – DPHP Chicken 100C 4H	–1035.825	139.9930	80.8250	–1383.5872	–688.0637	–12.82	2	.00	.01
Pair 34	DPHP Salmon 100C 0H – DPHP Salmon 100C 1H	68.8007	31.1826	18.0033	–8.6611	146.2626	3.82	2	.03	.06
Pair 35	DPHP Salmon 100C 0H – DPHP Salmon 100C 4H	3.1795	7.4245	4.2865	–15.2639	21.6230	.74	2	.27	.54
Pair 36	DPHP Salmon 100C 1H – DPHP Salmon 100C 4H	–65.6212	27.9930	16.1618	–135.1597	3.9173	–4.06	2	.03	.06
Pair 37	TDCIPP Honey 100C 0H – TDCIPP Honey 100C 1H	284.1431	134.3537	77.5691	–49.6100	617.8962	3.66	2	.03	.07
Pair 38	TDCIPP Honey 100C 0H – TDCIPP Honey 100C 4H	225.9066	106.7454	61.6295	–39.2637	491.0769	3.67	2	.03	.07
Pair 39	TDCIPP Honey 100C 1H – TDCIPP Honey 100C 4H	–58.2365	46.8003	27.0201	–174.4948	58.0218	–2.16	2	.08	.16
Pair 40	TDCIPP Chicken 100C 0H – TDCIPP Chicken 100C 1H	668.1016	926.3771	534.8441	–1633.147	2969.3499	1.25	2	.17	.34
Pair 41	TDCIPP Chicken 100C 0H – TDCIPP Chicken 100C 4H	–137.2336	1809.1748	1044.5276	–4631.473	4357.0057	–.13	2	.45	.91

Pair 42	TDCIPP Chicken 100C 1H – TDCIPP Chicken 100C 4H	–805.3352	1162.1766	670.9830	–3692.342	2081.6715	–1.20	2	.18	.35
Pair 43	TDCIPP Salmon 100C 0H – TDCIPP Salmon 100C 1H	353.4601	187.6946	108.3655	–112.7991	819.7193	3.26	2	.04	.08
Pair 44	TDCIPP Salmon 100C 0H – TDCIPP Salmon 100C 4H	234.6000	518.6970	299.4699	–1053.915	1523.1148	.78	2	.26	.52
Pair 45	TDCIPP Salmon 100C 1H – TDCIPP Salmon 100C 4H	–118.8601	348.8024	201.3812	–985.3333	747.6131	–.59	2	.31	.61
Pair 46	BDCIPP Honey 100C 0H – BDCIPP Honey 100C 1H	478.4704	21.1057	12.1854	426.0410	530.8998	39.27	2	<.001	<.001
Pair 47	BDCIPP Honey 100C 0H – BDCIPP Honey 100C 4H	722.9036	10.4897	6.0562	696.8458	748.9613	119.4	2	<.001	<.001
Pair 48	BDCIPP Honey 100C 1H – BDCIPP Honey 100C 4H	244.4331	17.6522	10.1915	200.5827	288.2836	23.98	2	<.001	.00
Pair 49	BDCIPP Chicken 100C 0H – BDCIPP Chicken 100C 1H	165.3163	65.6336	37.8936	2.2734	328.3593	4.36	2	.02	.05
Pair 50	BDCIPP Chicken 100C 0H – BDCIPP Chicken 100C 4H	630.4271	69.8316	40.3173	456.9559	803.8983	15.64	2	.00	.00
Pair 51	BDCIPP Chicken 100C 1H – BDCIPP Chicken 100C 4H	465.1108	26.9066	15.5345	398.2710	531.9505	29.94	2	<.001	.00
Pair 52	BDCIPP Salmon 100C 0H – BDCIPP Salmon 100C 1H	322.4642	28.8544	16.6591	250.7857	394.1426	19.36	2	.00	.00
Pair 53	BDCIPP Salmon 100C 0H – BDCIPP Salmon 100C 4H	695.2868	52.4550	30.2849	564.9813	825.5922	22.96	2	<.001	.00
Pair 54	BDCIPP Salmon 100C 1H – BDCIPP Salmon 100C 4H	372.8226	52.6083	30.3734	242.1364	503.5089	12.27	2	.00	.01
Pair 55	TBOEP Honey 70C 0H – TBOEP Honey 70C 1H	114.8562	24.3014	14.0304	54.4883	175.2242	8.19	2	.01	.01
Pair 56	TBOEP Honey 70C 0H – TBOEP Honey 70C 4H	132.2984	19.4081	11.2053	84.0860	180.5107	11.81	2	.00	.01

Pair 57	TBOEP Honey 70C 1H – TBOEP Honey 70C 4H	17.4421	27.8934	16.1042	–51.8488	86.7330	1.08	2	.20	.39
Pair 58	BBOEP Honey 70C 0H – BBOEP Honey 70C 1H	50.1455	40.8429	23.5807	–51.3139	151.6049	2.13	2	.08	.17
Pair 59	BBOEP Honey 70C 0H – BBOEP Honey 70C 4H	222.1213	47.6306	27.4995	103.8004	340.4422	8.08	2	.01	.01
Pair 60	BBOEP Honey 70C 1H – BBOEP Honey 70C 4H	171.9758	65.0103	37.5337	10.4812	333.4704	4.58	2	.02	.04
Pair 61	TPHP Honey 70C 0H – TPHP Honey 70C 1H	118.1624	2.7601	1.5936	111.3059	125.0190	74.15	2	<.001	<.001
Pair 62	TPHP Honey 70C 0H – TPHP Honey 70C 4H	109.0017	19.7958	11.4291	59.8262	158.1771	9.54	2	.01	.01
Pair 63	TPHP Honey 70C 1H – TPHP Honey 70C 4H	–9.1608	20.3036	11.7223	–59.5976	41.2761	–.78	2	.26	.52
Pair 64	DPHP Honey 70C 0H – DPHP Honey 70C 1H	6.1724	14.2440	8.2238	–29.2117	41.5565	.75	2	.27	.53
Pair 65	DPHP Honey 70C 0H – DPHP Honey 70C 4H	–17.5504	23.0974	13.3353	–74.9276	39.8268	–1.32	2	.16	.32
Pair 66	DPHP Honey 70C 1H – DPHP Honey 70C 4H	–23.7228	10.0559	5.8058	–48.7032	1.2576	–4.09	2	.03	.06
Pair 67	TDCIPP Honey 70C 0H – TDCIPP Honey 70C 1H	16.0727	31.6654	18.2820	–62.5885	94.7340	.88	2	.24	.47
Pair 68	TDCIPP Honey 70C 0H – TDCIPP Honey 70C 4H	–47.7921	97.0752	56.0464	–288.9402	193.3560	–.85	2	.24	.48
Pair 69	TDCIPP Honey 70C 1H – TDCIPP Honey 70C 4H	–63.8648	124.1861	71.6989	–372.3603	244.6306	–.89	2	.23	.47
Pair 70	BDCIPP Honey 70C 0H – BDCIPP Honey 70C 1H	43.3435	19.9250	11.5037	–6.1530	92.8401	3.77	2	.03	.06
Pair 71	BDCIPP Honey 70C 0H – BDCIPP Honey 70C 4H	61.5904	30.0531	17.3511	–13.0655	136.2464	3.55	2	.04	.07

Pair 72	BDCIPP Honey 70C 1H – BDCIPP Honey 70C 4H	18.2469	11.6866	6.7473	–10.7843	47.2781	2.70	2	.06	.11

Notations:

100C: Heated at 100 °C

70C: Heated at 70 °C

0H: Heated for 0 hours (non-heated)

1H: Heated for 1 hour

4H: Heated for 4 hours

Table S6.12 – SPSS output of paired-sample t-test for comparison of concentrations of OPEs spiked in heated and non-heated chicken and salmon matrices.

		Paired Differences					*t*	df	Significance	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				One–Sided *p*	Two–Sided *p*
					Lower	Upper				
Pair 1	TBOEP Chicken Non-heated – TBOEP Chicken Heated	–66.6872	2.3366	1.3490	–72.4916	–60.8829	–49.43	2	<.001	<.001
Pair 2	TBOEP Salmon Non-heated – TBOEP Salmon Heated	–13.9069	1.9363	1.1179	–18.7169	–9.0969	–12.44	2	.00	.01
Pair 3	BBOEP Chicken Non-heated – BBOEP Chicken Heated	–5.8215	12.7940	7.3866	–37.6035	25.9605	–.79	2	.26	.51
Pair 4	BBOEP Salmon Non-heated – BBOEP Salmon Heated	–.8254	8.3199	4.8035	–21.4931	19.8424	–.17	2	.44	.88
Pair 5	TPHP Chicken Non-heated – TPHP Chicken Heated	2.5959	2.8047	1.6193	–4.3715	9.5632	1.60	2	.13	.25
Pair 6	TPHP Salmon Non-heated – TPHP Salmon Heated	–3.9471	2.9927	1.7279	–11.3814	3.4873	–2.28	2	.07	.15
Pair 7	DPHP Chicken Non-heated – DPHP Chicken Heated	2.5296	16.9767	9.8015	–39.6429	44.7022	.26	2	.41	.82
Pair 8	DPHP Salmon Non-heated – DPHP Salmon Heated	–40.0590	6.8176	3.9361	–56.9948	–23.1231	–10.18	2	.00	.01
Pair 9	TDCIPP Chicken Non-heated – TDCIPP Chicken Heated	–85.2366	327.1904	188.9035	–898.0227	727.5494	–.45	2	.35	.70
Pair 10	TDCIPP Salmon Non-heated – TDCIPP Salmon Heated	–30.6349	68.4550	39.5225	–200.6866	139.4168	–.78	2	.26	.52
Pair 11	BDCIPP Chicken Non-heated – BDCIPP Chicken Heated	5.0872	4.5571	2.6311	–6.2333	16.4077	1.93	2	.10	.19
Pair 12	BDCIPP Salmon Non-heated – BDCIPP Salmon Heated	1.3108	5.3152	3.0687	–11.8929	14.5144	.43	2	.36	.71

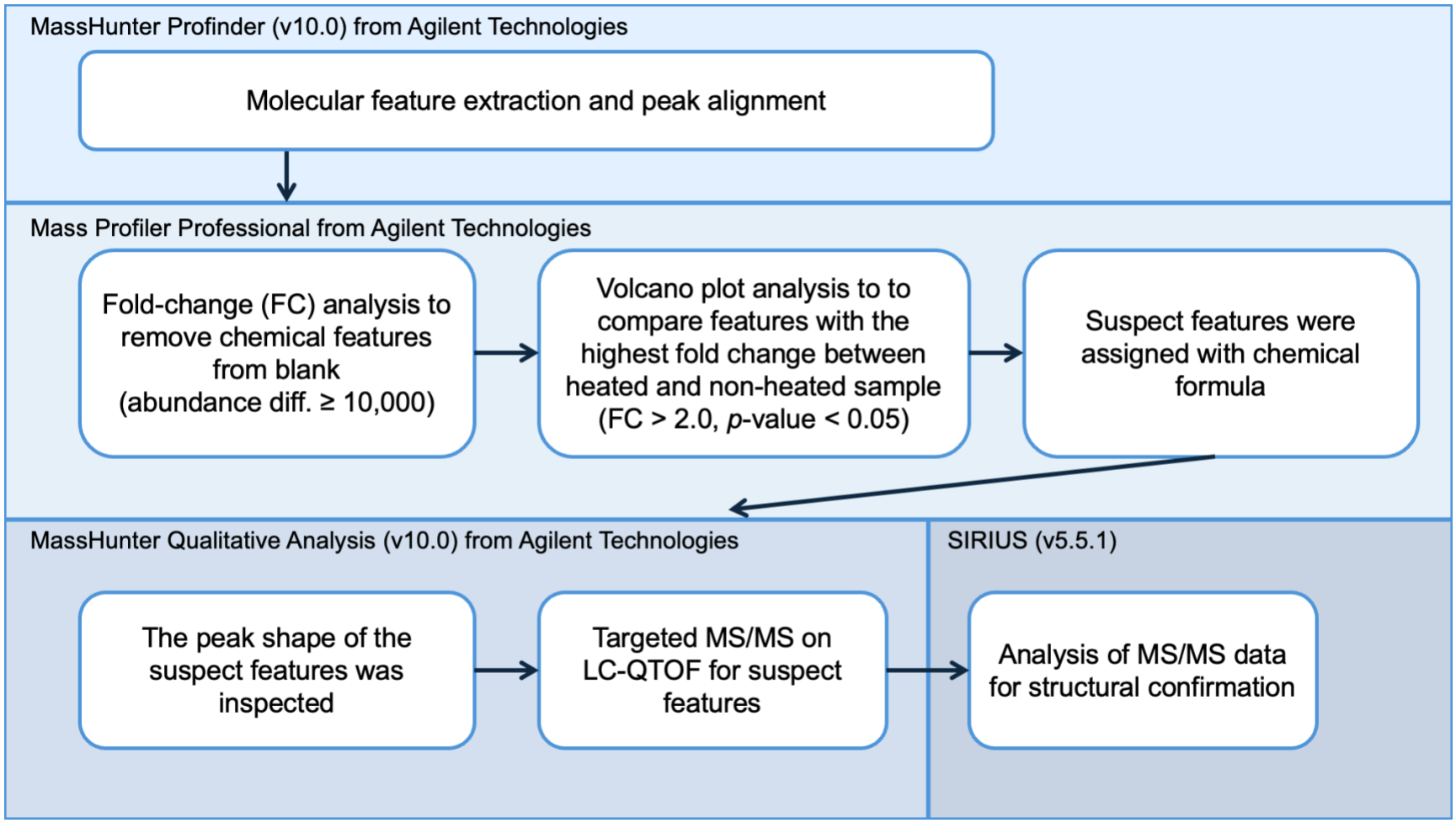


Figure S6.1 – Non-targeted workflow for identification of unknown OPE degradation products.

