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SANTÉ ET ALIMENTATION AU CŒUR DE LA VIE

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Développement d'une stratégie analytique dédiée aux esters organophosphorés.

Contribution à l'évaluation de l'exposition de l'homme à ces contaminants ré-émergents *via* l'alimentation.

JURY

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DEDICATION

To the Loving Memory of My Brother…

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To the esteemed members of the jury of this thesis,

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RÉSUMÉ DES TRAVAUX

Le développement de nos sociétés industrielles, vecteur de nombreux progrès, a profondément modifié non seulement notre mode de vie, dont notre alimentation, mais également notre environnement. De nouvelles substances synthétiques ont été intégrées dans les produits de consommation courante afin d'exploiter leurs diverses propriétés physico-chimiques. Parmi ces substances, les retardateurs de flamme (RF) sont utilisés couramment depuis les années 1960 pour assurer des degrés divers de protection contre l'inflammabilité, notamment des polymères d'origine pétrochimique. Les RF sont en effet en mesure de contribuer, de manière notable, à la réduction des risques de départ de feu et à l'amélioration de la sécurité dans les habitations et les lieux publics.

Chimiquement parlant, les RF sont des composés divers, incorporés dans les polymères et largement utilisés dans des objets usuels tels que les équipements électroniques, les textiles, *etc.*.. Ils améliorent la réaction au feu des polymères et permettent leur utilisation en conformité avec la réglementation incendie. Le bénéfice qu'ils apportent doit cependant être mis en balance avec les risques chimiques associés à leur usage, qui peuvent être considérés à différents stades du cycle de vie des produits et des matériaux polymères les composants. Comme dans beaucoup d'autres domaines d'application de la chimie, les risques et bénéfices doivent alors être considérés.

Historiquement, les RF les plus largement utilisés sont les RF bromés (RFB), par exemple les polybromodiphényléthers (PBDE), les hexabromocyclododécanes (HBCDD) ou le tétrabromobisphénol A (TBBP-A). Cependant, certains de ces RFB ont montré des effets délétères sur l'environnement et la santé de l'Homme (leur toxicité sur le plan du développement, de leur neurotoxicité, de leur immunotoxicité, de leurs effets perturbateurs endocriniens, etc.) et se sont révélés être persistants et bioaccumulables dans l'environnement. Par conséquent, du fait des effets toxicologiques de ces composés, des restrictions ont été fixées par les Nations Unies et l'Union Européenne sur certains RFB, notamment les polybromodiphényléthers (PBDE), RFB majoritairement utilisés qui ont graduellement été éliminés du marché. La demande actuelle pour des composés aux propriétés de retardateur de flamme tend alors à se diriger vers des composés alternatifs, tels que de nouveaux RFB ou encore les retardateurs de flammes phosphorés (RFP), dont certains sont halogénés. Avec l'application des nouvelles règles environnementales et sanitaires, les RFP trouvent une place privilégiée dans le marché de ces additifs. En conséquence, l'une des principales classes de RF aujourd'hui utilisées dans les plastiques et les textiles est celle des RFP. Ces derniers peuvent être divisés en (i) RFP inorganiques, tels que le phosphore rouge et le polyphosphate d'ammonium et (ii) les RF organophosphorés (OPFR) comprenant trois sous-groupes: les phosphinates, les phosphonates et les esters d'organophosphate (OPE).

En pratique, les OPE sont utilisés dans la technosphère pour deux raisons: (i) les non halogénés sont surtout employés comme plastifiants, bien que dans certains cas, ils soient aussi utilisés comme RF, tandis que (ii) les chlorés et bromés sont fréquemment utilisés comme RF dans les polymères. Parce qu'ils présentent à la fois des propriétés de RF et de plastifiants, les OPE constituent donc un groupe important d'additifs pour polymères sur le marché, comprenant au moins 22 composés.Cependant, leur utilisation en tant qu'additifs à des fins diverses pose un risque puisqu'ils peuvent migrer des produits dans lesquels ils sont incorporés et être transférés par la suite vers l'environnement. L'Homme peut alors y être exposé principalement par une combinaison de voies orale et par inhalation.

Des études récentes ont révélé que plusieurs OPE présentaient des effets potentiels de perturbation endocrinienne. Dans les environnements intérieurs, divers articles rapportent pour ces substances des concentrations comparables ou supérieures à la concentration de RFB qu'elles remplacent. De plus, la détection récemment rapportée de ces composés dans des régions polaires reculées suggère qu'ils sont sujets à un transport sur de longues distances dans l'environnement et plus persistants qu'on ne le pensait autrefois.

Dans ce contexte les OPE sont désormais considérés comme des polluants ré-émergents, avec une consommation mondiale croissante qui suscite de ce fait un intérêt grandissant de la communauté scientifique et des autorités sanitaires afin de statuer quant au risque associé. Si quelques données relatives à l'analyse des ces contaminants dans différents compartiments de l'environnement (par exemple la poussière, l'air et les sédiments) sont rapportées dans la littérature, peu d'information en revanche sur leur présence dans le biote est disponible, notamment en raison de l'absence de stratégie analytique efficace. Par conséquent, la nécessité d'approfondir les connaissances sur ces composés est très clairement ressentie, en particulier en France, où ce type de données fait particulièrement défaut.

Dans ce contexte et afin de contribuer à l'évaluation du risque associé à ces contaminants ré-émergents, un travail de doctorat a été élaboré entre le Laboratoire d'Étude des Résidus et Contaminants dans les Aliments (LABERCA) et le Laboratoire d'Analyse des Composés Organiques (LACO). Une convention de co-tutelle internationale a été formalisée entre les structures d'appartenance respectives: l'Ecole Nationale Vétérinaire, Agroalimentaire et de l'Alimentation Nantes Atlantique (Oniris) située à Nantes (France) et l'Université Libanaise de Beyrouth (Liban).

D'une part, le LACO installé à la faculté des Sciences à l'Université Libanaise à Hadtah est un laboratoire attaché à la Commission Libanaise de l'Energie Atomique - Conseil National de la Recherche

Scientifique Libanais (CLEA – CNRSL) et s'intéresse aux domaines de la chimie analytique et de la chimie analytique appliquée. Le laboratoire assure le développement et l'application des méthodes d'analyse pour la recherche des résidus des pesticides dans les produits alimentaires et dans l'environnement et pour la recherche des hydrocarbures Aromatiques Polycycliques dans les aliments d'origine animale, dans l'eau et dans le sol. Le laboratoire surveille également les résidus d'antibiotiques dans la viande et dans le miel au Liban. Les méthodes d'analyse de routine appliquées sont optimisées suivant les exigences de la norme ISO 17025.

Le présent travail de thèse qui se trouve dans le cadre de l'analyse des contaminants organiques environnementaux, fait partie de l'objectif principal du laboratoire pour l'élaboration de stratégies analytiques nécessaires à l'analyse des contaminants organiques éventuellement présents dans la chaîne alimentaire et dans l'environnement.

D'autre part, le LABERCA est une Unité de Recherche labellisée par la Direction Générale de l'Enseignement et de la Recherche (DGER, MAAP) et l'Institut National de la Recherche Agronomique (INRA, département AlimH). Il est par ailleurs Laboratoire National de Référence (LNR) conventionné par la Direction Générale de l'Alimentation (DGAl, MAAP) et la Direction Générale Health and Consumer (Commission Européenne) pour ce qui est de l'accompagnement de l'autorité compétente en matière de gestion de risque dans ses activités liées aux substances chimiques dans les denrées alimentaires. En parallèle, aux côtés des agences d'évaluation, il contribue à l'évaluation du risque chimique dans les aliments en produisant des données originales de contamination. Dans le cadre de son programme stratégique de recherche, le LABERCA s'intéresse depuis plusieurs années aux RFB. Avec la diversification des RF, il est alors apparu nécessaire de combler le manque de données sur les niveaux de contamination en OPE le long de la chaîne alimentaire. C'est dans cette problématique générale de santé publique que s'inscrit le présent travail de thèse pour lequel deux grands objectifs de recherche ont été définis comme suit:

- **Objectif 1-** Étudier, développer et optimiser les stratégies analytiques les plus adaptées pour identifier et quantifier les OPE à l'état de traces dans des matrices biologiques complexes.
- **Objectif 2-** Évaluer les niveaux de contamination en ces composés dans un ensemble d'échantillons de denrées alimentaires prélevés dans différentes régions françaises afin de contribuer à l'évaluation de l'exposition de la population.

Pour répondre à ces objectifs, ce mémoire de thèse a été organisé en quatre chapitres:

➢ Le **chapitre** 1, bibliographique, traite de la thématique générale associée aux RF puis focalise sur les OPE (Figure A) et présente les éléments relatifs à l'évaluation du risque associé. Il apparait que les OPE peuvent être distingués selon leur appartenance à trois familles chimiques différentes que sont les alkyles, les aryles et les composés halogénoalkyles. Le présent travail se concentre sur 18 OPE appartenant à ces trois familles, comme illustré Figure 1-9.

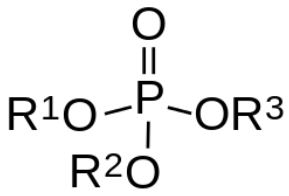

Figure A: Structure chimique des esters d'organophosphate (OPE)

❖ Les différences de taille et de structure des OPE etudiée peuvent avoir une grande influence sur leurs propriétés physico-chimiques. La nature lipophile des OPE est confirmée par les valeurs de log Kow qui sont positives mais qui varient considérablement entre les composés (gamme d' environ 1 à 10). Il existe également une grande variété en termes des facteurs de bioconcentration (BCF) qui varient de 1,37 pour le phosphate de tris-2-chloroéthyle (TCEP) à 10^6 pour le phosphate de tris(éthylhexyle) (TEHP). Le BCF augmente généralement avec l'augmentation de la masse moléculaire, à l'exception des composés contenant du chlore. Les OPE non halogénés ayant des masses moléculaires supérieures sont donc plus susceptibles d'être trouvés dans la nature que ceux avec des masses moléculaires inférieures. Pour les OPE contenant du chlore, aucune relation ne peut être trouvée entre le BCF, la masse moléculaire ou la proportion de chlore dans la molécule. Selon le critère de la réglementation de l'Union européenne sur l'enregistrement, évaluation, autorisation et restriction des produits chimiques (REACH) (BCF ≥ 500), les OPE aryliques peuvent être classés comme composés bioaccumulables. Cependant, le potentiel réel d'accumulation des OPE est variable d'une espèce à l'autre. De plus, chaque composé est transféré différemment le long de la chaîne alimentaire en raison de la variation des propriétés physico-chimiques, la biodisponibilité et de la transformation métabolique au sein des organismes. Dans les milieux aquatiques, les facteurs de bioamplification (BMF) des OPE semblent plus importants à travers le réseau trophique benthique qu'à travers le réseau trophique pélagique. Cela est vraisemblablement dû au fait que les OPE sont généralement adsorbés sur les particules et donc probablement plus abondants dans le sédiment que dans la colonne d'eau.Les données sur la persistance

dans l'environnement et la toxicité associée sont limitées. Les résultats des travaux précédents ont montré une dégradation rapide des phosphates de tris-2-butoxyéthyle (TBEP) et de tributyles (TnBP et TiBP) par la lumière solaire. Par contraste, les OPE chlorés semblent être plus résistants à la dégradation par la lumièresolaire. Les phosphates de tris(2-chloroisopropyle) (TCPP), de tris(1,3-dichloro-2-propyle) (TDCIPP) et le TBEP sont suspectés d'être cancérigènes, tandis que des effets neurotoxiques ont été observés pour le TCEP et le phosphate detriphényle (TPP). En outre, le phosphate de 2-éthylehexylediphényle (EHDP) est considéré comme très toxique pour les poissons et les plantes aquatiques. En conséquence, l'apparition d'OPE dans l'environnement peut constituer une menace pour la santé humaine via diverses voies d'exposition, par exemple par inhalation ou prise alimentaire.

- Les études toxicologiques détaillées, et en particulier celles à long terme, sont limitées et n'ont pas encore été entièrement exploitées, ce qui ne permet pas de conduire une évaluation du risque pertinente. Les valeurs de référence toxicologiques (VTR) pour l'exposition orale chronique sont très rares et n'existent que pour quelques composés (TBP, TBEP, TCP, TCEP, TDCPP).
- Les OPE ont précédemment été observés dans diverses matrices, comme dans l'air intérieur, la poussière en milieu domestique, l'eau potable, les sédiments et le biote. Les OPE ont été détectées dans des poussières d'intérieur à des niveaux variant de 0,02 à 100 µg/g. Ces niveaux semblent être variables d'une région à l'autre, mais également selon l'endroit considéré dans les études (extérieur, intérieur, type de pièce, …). Des concentrations comparables (jusqu'au µg/g) d'OPE ont été rapportées dans des échantillons de poussières en milieux domestiques en UE (Pays-Bas, Danemark, Allemagne et Espagne), en Amérique du Nord (Canada, Californie et Caroline du Nord) et en Asie (Chine et Japon). Les principaux OPE mesurés se sont avérés être les suivants: TBEP, TCPP, TnBP, TCEP, TPP et TDCIPP. Parmi les particularités, il peut être noté le niveau significativement plus élevé de TBEP dans les échantillons japonais, ce qui s'explique par l'utilisation plus fréquente de polissage en raison des planchers de bois dans les maisons japonaises.
- Par ailleurs, des études sur leur présence dans des compartiments biotiques tels les poissons ou les oiseaux sont rares et non exhaustives en termes de nombre de composés d'intérêt mesurés. Il apparait que les principales études relatives à la caractérisation de la contamination des poissons en OPE ont été menées en Asie (Philippine, Chine) et en Europe (Suède). Des valeurs de contamination de l'ordre de la dizaine de µg/g de matière grasse en TBEP ou EHDP ont ainsi été décrites.
- En ce qui concerne les denrées alimentaires autres que le poisson, là encore peu de données sont disponibles. Les rares études semblent indiquer une contamination *via* les matériaux

d'emballage. Il convient ici de souligner l'intégration des OPE dans des Etudes d'Alimentation Totale (EAT) menées en Suède (2015) et aux Etats Unis (entre 1991 et 2003), et notamment la détection de l'EHDP à des teneurs maximales de l'ordre du µg/g dans des bonbons et caramels emballées. Les données disponibles indiquent ainsi que ces contaminants sont ubiquitaires et peuvent être retrouvés dans la chaîne alimentaire en général, le poisson en particulier (qui est connu comme une source alimentaire essentielle pour les gens).à des teneurs non négligeables jusqu'à la dizaine de ppm (µg/g) rapporté au taux de matière grasse. Cependant, ces données apparaissent limitées et non exhaustives, notamment en France, et il convient de combler ces lacunes afin de pouvoir évaluer de manière appropriée et rigoureuse le potentiel risque associé à la contamination des denrées par ces contaminants chimiques.

- L'existence de stratégies analytiques robustes et adaptées à la caractérisation d'une large gamme de composés OPE constitue alors un des leviers nécessaire à la génération de données de contamination fiables. Si différentes méthodes dédiées à l'analyse des OPE ont été décrites dans la littérature, l'étape de préparation des échantillons implique généralement l'extraction liquide sous pression (PLE) associée à l'extraction sur phase solide (SPE) ou la chromatographie par perméation de gel (CPG) comme principales techniques respectivement utilisées pour les étapes d'extraction et de purification des matrices biologiques complexes. Aucune méthode normalisée pour l'extraction et le purification d'OPE à partir de diverses matrices n'est décrite dans la littérature.
- En ce qui concerne l'analyse des extraits, la chromatographie en phase gazeuse couplée à la détection d'azote-phosphore (GC-NPD) ou à la spectrométrie de masse (GC-MS) et la chromatographie liquide couplée à la spectrométrie de masse (LC-MS) ont été les techniques les plus fréquemment utilisés pour l'analyse de ces composés dans des échantillons environnementaux. Selon la littérature, le système GC- NPD souffre d'une sélectivité peu satisfaisante pour les OPE. La MS, d'autre part, est un outil d'identification plus puissant. Un inconvénient de l'utilisation de la source d'ionisation par impact électronique (EI) est la fragmentation étendue des OPE alkylés qui limite la sélectivité d'ions précurseurs choisis en MS en tandem (MS/MS). En outre, l'ionisation chimique en mode positif (PCI) a une sensibilité limitée. Lors de l'utilisation de la LC-MS pour l'analyse d'OPE, la formation d'adduits stables avec des cations métalliques tels que Na^+.

A l'issue de ce premier chapitre, il a ainsi été possible de définir les composés d'intérêt et d'orienter les choix analytiques à mettre en œuvre pour développer une stratégie judicieuse permettant d'intégrer une large gamme d'OPE représentatifs de ce type de contaminants chimiques. Il convient ici de souligner que cette ambition d'une approche multi-résidus constitue l'un des défis de ce travail.

Cette revue bibliographique détaillée a également été utile pour définir les niveaux de prévalence déjà signalés dans différentes régions du monde et ainsi pressentir la sensibilité requise de la méthode à développer. Comme illustré ci-après sur la Figure B, les chapitres suivants sont consacrés à présenter les travaux réalisés et l'investigation de différentes stratégies analytiques pour répondre aux deux objectifs définis, ainsi qu'à la discussion des résultats obtenus.

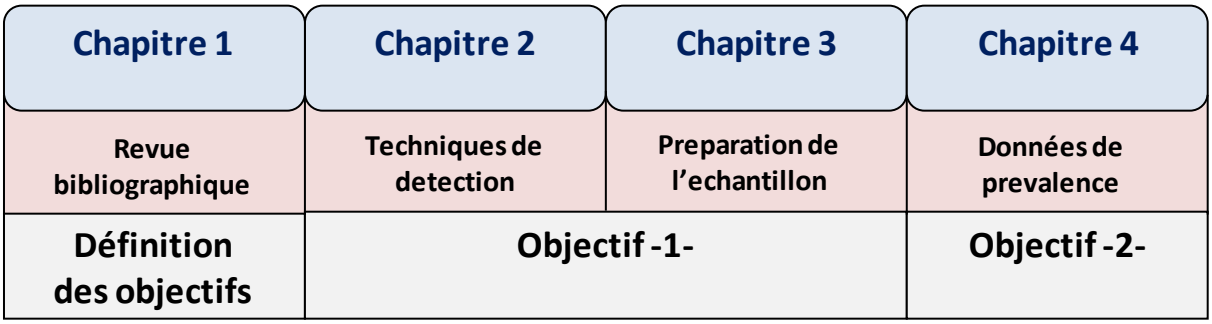

Figure B: Représentation schématique du plan de thèse, à la lumière d'objectifs ciblés.

➢ Le **chapitre 2** décrit les deux approches instrumentales investiguées. Devant le peu de données de référence disponibles dans la littérature, nous avons en effet souhaité dans un premier temps évaluer conjointement l'intérêt de la LC-ESI(+)-MS/MS et de la GC-MS/MS. Malheureusement, la séparation par LC a souffert de co-élutions qui n'ont finalement pu être résolues. Nous avons alors poursuivi le développement en utilisant la GC et l'investigation approfondie de différents modes d'ionisation. Non seulement les principales techniques d'ionisation de GC-MS/MS, c'est-à-dire l'impact électronique (EI), l'ionisation chimique en modes négatif et positif (NCI et PCI), ont été étudiées mais également le mode d'ionisation chimique à pression atmosphérique positive (APCI). Les techniques retenues sont l'EI et l'APCI, pour lesquelles des acquisitions sélectives en utilisant le mode «Selective Reaction Monitoring» (SRM) ont été optimisées. Ici, il convient de souligner que le travail présente, pour la première fois, une méthode instrumentale par GC-APCI-MS/MS pour l'analyse d'une large gamme d'OPE (n=18).