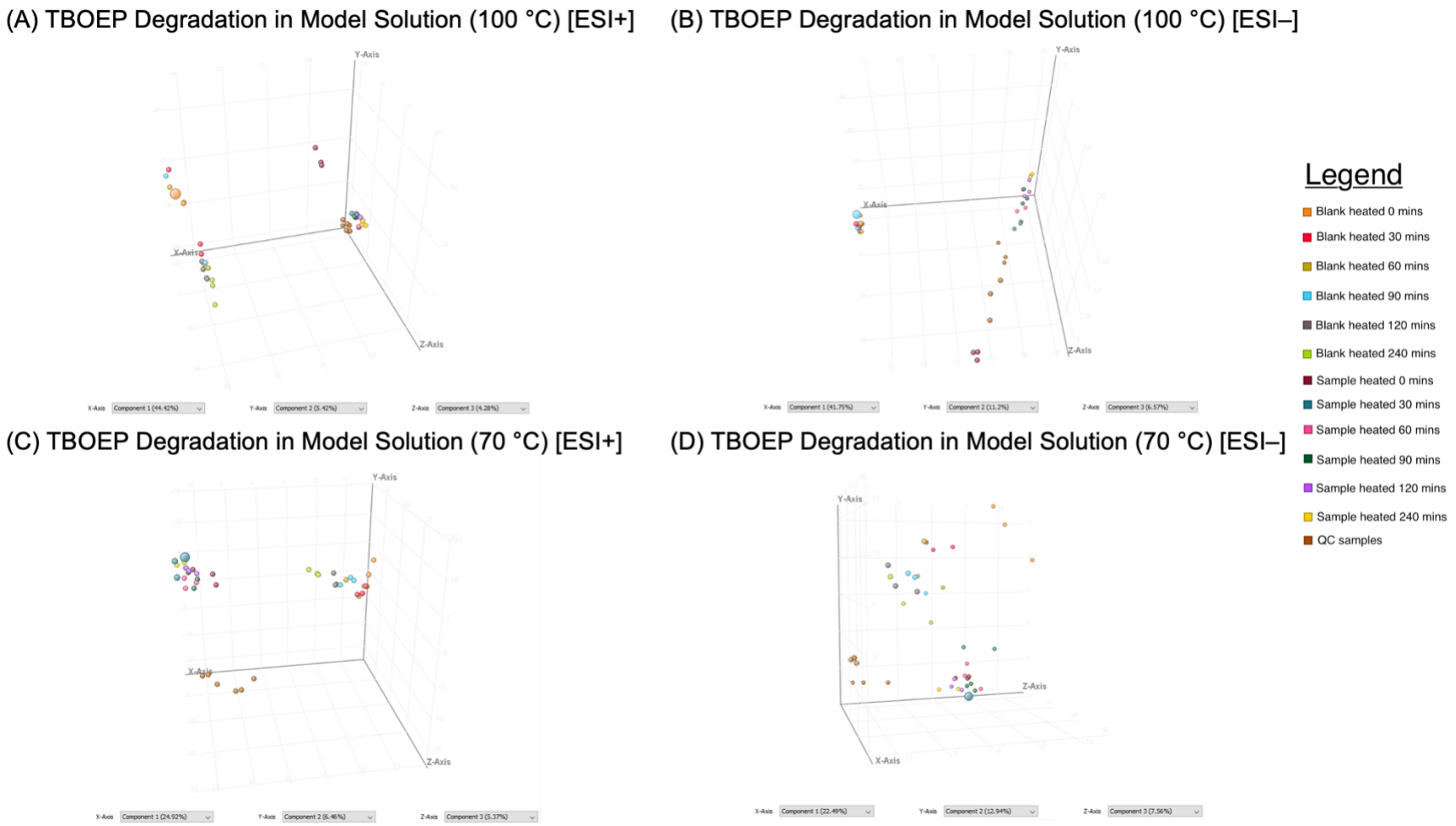


Figure S6.2 – PCA plots for chemical features extraction from samples in the degradation experiment in the model solution for TBOEP (A) at 100 °C and analyzed on ESI+ mode, (B) at 100 °C and analyzed on ESI– mode, (C) at 70 °C and analyzed on ESI+ mode, (D) at 70 °C and analyzed on ESI– mode.

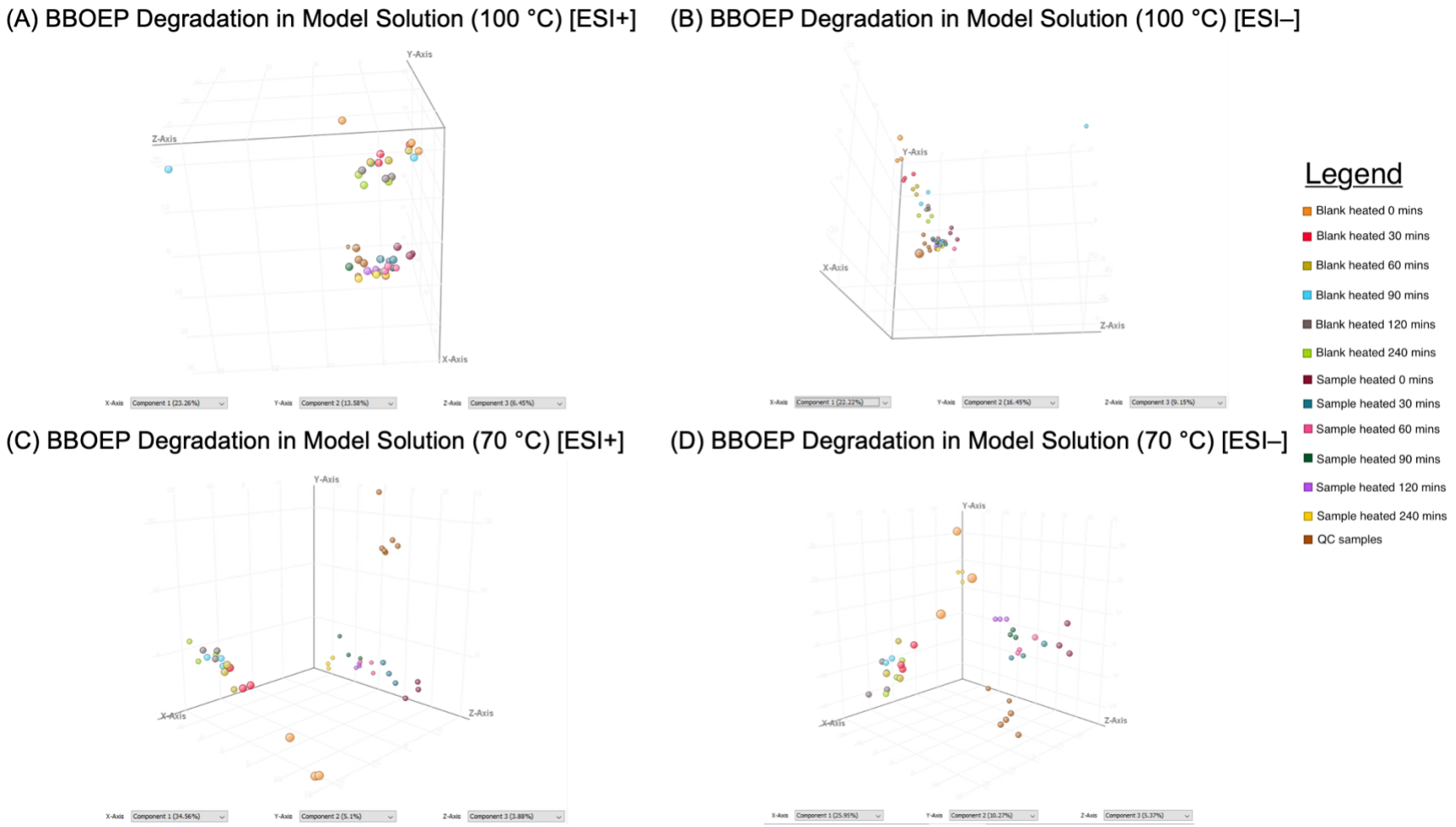


Figure S6.3 – PCA plots for chemical features extraction from samples in the degradation experiment in the model solution for BBOEP (A) at 100 °C and analyzed on ESI+ mode, (B) at 100 °C and analyzed on ESI– mode, (C) at 70 °C and analyzed on ESI+ mode, (D) at 70 °C and analyzed on ESI– mode.

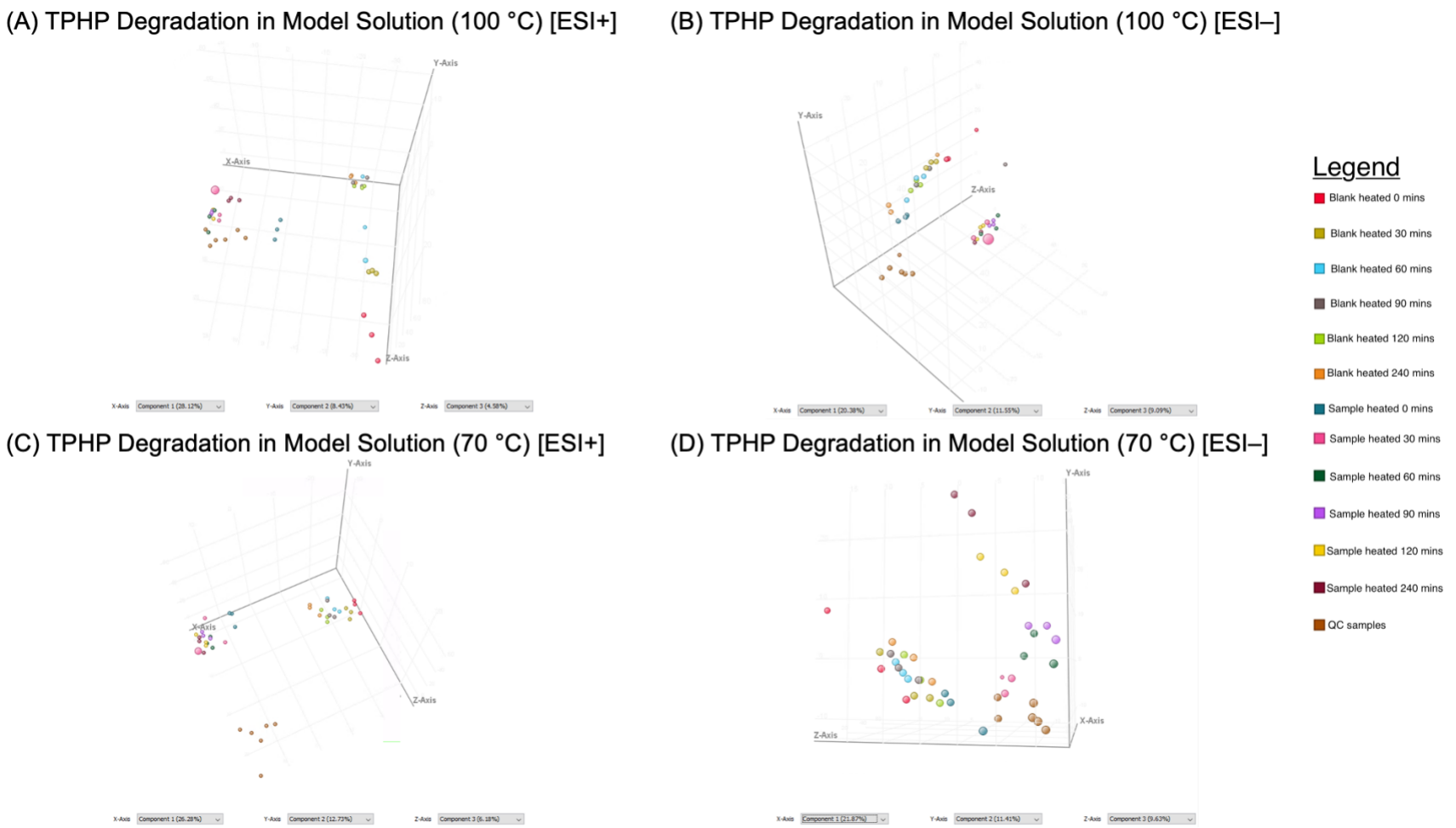


Figure S6.4 – PCA plots for chemical features extraction from samples in the degradation experiment in the model solution for TPHP (A) at 100 °C and analyzed on ESI+ mode, (B) at 100 °C and analyzed on ESI– mode, (C) at 70 °C and analyzed on ESI+ mode, (D) at 70 °C and analyzed on ESI– mode.