Le développement de la stratégie, comme c'est souvent le cas pour les méthodes multi-résidus qui visent à couvrir en une seule acquisition une large gamme de composés présentant des propriétés physico-chimiques significativement différentes, s'est révélé être une tâche complexe. Nous avons ainsi développé une méthode dédiée à l'analyse de 16 OPE (alkyles, aryles et chloroalkyles) et une autre méthode pour les 2 OPE bromoalkyles. La séparation chromatographique de 16 OPE a pu être réalisée en moins de 25 minutes en utilisant une colonne capillaire longue (30 m x 0,25 mm, 0,25 µm). Dans le même schéma, la séparation chromatographique des 2 OPE bromés a pu être obtenue en moins de 10 minutes en utilisant une colonne capillaire courte (15 m x 0,25 mm, 0,10 µm).

➢ Le **chapitre 3** décrit le développement des procédures de traitement des échantillons de poisson permettant l'extraction optimale d'OPE et une séparation supplémentaire des lipides et d'autres substances interférentes. Plusieurs techniques de préparation ont été investiguées et testées, d'abord sur des solutions constituées de standards analytiques, puis sur la matrice poisson retenue dans ce travail comme représentant d'échantillons biologiques.

L'extraction liquide sous pression (PLE) est la technique la plus utilisée pour l'extraction d'OPE de matrices biologiques telles que les poissons. Par conséquent, cette stratégie a également été sélectionnée. Les principaux paramètres qui influencent l'efficacité d'extraction sont la température, la durée d'extraction, le nombre de cycles d'extraction et le type des solvants. La sélection de la plupart de ces paramètres était basée sur la littérature. La température a été fixée à 100 °C afin d'obtenir un compromis entre le bénéfice de la température élevée et la stabilité des composés ciblés. Le temps d'extraction a été fixé à 5 minutes de temps statique avec un flush dynamique à 100%. Une purge d'azote finale (180 s) a été incluse afin de garantir l'élimination complète du solvant du système PLE. La pression a été fixée à 100 bar. Un seul paramètre, la nature du solvant d'extraction, a été optimisé, par comparaison entre le n-hexane et des mélanges cyclohexane/acétate d'éthyle et *n*-hexane/acétone. Le mélange cyclohexane/acétate d'éthyle a été sélectionné pour sa capacité à extraire une faible quantité de lipides tout en extrayant efficacement touts les OPE.

Diverses techniques de purification ont été testées, notamment:

- La SPE pour laquelle l'efficacité de plusieurs solvants d'élution a été évaluée: toluène, *n*-hexane, dichlorométhane, acétate d'éthyle, *n*-hexane/dichlorométhane 1:1 (v/v) et acétate d'éthyle/acétone 1:1 (v/v). Différents adsorbants ont également été testés, notamment les phases silice et Florisil®, et ce sous différentes formes: pur, désactivé par H_2O et acidifié par H_2SO_4. A l'issue de ce travail, l'acétate d'éthyle a été retenu puisque présentant la force d'élution la plus élevée sur tous les adsorbants étudiés. La silice et le Florisil sous leurs formes désactivées (3% H_2O) ont permis d'atteindre des taux de récupération légèrement plus élevés pour la plupart des composés que celles obtenues avec les formes activée. L'acidification de la silice a conduit à observer des taux de récupération médiocres pour presque toutes les OPE d'intérêt. Il convient de souligner à ce stade que le principal inconvénient de ces protocoles s'est avéré être lié à la contamination des échantillons pendant le protocole analytique, phénomène principalement observé pour les composés TCPP, TEHP et TBEP.
- L'utilisation de l'extraction liquide-liquide (LLE) quant à elle a permis d'observer de bons recouvrements pour la plupart des OPE ciblés, sauf pour le TEHP.

- La chromatographie par permeation de gel (GPC) a ensuite été évaluée. Le recours à cette stratégie a permis une bonne purification de la majorité des OPE en les séparant des lipides présents dans les matrices biologiques, tel le poisson. Ce développement méthodologique a toutefois été réalisé au détriment du taux de récupération du TEHP qui n'a pu être complètement séparé des lipides interférents.

  Les techniques de GPC et de LLE ont abouti à des rendements comparables en termes de taux de récupération des composés, mais pas en termes d'épuisement des lipides où le GPC a montré une capacité beaucoup plus élevée (> 98%).
- L'extraction liquide pressurisée sélective (SPLE) a également été testée par l'ajout d'une phase de purification directement dans la cellule d'extraction par PLE, afin d'améliorer l'élimination des substances interférentes. L'utilisation de 15 g de Florisil® permet d'épuiser environ 60% des lipides contenus dans l'échantillon, tout en maintenant un taux d'extraction satisfaisant pour la plupart des composés ciblés.

Finalement, les approches impliquant la SPE et la LLE n'ont pas été retenues. La stratégie de préparation des échantillons optimisée implique une combinaison de SPLE en tant qu'étape d'extraction et de première purification, suivie d'une étape de purification supplémentaire sur colonne chromatographique par perméation de gel (GPC).

Les performances de la méthode ont alors été évaluées. Pour ce faire, un échantillon composite de poissons, considéré comme un échantillon de contrôle qualité, a été analysé à 20 reprises. Les limites de sensibilité ainsi que d'autres paramètres de performance de la méthode ont été évalués par EI et APCI.

➢ **Le chapitre 4** présente la mise en œuvre de la méthode développée pour l'analyse de différents types d'échantillons alimentaires (n=97 poissons d'eau douce et marins et d'autres produits alimentaires emballés) collectés dans le cadre de divers projets de recherche au sein du LABERCA et précédemment analysés pour d'autres classes de contaminants environnementaux.

De manière synthétique, les échantillons de poisson analysés ont révélé des taux en OPE à des niveaux cumulés (n=18 OPE) inférieurs à 10 ng/g de poids frais. Les échantillons d'aliment analysés ont quant à eux présenté des taux cumulés plus élevés, jusqu'à 100 ng/g poids frais (sauf une exception). Les fréquences de détection des composés se situent entre 3-49%, 5-80% et 5-85% pour les poissons d'eau douce, les poissons marins et les échantillons d'aliment, respectivement. Les profils de contamination sont dominés principalement par le TCPP, le TiBP, le TnBP, le TCEP, le TDCIPP, le TPP et l'EHDP. D' autres composés OPE ont été détectés, mais à des fréquences et aux niveaux beaucoup plus bas.

De manière inattendue, un taux cumulé de l'ordre de 5000 ng/g poids frais a été observé dans un échantillon de gâteau marbré, avec une nette dominance de l'EHDP, un OPE autorisé pour la fabrication de matériaux au contact des denrées alimentaires (MCDA) avec une limite de migration spécifique de 2400 ng/g. Ces données de contamination sont les premiers résultats disponibles au niveau français et contribuent de ce fait à l'originalité de ce travail. Ces premières données sont une première étape vers l'évaluation de l'exposition de la population française pour ces composés ré-émergents.

Pour finaliser ce travail de doctorat et tenter une interprétation plus poussée des résultats observés, nous nous sommes finalement prêtés à un exercice approximatif d'évaluation quantitative des risques en calculant les quotients de risque. Les données préliminaires produites, comparées à des valeurs toxicologiques de référence choisies ou calculées, indiqueraient des ratios de risques faibles au regard des données toxicologiques disponibles. De telles observations conduiraient alors à conclure à l'absence de risque chez l'adulte relatif à ces composés dans les aliments. Néanmoins, des données supplémentaires sur l'exposition et la toxicologie sont nécessaires avant de pouvoir dresser des conclusions plus fines concernant les implications en matière de santé publique.

En résumé, la présente thèse porte sur le développement d'une stratégie analytique dédiée à l'identification et à la quantification des OPE, des contaminants chimiques considérés comme ré-émergents et nécessitant la production de données nouvelles, notamment à l'échelle française. Il s'est ainsi agit d'optimiser les étapes de préparation et d'analyse des échantillons par spectrométrie de masse pour une large gamme d'OPE (comportant des chaînes alkyles, aryles, chlorées ou bromées) à l'état de traces (de l'ordre du ng/g poids frais) dans le poisson et d'autres produits alimentaires emballés, ceci dans un contexte de sécurité chimique des aliments. La procédure de préparation de l'échantillon retenue est basée sur extraction sélective par liquide pressurisé (SPLE) avec du Florisil® en tant qu'adsorbant de lipides, suivie d'une purification supplémentaire par chromatographie par perméation de gel (GPC). La technique de séparation et de détection retenue consiste en une séparation chromatographique en phase gazeuse couplée à la spectrométrie de masse en tandem (GC-MS/MS), avec ionisation par impact électronique (EI) ou par ionisation chimique à pression atmosphérique (APCI), cette dernière figurant comme une approche innovante pour améliorer la détection d'une telle gamme d'OPE. Ces approches analytiques ont été appliquées à l'analyse d'échantillons de poissons et d'autres échantillons alimentaires. Il est attendu que de cette approche innovante contribue à l'évaluation des risques de ces contaminants ré-émergents, à travers la production de données originales sur l'exposition alimentaire à l'échelle française.

Des perspectives à ce travail sont d'ores et déjà envisagées, notamment la validation complète de la méthode analytique développée afin de pouvoir préparer sa mise en œuvre dans un contexte élargi telle que pourrait l'être une future Etude Alimentation Totale, dont le principal objectif est d'atteindre un degré de représentativité plus élevé et plus robuste en termes d'évaluation des risques. Une deuxième perspective sera de communiquer ces premières données aux agences chargées de l'évaluation du risque la sécurité sanitaire des aliments (ANSES, EFSA) pour mieux cerner l'exposition d'une population particulière (forts consommateurs de poisson par exemple) ainsi que d'autres populations de la catégorie adulte. Enfin il serait intéressant d'étendre l'application de la stratégie à la caractérisation de l'exposition interne du consommateur, c'est-à-dire l'étude de son imprégnation aux OPE ainsi que la caractérisation d'éventuels métabolites, ce qui constitue une question importante pour évaluer les risques associés chez l'Homme.

Dans le cadre de ce travail de recherche, plusieurs articles, communications orales et écrites ont été réalisés et présentés comme suit:

- Articles

✓ **Halloum W,** Cariou R, Dervilly-Pinel G., Jaber F, Le Bizec B (2017). APCI as an innovative ionization mode compared with EI and CI for the analysis of a large range of organophosphate esters using GC-MS/MS. Journal of Mass Spectrometry, DOI 10.1002/jms.3899.

✓ **Halloum W,** Cariou R, Dervilly-Pinel G., Jaber F, Le Bizec B (2017). Organophosphate esters in fish and packaged foodstuffs at the French level. En préparation.

- Actes à congrès

✓ **Halloum W**, Cariou R, Vénisseau A, Marchand P, Dervilly-Pinel G, Jaber F and Le Bizec B. Analysis of organophosphorus flame-retardants using gas chromatography coupled to tandem mass spectrometry. 34st International Symposium on Halogenated Environmental Organic Pollutants and POPs, Madrid, Spain, 31st August – 05th September 2014.

✓ **Halloum W**, Cariou R, Dervilly-Pinel G, Jaber F and Le Bizec B. Analysis of two bromine containing organophosphorus flame retardants using GC-EI(+)-MS/MS and LC-ESI(+)-MS/MS. 7th International Symposium on Flame Retardants, Beijing, China, 21–24 April 2015.

✓ **Halloum W**, Cariou R, Dervilly-Pinel G, Jaber F and Le Bizec B. Development of preparation procedure based on in-cell PLE followed by GPC for the analysis of OPEs by GC-EI/APCI-MS/MS. 36th International Symposium on Halogenated Environmental Organic Pollutants and POPs, Florence, Italy, 28th August – 02nd September 2016.

- Communications orales

✓ **Halloum W**, Cariou R, Dervilly-Pinel G, Jaber F, Le Bizec B. Development of preparation procedure based on in-cell PLE followed by GPC for the analysis of OPEs by GC-EI/APCI-MS/MS. 36th International Symposium on Halogenated Environmental Organic Pollutants and POPs, Florence, Italy, 28th August – 02nd September 2016.

✓ **Halloum W**, Cariou R, Dervilly-Pinel G., Jaber F, Le Bizec B. Analysis Of 18 Organophosphate Esters By GC-MS/MS via different ionization techniques (EI & APCI). Forum Doctoral 2016, Ecole Doctorale des Sciences et Technologie, Université Libanaise, Beyrouth, 18–19 mai 2016.

✓ **Halloum W,** Cariou R, Dervilly-Pinel G, Jaber F, Le Bizec B. Development of analytical strategies dedicated for the analysis of organophosphorus flame retardants in biological samples. Journées scientifiques de l'Ecole Doctorale Biologie Santé, La Chapelle-sur-Erdre, France, 9–10 décembre 2014.

➢ Affiches

- ✓ **Halloum W**, Cariou R, Dervilly-Pinel G., Jaber F, Le Bizec B. Analysis of organophosphorus flame-retardants by GC-MS/MS with EI and APCI ionisation techniques. 26th Annual Meeting of Society of Environmental Toxicology and Chemistry, SETAC Europe 2016, Nantes, France, 22nd – 26th May 2016.
- ✓ **Halloum W**, Cariou R, Dervilly-Pinel G, Jaber F, Le Bizec B. Analyse des retardateurs de flamme organophosphorés dans les poissons basés sur la GC-MS/MS, avec ionisation chimique à pression atmosphérique ou par impact électronique. «Les Troisièmes Journées Franco-Libanaises» (JFL3), Hadath, Liban, Octobre 2015.
- ✓ **Halloum W,** Cariou R, Dervilly-Pinel G, Jaber F, Le Bizec B. Mass spectral profiles of 18 organophosphorus flame retardants using various ionization modes (EI, CI, APCI) on GC-MS/MS. Congrès français de Spectrométrie de Masse et d'Analyse Protéomique, SMAP 2015, Ajaccio, France, 15–18 Septembre 2015.
- ✓ **Halloum W,** Cariou R, Dervilly-Pinel G, Jaber F. Le Bizec B. Analysis of two bromine containing organophosphorus flame retardants using GC-EI(+)-MS/MS and LC-ESI(+)-MS/MS. 7th International Symposium on Flame Retardants, Beijing, China, 21–24 April 2015.
- ✓ **Halloum W**, Cariou R, Vénisseau A, Marchand P, Dervilly-Pinel G, Jaber F. Le Bizec B. Analysis of organophosphorus flame retardants using gas chromatography coupled to tandem mass spectrometry. 34th International Symposium on Halogenated Environmental Organic Pollutants and POPs, Madrid, Spain, 31st August – 05th September 2014.

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LIST OF SYMBOLS

AND ABBREVIATIONS

AlO_2H	Boehmite
AED	Atomic Emission Detector
ADI	Acceptable Daily Intake
ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail
APCI	Atmospheric Pressure Chemical Ionisation
APP	Ammonium Polyphosphate
ATSDR	Agency for Toxic Substances and Disease Registry
BBOEP	Bis(2-ButoxyEthyl) Phosphate
BCEP	Bis(2-ChloroEthyl) Phosphate
BCPP	Bis(2-ChloroisoPropyl) phosphate
BCF	Bioaccumulation factor
BDCIPP	Bis(1,3-DiChloro-2-Propyl) Phosphate
BFR	Brominated Flame Retadant
Br	Bromine
bw	Body weight (unit)
CFR	Chlorinated FR
Cl	Chlorine
CO	Carbon monoxide
CoRAP	Community Rolling Action Plan
DAP	DiAlkyl Phosphate
DBP	DiButyl Phosphate
DBPhP	DiButyl Phenyl Phosphate
DPhBP	DiPhenyl Butyl Phosphate
DPP	DiPhenyl Phosphate
d-SPE	dispersive Solid Phase Extraction
dw	dry weight
EC	European Commission
ECHA	European Chemical Agency
EFRA	European Flame Retardant Agency

EFSA	European Food Safety Authority
EHDP	2-EthylHexyl Diphenyl Phosphate
EI	Electron Ionisation
EMR	Enhanced Matrix Removal
EPA	Environmental Protection Agency
ESI	ElectroSpray Ionisation
EU	European Union
F	Fluorine
FDA	Food and Drug Administration
FPD	Flame Photometric Detector
FR	Flame Retardant
fw	fresh weight
GC	Gas Chromatography
GCB	Graphitised Carbon Black
GPC	Gel Permeation Chromatography
HBCD	HexaBromoCycloDodecane
HCN	Hydrogen cyanide
HFR	Halogenated Flame Retardant
HRMS	High Resolution Mass Spectrometry
I	Iodine
ICP	Inductively Coupled Plasma
IER	Individual Excess Risk
IFR	Inorganic Flame Retardant
INCA2	l'étude Individuelle Nationale des Consommations Alimentaires 2
LABERCA	LABoratoire d'Etude des Residus et Contaminants dans les Aliments
LACO	Laboratoire d'Analyse des Composés Organiques
LC	Liquid Chromatography
LD50	Lethal dose (50%)
LOAEL	Lowest Observed Adverse Effect Level
LOD	Limit of Detection

LoR	Limit of Reporting
LOQ	Limit of Quantification
LLE	Liquid Liquid Extraction
MAE	Microwave Assisted Extraction
MAC	Maximum Allowed Concentration
MAP	MonoAlkyl Phosphate
MRL	Maximum Residue Limit
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
m-TCP	meta-TriCresyl Phosphate
NCI	Negative Chemical Ionisation
NFR	Nitrogen FR
NOAEL	No Observed Adverse Effect Level
NPD	Nitrogen Phosphorus Detector
OPE	OrganoPhosphate Ester
OPFR	OrganoPhosphorus FR
OR	Odd Ratio
o-TCP	ortho-TriCresyl Phosphate
PAH	PolyAromatic Hydrocarbon
PBDE	PolyBrominated Diphenyl Ether
PCA	Principal Component Analysis
PCI	Positive Chemical Ionisation
PFR	Phosphorus FR
PLE	Pressurized Liquid Extraction
PO_3	Phosphite
PSA	Primary Secondary Amine
p-TCP	para-TriCresyl Phosphate
QRA	Quantitative Risk Assessment
QuEChERS	Quick Easy Cheap Effective Safe Rugged
RP	Red Phosphorus

Sb_2O_3	Antimony Trioxide
SIM	Single Ion Monitoring
SRM	Selective Reaction Monitoring
SPE	Solid Phase Extraction
SPLE	Selective Pressurised Liquid Extraction
TBBPA	TetraBromoBisPhenol A
TBEP	Tris(2-ButoxyEthyl)Phosphate
TDS	Total Diet Study
TiBP	Tri-*i*-Butyl Phosphate
TnBP	Tri-*n*-Butyl Phosphate
TCEP	Tris(2-ChloroEthyl)Phosphate
TCP	TriCresyl Phosphate
TCPP	Tris (1-Chloro-2-Propyl) Phosphate
TDCIPP	Tris(1,3-DiChloro-2-Propyl)Phosphate
TDBPP	Tris(2,3-DiBromoButyl) Phosphate
TEHP	Tris(2-EthylHexyl) Phosphate
TEP	TriEthyl Phosphate
TRV	Toxicolological Reference Value
TPrP	TriPropyl Phosphate
TPP	Triphenyl phosphate
TTBNPP	Tris(TriBromoNeoPentyl) Phosphate
WEEE	Waste Electrical and Electronic Equipment
WHO	World Health Organisation

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GENERAL INTRODUCTION

With the important progress of the industrial societies, changes have been incorporated not only in our life style, but also in environment and food. New substances have been synthesized by human and integrated into numerous industrial and consumer products aiming to exploit their interesting physico-chemical properties. In this trend, and to meet fire regulations, flame retardants (FRs) are commonly used in consumer products since the 1960s in order to provide varying degrees of flammability protection (van der Veen and de Boer, 2012). The use of FRs have been found to be life saving and also a key factor preventing damages and loses (Iqbal *et al.*, 2017).