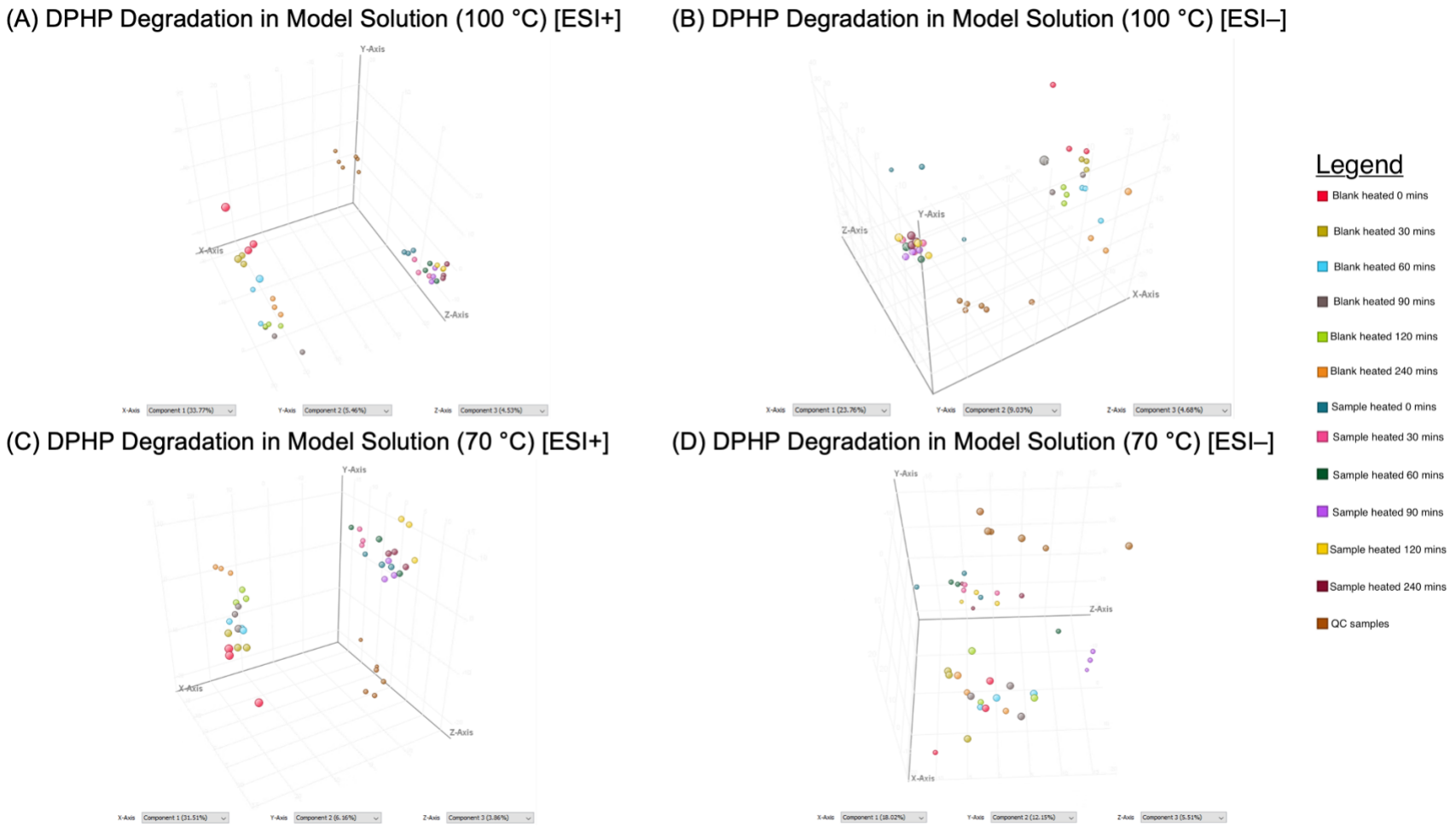


Figure S6.5 – PCA plots for chemical features extraction from samples in the degradation experiment in the model solution for DPHP (A) at 100 °C and analyzed on ESI+ mode, (B) at 100 °C and analyzed on ESI– mode, (C) at 70 °C and analyzed on ESI+ mode, (D) at 70 °C and analyzed on ESI– mode.

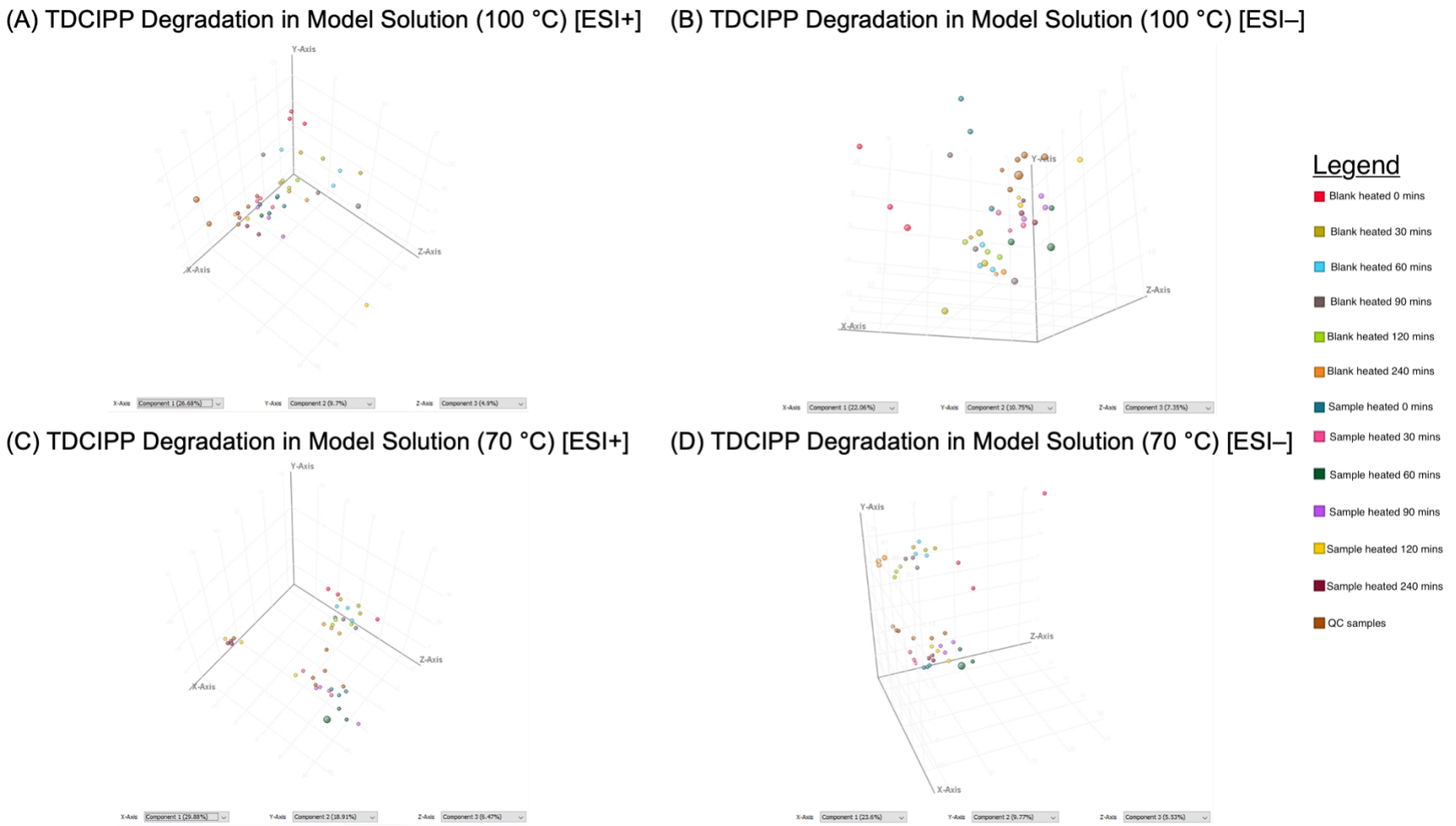


Figure S6.6 – PCA plots for chemical features extraction from samples in the degradation experiment in the model solution for TDCIPP (A) at 100 °C and analyzed on ESI+ mode, (B) at 100 °C and analyzed on ESI– mode, (C) at 70 °C and analyzed on ESI+ mode, (D) at 70 °C and analyzed on ESI– mode.

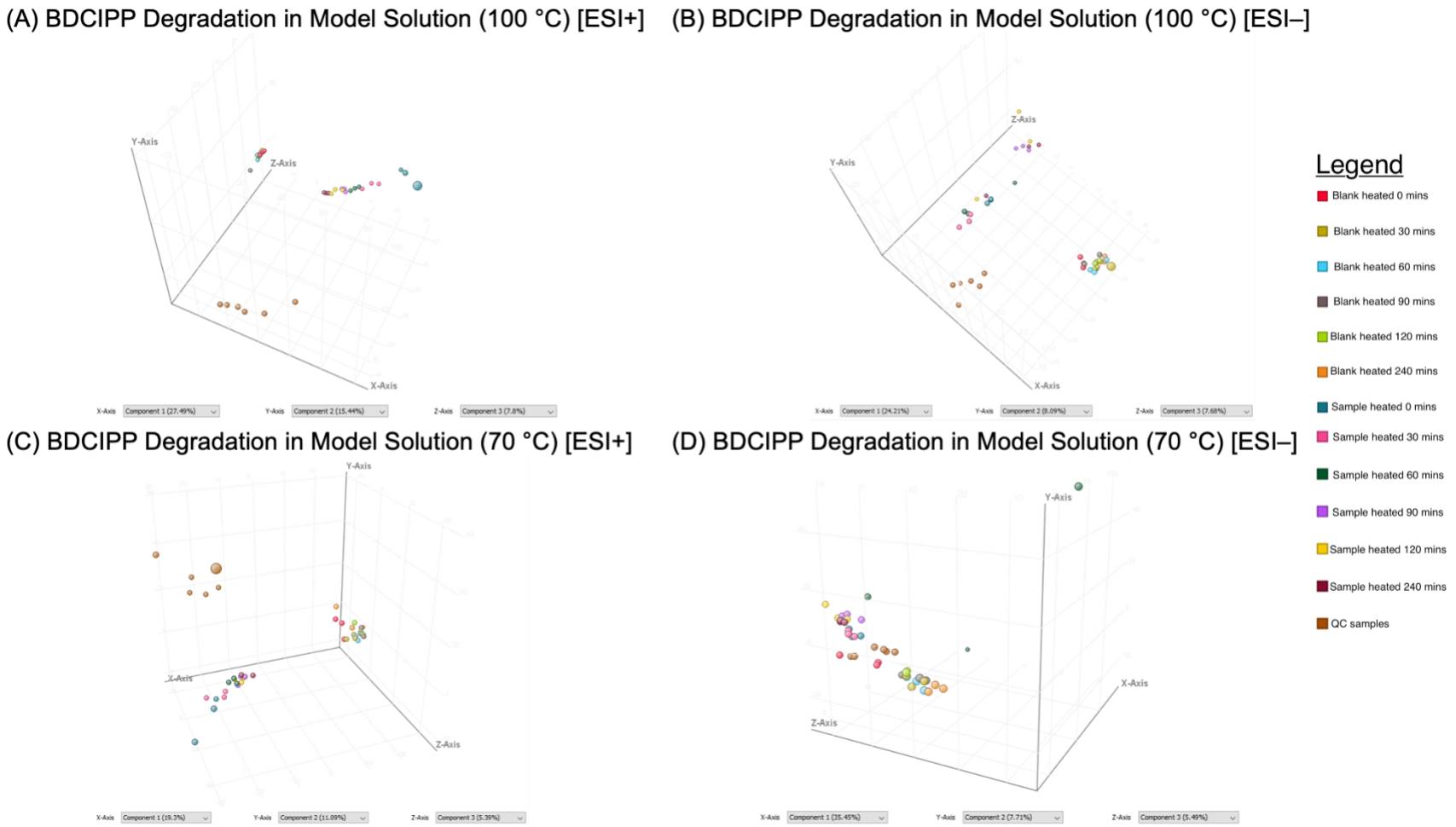


Figure S6.7 – PCA plots for chemical features extraction from samples in the degradation experiment in the model solution for BDCIPP (A) at 100 °C and analyzed on ESI+ mode, (B) at 100 °C and analyzed on ESI– mode, (C) at 70 °C and analyzed on ESI+ mode, (D) at 70 °C and analyzed on ESI– mode.