Historically, the most extensively and widely used FRs are brominated FRs (BFRs), *e.g.* polybrominated diphenyl ethers, hexabromocyclododecanes and tetrabromobisphenol A. However, some of these BFRs have deleterious effects on humans and environment and proved to be persistent and can bioaccumulative in the environment. Therefore, BFRs were gradually and partly being phased out of market due to their developmental toxicity, neurotoxicity, immunotoxicity, endocrine-disrupting effects and so on (Zhang *et al.*, 2016a). The definition of an endocrine disruptor according to the International Programme on Chemical Safety is: “an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations” (WHO, 2002).The strict bans and increasing regulatory pressure to on the worldwide use of this family of compounds has paved the way for the use of replacements and hence the emergence of novel brominated families but also the re-emergence of organophosphate ester (OPE) FRs, including halogenated and non-halogenated ones.

From here, the global production and usage of these OPEs have increased substantially in recent years (van der Veen and de Boer, 2012; Zhang *et al.*, 2016a) mainly because the industries thought that these OPEs would break down in the environment and not pose much harm. However, their use as additives (not being covalently bound to materials) for FR as well as plasticising purposes poses a risk as they might migrate from the products in which they are incorporated and be further transfered into the surrounding environment. Humans can then be exposed by a combination of oral, inhalation and dermal routes.

Recent detections of OPEs in remote areas suggest they are more persistent than once thought. The publications of various scientific articles, which highlight the global occurrence of these alternatives showed concentrations of 2-3 of magnitude higher than the concentration of BFRs they are replacing, demonstrating that these OPEs are persistent, bioaccumulative and subjected to long range transport (Gramatica *et al.,* 2016). Moreover, recent studies have revealed that several OPEs exhibited potential endocrine-disrupting effects (Zhang *et al.*, 2016a). Unfortunately, the chemical and toxicological properties, the environmental behaviour of majority of these OPEs are often little known (Gramatica

et al., 2016), with a large occurrence data gaps especially in biotic compartments. From here, the objectives were plotted for the present thesis work which was laid down under an international joint agreement between Oniris, and the Lebanese University. In the two institutions, the host laboratories were 'LABoratoire d'Etude des Résidus et Contaminants dans les Aliments' (LABERCA) and 'Laboratoire d'Analyse des Composés Organiques' (LACO), respectively.

LACO is concerned mainly in the domains of analytical chemistry and applied analytical chemistry. The LACO has been working for several years on the structural identification and the elaboration of analytical strategies for the trace analysis of several organic contaminants (e.g., pesticides, PAHs and antibiotics residues) in different food and environmental samples. This study will provide the Lebanese laboratory a developed and optimised method for the determination of such new re emerging organic pollutants, the OPEs, in environmental samples.

The present work has been realised within the French National Reference Laboratory 'LABoratoire d'Etude des Résidus et Contaminants dans les Aliments' (LABERCA) which contributes to chemical food safety assessment and management in relation with residues of growth promoters and contaminants such as dioxins, PCB, PAH and other related persistent organic pollutants. As part of its strategic scheme of research, LABERCA has been working for several years on BFRs according to their recognition of potential risk to human health through food ingestion. With the new thinking on FRs, the challenge is to fulfil the data gap on the contamination levels of OPEs. To attain this innovation challenge, it was interesting to extend the research into the investigation and development of a new and highly sensitive and selective analytical methods for the determination of trace levels of OPE flame retardants and plasticizers. This research work, as others in the unit's historical field, is classified in the general domain of public health and particularly in the context of food chemical safety which is a part of an overall and integrated approach of exposure characterisation, from agricultural supplies to man and his descendants.

The present manuscript presents in the first chapter (Chapter 1) a bibliographic background which focuses in its first part on the importance of FRs to meet the fire safety standards, the classifications of these FRs, their intervention with the fire cycle, the main applications and uses. After this, the chapter will talk about the risk characterisation of OPEs group by describing the physical chemical properties and hence their toxicity and their ubiquitous occurrence in various compartments (*e.g.* biota, water, air). The end of the chapter will present the analytical strategies for OPEs, as described in the literature, including the sample preparation, the identification and detection techniques.

This first part would permit to have a better vision for the chemical aspects of these contaminants as well as the possible analytical strategies enabling their detection. It would also aid in defining the data gaps in the field and hence the scopes of the present work in fulfilling these gaps. The next chapters are dedicated to the PhD work and the investigation of different analytical strategies to respond to the defined problematic as well as the discussion of the obtained results.

In chapter two is described the development and optimisation of the selected instrumental detection methods by gas chromatography coupled to tandem mass spectrometer (GC-MS/MS). This included the optimisation of the chromatographic separation conditions by GC and the spectrometric conditions by MS/MS *via* electron impact (EI) and atmospheric pressure chemical ionisation (APCI) modes. This particular part of work is preceded by the detailed investigation of other ionisation modes on GC (negative and positive chemical ionisation) as well as on LC (electron spray ionisation). It is worth noting that the work presents for the first time an instrumental method for the analysis of a large range of these chemical contaminants based on GC-MS/MS fitted with a positive APCI source.

In chapter three, the comparison and choice of sample handling procedure is described by focusing mainly on the cleanup technique. The experiments were done on pure standard solutions as well as on fish matrix. The chapter is concluded by the investigation of the efficiency of the defined procedure in terms of compounds extractability and lipids contained in the final extract. To evaluate the reliability of our results, a quality control practice was performed by creating the control charts corresponding for each compound based on fish pool, as representative of the analysed samples. Other method performances parameters were also investigated for the whole/complete strategy.

In chapter four, the whole developed method was implemented for the analysis of a series of different food samples (*i.e.* fish and other foodstuffs). We also wanted to study the particular case of exposure potential resulting from the possible transfer of OPEs into food from food contact materials treated with these compounds. For each application (FR and plasticizer), a number of samples was analysed in order to release the first national occurrence data survey. We finally attempt to exercise a risk assessment approach to interpret our results in order to make conclusion on the possible problems/risks associated with the studied OPEs.

As a conclusion, all the results of this innovative approach are foreseen to contribute to the risk assessment of these re-emerging contaminants, through the production of original food exposure occurrence data at the French level.

Within the frame of this research work, several articles, oral and written communications have been realised, and presented as follows:

- Articles
  - ✓ **Halloum W**, Cariou R, Dervilly-Pinel G, Jaber F, Le Bizec B (2017). APCI as an innovative ionization mode compared to EI and CI for the analysis of a large range of organophosphate esters using GC-MS/MS. Journal of Mass Spectrometry, 52, 54–61.
  - ✓ **Halloum W**, Cariou R, Dervilly-Pinel G, Jaber F, Le Bizec B. Organophosphate esters in fish and packaged foodstuffs at the French level, *In Preparation*.

- Oral communications
  - ✓ **Halloum W**, Cariou R, Dervilly-Pinel G, Jaber F, Le Bizec B. Analysis of OPEs by GC-EI/APCI-MS/MS. Application to fish samples. 36th International Symposium on Halogenated Persistent Organic Pollutants (Dioxin 2016), August 2016, Firenze, Italy.
  - ✓ **Halloum W**, Cariou R, Dervilly-Pinel G, Jaber F, Le Bizec B. Analysis Of 18 Organophosphate Esters By GC-MS/MS via Different Ionization Techniques (EI & APCI). Forum Doctoral (FD1EDST16), May 2016, Beirut, Lebanon.
  - ✓ **Halloum W**, Cariou R, Dervilly-Pinel G, Jaber F, Le Bizec B. Development of analytical strategies dedicated for the analysis of organophosphorus flame retardants in biological samples. Scientific days of the doctoral school - Ecole Doctorale Biologie Santé, December 2014, La Chapelle sur Erdre, France.

- Proceedings
  - ✓ **Halloum W**, Cariou R, Dervilly-Pinel G, Jaber F, Le Bizec B. Analysis of OPEs by GC-EI/APCI-MS/MS. Application to fish samples. 36th International Symposium on Halogenated Persistent Organic Pollutants (Dioxin 2016), August 2016, Firenze, Italy.
  - ✓ **Halloum W**, Cariou R, Vénisseau A, Marchand P, Dervilly-Pinel G, Jaber F, Le Bizec B Analysis of organophosphorus flame retardants using gas chromatography coupled to tandem mass spectrometer, Dioxin 2014, Madrid, Spain.

- Posters
  - ✓ **Halloum W**, Cariou R, Dervilly-Pinel G, Jaber F, Le Bizec B. Analysis of organophosphorus flame retardants by GC-MS/MS with EI and APCI ionisation techniques.'The society of Environmental Toxicology and Chemistry' (SETAC) Europe 26th Annual Meeting, May 2016 in Nantes, France

- ✓ **Halloum W**, Cariou R, Dervilly-Pinel G, Jaber F, Le Bizec B. Analyse des retardateurs de flamme organophosphorés dans les poissons basés sur la GC-MS/MS, avec ionisation chimique à pression atmosphérique ou par impact électronique. 'Les Troisièmes Journées Franco-Libanaises' (JFL3), October 2015, Hadath, Lebanon.
- ✓ **Halloum W**, Cariou R, Dervilly-Pinel G, Jaber F, Le Bizec B. Mass spectral profile of 18 organophosphorus flame retardants using various ionization modes (EI, CI & APCI) on GC-MS/MS. 'Spectrométrie de Masse et d'Analyse Protéomique' (SMAP), September 2015, Ajaccio, France.
- ✓ **Halloum W**, Cariou R, Dervilly-Pinel G, Jaber F, Le Bizec B. Analysis of two bromine containing organophosphorus flame retardants using GC-EI(+)-MS/MS and LC-ESI(+)-MS/MS, the 7th international symposium on Brominated Flame Retardants 2015, Beijing, China.
- ✓ **Halloum W**, Cariou R, Vénisseau A, Marchand P, Dervilly-Pinel G, Jaber F, Le Bizec B Analysis of organophosphorus flame retardants using gas chromatography coupled to tandem mass spectrometer, Dioxin 2014, Madrid, Spain.

CHAPTER ONE

LITERATURE REVIEW-

FROM ADDITIVES TO FLAME RETARDANTS AND ORGANOPHOSPHATE ESTERS

1. FROM ADDITIVES TO FLAME RETARDANTS AND ORGANOPHOSPHATE ESTERS

1.1. **OPES: ONE ADDITIVE FAMILY, TWO INDUSTRIAL PURPOSES**

Polymeric materials are produced in large quantities and comprise a large number of materials such as plastics, rubbers, surface coatings, *etc*. Polymeric materials are made by forming covalent bonds between a large number of small molecules (monomers) to produce long chains. This process is referred to as polymerization. Most polymeric materials are organic materials and are thus combustible. As such, they have to satisfy fire resistance requirements when used in various applications. Additives are frequently added to polymers during processing or end-use application to produce a specific result like resistance to heat or flames, and improvement of physical and mechanical properties, particularly plasticization and impact resistance (Ambrogi *et al.*, 2017). From here, two important groups of additives are plasticisers and Flame Retardants (FRs) (Bergh, 2011).

Plasticizing compounds may be added to a polymer to reduce its stiffness and improve polymers' processability, flexibility, elasticity and durability. Plasticisers should generally have a solubility level close to that of the polymer itself, and multiple plasticising additives can be used in a single mixture as long as they are compatible with each other and the polymer. Organophosphate triesters (also known as organophosphate esters, OPEs) represent a group of compounds that are used for this purpose (Bergh, 2011). OPEs are used in the technosphere for two reasons: the non-halogenated ones are mostly employed as plasticisers although in some cases, they are also used as FRs, while the chlorinated and brominated ones are frequently used as FRs (Andresen *et al.*, 2004; Wei *et al.*, 2015) for improving the polymers' fire resistance (Bergh, 2011). Because they have both flame retarding and plasticizing properties, OPEs are an important group of polymer FRs on the market.

1.2. **A GENERAL VISION ON CHEMICAL FRs**

1.2.1. FLAME RETARDANTS: A BURNING ISSUE

The use of FRs is essential to improve the fire safety of combustible products and materials (Stapleton *et al.*, 2014) and therefore to save lives. They are typically added to industrial and consumer products to meet specifications regarding flammability, described in international and national standards. In United States, the UL 94 standard (for safety of flammability of plastic materials for parts in devices and appliances testing) is a plastic flammability standard released by

the Underwriters Laboratories of the United States. Other standards exist in US depend on the product and the industry, like the California Technical Bulletin (TB) 117 for upholstered furniture. Additionally, in Europe, the IEC 65 (International Electrotechnical Commission: Households and similar electrical appliances- Safety) approved the flammability requirements for plastic materials at a certain distance from specified potential ignition sources.

To meet such regulations, manufacturers have to add FR chemicals to a wide range of products used every day. According to the market forecasting from the British Chambers of Commerce (BCC) which was published in April 2015, the worldwide consumption of flame retardant chemicals reached nearly 3.9 billion and 4.2 billion pounds in 2013 and 2014, respectively. The consumption was expected to reach a compound annual growth rate (CAGR) of 6.7%, increasing to a total of 5.7 billion pounds in 2019.

Besides and according to the study recently published in 2016 by the market research institute Ceresana, a leading international market research and consultancy company for the industrial sector, more than 2 million tons of FRs were consumed worldwide in 2013 and the forecasts revenues of approximately US $ 7.15 billion to be generated in 2021.

Still in the terms of worldwide consumption of FRs, a study was published in November 2014 by IHS MARKIT Ltd., a company based in London, UK. Of the total 2013 volume of FRs, about 27% was consumed in China, followed by 22% for Western Europe and 22% for North America. China is expected to remain the largest consumer, with 30% of global consumption in 2018.

Indeed, FRs are applied in all sectors of our everyday life:

- Electronics and electrical devices: television, computers and laptops, including monitors, keyboards and portable digital devices, telephones, refrigerators, washers and dryers, electronic circuit boards...;

- Building and construction materials: electrical wires and cables, including those behind walls, insulation materials (*e.g.* polystyrene and polyurethane insulation foams), paints and coatings which are applied to a variety of building materials...;

- Furnishings: natural and synthetic filling materials and textile fibers, foam upholstery, curtains and carpets...;

- Transportation: airplanes, trains, automobiles, including seat covers and fillings, roof liners, textile carpets, curtains, internal structures, including dashboards and instrument panels, electrical and electronic cable coverings, stereo components...

1.2.2. FLAME RETARDANTS AND FIRE CYCLE

In all their forms, FRs interact with the fire cycle in order to prevent, delay or stop it. First, it is important to understand the fire cycle. Indeed, materials can burn whenever the three fire factors (fuel, heat and oxygen) coincide. Then, the course of a fire can be split into three phases; the initiating, the fully developed and the decreasing fire.

The fire cycle (Figure 1-1) comprises a multitude of single steps and can be divided into seven main stages (EFRA, 2014). When the material is in its condensed phase, the fire can be initiated by any energy source (heat, incandescent material or a small flame). This basically creates endothermic heating (absorption of the energy) and decomposition of polymer, resulting in an inert carbonized material (called char). Pyrolysis degrades the polymers' long-chain molecules into smaller hydrocarbon molecules, the flammable gases, which are emitted into the gas phase, are mixed with the atmospheric oxygen and ignite, initiating the exothermic processes of flame propagation. The proper mix of oxygen and fuel is reached in the combustion zone, where exothermic processes take place releasing high-energy radicals (*e.g.* $H^{\bullet}$ and $OH^{\bullet}$). The flame spreads over the decomposed polymer surface and the diffusion is supported by extremely high energy $H^{\bullet}$ and $OH^{\bullet}$ radicals which confer a high velocity on the flame front. Incomplete combustion products are also emitted during a fire (carbon monoxide, polycyclic aromatic hydrocarbons, hydrogen cyanide, *etc*) (Wakefield, 2010). Energy emitted during exothermic reactions is transmitted to the polymer and reinforces pyrolysis, which allows the reaction to sustain itself (Troitzsch, 1990).

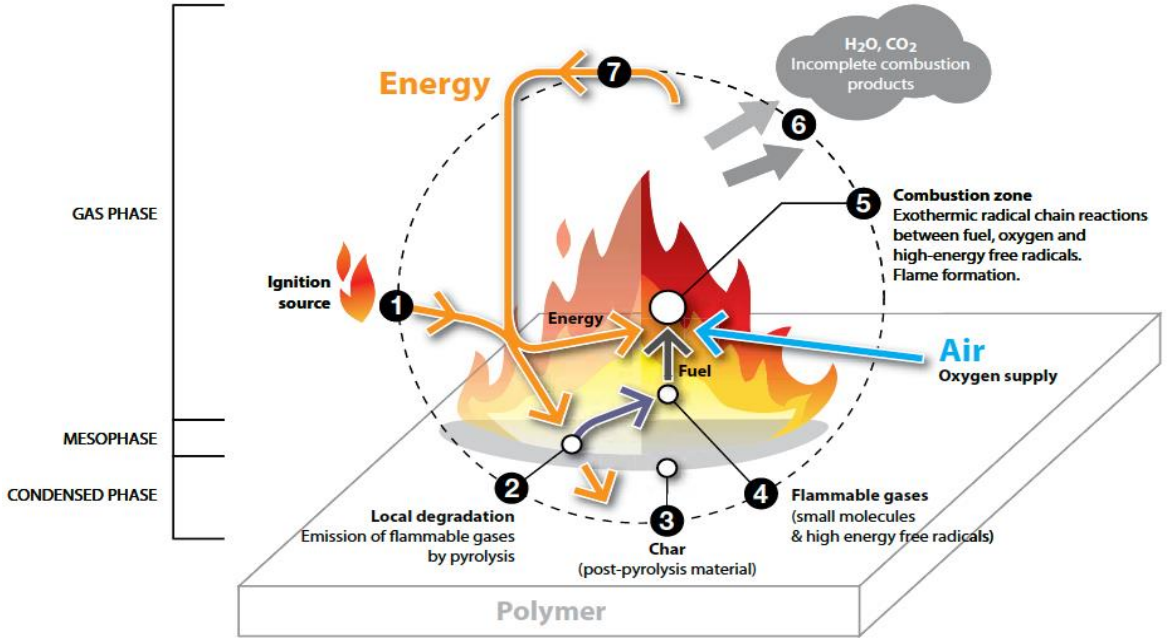


Figure 1-1: Schematic representation of the fire cycle with its main steps (EFRA, 2014).

Successful strategies to reduce the flammability of polymeric material involve interrupting the complex stages of the combustion process at one or more points of the fire cycle (Joseph and Ebdon, 2001). FRs act at different stages, depending on their chemical basis.

Whatever the mechanism used, the end effect is to hinder the fire initiation, limit its propagation and if possible to exclude the flashover. Flashover is the "fireball" that can quickly occur when the combination of heat and the release of flammable gases cause automatic combustion. As shown in Figure 1-2, the use of FRs delays flashover, reduces the rate and intensity of burning and can extend the escape time by a factor 10 or even more (Hofland, 2010).

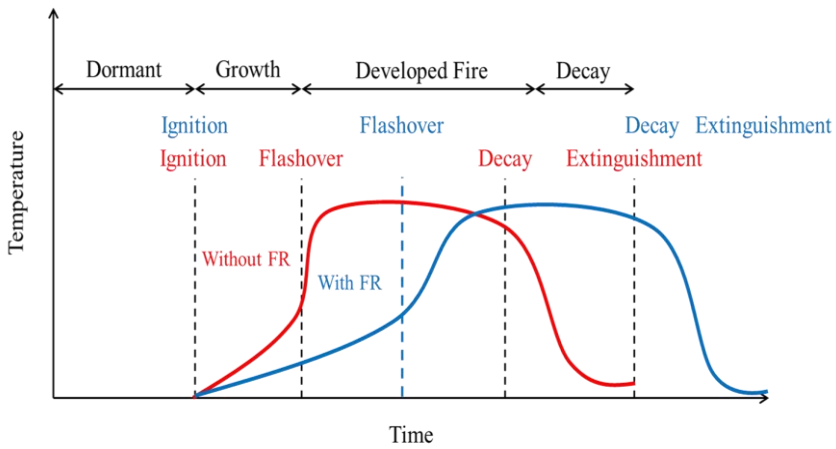


Figure 1-2: Delayed time of flashover upon the use of FRs along the fire phases (On a Quasi-Related Note).