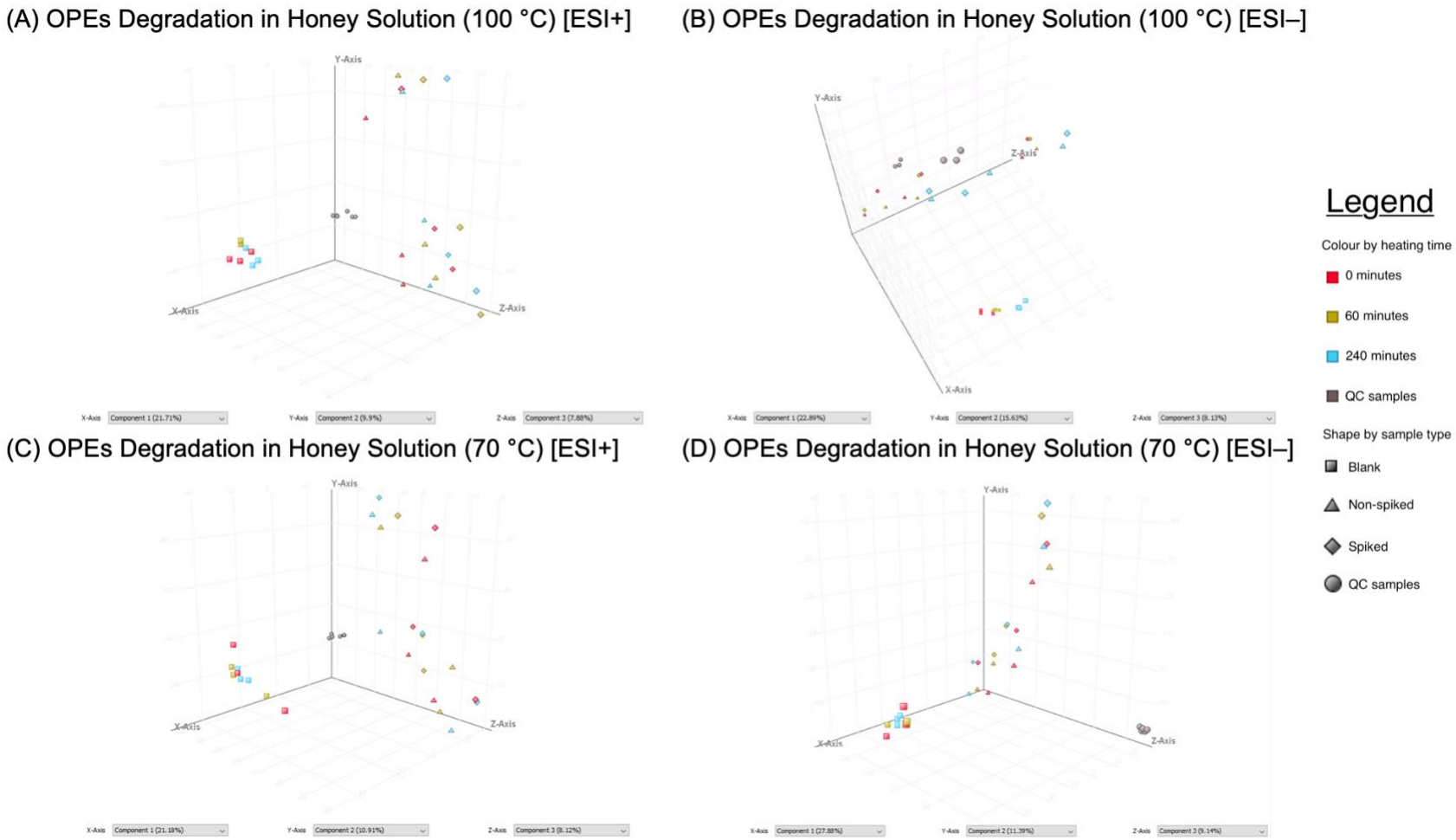


Figure S6.8 – PCA plots for chemical features extraction from samples in the degradation experiment in the honey matrix (A) at 100 °C and analyzed on ESI+ mode, (B) at 100 °C and analyzed on ESI– mode, (C) at 70 °C and analyzed on ESI+ mode, (D) at 70 °C and analyzed on ESI– mode.

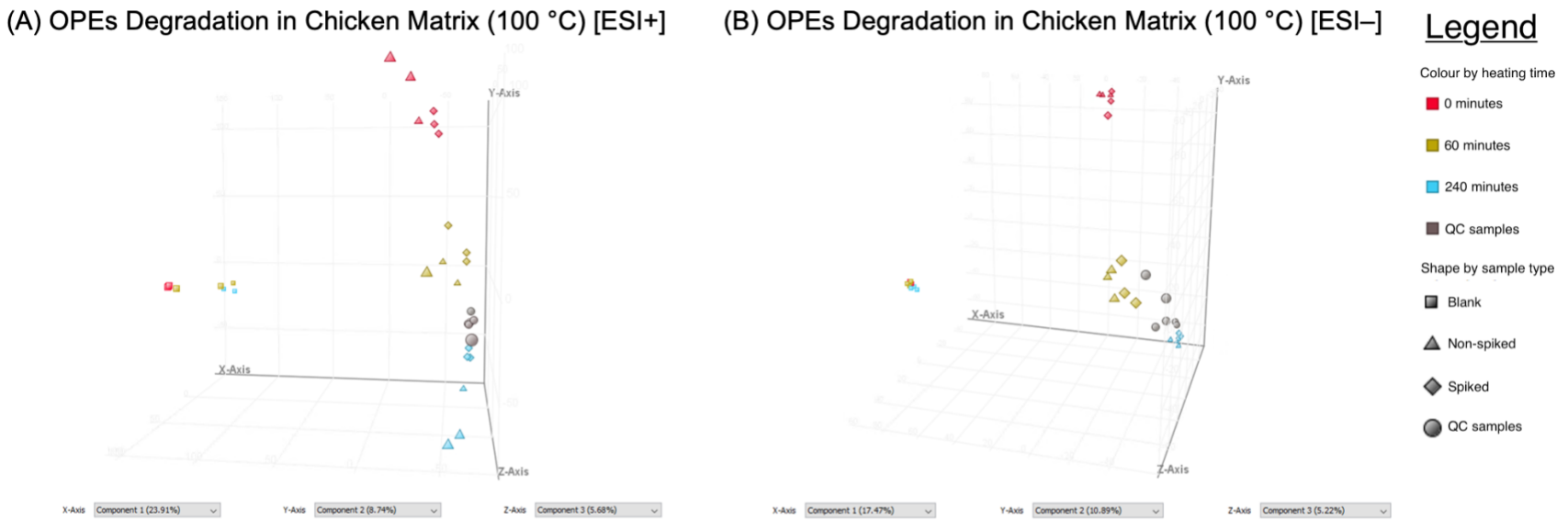


Figure S6.9 – PCA plots for chemical features extraction from samples in the degradation experiment in the chicken matrix (A) at 100 °C and analyzed on ESI+ mode, (B) at 100 °C and analyzed on ESI– mode.

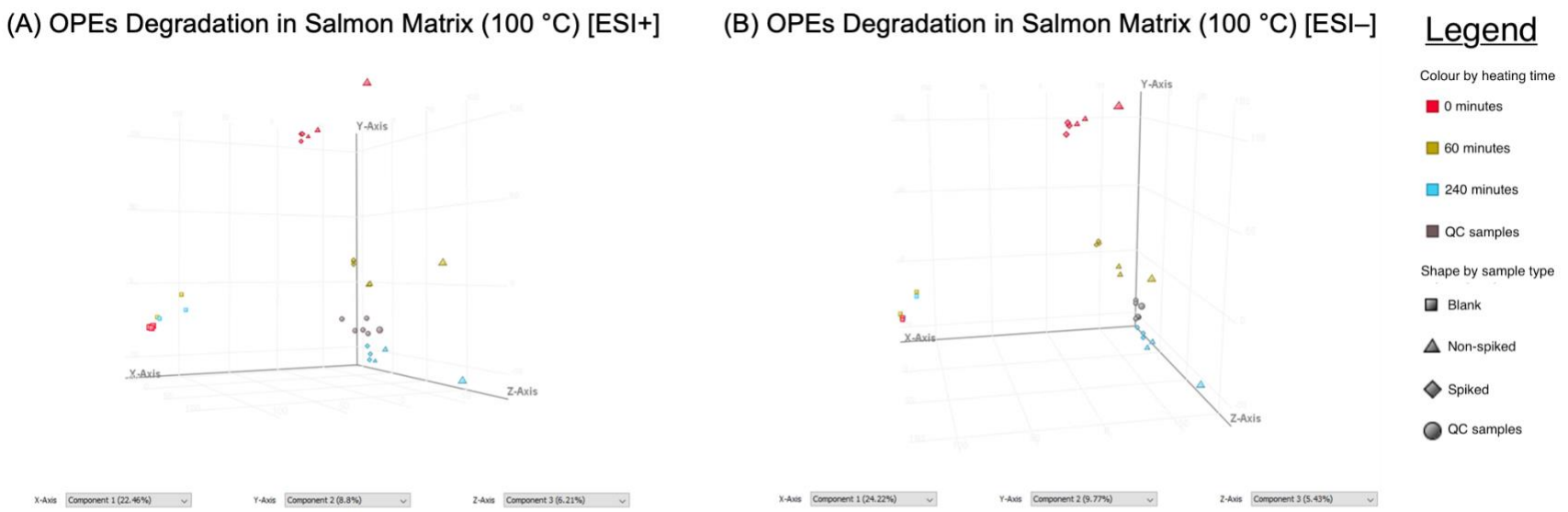


Figure S6.10 – PCA plots for chemical features extraction from samples in the degradation experiment in the salmon matrix (A) at 100 °C and analyzed on ESI+ mode, (B) at 100 °C and analyzed on ESI– mode.

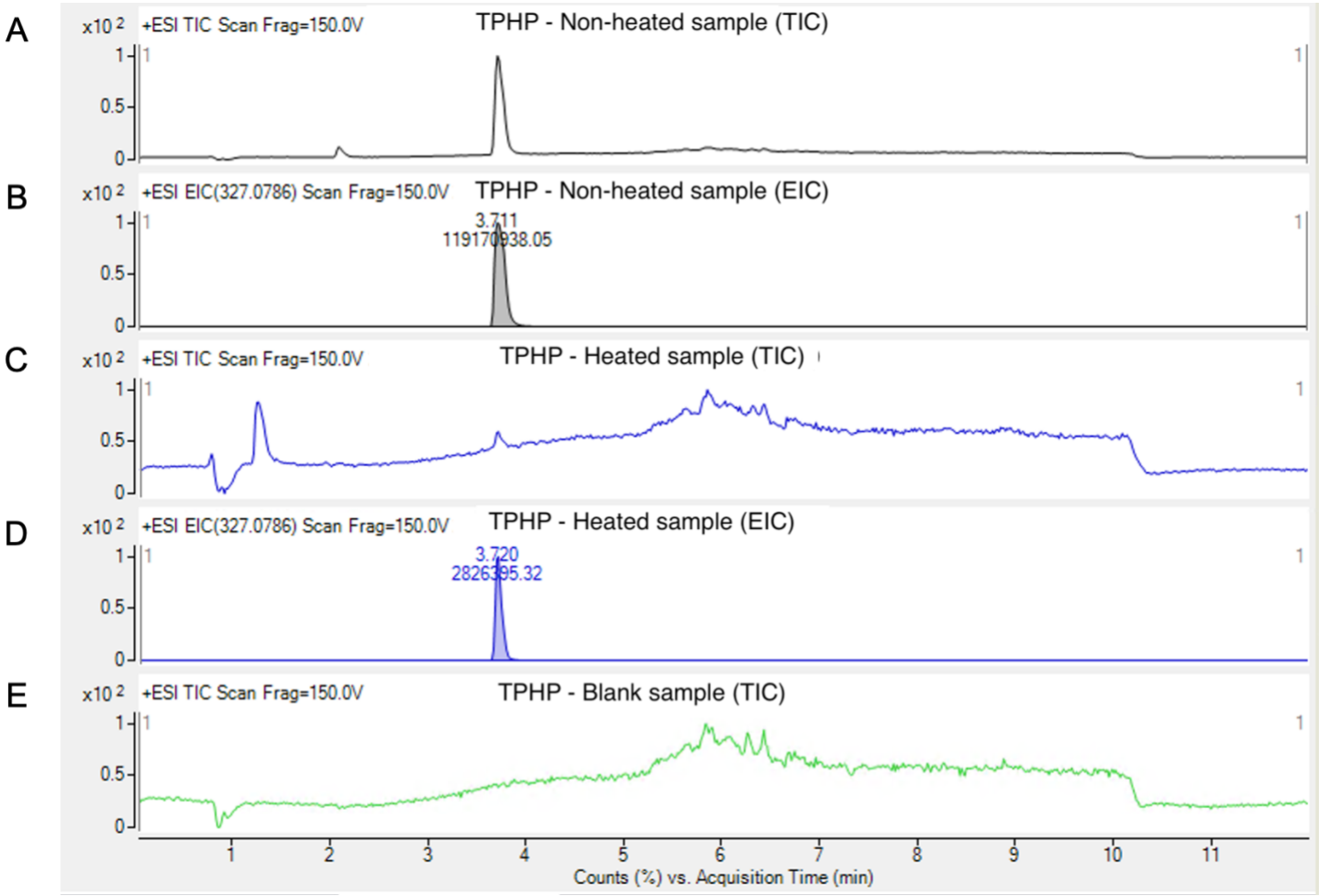


Figure S6.11 – Total ion chromatograms (TIC) and extracted ion chromatograms (EIC) of TPHP. (A) TIC for non-heated sample, (B) EIC for non-heated sample, (C) TIC for heated sample, (D) EIC for heated sample and (E) TIC for blank sample.

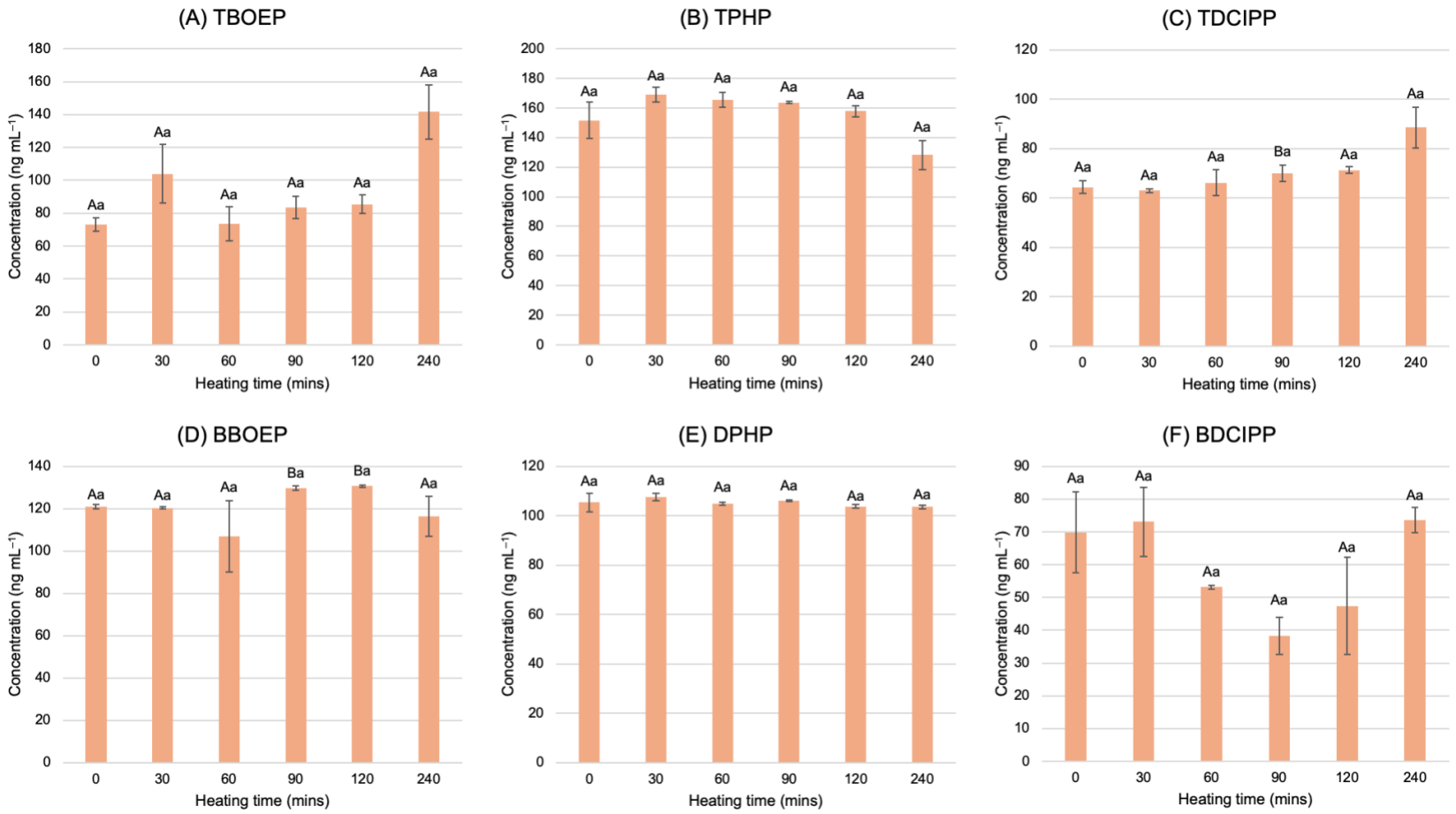


Figure S6.12 – The concentration of target OPEs heating at 70 °C over 240 minutes in the model solution. (A) TBOEP, (B) TPHP, (C) TDCIPP, (D) BBOEP, (E), DPHP and (F) BDCIPP. Statistical significance between time t and 0 was denoted by uppercase letters; statistical significance between t and its previous time point was denoted by lowercase letters. (n = 3, error bars represent the SD).

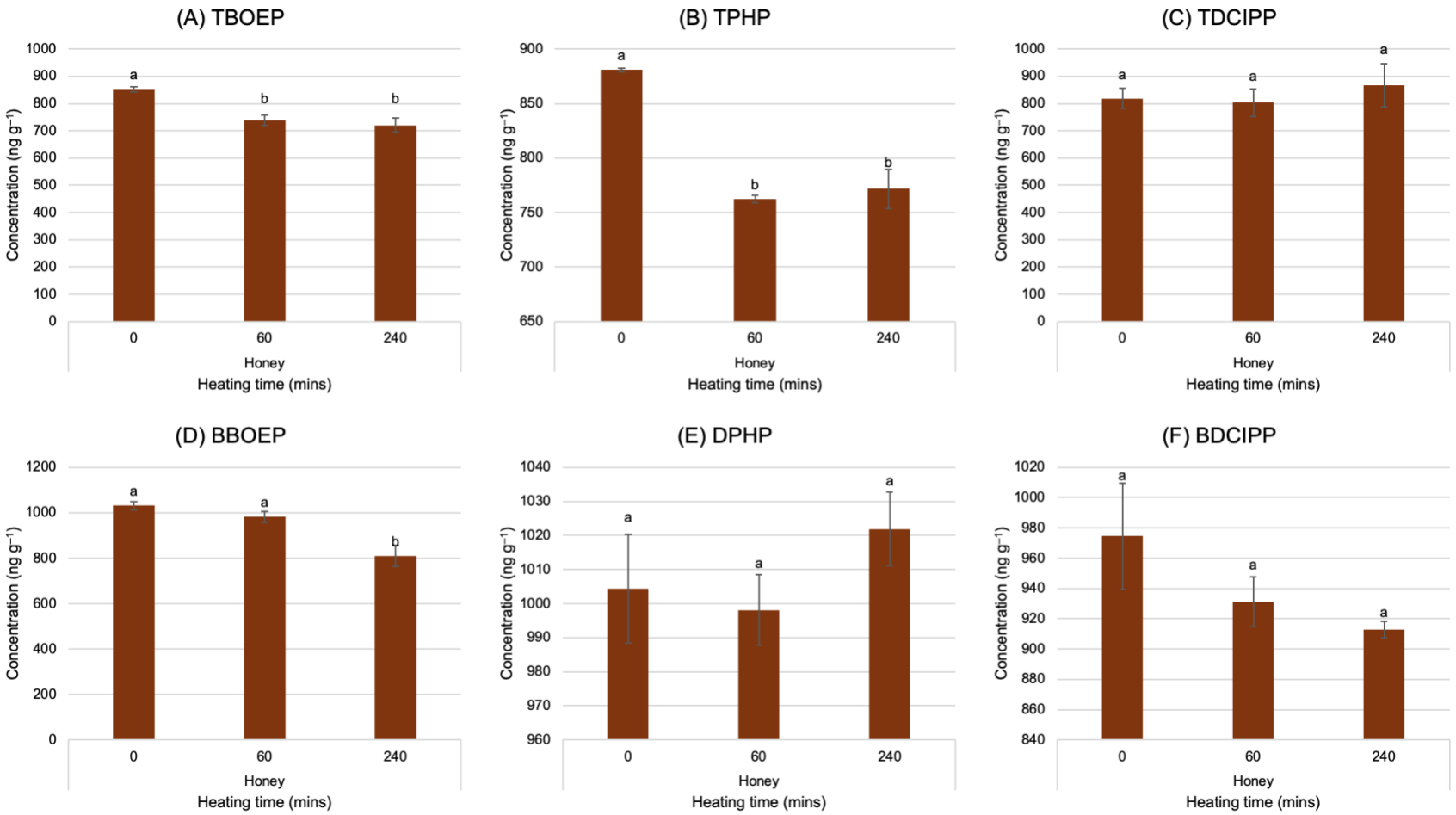


Figure S6.13 – The concentration of target OPEs heating at 70 °C over 240 minutes in the honey matrix. (A) TBOEP, (B) TPHP, (C) TDCIPP, (D) BBOEP, (E), DPHP and (F) BDCIPP. Statistical significance was denoted by the letters on the bars (n = 3, error bars represent the SD).

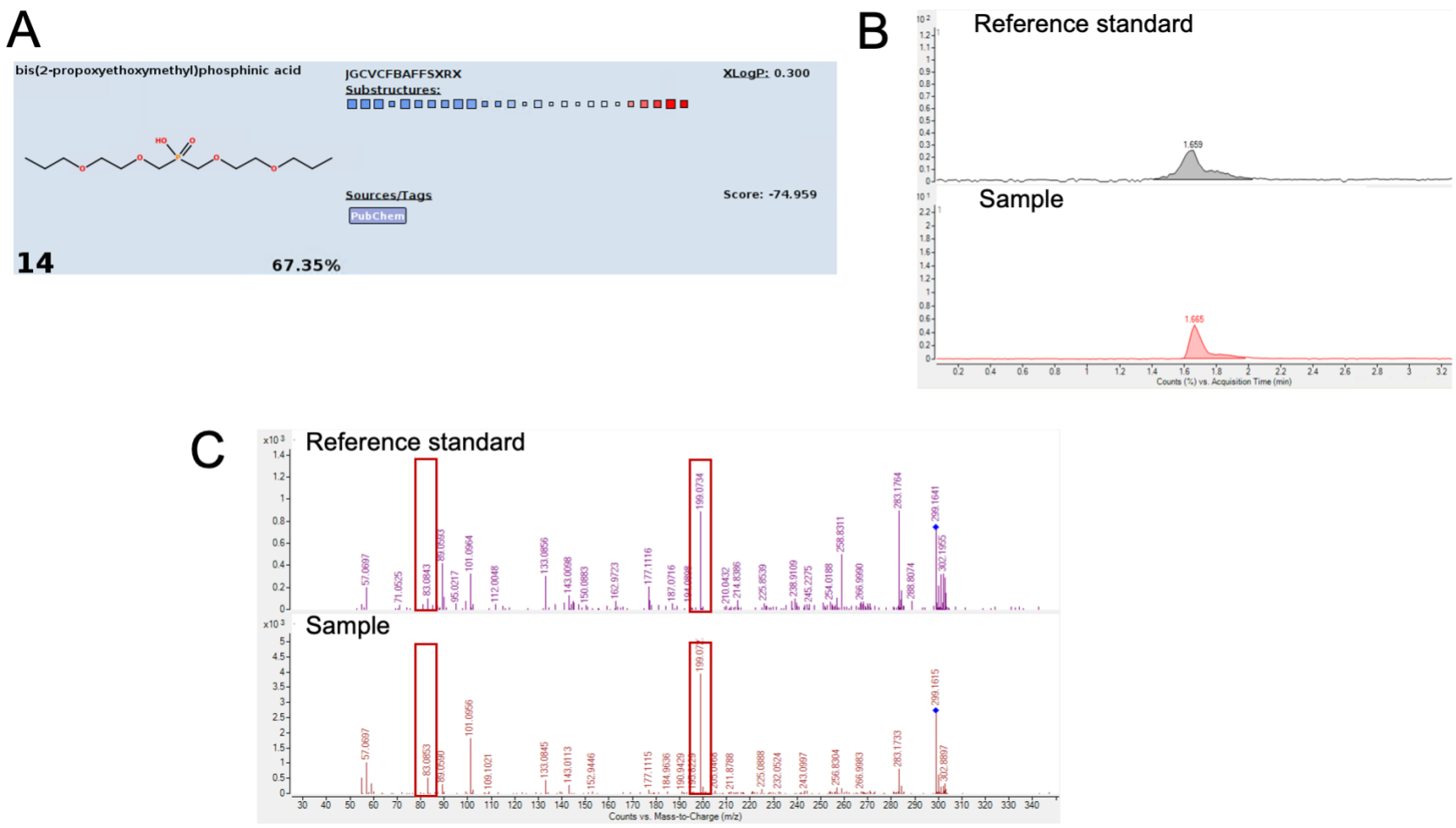


Figure S6.14 – Identification of BBOEP as a degradation product in TBOEP samples in the model solution, heated at 100 °C for 240 minutes (A) Structural identification of with SIRIUS. (B) Extracted ion chromatogram in sample and reference standard. (C) MS/MS spectrum of BBOEP in sample and reference standard at CE 10V.