Depending on their nature, FRs act to break the fire cycle at different stages. They can act chemically and/or physically in the condensed or gas phase (Troitzsch, 1998; EFRA, 2014; Flame retardants-online, 2016).

Physical action is reached by cooling the substrate to a temperature that is unable to sustain the burning process, *e.g.* aluminium hydroxide. It can also be achieved by diluting the substrate in the gas phase (*i.e.* formation of water) and the solid phase (alumina trihydrate and magnesium hydroxide). This can also be reached by coating the substrate (shielding it with either a solid or gaseous layer, protecting it against the attack of oxygen and heat, *e.g.* phosphorus and nitrogen compounds). Chemical action in the gas phase interferes with the combustion processes by interrupting the radical mechanisms through eliminating the high energy $H^{\bullet}$ and $OH^{\bullet}$ radicals by halogen halides from halogenated FRs, resulting in a cooling of the system, a reduction and suppression of the supply of flammable gases. In the condensed phase, the FR forms a char layer on the surface of the polymer, this occurs through dehydration action generating double bonds in the polymer. These form the carbonaceous layer by cyclising and cross-linking the smothering the material and inhibiting the oxygen supply, thereby providing a barrier against the heat source (*e.g.* phosphorus and nitrogen compounds).

Chemically speaking, FRs represent a group of very diverse substances which may strongly differ in chemical and physical properties, mode of action, toxicology and environmental behaviour (Stapleton *et al.*, 2014). Based on their chemical nature, FRs consist of inorganic and organic compounds. From these FRs, brominated and chlorinated FRs (BFRs and CFRs, respectively) and phosphorus FRs (PFRs) cover the major proportion of organic FRs (Bergman *et al.*, 2012). These chemical elements are responsible to provide their effectiveness. Besides, FR properties can also be achieved by other means than FR chemicals through materials design and barrier technologies (intumescent systems) (The Norwegian Pollution Control, 2009).

Within these groups, FRs can be classified as either reactive or additive (Stapleton *et al.*, 2014) with the aim of increasing the fire resistance of materials (Bergman *et al.*, 2012). On one hand, reactive FRs are covalently bound to materials either by incorporating them into the polymer backbone during the polymerization reaction or by grafting them onto it, which result in a modified polymer with fire proof properties and different molecular structure compared to the original polymer molecule. This enables the polymer to keep the FR properties intact over time with very low emissions to the environment. On the other hand, additive FRs used in thermoplastics are typically incorporated after the manufacturing of the polymer and during the processing of the end product (The Norwegian Pollution Control, 2009).

1.2.2.2. **Halogen-containing FRs**

The effectiveness of HFRs depends on the halogen atoms contained. The energies of halogen-carbon bonds decreases in the order F > Cl > Br > I. Fluorine- and iodine-based FRs are not used in practice for FR applications because the bonds with carbon are either too strong (too thermally stable) or too weak (decompose at low temperature). Bromine is the most effective since its bonding to carbon enables it to interfere at a more relevant temperature in the combustion and it is assumed that HBr is liberated at high concentration in the flame zone. Chlorine containing FRs are considered as slightly less effective, because it can release HCl over a wider range of temperature and hence the latter is present at lower concentration in the flame zone (Troitzsch, 1998). Halogen-containing FRs act by interfering with the radical chain mechanism in the gas phase. The high energy radicals are formed by chain branching as following (Flame retardants-online, 2016): $H^{\bullet} + O_2 \quad OH^{\bullet} + O$

These radicals are removed by the halogen-containing FRs as follows:

(1) Release of halogen radicals ($X^{\bullet}$) from the FR (RX): $R\text{-}X \longrightarrow R^{\bullet} + X^{\bullet}$

(2) Formation of hydrogen halides (HX): $X^{\bullet} + RH \longrightarrow R^{\bullet} + HX$

(3) Neutralization of high energy radicals (by low energy $X^{\bullet}$): $HX + H^{\bullet} \longrightarrow H_2 + X^{\bullet}$,

$HX + OH^{\bullet} \longrightarrow H_2O + X^{\bullet}$

(4) Regeneration of hydrogen halide by reaction with hydrocarbon: $X^{\bullet} + RH \longrightarrow R^{\bullet} + HX$

1.2.2.3. **Phosphorus-containing FRs**

Phosphorus-containing FRs (PFRs) act mainly in the solid phase of burning materials by forming a charred surface layer of phosphorus compounds (Figure 1-3). Upon heating, the phosphorus reacts and gives a polymeric form of phosphoric acid. This acid causes the material to char, forming a glassy layer, which shields the material from oxygen and prevent the formation of flammable gases.

Non-halogenated PFRs act in the solid phase of burning materials, while halogenated ones can also act in the gas phase by interrupting the radical chain process. In this case, halogens and phosphorus act independently and thus combining the different flame retarding mechanisms of these elements (Steukers *et al*. 2004; van der Veen and de Boer, 2012).

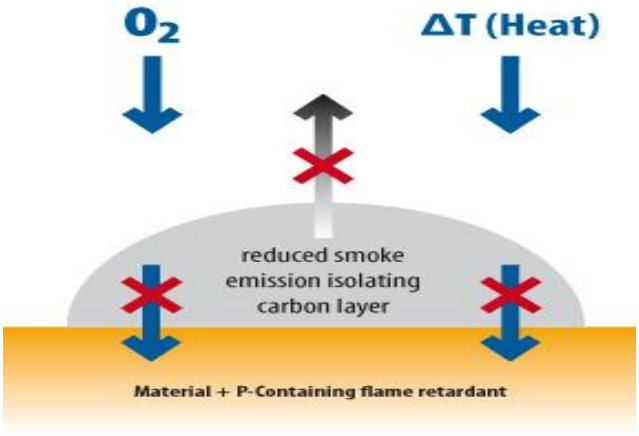


Figure 1-3: Mode of action of PFRs (EFRA, 2014).

1.2.2.4. **Nitrogen-containing FRs**

Nitrogen-containing FRs (NFRs) may act by the release of inert gases (ammonia, nitrogen) into the gas phase, which dilute volatile polymer decomposition products. NFRs can also act by condensation reactions in the solid phase, where melamine is transformed into cross-linked molecular structures promoting char formation (Flame retardants-online, 2016). Melamine-based products are the most widely used type of NFRs and are used for example in polyurethane foams for furniture (Troitzsch, 1998).

1.2.2.5. **Inorganic FRs**

The most important inorganic FRs (IFRs) are aluminum trihydrate or aluminum hydroxide ($Al(OH)_3$) and magnesium hydroxide ($Mg(OH)_2$). As seen in Figure 1-4, IFRs operate by interfering with the burning process through three main physical processes (EFRA, 2014):

(1) Release of inert gases such as water vapor that cool the material surface and dilute the gases feeding the flame;

(2) Energy absorption through endothermic decomposition, retarding the pyrolysis process;

(3) Production of non-flammable and resistant charred layer on the surface of the material.

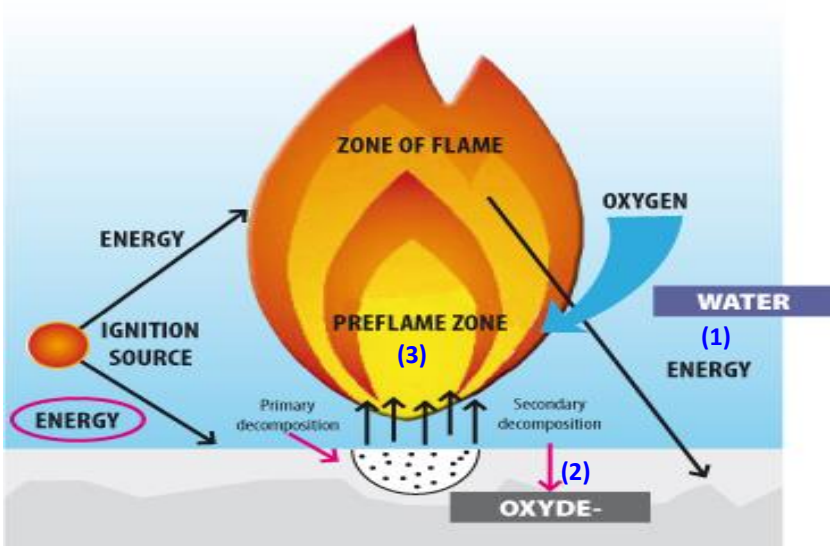


Figure 1-4 : Mode of action of IFRs (EFRA, 2014).

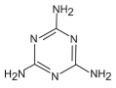
1.2.2.6. **Intumescing FR systems**

Intumescing FR systems undergo a thermal degradation process on heating, which produces a thermally stable, foamed, multicellular residue called 'intumescent char', providing insulation to the underlying polymeric materials and partially protecting it against the attack of heat and fire (Camino, 1998). Basically, intumescent systems consist of 'carbon' donors (*e.g.* polyalcohol), 'acid' donors (*e.g.* ammonium polyphosphate) and 'spumific/blowing' agent (helps in swelling as a result of heat exposure, *e.g.* melamine). The intumescing mechanism proceeds in six main steps:

(1) Softening of polymer;

(2) Release of an inorganic acid; $(NH_4PO_3)_n \xrightarrow[-n\ NH_3]{>250\ ^oC} (HPO_3)_n$

(3) Carbonization; $(HPO_3)_n + C_x(H_2O)_m \longrightarrow [C]_x + (HPO_3)_n x m H_2O$

(4) Gas formation by the spumific compound;  $\xrightarrow{\Delta} NH_2 \xrightarrow{O_2} N_2 + H_2O$

(5) Foaming of the mixture;

(6) Solidification through cross-linking reactions.

1.2.2.7. **Synergism**

Many synergistic FR systems based on phosphorous and nitrogen, metal hydroxides and salts have been developed in recent years. A typical example is the synergy of antimony trioxide (Sb_2O_3) with brominated or chlorinated compounds. Sb_2O_3 alone has no flame retardancy effect. With Br/Cl compounds, however, it acts as a catalyst, facilitating the breakdown of these halogenated FRs to

achieve free radicals. It also reacts with halogens to produce volatile antimony halogen compounds, which are themselves directly effective in removing high energy $H^{\bullet}$ and $OH^{\bullet}$ radicals (EFRA, 2014). Aluminum oxide hydrate (AlO_2H or Boehmite) also acts as synergist in conjunction with metal phosphinates (EFRA, 2015; Flame retardants-online, 2016).

1.2.3. TIME TREND AND REGULATORY STATUS

Even though the history of FRs dates back thousands of years, it is the recent developments, and in particular the use of organic FRs, that is of current concern (Bergman *et al.*, 2012). Polychlorinated biphenyls (PCBs) were manufactured and applied as FRs from the late 1920s until the mid-1980s, although PCBs were also used in a multitude of other applications, particularly in electrical equipment. Other chlorinated compounds came into use as FR, probably from the 1960s onwards, sometimes also including a phosphate group, such as the tris (2,3-dichloropropyl)phosphate (TDCPP) and tris (1,3-dichloro-2-propyl) phosphate (TDCIPP) (Gold *et al.,* 1978). The brominated analog of the former compound, tris (2,3-dibromopropyl) phosphate (TDBPP) made the headlines in the 1970s due to its use in children's pajamas (Gold *et al.,* 1978). In the beginning of the 1970s, an increasing number of BFRs, *e.g.* polybrominated biphenyls (PBBs) and polybrominated diphenyl ethers (PBDEs), came to the market (Bergman *et al.*, 2012). In 1997, the World Health Organization contributed to list all major FRs, also including any inorganic chemicals used in that role (WHO, 1997). For over four decades, HFRs have been in the focus of concern for public health. (Pijnenburg *et al.,*1995) made the first review of BFRs, including what was known of their analysis, toxicity and environmental occurrence, and numerous other reviews and/or assessment documents have been published since then. Some HFRs have proven to be persistent, bioaccumulative and/or toxic compounds and then were established as a threat to environment, to animals and humans (van der Veen and de Boer, 2012).These properties led the governments to adopt restrictions on the production and use of these compounds (Cristale and Lacorte, 2013).

In 2009, the United Nations Environment Programme (UNEP) has decided in a meeting of the parties of the Stockholm Convention on Persistent Organic Pollutants (POPs) that octaBDE and pentaBDE are officially labeled as POPs (Decision SC-4/14, SC-4/18). The US Environmental Protection Agency (EPA) announced the phase out of decaBDE by the end of 2013 (Brandsma *et al.*, 2013). HBCD was also added to the list of 22 other substances targeted for global elimination under the Stockholm Convention on Persistent Organic Pollutants. However, it can continue to be used in expanded or extruded polystyrene insulation for buildings until 2019.

At the EU level and since 1995, the European Commission listed HBCD as one of the priority substances for risk assessment. More recently, the Commission Recommendation recommended the monitoring

of traces of brominated FRs in different food commodities in order to investigate and assess the presence of some PBDEs, HBCDDs, tetrabromobisphenol A as well as some brominated phenol and their derivatives (2014/118/EU).

Still at the EU level and according to the RoHS Directive, "the Restriction of the use of certain Hazardous Substances in electrical and electronic equipment", the use of Penta-BDE and Octa-BDE in electrical equipments is banned in the European Union since August 2004. The directive on the Waste Electrical and Electronic Equipment (WEEE) prescribes the removal or the separate treatment of certain substances, mixtures and components, including plastic containing brominated FRs, from any separately collected WEEE. The directive has been in force since February 2003.

The market for FR chemicals is being driven by these globally tightening fire safety regulations. The phase-out of several high-production volume BFRs has led to an increase in the production and application of alternative FRs, *e.g.* PFRs (Brandsma *et al.*, 2013). Figure 1-5 presents the distribution per region of the sales of FRs (with a total of 4.2 billion US $) classified in terms of their chemical nature. As previously mentioned in section 1.2.1., about the global demand for the FRs , the Asia region presented the largest market for the sale of FRs but BFRs is the mostly selling types. In parallel, we can see that in Europe and US the PFRs sales exceeded that of BFRs in 2007.

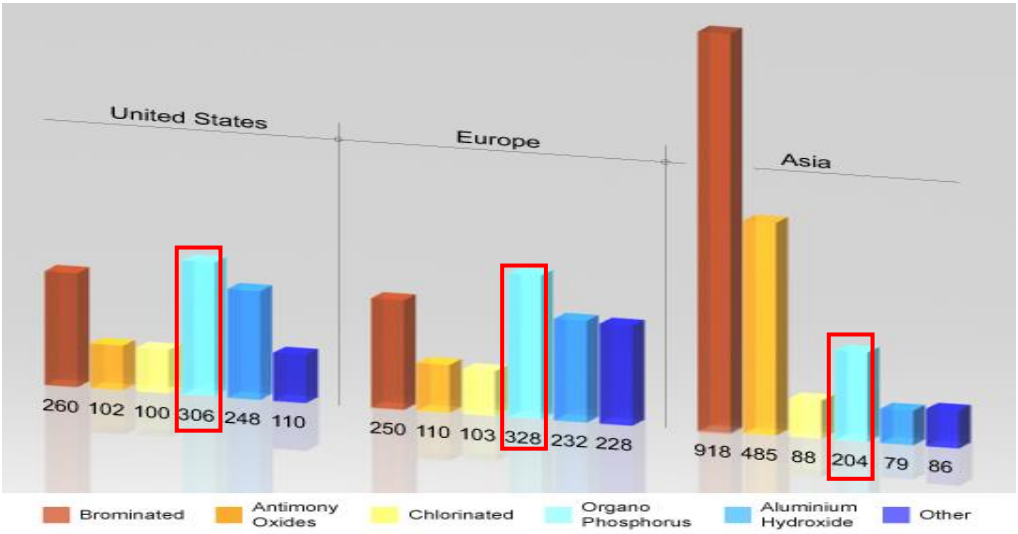


Figure 1-5: Sales of FRs by Region for 2007, in million US $ (Flame retardants-online, 2016).

According to the FR profile consumption per group, Figure 1-6 presents the estimates from Towsend Solutions (USA) for the global consumption (by type) of FRs in plastics in 2011 which amounts to around 2 Million tons. The PFRs contributed to 15% of the global consumption.

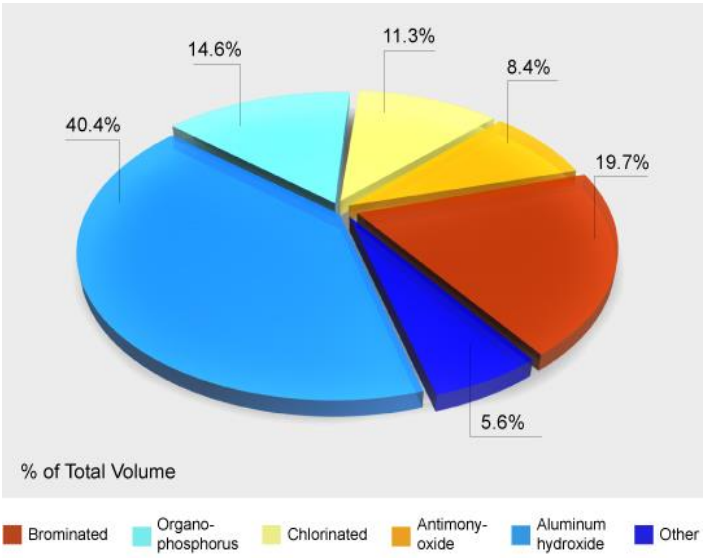


Figure 1-6: Global consumption of FRs in plastics by type in 2011, 2 Million Tonnes (Flame retardants-online, 2016).

Figure 1-7 presents Sweden as an example of the decline in the use in polymeric materials (1999-2010) of the mostly employed BFRs including PBDE, TBBP A, HBCD as well as other BFRs.