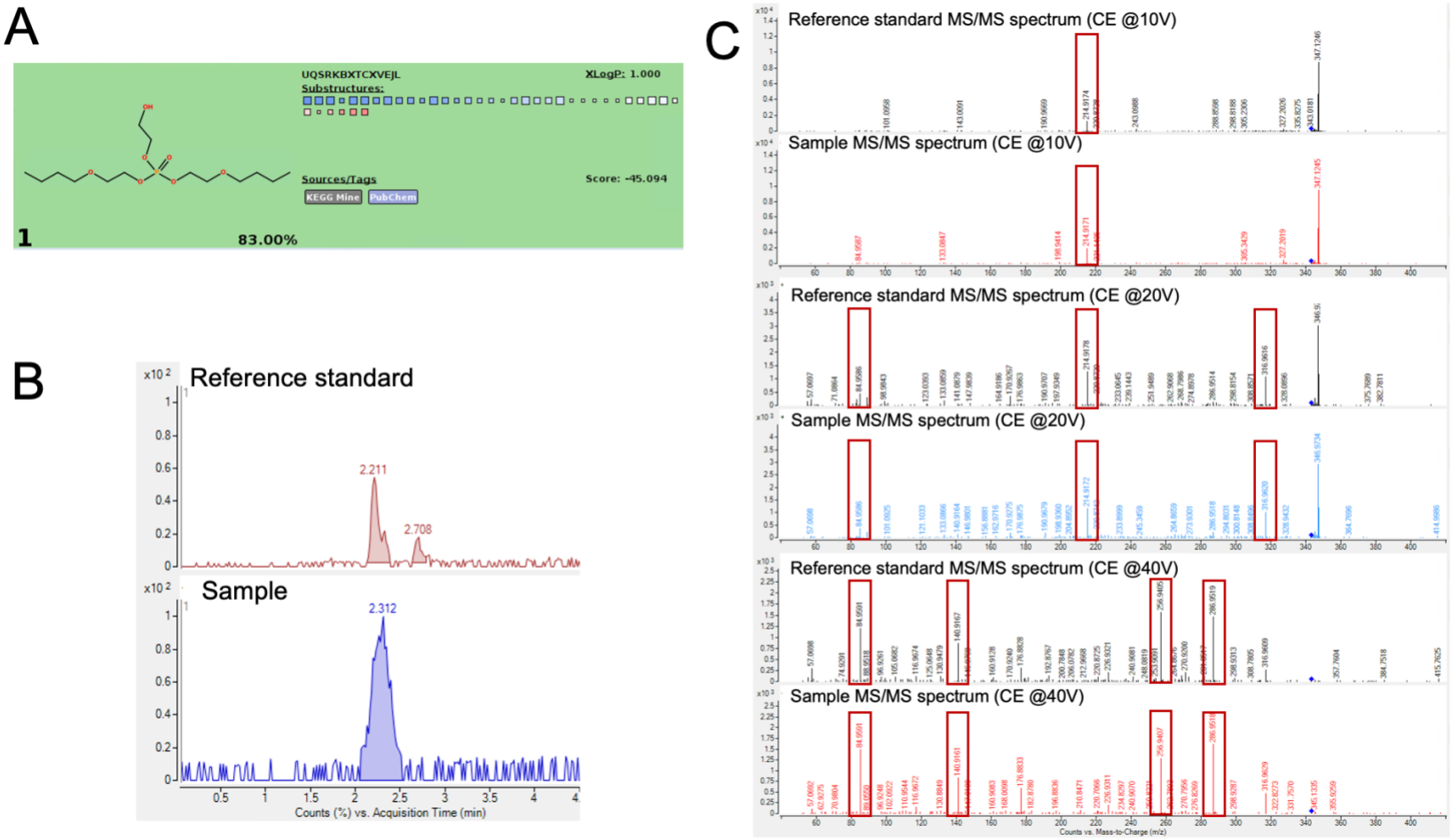


Figure S6.15 – Identification of BTBOEP as a degradation product (TBOEP-DP2) in TBOEP samples in the model solution, heated at 100 °C for 240 minutes (A) Structural identification of with SIRIUS. (B) Extracted ion chromatogram in sample and reference standard. (C) MS/MS spectrum of BTBOEP in sample and reference standard at CE 10, 20 and 40V.

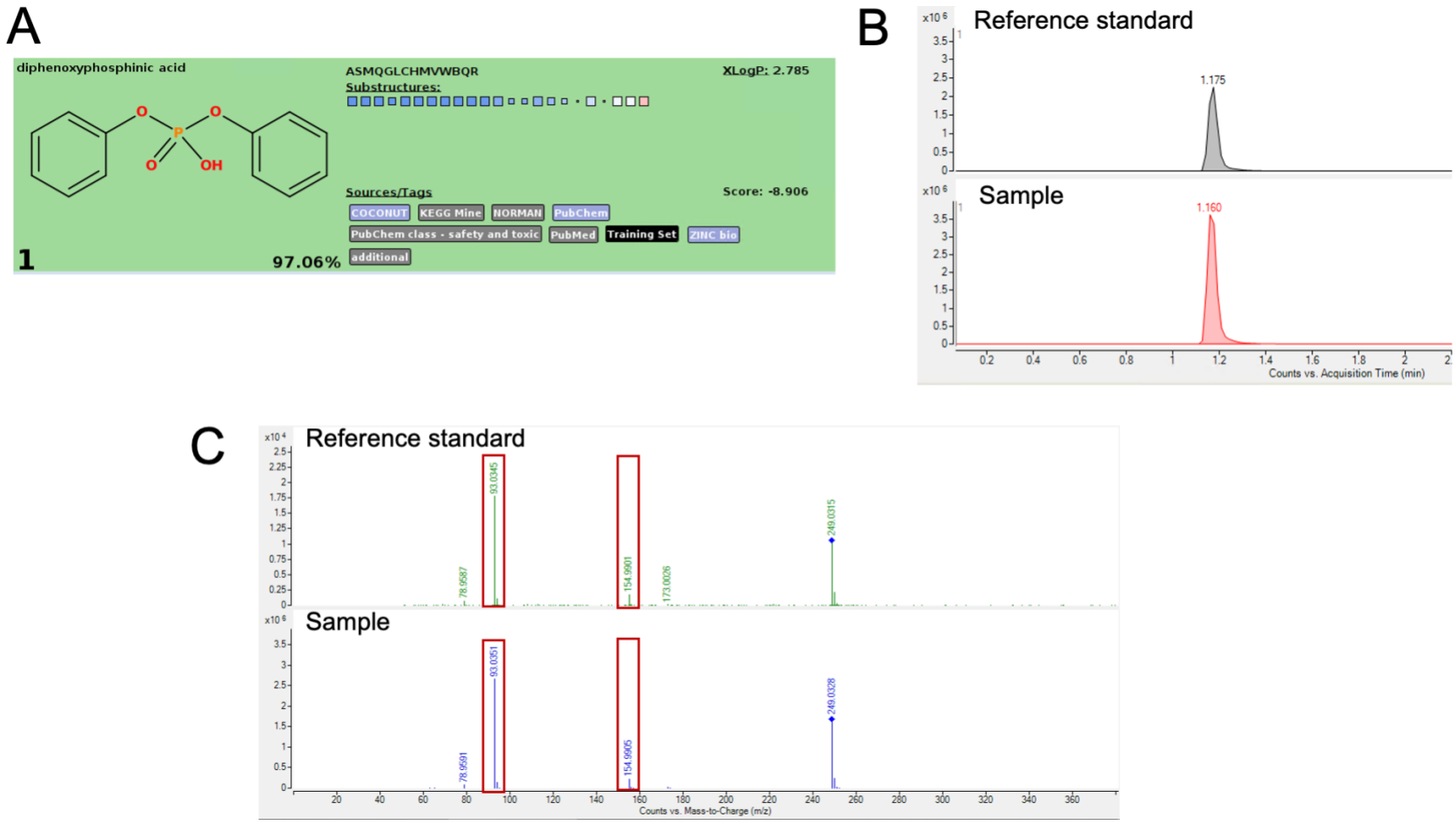


Figure S6.16 – Identification of DPHP as a degradation product in TPHP samples in the model solution, heated at 100 °C for 240 minutes. (A) Structural identification of with SIRIUS. (B) Extracted ion chromatogram in sample and reference standard. (C) MS/MS spectrum of DPHP in sample and reference standard at CE 20V.

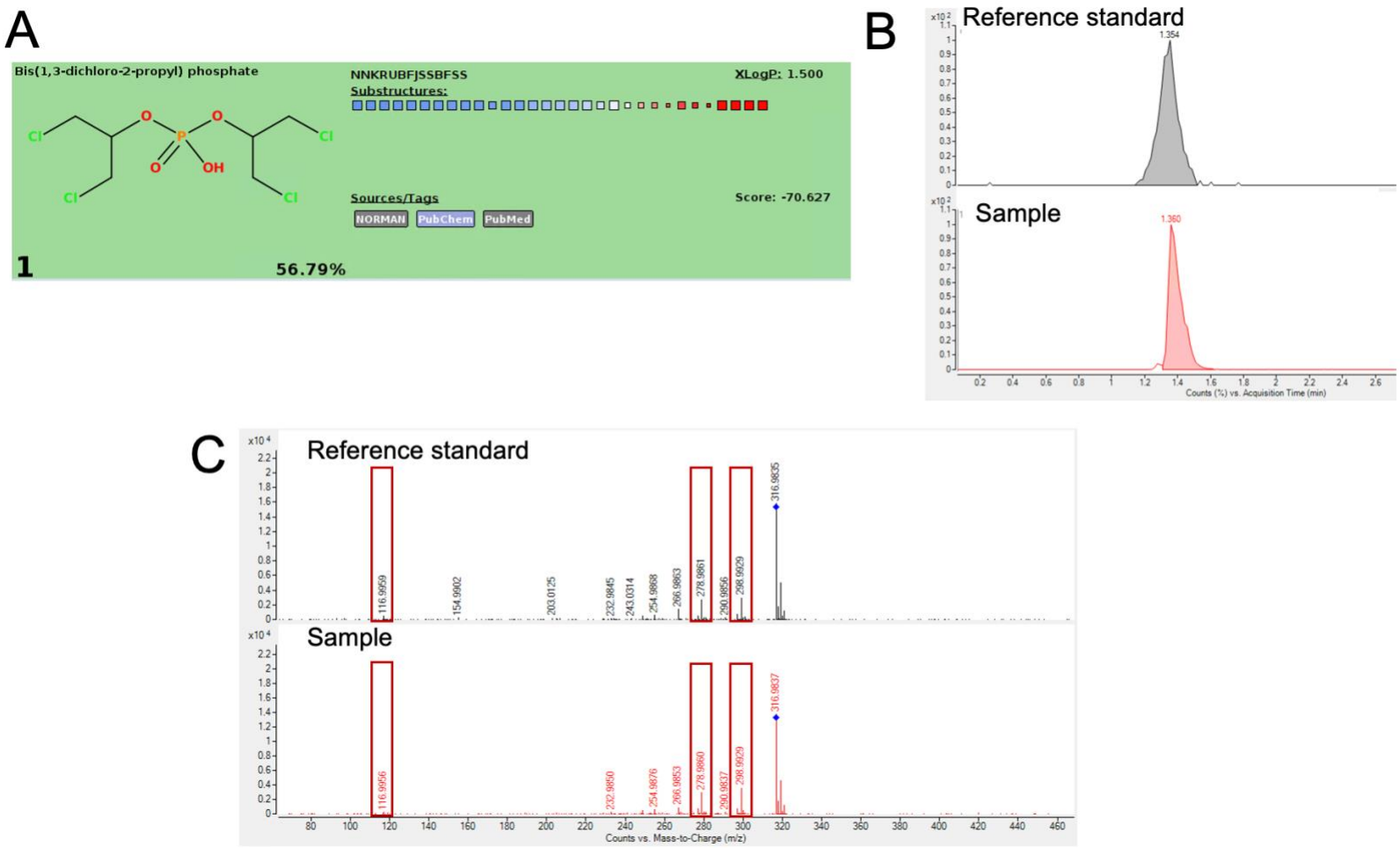


Figure S6.17 – Identification of BDCIPP as a degradation product in TDCIPP samples in the model solution, heated at 100 °C for 240 minutes. (A) Structural identification of with SIRIUS. (B) Extracted ion chromatogram in sample and reference standard. (C) MS/MS spectrum of BDCIPP in sample and reference standard at CE 10V.