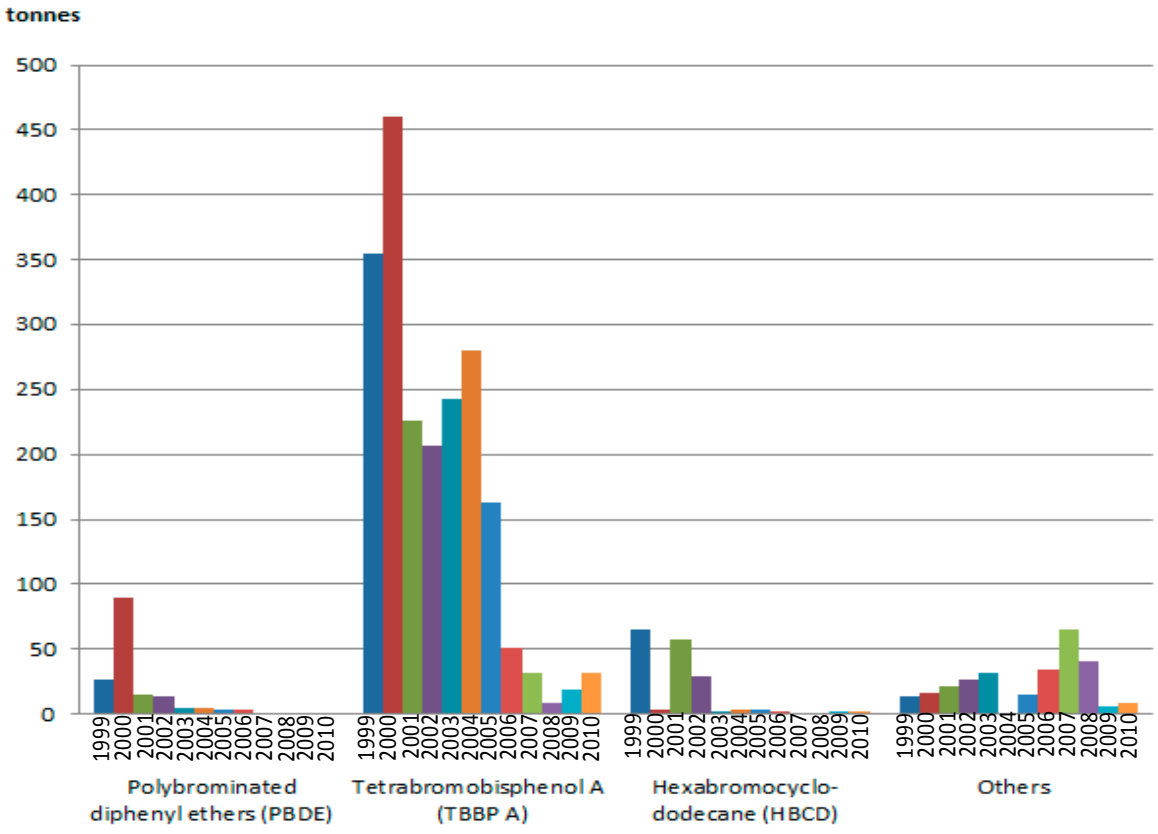


Figure 1-7: Turnover of some brominated FR compounds used in chemical products 1999-2010, Sweden (KEMI, 2016).

According to the EFRA, the consumption of PFRs in EU increased from 84,000 tons in 2004 to 91,000 tons in 2006 (20% of the FR consumption of 465,000 tonnes/year). Of this percentage, 11% were chlorinated phosphates, Tris(1-chloro-2-propyl) phosphate (TCPP) representing 80% of the chlorinated PFRs) and 9% were non-halogen PFRs. The BFRs contributed to 10% of this FR consumption.

Indeed and according to their presence in the environment, FRs can be divided into established, emerging, potential and novel FRs. Established FRs include chemicals which are extensively documented regarding production and use as FRs, chemistry, fate, exposures, environment and toxicity. Emerging FRs are chemicals which are documented regarding production and use as FRs and have been recently shown to occur in environment/wildlife. Novels FRs are chemicals which are documented as potential FRs and have been shown to be present in materials or products but not in the environment. Potential FRs are chemicals reported to have applications as FRs but not observed in the environment or products (Bergman *et al.*, 2012). In this context, PFRs are then considered as re-emerging pollutants because of their increased production and use more and more after PBDE bans and their ubiquitous occurrence/distribution in environmental compartments (Cristale and Lacorte, 2013).

1.3. **ORGANOPHOSPHATE ESTERS**

As mentioned since the beginning of this chapter, the additives to polymers comprise several families of compounds including the BFRs and PFRs. The restrictions on BFRs (as illustrated in the previous sections) led to the increase of the production and use of PFRs of which OPEs represent an important group in the market.

1.3.1. OPEs- A GROUP OF PFRs

Nowadays, one of the principal classes of flame retardants used in plastics and textiles is that of phosphorus compounds. PFRs can be divided into (i) inorganic PFRs, such as red phosphorus (RP) and ammonium polyphosphate (APP), and (ii) organophosphorus FRs (OPFRs), including three subgroups (Figure 1-8): the phosphinates, the phosphonates and the organophosphate esters (OPEs) (van der Veen and de Boer, 2012).

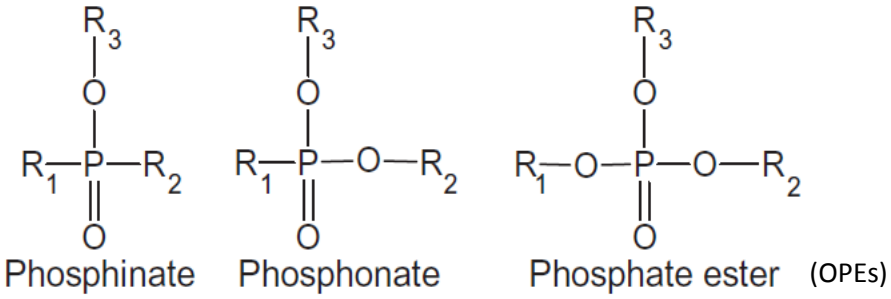


Figure 1-8: Chemical structures of three subgroups of OPFRs.

Phosphate esters, with or without halogen, are the predominant phosphorus-based flame retardants in use (WHO, 1997). As such, the present study focuses on the most common OPE-type FRs and plasticizers.

1.3.2. LIST OF MAIN OPEs

OPEs are synthetic phosphoric acid derivatives. They possess a central phosphate molecular group, but their structures vary depending on different ester linkages and can roughly be divided into three types: alkyl OPEs, aryl OPEs and halogenated (chlorinated and brominated) OPEs. The targeted compounds in our work are presented in Figure 1-9 in which the chemical structures, the names, the abbreviations and the molecular weights are presented. It is worth to note that the compounds are the mostly used OPEs in the market as well as the previously mentioned ones in the literature.

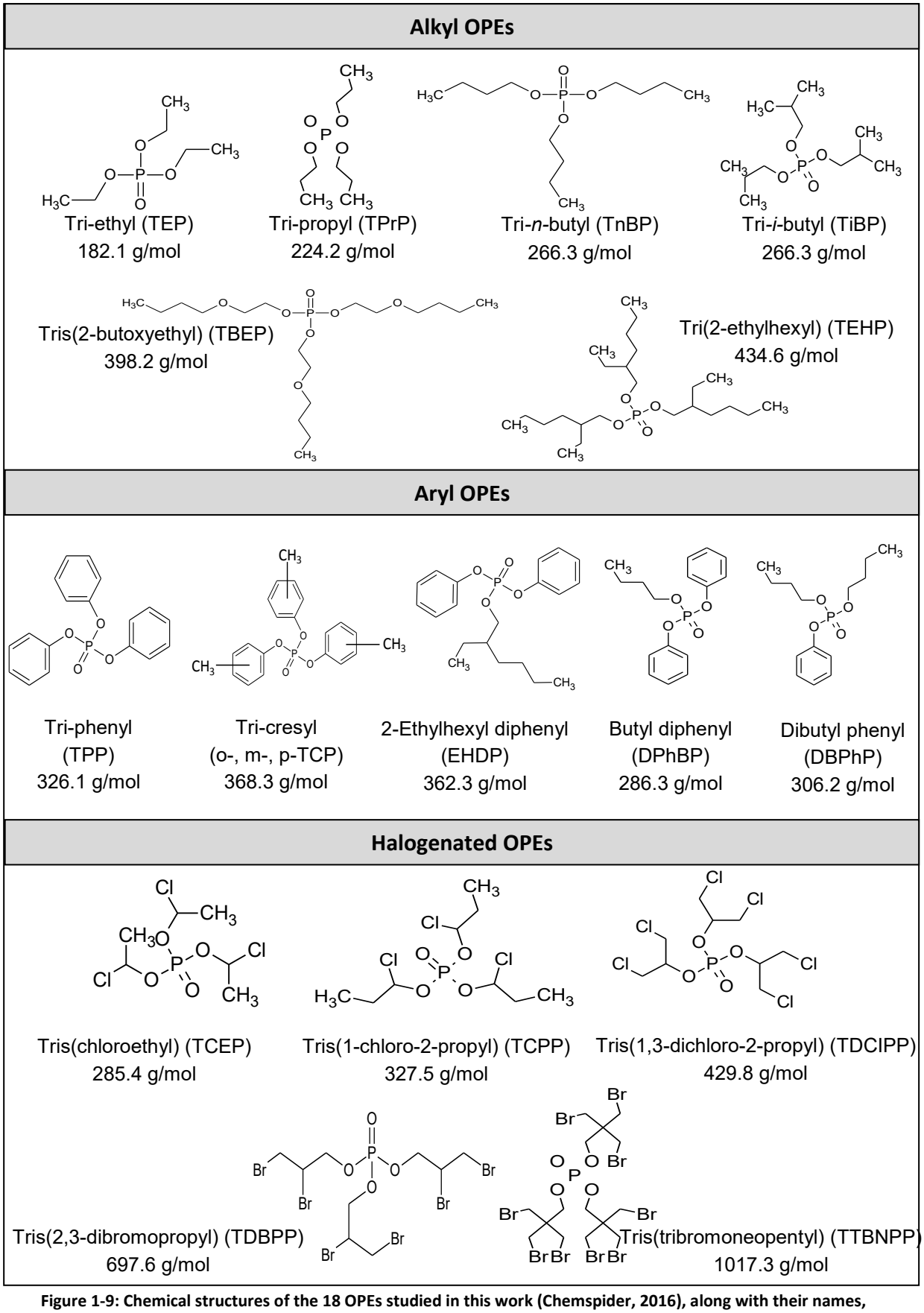


Figure 1-9: Chemical structures of the 18 OPEs studied in this work (Chemspider, 2016), along with their names, abbreviations and molecular weights (the most abundant isotopologue in case of the halogenated OPEs)

1.3.3. APPLICATIONS AND REGULATIONS RELATED TO OPEs

OPEs have been extensively used for several decades. They are mainly used for two purposes, depending greatly on the type of side chain of the phosphate ester (Stapleton *et al.*, 2009; Wei *et al.*, 2015). (i) Halogenated compounds are applied as FRs and (ii) the non-halogenated compounds are mostly used as plasticisers (the non-branched alkyl phosphates such as TnBP, TiBP, TPP and TBEP are predominantly used as plasticisers, lubricants, antifoaming, though in some cases, they are also used as FRs) (Andresen *et al.,* 2004; Leonards, 2011). They are used in many products, *e.g.* furniture, textiles, cellulose, rubber, cables, building materials, insulation materials, paints, floor polishes, hydraulic fluids and electronic (Brandsma *et al.*, 2013).

In this trend, TEP, TnBP and TiBP are mostly used for their plasticising properties in unsaturated polyester resins, cellulose acetate and synthetic rubber. Other specific uses include the use of TnBP, TPP and TCP as lubricants in hydraulic fluids; TnBP is also used as an antifoaming agent in concrete, as a wetting agent in casein glue and as a pasting agent in pigment paste (Wei *et al.*, 2015). TBEP is often used in floor wax and rubber stoppers (Van den Eede *et al.,* 2011). Several OPEs are also added to polyurethane foam (*e.g.* TPP with pentabromodiphenyl ether BDE mixture) as well as in hydraulic fluids (*e.g.* TPP, TCP, TnBP). TCEP, TCPP and TPP are used in flexible and rigid polyurethane foams, plastics, and textiles (Cristale and Lacorte, 2013; van der Veen *et al.,* 2012). TDCIPP and TCEP are prohibited in Washington State (USA) according to the "Toxic Free Kids Act". EU Directive 2014/81/EU also introduced specific limits (5 mg/kg) for TCEP, TCPP and TDCIPP in certain toys.

According to van der Veen *et al.* (2012), it is very important to avoid OPEs compounds which may be more persistent, bioaccumulative and toxic to humans and to environment than BFRs. The number of regulations on these compounds is however still limited. The regulation (EC) No 1223/2009 on cosmetic products listed TCP, TCEP and TBP as prohibited substances in cosmetics since their use can raises a potential risk to human health. This was based on the hazardous properties of these substances classified as carcinogenic, mutagenic or toxic for reproduction (CMR), pursuant to Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures. On the other hand, the Commission Directive 2014/81/EU on the safety of toys specified limited values for TCEP, TCPP and TDCP at 5 mg/kg (content limit). In the Community Rolling Action Plan (CoRAP) update covering years 2014, 2015 and 2016, the European Chemical Agency (ECHA, 2016) listed TPP as compound to be evaluated in 2017 by the United Kingdom. EHDP is approved for use in plastic food contact material (Wei *et al.,* 2015).

As a conclusion of this section, Figure 1-10 is used to illustrate the classification of OPEs, the focus of our study, in the large family of additives.

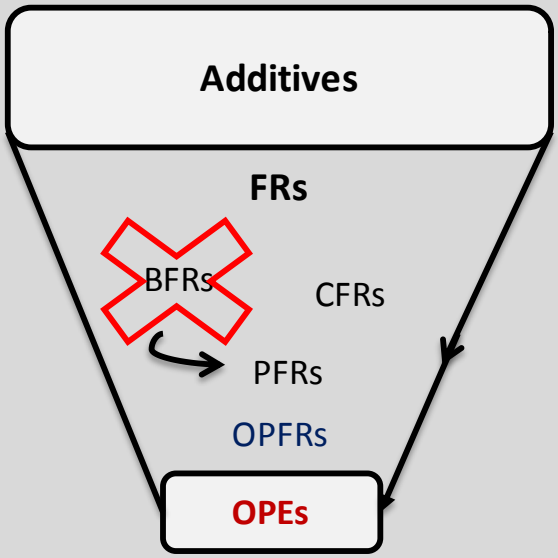


Figure 1-10: The focus of study on OPEs, a subgroup from additive compounds

As a perspective, the recognition of generation of these re-emerging contaminants, the OPEs due to the industrial and other activities and transport and their persistence in the environment and biological activities brings out the necessity and importance of their assessment of risk they pose to the surrounding environment and the humans. This would be possible through the development of necessary risk assessment tools.

1.4. **OPEs RISK ASSESSMENT**

Risk Assessment is a systematic and well documented process to define and quantify potential human health risks and the adverse effects resulting from the exposure to a toxic chemical (WHO, 2010). The European Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances aims to ensure the protection of man, including workers and consumers, and of the environment through risk evaluation. This European Union (EU) risk assessment investigates the adverse effects of a substance resulting from human exposure to a particular hazard, which is then placed against the exposure of this substance to humans or different environmental compartments. The concentration in the environment, or to which human beings are exposed, must not be greater than the non observable adverse effect level (*i.e*. the concentration at which the substance causes no toxic effect). Risk is usually defined as a function of both hazard and exposure. While risk assessment cannot change the hazard properties of a chemical, it can identify risk levels expected to be associated

with different exposure routes (Howard, 2014). A health risk assessment can be divided into two types, qualitative and quantitative. Quantitative Risk Assessment (QRA) is the use of measurable, objective data to determine asset value and associated risk(s). It is characterised by assigning a numerical value to the risk, in contrast with qualitative risk analysis, which is typified by risk ranking or separation into descriptive categories of risk. This methodology can be used for different purposes. The first is for predictive purpose, which permits the assessment of the health risks associated with potential future exposure. It can also be used to predict risks either on long-term (effects not expressed at time t) or short-term after a past exposure.

The results are expressed in two different ways depending on the available information. In the case of substances without a threshold (*e.g.* carcinogenic substances), the phenomenon estimated the increased probability of developing cancer, expressed in Individual Excess Risk (IER). In the case of substances with a threshold, the risk is measured by performing a dose ratio, called danger quotient or risk ratio between the population exposure doses to the reference dose (at which health effects may occur).

The advantage of this type of study is to calculate a health risk when epidemiological studies are not feasible (effective population is too low, no reported effects). These results guide decision making for health monitoring of populations and in particular the implementation of actions to limit exposure. This method aims to provide results in a relatively short time with reduced costs compared to other studies, such as epidemiological studies, for example. The limitations of this tool rest in the dependence on other sciences (epidemiology, toxicology), namely the availability of toxicological reference values (TRVs) for the studied pollutants. These values are usually delivered by agencies in charge of food safety, like the 'Joint FAO/WHO Expert Committee on Food Additives' (JECFA), 'European Food Safety Authority' (EFSA) and the 'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail' (ANSES). In addition, data on the effects of the range or combination of these compounds are rarely available. However, the observed health effects were likely due to the combined action of different substances.

In this part, we are going to present the four main steps of the risk assessment process consisting of hazard identification, hazard characterisation, exposure assessment and risk characterisation as well as the application of quantitave risk assessment to human.

1.4.1. HAZARD IDENTIFICATION

Hazard identification is the first step of the risk assessment process. It determines whether the exposure to a chemical can increase the incidence of a particular adverse health effect and determines

the likelihood of occurrence in humans. This step requires firstly, understanding the impacts of these compounds on the environment, including the organisms which live on it. At this stage of risk assessment process, it is crucial to identify and document the contaminant characteristics (*i.e.* physical/chemical properties and environmental behavior) (Iscan, 2004).

1.4.1.1. **Which substances?**

While OPEs are currently in use, still little information is available regarding their physical-chemical properties and environmental fate. High quality physical-chemical property data are needed not only to inform on the potential fate and hazards of these pollutants, but also to provide the foundation in regulatory risk assessment for data interpretation of all endpoints (*e.g.*, fate and behavior, toxicity and exposure) (van der Veen and de Boer, 2012; Zhang *et al.*, 2016a).

Indeed, OPEs have a wide range of physical chemical properties in the environment. For example, their solubility, log Kow values, persistence, bioconcentration factors (BCFs) are quite different. The structural differences among OPEs lead to a variety of chemical and physical properties within this family (Leonards, 2011, Brandsma *et al.,* 2015). Table 1-1 listed some available physicochemical properties for the targeted OPEs compounds

The molecular masses of the 18 selected OPEs have a wide range from 182.1 to 1017.3 $g.mol^{-1}$. In general, the solubility in water decreases by increasing molecular mass and this is confirmed by the octanol-water partitioning coefficients (log kow) values (van der Veen and de Boer, 2012). The positive log K_{ow} for most of the OPEs reflect the lipophilic than hydrophilic nature of these compounds. The log K_{ow} values range from 1.08 for TEP to 10.09 for TEHP. Volatile OPEs with higher vapor pressures, such as TEP, TBP and TCEP, tend to be more likely to emit into air and settled onto dust than heavier OPEs which can strongly be adsorbed to particulate matter (Wei *et al.,* 2015; Brandsma *et al.,* 2015). On the other hand, the structural differences also influence the persistence of various OPEs in the environment. Chlorinated OPEs are more resistant to biodegradation than the alkyl and aryl phosphates. Both the vapor pressures and bioconcentration factors (BCF) vary greatly between compounds, where the BCF generally increases with the increase in the molecular mass (except of chlorine containing compounds). Aryl and alkyl-OPEs with higher molecular mass are more hydrophobic, have similar BCFs and have an affinity for sediment and soil (van der Veen and de Boer*.,* 2012; Wei *et al.,* 2015). Chlorinated OPEs have been shown to be more water soluble and are considered to be persistent threats to aquatic animals (Hou *et al.,* 2016).

Table 1-1: List of OPEs along with their main physical-chemical properties (Bergman *et al.*, 2012; van der Veen and de Boer, 2012; Wei *et al.*, 2015)(Chemspider, 2014). BCF: Bioconcentration factor, *BP at 760 mm Hg, NF: Not Found.