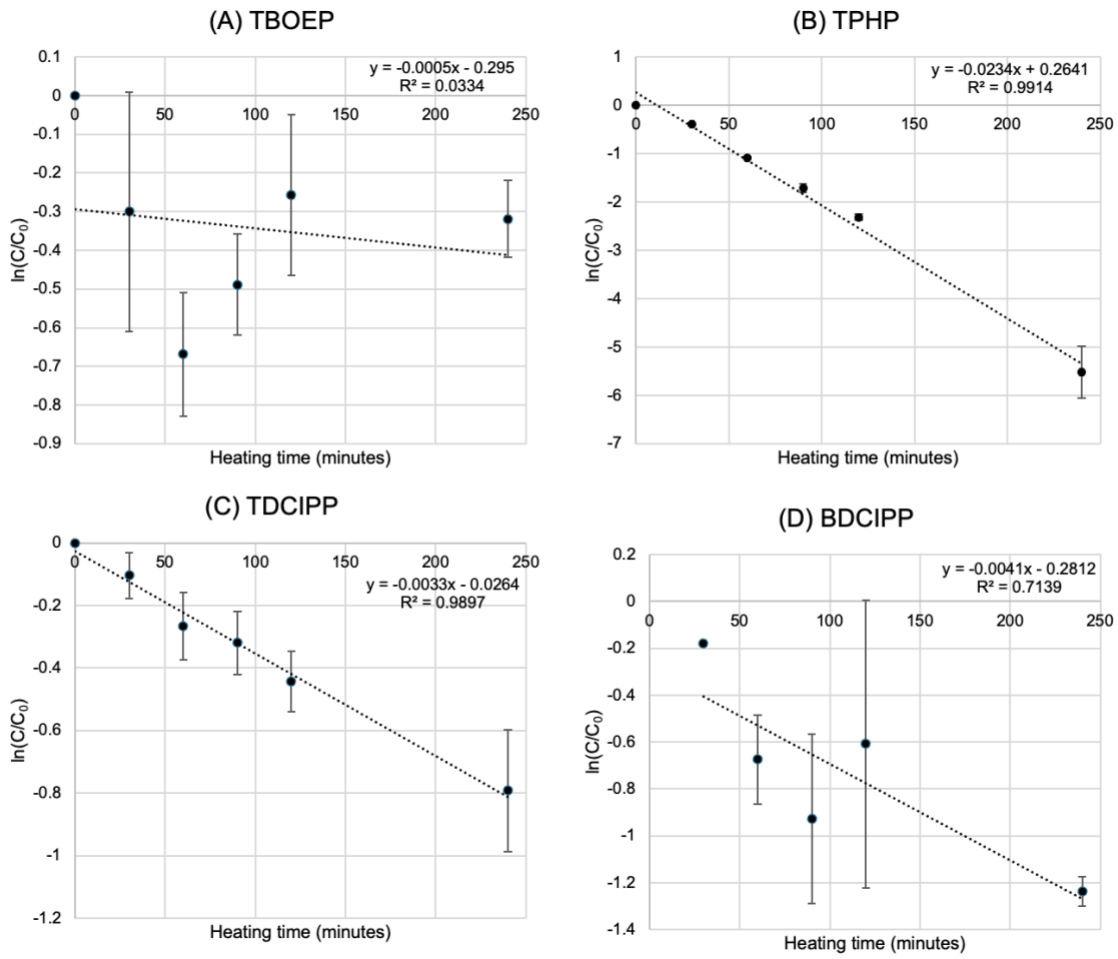


Figure S6.18 – Degradation kinetics in model solution at 100 °C for (A) TBOEP, (B) TPHP, (C) TDCIPP and BDCIPP.

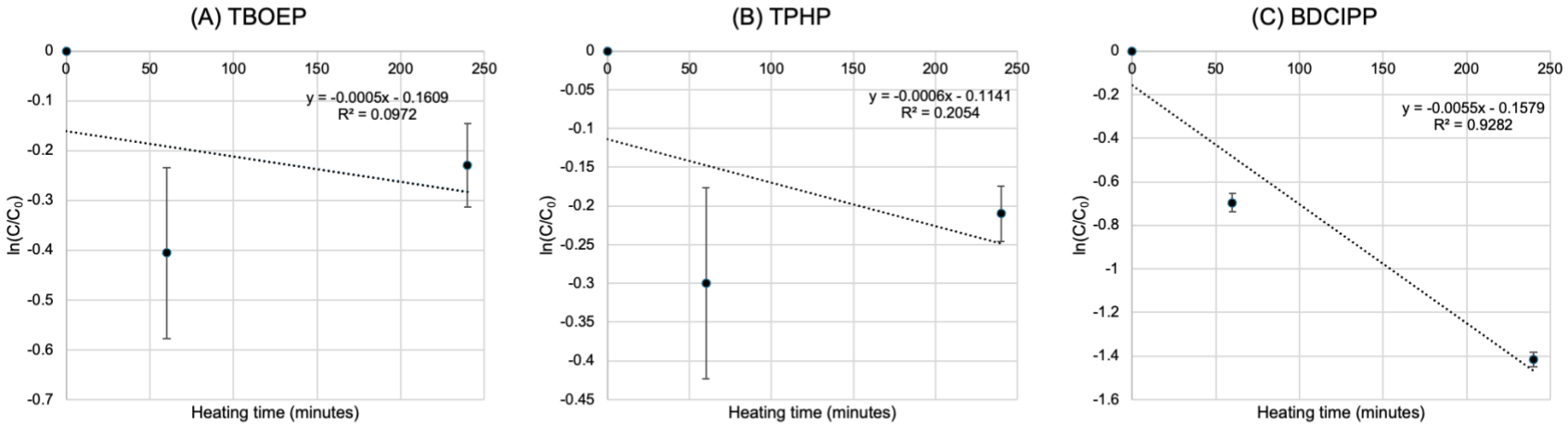


Figure S6.19 – Degradation kinetics in honey solution at 100 °C for (A) TBOEP, (B) TPHP, and (C) BDCIPP.

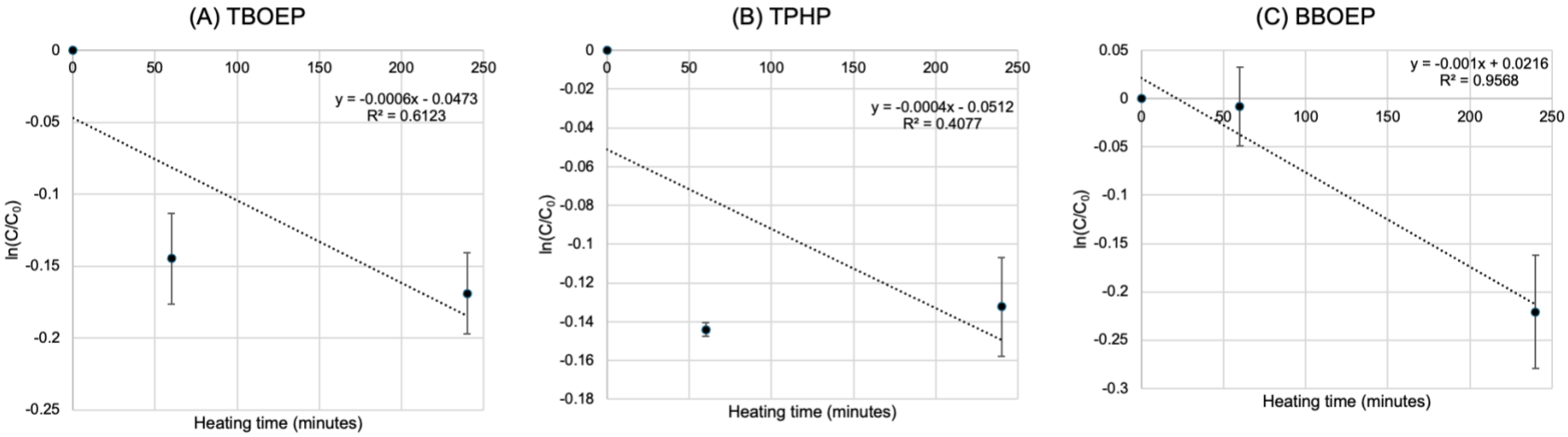


Figure S6.20 – Degradation kinetics in the honey matrix at 70 °C for (A) TBOEP, (B) TPHP, and (C) BBOEP.

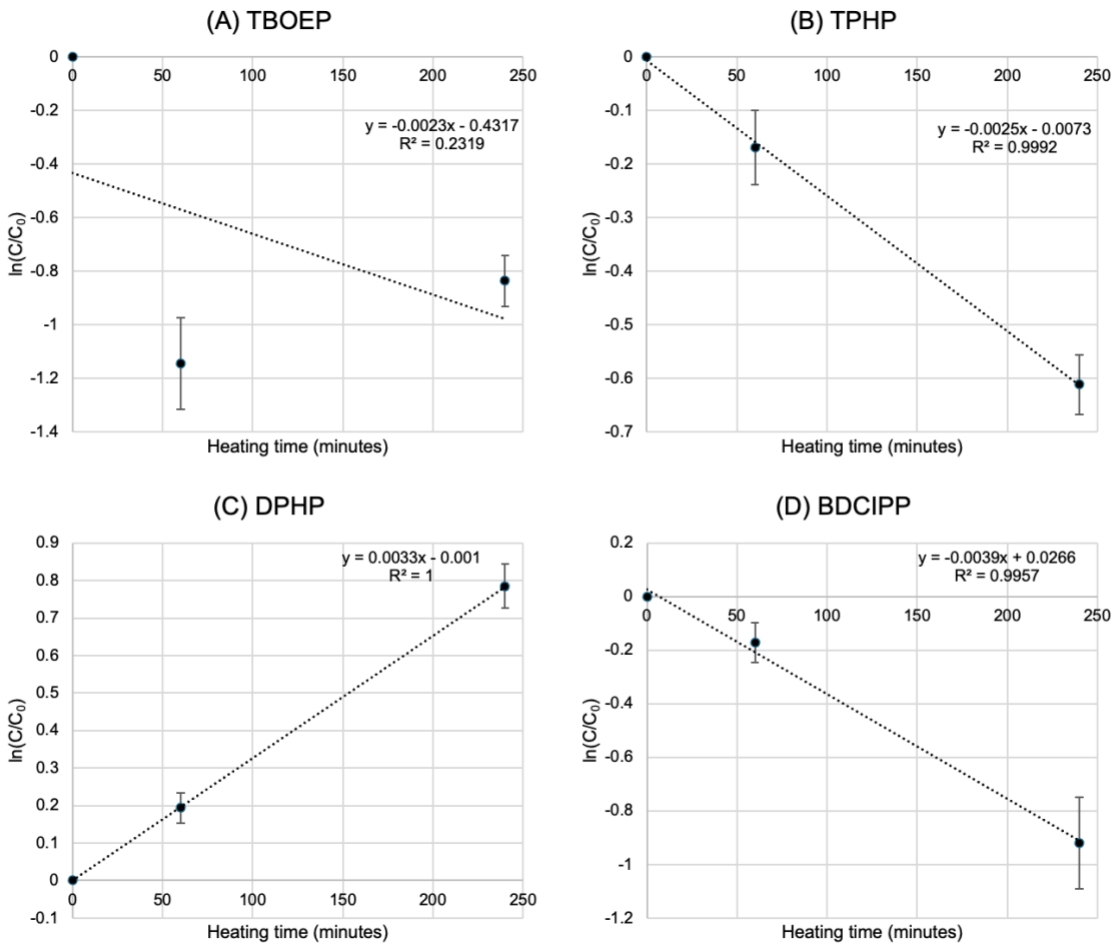


Figure S6.21 – Degradation kinetics in the chicken matrix at 100 °C for (A) TBOEP, (B) TPHP, (C) DPHP and (D) BDCIPP.

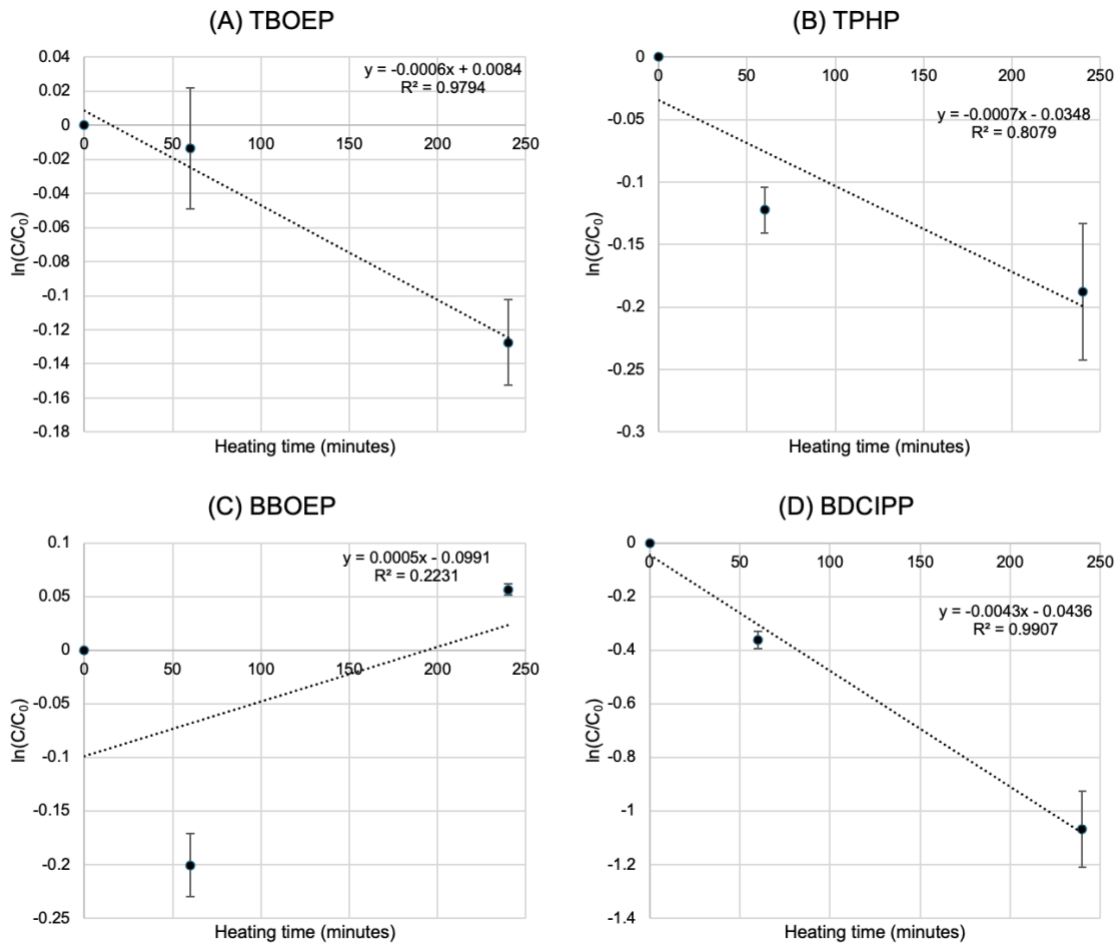


Figure S6.22 – Degradation kinetics in the salmon matrix at 100 °C for (A) TBOEP, (B) TPHP, (C) BBOEP and (D) BDCIPP.

Chapter 7. General Conclusions

7.1 Conclusions

This main research objective of this project was to investigate the additional sources and fate of OPEs and NFRs in food. With a workflow that combines targeted and non-targeted analysis, contaminants present in the samples could be detected and identified at trace levels with LC-MS, this included unreported FRs and their degradation products under heating. The workflow also incorporated a machine-learning software, *SIRIUS*, which was used for structural identification for unknown compounds. The studies in this project have shown that this workflow could be comprehensive for detecting unknown compounds present in the samples. Regarding the current exposure assessment for FRs, regulatory agencies depend mostly on the surveillance of raw food samples, but the level of contaminants could have been influenced by industrial processes or thermal treatment. Therefore, this research project bridged the knowledge gaps identified in the current literature, which concerns the additional sources and fate of OPEs and NFRs.

Regarding the additional sources of FRs in food, a non-targeted screening workflow was developed for the detection of FRs in honey and plastic food packaging materials, involing a self-built screening library. In both types of samples, simple sample preparation methods were involved: the former used a direct injection method, while the latter used a direct extraction method. The results demonstrated that with the developed non-targeted screening workflow for FRs, unreported compounds present at trace levels (from 2.77-1290 ng g^{-1} in honey, and from 1-560 ng cm^{-2} in packaging samples) could be detected and identified correctly for a large number of samples. This was also beneficial to apprehend the trend of occurrence of FRs in the samples. For instance, the difference in FR profile between rural and urban honey samples could be recognized, using the chemical features extracted based on the FR screening library on PCA plots.

For additional sources of FRs from plastic food packaging materials, the potential for migration of OPEs was also determined. Based on the fact that the highest concentration of the target OPEs was detected in thermal labels used for fresh food products, the migration of OPEs was examined in both food simulant and real food matrix, which was chicken breast. The results revealed that TBOEP could migrate from the thermal label to both food simulant and fresh food, but it was below the specific migration limit. The results confirmed the hypothesis that some of these OPEs used as additives in the plastic packaging materials may lead to a higher exposure level due to chemical migration, if the food had close contact with the thermal label before consumption. Currently, not all OPEs have a reference for specific migration limits, the lack of this relevant information also implies that the current knowledge may not be comprehensive for an accurate exposure assessment.

In terms of the fate of the FRs in food, a literature review on the current findings was first conducted. This review summarized the abiotic degradation studies done in the literature on the four major classes of FRs in both the environment and food. It was concluded that there was more information on the degradation behaviour of legacy FRs, i.e. PBDEs. The degradation for replacement FRs, including NBFRs and OPEs, was not researched extensively, possibly due to their recent introduction in the industry. Nonetheless, this showed that there is very limited knowledge of the degradation in typical environmental and food processing settings. The scarce information on the degradation in food matrices was also concerning, given the fact that some FRs, especially OPEs, have been detected in food samples across the globe in recent years. Based on the knowledge gaps, it was concluded that it is essential to conduct studies to understand how these

FRs present in food could degrade under heating and if the unknown degradation products are more toxic than the parent compound.

Accordingly, the degradation of OPEs was studied in the model solution and three selected food matrices, at typical cooking temperatures. Based on the results, triester OPEs were observed to degrade in water, but the rate was lowered in the food matrices. In terms of exposure assessment, this worst-case-scenario experimental design could signify that the actual consumption of OPEs from diet could be overestimated, since it does not account for the degradation from heating. However, this neglected the toxicities of the unknown degradation products, meaning that if these degradation products are more toxic, the risk associated with the consumption would be worse. These degradation products include the confirmed diester OPEs, and BTBOEP degraded from TBOEP under heating in water at 100 °C. For other degradation products, although there were proposed structures derived from the non-targeted analysis workflow, they were not confirmed with the reference standards. Although some of these degradation products resembles the parent OPE structures, they were not present in the chemical online database, according to the MS/MS spectra matching algorithm with these databases by *SIRIUS* . This means that unknown compounds were produced during the heating process, and the identification was limited in this workflow without authentic reference standards. Moreover, the inconsistent degradation trends of these OPEs, under different food matrices and heating temperatures were observed, and different mechanisms of degradation could occur. This shows that the thermal degradation reactions of OPEs are compound- and matrix-dependent. More studies on the interaction between OPEs and the food matrices may be able to offer a better explanation of the results.

Overall, the additional sources of OPEs and NFRs and their fate in different scenarios were studied, and the results demonstrated that there are limitations in the current exposure assessment of FRs in food. For example, these FRs detected from raw food samples could have originated from the migration of food packaging materials, not from the environment (incurred in the food); and these FRs present may degrade after cooking. Besides FRs, this hypothesis can be applied to other contaminants as well. Overall, this thesis suggests that, with the additional sources and fate of OPEs and NFRs in food, regulatory agencies could refine the process of assessing the intake levels, which could reflect a more realistic situation of how the general population are exposed to these contaminants from food. Hence, this could also improve the overall accuracy of the risk assessment of these FRs via diet.

7.2 Contribution to knowledge

This research project has various aspects of novelty in analytical chemistry, food safety and risk assessment.

From the analytical chemistry perspective, this study was the first to apply a non-targeted screening workflow to study the presence of unknown compounds in honey and food packaging samples. With the in-house FR screening library, this workflow could be applied to detect FR contaminants in other samples; or apply screening on other classes of contaminants from the samples analyzed. Additionally, this non-targeted workflow incorporated a machine-learning software, *SIRIUS*, which utilizes MS/MS data and online database for structural identification for unknown compounds. This improves the confidence level of the identification of unknown copomunds before confirmation with authentic reference standards.

This was also the first study to apply non-targeted analysis to determine the degradation products of OPEs, under cooking temperatures in food matrices. With the emergence of non-targeted analysis in the field of food and toxicant analysis, this project demonstrated that data acquired from HRMS could be processed and could offer a holistic concept of extractable chemical compounds. This complements the traditional approach of targeted analysis to uncover the presence of unexpected compounds in the samples. This application of non-targeted analysis also permitted the identification of unknown compounds present in the food matrices, including proposing structures and chemical formulae for the degradation products. It has been known that one of the main challenges of non-targeted workflow is the data processing technique – it is necessary to establish a standardized workflow to handle thousands of chemical features extracted. Despite the fact that this current study depended on a lot of manual feature inspection, the

workflows proposed could aid the future development of a more automated method for data processing.

Regarding the food safety aspect, this study was the first to study the migration of FRs from plastic packaging materials used for fresh food products. More specifically, this was the first study to compare the FRs detected in a range of packaging materials from fresh food products, and revealed that thermal labels used could be a major source of OPE contamination in food, in terms of the concentration. While the current studies focused on investigating different types of polymers used in plastic packaging materials, this study provided a novel angle of looking into the source of OPE contamination by the type of packaging used. Other non-thermal labels on fresh food, such as stickers on fresh produce, or processed food could also be the next target of investigation. This study was also the first time a controlled migration study was reported in the literature to determine the specific migration limit of OPEs in food, an extension of using real food samples for migration studies in the field could bridge the gap of extrapolating the results from using food simulants.

Concerning the exposure and risk assessment, this was the first study of the degradation of OPEs in water and food matrices under heating, which demonstrated that using raw food for exposure assessment may not be reliable enough for the actual exposure level from diet. The same conclusion has been drawn from previous degradation studies done on antibiotics and fungicides, where these contamaints have shown trends of degradation under heating. Hence, this study could facilitate adjustments to the current exposure assessment, for instance, there could be additional sources of contamination from food packaging materials, also, the concentration of some OPEs may decrease after heating. With the identification of unknown degradation products of OPEs produced during the heating process, this study may also suggest compounds that could be

prioritized for toxicity testing. For example, the overall toxicities of diester OPEs are not well-studied currently in the literature, but they were determined to be one of the degradation products in both the aqueous model solution and food matrices. Hence, the risk assessment could also be revised, by incorporating knowledge of a more accurate exposure assessment, as well as the toxicities of the unknown degradation products identified.

In terms of other applications, this was the first time the half-life of the target OPEs was determined under actual cooking conditions in both the aqueous model solution and food matrices. The trend observed could aid the prediction of the half-life of other OPEs based on the side-chain chemistry, with more OPEs studied. This study was also the first to report the difference in degradation trend between spiking in raw and cooked food, where it showed that the degradation observed for most OPEs was not due to the conformational changes in the food matrix and trapping these OPEs within, and hindering the extraction process.

7.3 Recommendations for future studies

Regarding the sources and fate of OPEs in food, this project could be expanded for other future studies. For the FRs detected from honey samples, it could be determined if these contaminants originated from the atmospheric air, where they attached to the body of the honeybees directly, or from the flowers honeybees travelled to collect nectar. It could also determine if these FRs could be metabolized in the honeybees before being transferred to the honey. Other classes of contaminants that could occur in atmospheric air could also be screened in honey samples, such as, polycyclic hydrocarbons. Some of these contaminants are more suitable to be analyzed by GC-MS, therefore, this workflow could be optimized based on the current study. Concerning the source of contamination of OPEs in honey samples, other factors could also be taken into consideration, such as the landfill or waste management locations, or further dividing the production sites into rural, sub-urban and urban areas. This may provide further insight for the sources of contamination. Additionally, as some of the OPEs could attached to dust particulates in the air, it would also be useful to determine the particulate content in the honey samples.

For the study on detecting FRs in plastic food packaging materials, knowing that these OPEs used as adhesive could potentially migrate from the thermal labels to the food, non-targeted screening could be applied to screen for other chemicals used as adhesive or coating agents that are not FRs. More OPEs or other additives with available reference standards could also be added in the targeted analysis, to provide a more comprehensive picture of the contaminant profiles in different types of fresh food packaging. More target OPEs could also. provide a better understanding of the migration behaviour of other OPEs and additives used in food packaging materials, especially in the controlled migration experiment with real food matrices. More food

matrices could also be used in the controlled migration experiment, to understand if these FRs would have different migration behaviour depending on the food, such as the lipid or water content. In addition, melamine was identified correctly in the non-targeted screening workflow as one of the contaminants with high signals in the thermal labels. Consequently, an extraction method and LC-MS method for melamine would be useful to determine the migration behaviour from the thermal label to food. This could be applied to other compounds identified in the non-targeted screening workflow as well. Knowing that these OPEs and NFRs are used in specific plastic packaging materials, understanding the types of plastic in thermal labels or the wrapping film may also be useful to know if they are contributing factors to the chemical migration of OPEs or NFRs in food.

In the degradation study of OPEs in food, only three food matrices were selected. The study could be extended to other types of food matrices with a wider range of characteristics, such as water or lipid content. Interactions between contaminants and the food matrices was not well-studied in the literature, this could be determined by mimicking the food matrices or isolating significant compositions, and thus provide a better understanding of the interactions between OPEs and the food matrices. Alternatively, the use of ^{1}H or ^{13}C labelled standards for the degradation reaction could also offer some insights into the degradation mechanisms or their interactions with the food matrices. Advanced knowledge of these interactions could also help predict the degradation behaviour of other classes of contaminants found in food. Other cooking methods could also be studied, including microwave, or frying which involves a higher temperature. This degradation study also relied on the spiking of OPEs in food samples, it would be appropriate to use incurred samples as well. The heating experiments conducted also involved extreme conditions,

such as using prolonged heating period. Additional experiment with common cooking practices, such as applying a normal cooking duration, temperature, and method could also offer a provide a realistic degradation trend of OPEs. Moreover, the current risk assessment in Canada involves grouping of OPEs based on the side-chain chemistry, such as aryl-OPEs. While the grouping for assessment could improve the overall efficiency, given that there is a large number of OPEs, the current study also revealed that the degradation reactions are compound- and matrix-dependent. Therefore, a suitable model would be useful to determine appropriate extrapolation factors for the prediction of behaviour of individual OPEs.

Overall, this project has shown the workflows adopted could effectively analyze FRs as contaminants in food samples, for instance, non-targeted analysis to screen for unreported contaminants and degradation products, as well as controlled migration study for fresh food samples. These analyses could be applied and optimized for other classes of contaminants found in food, to improve the accuracy of the general exposure assessment from diet.

General Reference List

Note: In accordance with the Guidelines for Thesis Preparation, each of the manuscript chapters (i.e., Chapters 3-6) contain their own reference list. Hence, the following list corresponds to the references included in the remaining chapters of the thesis (i.e., Chapters 1 and 2)

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