Compound	Common name	Abbreviation	CAS number	Molar mass (g/mol)	Boiling point (°C)*	Log Kow	Vapor Pressure (mm Hg) at 25°C	BCF
Alkyl phosphates	Triethyl	TEP	78-40-0	182.1	216	1.08	2.9×10^{-1}	3.88
	Tripropyl	TPrP	513-08-6	224.2	254	1.87	2.9×10^{-2}	63.1
	Tri-n-butyl	TnBP	126-76-8	266.3	289	4.00	1.1×10^{-3}	1.03×10^{3}
	Tri-iso-butyl	TiBP	126-71-6	266.3	264	3.60	1.3×10^{-2}	391
	Tris(2-butoxyethyl)	TBEP	78-51-3	398.4	414	3.75	2.1×10^{-7}	1.08×10^{3}
	Tris(2-ethylhexyl)	TEHP	78-42-2	434.6	220	10.09	2×10^{-6}	1×10^{6}
Aryl phosphates	Dibutyl phenyl	DBPhP	2528-36-1	286.3	333	4.08	NF	NF
	Butyl diphenyl	DPhBP	2752-95-6	306.2	368	4.41	NF	NF
	Triphenyl	TPP	115-86-6	326.1	370	4.59	1.2×10^{-6}	113
	2-Ethylhexyldiphenyl	EHDP	1241-94-7	362.3	421	6.64	2.5×10^{-7}	6.49×10^{4}
	Tricresyl	o-TCP	78-30-8	368.3	410	5.48	1.8×10^{-7}	8.56×10^{3}
		m-TCP	563-04-2	368.3	442	6.34		
		p-TCP	78-32-0	368.3	439	5.11		
Chlorinated phosphates	Tris(chloroethyl)	TCEP	115-96-8	285.4	351	1.47	1.1×10^{-4}	1.37
	Tris[(2R)-1-chloro-2-propyl]	TCPP	-	327.5	359	2.59	1.9×10^{-6}	42.4
	Tris(1,3-dichloro-2-propyl)	TDCIPP	13674-87-8	429.8	457	3.27	7.4×10^{-8}	13.5
Brominated phosphates	Tris(2,3-dibromopropyl)	TDBPP	126-72-7	697.6	544	3.71	3.17×10^{-9}	NF
	Tris(tribromoneopentyl)	TTBNPP	19186-97-1	1017.3	595	7.55	1.41×10^{-17}	NF

1.4.1.2. **What adverse effects?**

The US-EPA performed an evaluation on the toxicity of alternatives to DecaBDE including the OPEs (US-EPA, 2014) and concluded that insufficient toxicity data were available on toxicity of these alternatives. The studies on the toxicity of OPEs, their impact in the environment and their effect on human health are still very limited particularly on the chronic effects. The prediction of health effects in humans relied on the animal laboratory experiments. This may be inappropriate since some toxic effects can significantly differ in terms of susceptibility of different species (ATSDR, 2012).

Based on the available toxicological studies, it has been shown that OPEs are toxic and have been directly linked to health problems and have the potential to cause adverse reproductive, endocrine and systemic effects in animals as a result of long term exposure to animals (Hou *et al.,* 2016).

Most OPEs show strong hemolytic effects (decomposition of red blood cells). Although these effects are mainly found in rats exposed *via* gavage, adverse biological effects related to humans, such as hemolytic and reproductive effects have also been reported (van der Veen and de Boer, 2012). Additionally, some OPEs can inhibit specific liver carboxylesterases and cause altered hepatic lipid metabolism and can be linked to dyslipidemia as well as OPEs-induced serum hyperglyceridemia in mice (Morris *et al.*, 2014).

Infact, the differences in size and polarity of OPEs can have a large influence on the physical and biochemical toxicity (Greaves and Letcher, 2016). Some alkyl OPEs like TnBP, TiBP and TBEP have been shown to contribute to adverse health effects.

According to Leonards (2011) screening report, TiBP is harmful if swallowed, irritating to the skin and eyes, and has not shown to be mutagenic. Neurotoxic effects associated with exposure to TnBP have been reported (van der Veen and de Boer, 2012). The maximum allowable concentration (MAC value) for TnBP for 8 hours is 5 mg/m^3 (= 0.459 ppm). The LD_{50} value for Killifish (*Oryzias latipes*) and Goldfish (*Carassius auratur*) were 9.6 and 8.8 mg/L, respectively. TiBP data on LD_{50} for rats and mice by oral exposure were 3,072-12,800 and 3,200-6,400 mg/kg bw respectively. TnBP was significantly associated with the prevalence of asthma (OR: 2.85 in floor dust, 5.34 in multi-surface dust) and allergic rhinitis (OR: 2.55 in multisurface dust) (Araki *et al.*, 2014).

TBEP is harmful by inhalation, if swallowed and by contact with skin. The toxicity of TBEP to aquatic organisms is moderate. The 48-h LC_{50} in *Daphnia magna* is 75 mg/L and the 96-h LC_{50} values in fish range between 16 and 24 mg/L. The 4-h LC_{50} for TBEP for rats by inhalation were > 4.43 mg/L. TBEP data on LD_{50} for rats by oral and dermal routes were 3,000 and 47,000 mg/kg bw, and for rabbits > 5,000 and > 100,000 mg/kg bw, respectively (WHO, 2000; Leonards, 2011). TBEP is possibly carcinogenic (Andresen *et al.,* 2004)

TEHP is irritating to the skin, but not to the eyes. It is not considered to be carcinogenic or mutagenic. The LD50 for rats by oral exposure was 10,000-37,080 mg/kg bw (US EPA, 2009). LD50 values for rabbits by oral and dermal route were ~ 20,000 and ~ 46,000 mg/kg bw, respectively. The 96-h exposure of fish resulted in LC50 values >100 mg/L (Leonards, 2011).

Besides, aryl OPEs have been shown to contribute to heart toxicity by disturbing the expression of transcriptional regulators in zebrafish (Du *et al.*, 2015). TPP is potentially problematic as replacement of DecaBDE (US EPA, 2014). TPP has been shown to cause contact dermatitis, and it can inhibit human blood monocyte carboxylesterase, which affects the immunologic defense system. It has a low impact on human health, but is very toxic to aquatic ecosystems (McPherson *et al.,* 2004). The acute toxicity of TPP for fish (96-h LC_{50}) ranges from 0.36 mg/L in rainbow trout to 290 mg/L in bluegills (*Lepomis macrochirus*) (TOXNET, 2016). The LC_{50} was 1.0-1.2 mg/L, 0.36-290 mg/L and 3,500-10,800 mg/kg for daphnia, fish and rats,

respectively (Wei *et al.*, 2015). Human exposure studies showed a slight significant reduction in blood cell cholinesterase activity in employees exposed to triphenyl phosphate (TPP) over a period of 8-10 years. Studies on TPP toxicity showed also a delayed peripheral neuritis involving motor neurons, resulting in flaccid paralysis, particularly of the distal muscles. A report indicated a case of allergic contact dermatitis associated with exposure to TPP that was contained in plastic eyeglass frames (TOXNET, 2016).

EHDP is considered highly toxic to fish and aquatic plants and has potential to bioaccumulate. The oral LD_{50} value for rabbit is 218 mg/kg bw and the dermal LD_{50} value for rabbit is > 7,900 mg/kg bw (Leonards, 2011). There is a significant difference in toxicity between the isomers of TCP. The o-isomer was initially considered to be the most toxic isomer in aircraft turbine engine oil, with a MAC value of 0.1 mg/m^3 for 8 h, and it has been removed as much as possible from commercial products. It is harmful if swallowed and in contact with skin. Studies have suggested it to be a possible reproductive toxin (McPherson *et al.,* 2004), and to be toxic to the central nervous system. The MAC value for DBPhP for 8 hours is 0.299 ppm and the oral LD_{50} for rat is 2,200 mg/kg. For DPhBP, the oral LD_{50} for rat is 2,100 mg/kg.

Chlorinated OPEs like TCEP, TCPP and TDCIPP have proven to be neurotoxic and carcinogenic. Based on California's proposition 65 for the list of chemicals known to the state to cause cancer or reproductive toxicity (on 21 October 2016); TCEP, TDCPP and TDBPP were listed as carcinogenic substances.

TDCIPP levels in house dust were found to be correlated with reduced concentrations of thyroid hormones levels and increased prolactin levels in males (Hou *et al.,* 2016). Araki *et al.* (2014) evaluated the correlation of 11 OPEs in indoor dust (floor and multi-surface dust) with asthma and allergies in 624 inhabitants from 182 family homes in Japan. Significant associations were found between the prevalence of atopic dermatitis and the presence of TCPP and TDCPP in floor dust with odds ratios (OR) of 2.43 and 1.84, respectively. Chlorinated OPEs were suspected carcinogens with observed tumor growth not only in kidney, liver and thyroid for TCEP and TCPP but also in brains and testes for TDCPP (WHO, 1998). TCEP is toxic to aquatic organisms and it may cause chronic adverse effects. It is carcinogenic for animals, is a neurotoxin in rats and mice, and has been showed to induce adverse reproductive effects in rats. Adverse biological effects related to humans have also been reported, such as skin irritation, hemolytic and reproductive effects like reduced fertility, a longer estrous cycle length, reduced sperm motility and reduced sperm density (Leonards, 2011). The LC50 values documented for fish (96-h) ranged from 6.3 to 250 mg/L (Wei *et al.*, 2015). TCPP is persistent and the compound might moderately accumulate in food chains. It is considered to be potentially carcinogenic. The acute (oral), inhalative and dermal toxicity have been tested in rats: LD50 values ranged 500 - 4,200 mg/kg bw > 4.6 mg/L - >17.8 mg/L, and 1,230 to 5,000 mg/kg bw, respectively. TCPP is reported to be irritating to skin and eyes of rats. TDCPP is considered harmful by inhalation and irritating to the skin and it is carcinogenic to rat (Leonards, 2011).

A risk assessment by the European Commission relies upon the work of EFSA, classified TDCIPP as safe for its intended use (EU risk assessment report, 2008). However, the US Consumer Product Safety Commission estimated that daily exposure to TDCIPP exceeds the acceptable daily intake for non-cancer toxicity by two to five times (Babich, 2006). Furthermore, TDCIPP was classified as a cancer-causing agent by the California EPA (OEHHA, 2011) and is predicted to increase cancer risk at current exposure levels (Babich, 2006). TDCPIP increased developmental abnormalities in zebrafish embryos (McGee *et al.,* 2012), showed neurotoxic properties in cultured neuroendocrine (PC12) cells (Dishaw *et al.,* 2011) and caused leg and wing weakness in chickens (Ulsamer *et al.,* 1980). Recent studies suggest that TDCIPP has endocrine-disrupting potential. It has been associated with reduced thyroxine (T4) levels in humans, chicken embryos, and zebrafish (Meeker and Stapleton, 2010; Farhat *et al.*, 2013; Wang *et al.*, 2015), disrupted sex hormone levels in zebrafish (Liu *et al.,* 2012) and dysregulated thyroid hormone (TH)-responsive genes in zebrafish (Wang *et al.,* 2013) and chicken embryo hepatocytes (Crump *et al.,* 2012).

1.4.1.3. **Which population?**

The exposure to OPEs includes general and occupational populations. For the general population, the most relevant exposure pathways for OPEs are inhalation, ingestion of dust and dermal contact. In addition, children may be orally exposed to fabrics treated with OPEs. A child's exposure may differ from an adult's exposure in many ways. Children drink more fluids, eat more food, breathe more air per kilogram of body weight, and have a larger skin surface in proportion to their body volume. A child's diet often differs from that of adults. The developing human's source of nutrition changes with age: from placental nourishment to breast milk or formula to the diet of older children who eat more of certain types of foods than adults. A child's behavior and lifestyle also influence exposure. Children crawl on the floor, put things in their mouths, sometimes eat inappropriate things (such as dirt or paint chips), and spend more time outdoors. Children also are closer to the ground, and they do not use the judgment of adults to avoid hazards (ATSDR, 2012).

Personnel who handle OPEs (occupational population) as pure chemicals, for example, in industries manufacturing OPEs, plastics, textiles, oil products, concrete, *etc.* are suspected to be the most heavily exposed. Other groups that may be more exposed to OPEs than the general population include, *inter* alia, personnel who handle large quantities of hydraulic fluids (*e.g.* aircraft and shipyard technicians), aircraft crew, professional drivers, construction workers and workers at recycling plants for electronic goods.

1.4.1.4. **What conditions of exposure**

Exposure to a contaminant can occur through multiple routes, simultaneously or at different times. In this part, we are going to present the emission sources, the potential transport pathways, the exposure routes of Human and the levels recorded in different compartments.

OPEs are used in very large quantities as FRs and plasticisers and the use is expected to increase because OPEs are currently replacing brominated FRs in many applications (Sundkvist *et al. ,* 2010). Most OPEs are not chemically bound to the original material; they are slowly released in different environmental compartments by abrasion and volatilization (Marklund *et al.,* 2003) and/or leaching during their lifetime, including production, usage, and disposal and recycling processes. The release of OPEs from products is likely to occur due to their moderate vapor pressure. The emission potential of these compounds from materials and vehicles and from e-waste recycling activities has been verified as the dominant sources in indoor and outdoor environments (Wei *et al*., 2015). As a result of these processes and their growing consumption, they are widely distributed in both indoor and outdoor environments. Furthermore, some of these chemicals (*e.g.* halogenated alkyl phosphates) have a low degradation potential and thus may be persistent (Reemtsma *et al.,* 2008; Van den Eede *et al.,* 2011). Therefore, their ubiquitous occurrence may pose a threat to human health through diverse exposure routes.

Despite their reported short atmospheric half lives, OPEs could reach the aquatic and terrestrial systems *via* washout from the atmosphere, *i.e.* precipitation, identified as an important entry pathway into the aquatic environment. Based on previous findings, the emission from materials containing OPEs, wastewater discharge, long range atmospheric transport and aerial deposition are the predominant transport mechanisms for the widespread occurrence of OPEs in various matrices on a global scale (Wei *et al*., 2015). This will be well illustrated in the coming sections in Figures 1-13 and 1-14.

- **External Exposure**

As mentioned in the paragraph 1.4.1.3., human can be exposed to OPEs by a combination of oral, inhalation and dermal routes. Among the non-occupational population, young children may be at a higher exposure risk than adults since they are more likely to put OPEs treated materials in their mouths. On the other hand, the inhaled proportion of dust/food is higher compared to their body weight than that corresponding to adults. Human exposure is highly susceptible, mainly by dust inhalation and ingestion *i.e.* by eating fish or by breastfeeding (Sundkvist *et al.,* 2010). Several studies have found that inhalation of indoor air and dust is one of the most important pathways for people living in indoor environments because building materials are considered to be a significant source and OPEs can directly be taken up by particles on the surface of furniture or equipment (de Boer *et al.*, 2016; Hou *et al.,* 2016). Moreover, dietary exposure of OPEs *via* food is a major concern for the general population. Skin also contributes to the total uptake of OPEs (Hou *et al.,* 2016). The human dermal exposure to OPEs was investigated by Abdallah *et al.* (2015).

Additionally, there are diverse pathways to uptake or absorb OPEs through ingestion, gill absorption, skin absorption and inhalation In an investigation of the distribution of OPEs in fish, researchers found that gill absorption may be one of the most common ways in which aquatic animals take up OPEs dissolved in the

water. Ingestion is assumed to be another critical pathway for the entrance of OPEs to the bodies of animals. All of these findings favor the emission from materials containing OPEs and to undergo different transport mechanisms for the widespread occurrence of OPEs in various matrices on a global scale (Wei *et al.,* 2015).

- **Metabolic processes**

Metabolism is considered to be a major determinant of the bioaccumulation of xenobiotics, their fate as well as indirect determinant of the toxicological effects of these compounds. Previous studies have demonstrated that OPEs can be rapidly metabolised through phase-I and phase-II biotransformation to metabolites, which are more hydrophilic and more readily eliminated (Hou et al., 2016). Animal *in vivo* and human *in vitro* studies have been recently investigated (Greaves and Letcher, 2016) and suggested that OPEs are mainly sensitive to two types of phase I biotransformation reactions — hydrolysis and oxidative metabolism (Van den Eede *et al.,* 2013a). These diesters of OPEs have already been considered as target metabolites in several biomonitoring studies. In vitro study on human liver fractions indicated a significant formation of hydroxylated metabolites for TCPP, TPP, and TBEP (Van den Eede *et al.,* 2013a). And more recently this was investigated by Abdallah *et al.,*2016 who studied the metabolic profile for TCEP, TCPP and TDCPP applied concomitantly to human hepatocyte cultures.

Information on the metabolism of alkyl OPEs exists only for TnBP in laboratory animals and TBEP in human liver microsomes (Hou *et al.,* 2016). The metabolic transformation of TnBP has been studied in male rats following oral administration of ^{14}C-labeled TBP. Following single or repeated oral dosing in rats, TBP was detected in the gastrointestinal tract, blood and liver. The first stage of metabolism appeared to be oxidation at the omega position on the butyl chains. The generated hydroxyl groups were further oxidized to produce carboxylic acids and ketone, respectively. The oxidized alkyl moieties were removed as glutathione conjugates. In the urine, the major metabolites were dibutyl hydrogen phosphate (DnBP), butyl dihydrogen phosphate (MnBP) and butyl bis(3-hydroxybutyl) phosphate (di-OH-TnBP) as well as other metabolites of hydroxylated derivatives of the butyl moieties (TOXNET, 2016; Hou *et al.,* 2016). No phase II metabolites of TnBP have been reported (Hou et al., 2016).

In a human liver microsome study, bis(2-butoxyethyl) phosphate (BBEP), bis(2-butoxyethyl) hydroxyethyl phosphate (BBEHEP), four isomers of mono-hydroxylated TBOEP (di-OHTBEP) and some ketone isomers were reported to be metabolites of TBEP (Van den Eede *et al.,* 2013a). For Phase-II metabolites, a glucuronide conjugate with BBEHEP was identified in human liver S9 fraction incubation (Van den Eede *et al.,* 2013a). In another study using human liver microsomes, the mono-hydroxylated metabolites of TBEP were confirmed to be 3-HO-TBEP (bis(2- butoxyethyl) 3-hydroxyl-2-butoxyethyl phosphate), 1-HO-TBEP (bis(2-butoxyethyl) 1-hydroxyl-2-butoxyethyl phosphate) and 2- HO-TBEP (bis(2-butoxyethyl) 1-hydroxyl-2-butoxyethyl phosphate) (Van den Eede *et al.,* 2013a; Hou *et al.,* 2016).

As for aryl-OPFRs, metabolites of TCP and TPP were investigated in laboratory animals. The metabolism of p-TCP was studied in the rat after administration of methyl-^{14}C p-TCP. The major metabolites were p-hydroxybenzoic acid, p-cresyl phosphate (DCP), and p-cresyl p-carboxyphenyl phosphate (di-COOH-TCP). Following the oral administration, p-TCP was absorbed from the intestine, distributed to the fatty tissues, and moderately metabolized to a variety of products of oxidation and dearylation of p-TCP, which were then excreted in the urine, feces, bile and expired air (TOXNET, 2016; Hou et al.2016). o-TCP is metabolized in rats, rabbits, mice, and chickens to form a neurotoxic esterase inhibitor. o-TCP is metabolized via three pathways. The first is the hydroxylation of one or more of the methyl groups, and the second is the dearylation of the o-cresyl groups. The third is further oxidation of the hydroxymethyl to aldehyde and carboxylic acid. o-TCP and its metabolites are eliminated *via* the urine and feces, together with small amounts in the expired air (TOXNET, 2016).

Su et al. (2014) studied the metabolism of TPP in chicken embryonic hepatocytes in vitro. The identified metabolites were DPP, hydroxylated TPP (OH-TPP) and dihydroxylated TPP isomers (di-OH-TPP). Su et al. (2015) then identified the hydroxylated TPP as p- and m-OH-TPP and found that the conjugate with glucuronic was primarily on p-OH-TPP. To date, the metabolism of TPHP has also been studied in human liver microsomes in vitro. In addition to the metabolites in the aforementioned chicken hepatocytes, mono-ester (MPP) and hydroxylated DPP (OH-DPP) have also been identified in the metabolism of TPP. Glucuronide conjugates and sulfate conjugates of TPP were observed in the human liver S9 fractions in the in vitro study of Phase-II metabolites (Van den Eede *et al.,* 2013a).

A general metabolic pathway of aryl was same as alkyl OPEs (Figure 1-11). The Phase-I metabolic processes included hydroxylation, dihydroxylation and carboxylation on the phenyl. Glucuronide and sulfate can only react on hydroxylated or dihydroxylated metabolites in Phase-II reaction. What worth mentioning is EHDP, who owns both alkyl and aryl. Major urinary metabolites of EHDP included diphenyl phosphate and phenol. Minor metabolites included p-hydroxyphenyl-phenyl phosphate and monophenyl phosphate (TOXNET, 2016).

Previous studies in rodents demonstrated that TDCPP was rapidly metabolized, and the primary metabolites identified were dialkyl metabolites, bis(1,3-dichloro-2-propyl) phosphate (BDCPP), and diphenyl phosphate (DPP), respectively. Furthermore, a recent study investigating the in vitro metabolism of these same two OPFRs in human liver microsomes demonstrated that the primary metabolites in humans were also likely BDCPP and DPP (Meeker *et al.,* 2011).

The metabolism of chlorinated OPEs (TDCIPP, TCEP and TCIPP) in human liver preparations was also examined recently (Van den Eede *et al.,* 2013a). Hydroxylated TDCIPP (OH-TDCIPP), carboxylated TDCIPP (COOH-TDCIPP), BDCIPP and hydroxylated BDCIPP (OH-BDCIPP) were all validated as major Phase-I

metabolites for TDCIPP. The identified TCEP metabolites included BCEP and hydroxyethyl 2-chloroethyl hydrogen phosphate (OH-TCEP). Similarly, TCIPP metabolized to hydroxylated TCIPP (OH-TCIPP), bis(1-chloropropyl) phosphate (BCIPP), hydroxylated BCIPP (OH-BCIPP) (this was confirmed by dosing experiments of animals with ^{14}C-radiolabeled TCPP (TOXNET, 2016)) and carboxylated TCIPP (COOH-TCIPP). For the Phase-II metabolites, glutathione-conjugated TDCIPP and TCEP were identified in all chlorinated OPEs, except for TDCIPP (Van den Eede *et al.,* 2013a).

The overall metabolic pathway of chlorinated OPEs is shown in (Figure 1-11). The Phase-I metabolic pathway of chlorinated OPEs involves cleavage of the ether bond (O-dealkylation) and oxidative dehalogenation of the terminal carbon atom. These reactions produce the formation of diesters (DAPs) and hydroxylated metabolites and ultimately form carboxylic acids. In addition, the formation of Phase-II metabolites (glutathione conjugate) can occur through direct substitution of Cl atoms in chlorinated OPEs, which are electrophilic or substitutive in molecules.

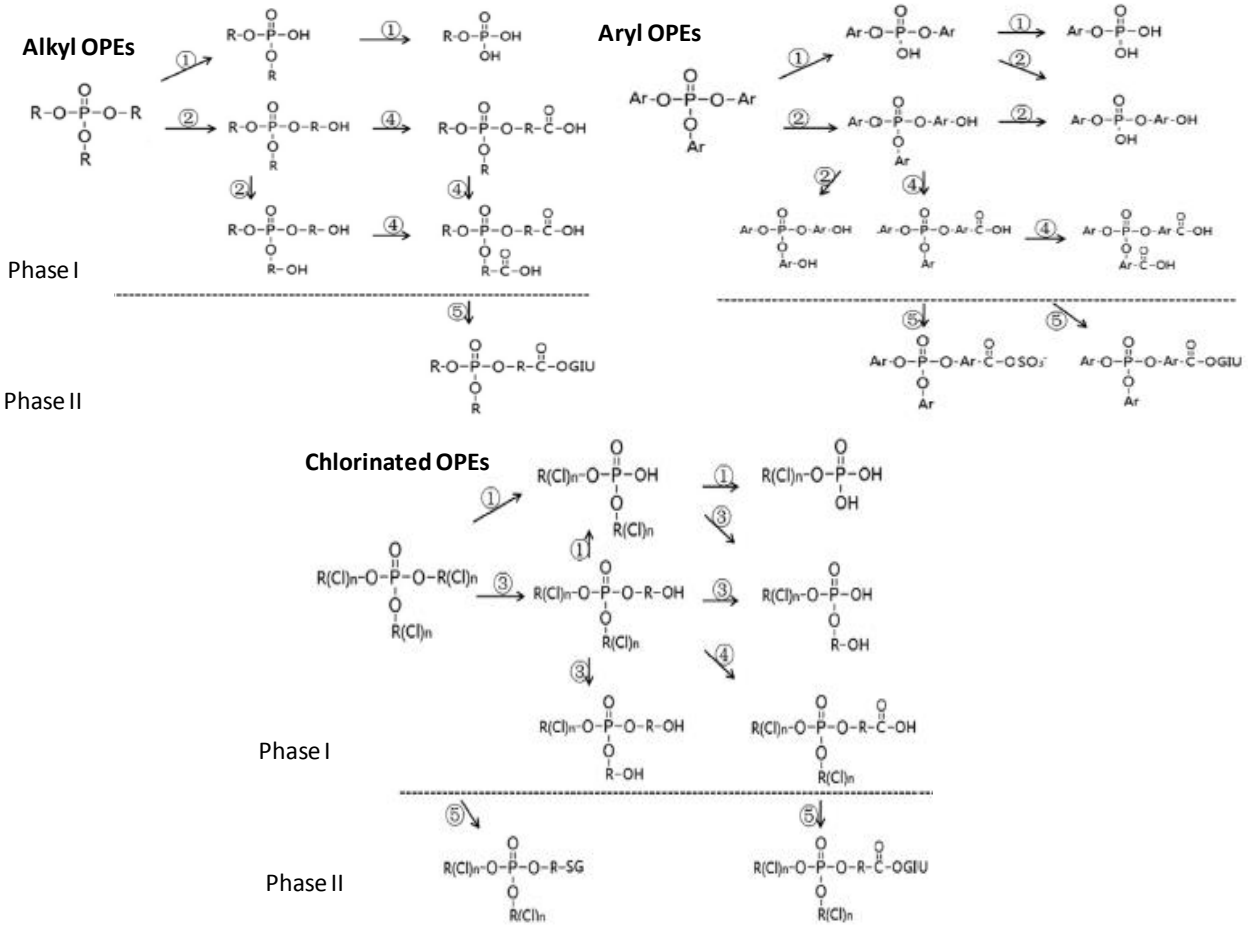


Figure 1-11: The proposed metabolic pathways (Phase I and Phase II) of alkyl, aryl and brominated OPEs. Reaction numbers refered to the following annotations (1: O-dealkylation, 2: Hydrocylation, 3: Oxidative dechlorination, 4: Oxidation and 5: Conjugation) (Hou et al., 2016).

The conclusion on the metabolic processes can be also illustrated in Figure 1-12, which represent the schematic representation of the OPEs fate in humans and animals and hence the position of metabolic processes (Hou et al., 2016).

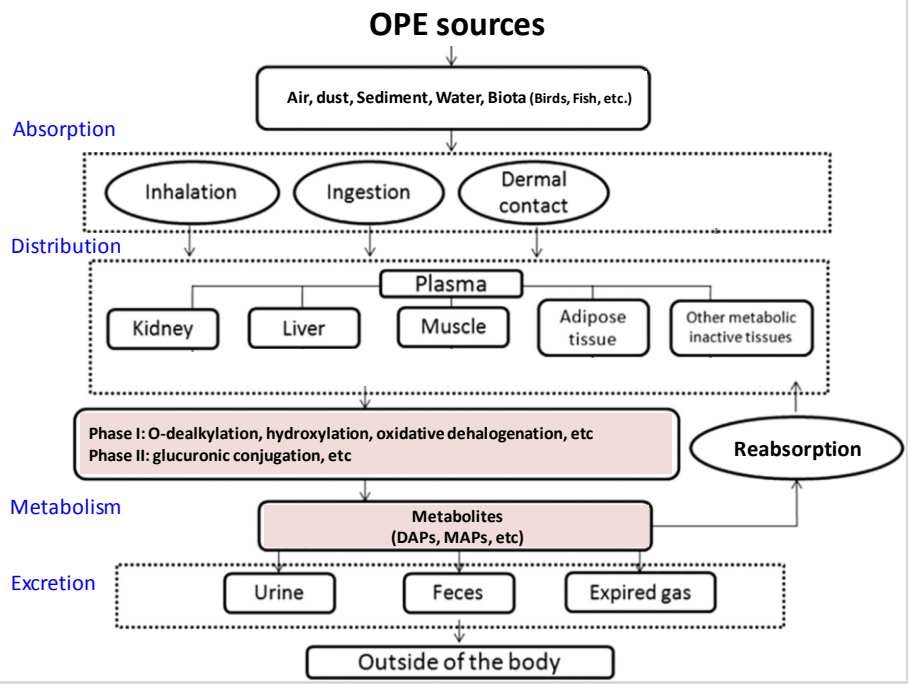


Figure 1-12: Schematic representation of the fate of OPEs in humans and animals (Hou et al., 2016).

➢ Internal Exposure

Biomonitoring is regarded as the gold standard in chemical exposure assessment. In contrast to external exposure, which concerns the source or pathway to the body, studies of internal exposure have focused on the total concentration of circulatory OPEs and their metabolites within the body, which indicates the total burden of exposure. Urine, plasma and saliva are the most frequently used matrices in human biomonitoring (Hou et al. , 2016). OPE diesters (DAPs) and monoesters (MAPs) are thought to be the major metabolites.

Furthermore it has to be considered that hydrolysis may continue ultimately, to phosphate. These were quantified in previous studies and identified as biomarkers to assess human exposure. In total, DAPs of bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), bis(1-chloro-2-propyl) phosphate (BCIPP), bis(2-chloroethyl) phosphate (BCEP), bis(2-butoxyethyl) phosphate (BBOEP), di-n-butyl phosphate (DBP), diphenyl phosphate (DPP), diethylhexyl phosphate (DEHP) and some MAPs of MEHP, MBP, MCIPP, MBOEP and MPP have been quantified in human urine, as detailed in the previous section on metabolism.

In their study, Van den Eede *et al.* (2014) wanted to characterise the average levels and age-related patterns of OPEs metabolites in urine in the general Australian population. DPP was found in several samples at levels

which were one order of magnitude higher than previously reported (up to 730 ng/mL). Weighted linear regression revealed a significant negative association between log-normalized BDCIPP and DPP levels and the age. Significantly greater levels of BDCIPP and DPP were found in children's urine compared to adults, suggesting higher exposure to OPEs in young children. Petropoulou *et al.* (2016) also quantified 4 DAPs in urine samples from 13 adult in California, collected from 8 females and 5 males in the morning hours. BCEP was detected at 0.4–15 ng/mL; BDCIPP at 0.5–7.3 ng/mL, DPhP at < MDL-5.6 ng/mL and BCIPP at < MDL-3.5 ng/mL.

The biomonitoring study by Cequier *et al.* (2015) reported the occurrence of DAPs in urine from a Norwegian mother–child cohort (48 mothers and 54 children). Median urinary concentrations of DPHP were 1.1 and 0.51 ng/mL in children and mothers, respectively, followed by BDCIPP with medians of 0.23 and 0.12 ng/mL, respectively. Median concentrations in urine from children and mothers were <0.18 ng/mL for BBEP and <0.12 ng/mL for DnBP. The concentrations of DPP and BDCIPP in urine from children were significantly correlated with those found for their parent compounds in air and dust from the households. For mothers, only the urinary concentration of BDCIPP was correlated to its precursor in dust from the households; which might indicate higher impact of the household environment on children than mothers. In the same issue, Van den Eede *et al.* (2013b) developed a method for the determination of 6 metabolites of OPEs in human urine. Target DAPs included DBP, DPHP, BBOEP, BCEP, BCPP, and BDCIPP. Set of urine samples from adult volunteers (n=59) from Belgian population was analysed, in which DPHP was the major DAP metabolite. A significant increase of DPHP levels was observed in the group of smokers (geometric mean of 1.55 ng/mL) compared to the non-smokers (geometric mean of 0.88 ng/mL), suggesting that an altered metabolism induced by tobacco smoke may be responsible for the formation of DPP from TPP than in non-smokers. Schindler *et al.* (2009) determined the internal body burden of 30 persons from the German general population. OPE-metabolite concentrations in their urine ranged from <LOD to 27.5 ng.g $^{-1}$ and <LOD to 4.1 ng.g $^{-1}$ for BCEP and DPP, respectively.

Human milk is considered as the best source of nutrition for infants. Breast milk contains the optimal balance of fats, carbohydrates and proteins for developing babies, and it provides a range of benefits for growth, immunity, and development. Unfortunately, breast milk is not pristine. Chemical contamination of human milk is widespread and is the consequence of decades of inadequately controlled pollution of the environment by toxic chemicals (Landrigan *et al.*, 2002). From here, Kim *et al.* (2014) determined the concentrations of 10 OPFRs in 99 human breast milk samples collected from the Kanagawa Prefecture, Japan (n=20) in 2009–2011, Malate (n=19) and Payatas (n=22), the Philippines in 2008, and Hanoi (n=7), Bui Dau (n = 10) and Trang Minh (n 9), Vietnam in 2008. Among the targeted OPEs, TCEP and TPhP were the predominant compounds and were detected in more than 60% of samples in all three countries. The concentrations of OPEs in human breast milk were significantly higher ($p < 0.05$) in the Philippines (median 70 ng/g lw) than

those in Japan (median 22 ng/g lw) and Vietnam (median 10 ng/g lw). In milk samples, TCPP (median 45 ng/g) and TBP (median 12 ng/g) were the most frequently occurring OPEs. In the same trend, Sundkvist *et al.* (2010) investigated the levels of 11 OPEs in human breast milk from Sweden. TCPP (45 ng/g) and TBP (12 ng/g) were the dominant compounds. The levels of TBEP tended to be higher in milk samples collected 10 years ago than in recently collected milk samples.

Figure 1-13 and 1-14 illustrate conclusions drawn for the exposure routes and conditions in both outdoor and indoor environments, respectively. The highlights in outdoor environment are (Figure 1-13):

- The effluents from industries fabricate or use the OPEs and the wastewater discharges were presumed to be the primary entry pathway of OPEs to surface water and aquatic compartment (Wei *et al.*, 2015).
- Monitoring studies of OPEs in air and precipitations from remote areas implied that certain OPEs were subject to long range atmospheric transport (**LRAT**) and **aerial deposition**.
- OPEs could reach the aquatic and terrestrial systems *via* washout from the atmosphere, *i.e.* precipitation, identified as an important entry pathway into the aquatic environment.

Indoors, the situation is entirely different... (Figure 1-14) OPEs are present at high concentrations where we live and work, in our computers and phones, in the upholstery we sit on, in cars as well as in the many products in the buildings we spend time in (de Boer *et al.*, 2016).

- OPEs can be emitted from the equipment and furniture through volatilization or abrasion (small particles breaking off from textile fibers, *etc.*).
- OPEs can directly be taken up by particles on the surface of furniture or equipment.
- The high chemical concentrations in indoor dust and air suggests that the major exposure routes are inhalation, dermal contact and especially for young children, hand-mouth contact.

Emission, migration and human exposure to OPEs from both outdoor and intdoor environment highlights are:

- **Predominant sources:**

Outdoor: Emission from materials containing OPEs, wastewater discharge, LRAT and aerial deposition

Indoor: Furniture, electronics, textiles, baby items, contaminated food, etc.

- **Exposed population:** occupational and general population including habitants
- **Main exposure routes:** Dermal by contacting OPEs treated materials (especially for small children), Inhalation of contaminated indoor air and dust and Ingestion of contaminated food. *The high OPEs concentrations in indoor dust and air now suggests that the inhalation is the major human exposure route (de Boer *et al.*, 2016; Schreder *et al.,* 2016)

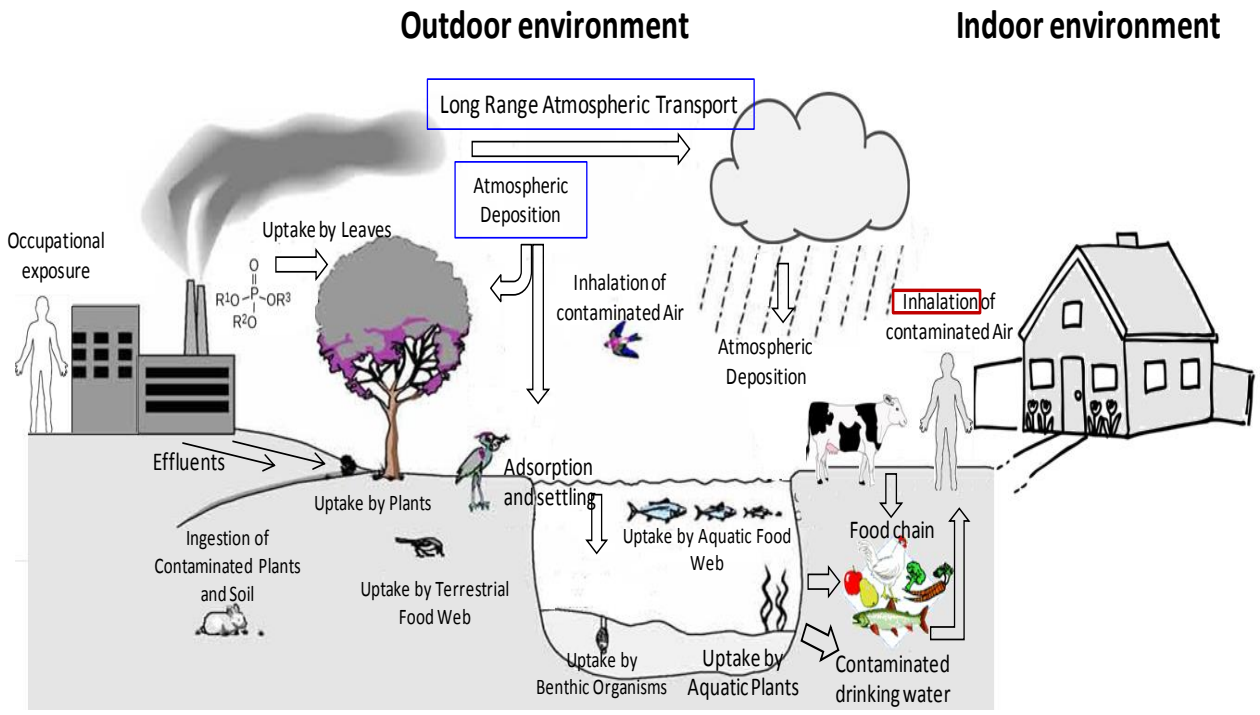


Figure 1-13: Potential transport pathways of OPEs in the outdoor environmental compartments and human exposure routes.

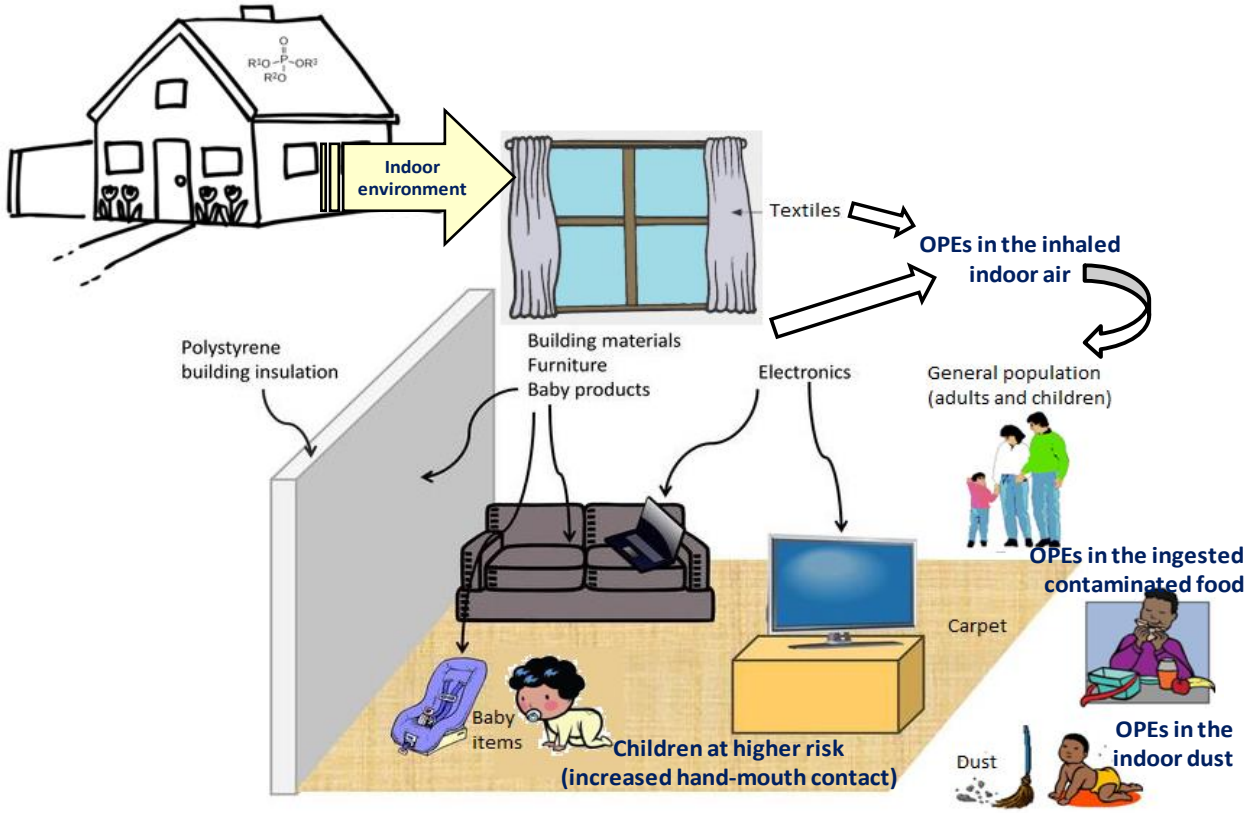


Figure 1-14: Potential transport pathways of OPEs in the indoor environmental compartments and human exposure routes.

As well illustrated, this step would include the determination of the danger potential of pollutants in function of different exposure routes. The limitation in this section is that Human epidemiology data are the most desirable and are given highest priority since they avoid the concern for species differences in the toxic response. Unfortunately, reliable epidemiology studies are rarely available. In practice, animal bioassay data are generally the primary data used in risk assessments. The use of laboratory animals to determine potential toxic effects in humans is a necessary and accepted procedure. It is a recognized fact that effects in laboratory animals are usually similar to those observed in humans at comparable dose levels. Exceptions are primarily attributable to differences in the pharmacokinetics and metabolism. Indeed, the information from studies conducted by the oral route exposure to OPEs was only available from animal studies which is still a gap knowing that the environmental monitoring data available suggested that the levels of some of these substances to which the general population might be exposed through contact or use of consumer products (including food), or that are commonly found in environmental media are generally orders of magnitude lower than those used in studies with experimental animals.

1.4.2. HAZARD CHARACTERISATION

This stage in the risk assessment process involves prediction of the frequency and severity of effects in exposed populations. The dose-response assessment step quantifies the hazards which were identified in the hazard identification phase. It determines the relationship between dose and incidence of effects in humans. There are normally two major extrapolations required. The first is from high experimental doses to low environmental doses and the second from animal to human species. This is a limitation and is presented by the extrapolation of observations in animal experiments at average dose to the weak exposure doses in humans and the transposition of population data animals to humans.

Indeed, the required data for OPEs risk assessment are still missed. There exist some estimates of exposure levels posing minimal risk to humans that have been made by ATSDR (2012). This level is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (non-carcinogenic) over a specified duration of exposure. These levels are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. They are based on noncancerous health effects only and do not consider carcinogenic effects. Individuals are often exposed to substances by more than one exposure pathway (*e.g.*, drinking of contaminated water, inhaling contaminated dust). In such situations, the total exposure will usually equal the sum of the exposures by all pathways. Hence, these levels can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes.

The procedures used is illustrated in Figure 1-15, it consists of extrapolate from high to low doses are different for assessment of carcinogenic effects and non-carcinogenic effects. Non-carcinogenic effects (*e.g.* neurotoxicity) are considered to have dose thresholds below which the effect does not occur. The lowest dose with an effect in animal or human studies is divided by Safety Factors (usually 100) to provide a margin of safety.

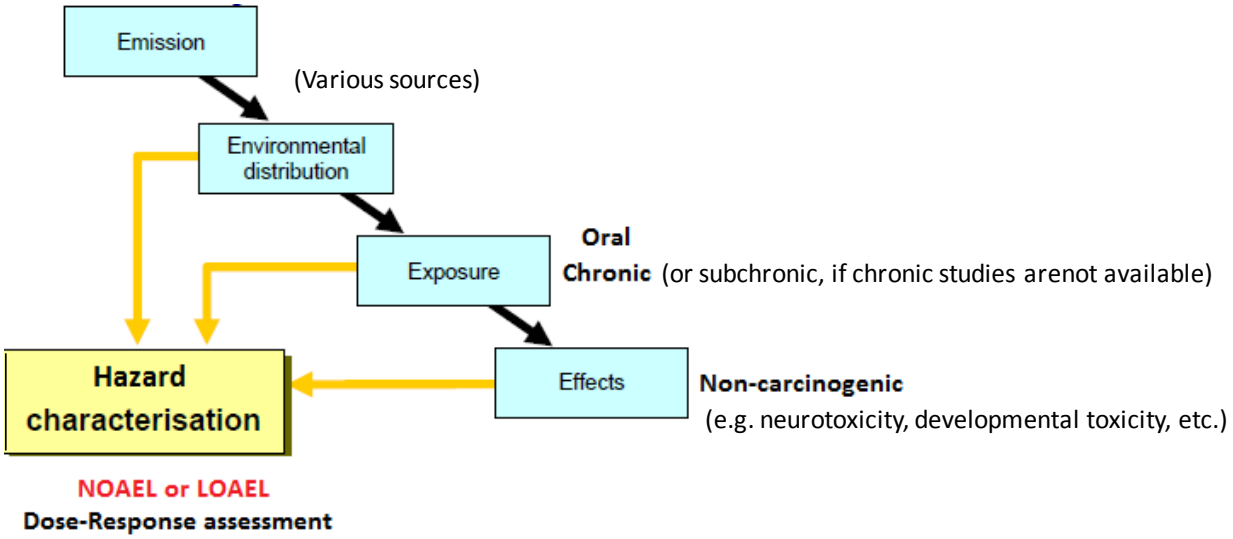


Figure 1-15: Schematic representation of the Dose-Response assessment required in the step of hazard characterisation, as well as our targeted approaches in this issue.

As previously described, this step can also be referred as dose-response assessment step during which one quantifies the hazards which were identified in the hazard identification phase. It determines the relationship between dose and incidence of effects in humans in terms of acceptable daily intake (ADI) in mg/kg/day.

The TRVs provide information on the occurrence of adverse health effects for a given exposure dose. TRVs are calculated from the available toxicological data type NOAEL ("No Observed Adverse Effect Level") and LOAEL ("Lowest Observed Adverse Effect Level") which are presented in Table 1-2, where we can see that these data are not available for all OPEs.

Table 1-2: Summary for the available toxicological information in the literature for the set of our targeted OPEs.

OPE	Experiment	NOEL/NOAEL orLOAEL for chronic/subchronic oral exposure (mg/Kg bw or mg/Kg bw/ day)	Endpoint-adverse health effects	Reference
TEP	Rat-	Subchronic 670 mg/kg bw	Retarded weight gain, elevated liver and adrenals weight	(OECD-UNEP, 2002)
TPrP	No record			
TnBP	Rats and mice	Chonic 8 mg/kg bw/day-	Renal- Systemic (Int.,chr.), Immunologic, Neurologic, Reproductive, Genotoxic	(ATSDR, 2012)
TiBP	No record			
TBEP	Rats	Subchronic 15 mg/kg bw/day	Hematological and clinical effects	(WHO, 2000)
TEHP	Rats	Intermediate 430 mg/Kg bw/day	weight loss	(WHO, 2000)
TPP	Rats	Subchronic 161 mg/kg bw- Reduce body weight 700 mg/kg bw- Immunotoxicity 690 mg/kg bw - Fertility	Slight depression in growth rates and increased liver weights (35 days)	(OECD-UNEP, 2002;, TOXNET, 2016)
EHDP	Rats-	Subchronic 165 mg/kg bw/day	Increase in kidney, teste and brain weight	(TOXNET, 2016)-
DBPhP	Rats	Subchronic 5 mg/Kg bw/day	Reduced liver hepatocyte vacuolation -	(TOXNET, 2016)
DPhBP	No record-			
o-, m-, p- TCP	Rats	Chronic 2 mg/Kg bw/day	Ovarian lesion- Systemic (Int.,chr.), Immunologic, Neurologic, Reproductive, Genotoxic	(ATSDR, 2012)
TCEP	Rats	Chronic 20 mg/Kg bw/day	Renal lesion- Systemic (Int.,chr.), Immunologic, Neurologic, Reproductive, Genotoxic. There is inadequate evidence for the carcinogenicity of TCEP in experimental animals and no data in humans	(ATSDR, 2012)
TCPP	Rats	Subchronic NOAEL (male rats) = 800 mg/kg bw	histopathologic changes -	(OECD-UNEP, 2000)
TDCIPP	Rats	Chronic 2 mg/Kg bw/day 14 mg/kg bw/day	Systemic (Int.,chr.),Immunologic, Neurologic, Reproductive, Genotoxic Hematological and clinical effects	(ATSDR, 2012) (TOXNET, 2016)
TDBPP	There is sufficient evidence in experimental animals for the carcinogenicity of TDBPP. It is probably carcinogenic to humans (Group 2A)			(TOXNET, 2016)
TTBNPP	Rats	1,358 and 1,685 mg/Kg bw for males and females, respectively	Treatment-related effects, uncertain potential for liver effects based on the bromo substituents. Estimated to have moderate potential for carcinogenicity.	(US EPA, 2014)

For oral exposure, the TRV is called ADI (Acceptable Daily Intake). It is expressed in amount (mg, µg or ng) per kilogram of body weight (bw) per day. The TRVs are established by the authorities' bodies such as the WHO, ATSDR, the US EPA or EFSA at the EU level.

For all these TRVs, general assumptions are applied, for example, "an effect observed in animals may also develop in men ... "or" ... Human is more sensitive than the animal ...". For the fetus, it is necessary to develop specific TRV because the exposure sources are different and the fetus is particularly sensitive to endocrine disruption. However, these data are extremely rare.

After looking for the available TRVs, it is important to estimate the ADI for these compounds as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects over a specified duration (for example chronic exposure of 365 days or more) of exposure through inhalation or oral routes. It is determined by applying safety factors (in order to account for the uncertainty in the data while providing a margin of safety for allowable human exposure) to the highest dose in human or animal studies which has been demonstrated not to cause toxicity (NOAEL). The Safety factor 100 was usually used (10 for animal to human extrapolation and 10 for human variability). The critical toxic effect used in the calculation of an ADI is the serious adverse effect which occurs at the lowest exposure level. It may range from lethality to minor toxic effects. It is assumed that humans are as sensitive as the animal species unless evidence indicates otherwise.

$$\boldsymbol{ADI}\ ((\boldsymbol{mg/kg\ bw})/\boldsymbol{day}) = \mathbf{NOAEL}\ /\ \mathbf{Safety\ Factor(s)}$$

As a conclusion, we have tried to summarize all available toxicological information about our targeted OPEs from different sources. We were interested in the non-carcinogenic toxic effect due the chronic (or subchronic if chronic studies are not available) oral exposure from laboratory animal experiments.

In this trend, we've looked for the available NOAEL or LOAEL, noting that the dose-reponse relationship is not yet assessed or well organized for all OPEs. All in all, it is important then to assess the exposure in order to compare it to these available toxicological reference values.

1.4.3. EXPOSURE ASSESSMENT

In order to better understand the environemtal fate and exposure to OPEs, this section is dedicated to describe the contamination levels reported in biotic and abiotic compartments. The abiotic matrices include sediments, air and dust, while the bioltic ones include fish and other foodstuffs.

1.4.3.1. **Contamination levels in abiotic compartments**

Due to their physical-chemical properties, FRs and hence OPEs tend to accumulate on organic carbon rich matter and have been detected in various abiotic environmental matrices, like sediments, air and dust. Many studies have discussed the occurrence of these OPEs in such abiotic compartments. In our thesis, we will focus on the most recent studies for the widespread occurrence findings at the global scale. From these findings, the geographical distribution, the levels and the dominance of compounds will be illustrated. These levels will be presented in dry or wet /fresh weight basis (dw and ww or fw, respectively). The use of dw based values, enables to calculate the concentration based on the percent of dry matter present in the sample. The fw based values compensate for the moisture content of the analysed sample.

- **Sediments**

Aquatic sediments are probable deposition sites for organic pollutants (Iqbal *et al.,* 2017) including OPEs. However, limited number of OPEs has been documented to date. In this part, we are going to present the occurrence data from the most recent studies in Norway, China and Spain, focusing mainly in industrialized areas.

Extremely high OPE levels were detected at 7,460 to 17,900 and 22,700 to 33,800 ng/g for sediments collected from the pump pit at landfill sites and automobile destruction sites in Norway, respectively. High OPE concentrations were also obtained in the river sediments from Norway ranging between 490 to 22,500 ng/g, with a dominance of TCPP, TDCPP, TCEP, TBEP and TPP, possibly assosciated with the massive consumption and use pattern of OPEs in Norway (Green et al., 2008). Sediments from other countries (such as Spain and China) had significantly lower OPE levels.

In 2016, Li *et al.* investigated the levels of 8 OPEs in river sediments collected in 2013 from Shiwuli and Tangxi rivers, two urban rivers in Hefei city. These are heavily polluted rivers according to Anhui Environmental Bulletin. The River Shiwuli presented a median of 58.6 ng/g dw with the dominance of TEHP, TEP, TPrP. The River Tangxi presented a median of 68 ng/g dw with the dominance of the chlorinated OPEs (TCEP, TCIPP and TDCIPP). Other studies were conducted for the analysis of OPEs in sediments from different regions in China. Lu J-X *et al.* (2014) analysed 7 OPEs in sediment samples collected in 2013 in Shanghai city. Among the detected OPEs, TCEP and TCPP were the prominent compounds with a sum of concentrations reaching 700 ng/g dw.

Cristale and Lacorte (2013) analysed sediment samples collected during spring 2012 from three rivers in Spain, suffering different anthropogenic pressures. TEHP, EHDP, TCP and TPP were frequently detected in concentrations ranging from 2.1 to 290 ng/g dw. TCPP was detected in most of the sediment samples, with concentrations ranging from 13 to 365 ng/g dw. TBP, TiBP, TDCPP and TCEP were detected in few sediment

samples, at concentrations from 2.2 to 13 ng/g dw. On the other hand, TBEP was not detected in sediments. The River Besòs presented the highest OPE sediment levels, with Σ_{10}OPE between 153 and 824 ng/g dw.

The results suggested that diffuse sources, such as dry and wet deposition, played an important role in the distribution of OPEs in these sediments. However, still limited data are available for OPEs in sediments while many studies have discussed the occurrence of these compounds in air and dust in different regions.

- **Air/ Atmosphere**

Air is an important compartment of the environment, and is regarded as a major reservoir for the OPEs released from consumer products, resulting in the ubiquitous detection of OPEs in the atmosphere (Wei *et al.,* 2015). Researchers have previously conducted surveys on the occurrence of OPEs in various indoor as well as outdoor atmospheric environments. In the following part, we are going to present the most recent studies on the occurrence data for the presence of OPEs in indoor air (China) as well as outdoor air in industrilised (Great lakes, German coast, Mediterrranean and Black Seas) and remote (ocean) areas.

The magnitude and the distribution pattern of OPEs varied significantly among various categories of indoor environments, mostly depending on the types and quantities of emission sources. Private homes tended to have lower OPE concentrations compared with work environments (Wei *et al.,* 2015).

Faiz *et al.* (2016) measured 7 OPEs in airborne particles and settled dust samples collected during the period of November 2014 to February 2015, from three different types of indoor spaces (offices, conference halls and laboratories) in an institute building in Nanjing University, China. TCEP and TCPP were the major OPEs found indoors. The results showed that use of certain products indoors such as cushion chairs and sofa, vinyl floors furnished with floor polish and computer/electronic equipment contributes to the presence of these OPEs.

The OPEs emitted into the indoor environment may eventually reach the outer environment through diverse processes, such as ventilation and disposal of dust bags at dumpsites (Marklund *et al.,* 2003). The sum of OPE concentrations in the outdoor air were detected at approximately 1-4 orders of magnitude lower than the reported results indoors (Wei *et al.,* 2015). Salamova *et al.* (2016) presented the measurements of 6 halogenated and non-halogenated OPEs in particle, vapour and precipitation samples collected in the US Great Lakes basin every 12 days between 2012 and 2014 at five US Integrated Deposition Network sampling sites. The results showed the presence of OPEs in all phases of the atmosphere with the highest contamination found in atmospheric precipitation samples (220 ± 38 pg/m^3) and the dominance of TPP. Their presence at both urban and remote location reveals that OPEs may undergo long range atmospheric transport. Besides, Shoeib *et al*. (2014) analysed a subset of 18 air samples at an urban site in Toronto, Canada

for the occurrence of 6 OPEs (3 aryl and 3 chlorinated). The samples were collected during the period of March 2010 to August 2011. The ∑OPE mean concentration was about 2643 pg/m^3. The order of contamination was as follows: TPhP> TCEP, TCIPP> EHDP> TDCPP.

Wolschke *et al.* (2016) reported the occurrence of 9 OPEs in the marine atmosphere of the German Coast. A number of 58 samples were collected from August 2011 to October 2012. The concentration (gas + particle phase) of total OPEs was on average 5 pg/m^3. A significant part of the sum OPE concentration (55%) was detected in the gas part and high contribution from the gas phase was observed for individual compounds such as TiBP. Still in Germany, MÖlller et al. (2011) presented the occurrence of 8 chlorinated and non-chlorinated OPEs in the marine atmosphere. For this purpose, air samples were collected in the German part of the North Sea from March to July 2010. The Σ_8OPEs concentrations ranged from 110 to 1 400 pg/m^3 where TCPP dominated all samples. Regarding their sites, samples of continental air masses showed the highest concentrations due to the high influence of industrialised regions on atmospheric emissions and concentrations.

MÖller *et al.* (2012) investigated the presence of OPEs in airborne particles over the Pacific, Indian, Arctic, and Southern Ocean. Samples were taken from East Asia to the high Arctic (collected from June to September 2010) and another from East Asia toward the Indian Ocean to the Antarctic (collected from November 2010 to March 2011). These were analysed for three halogenated OPEs (TCEP, TCIPP and TDCIPP), four alkyl OPEs (TnBP, TiBP, TBEP, TEHP) and TPP. The sum of the eight investigated OPEs in the two sampling sites ranged from 230 to 2,900 pg/m^3 and from 120 to 1,700 pg/m^3, respectively. The chlorinated compounds were the dominating compounds, with concentrations from 19 to 2,000 pg/m^3 and 22 to 620 pg/m^3, respectively.

The studies concerned in the analysis of air samples showed the presence of OPEs in indoor, urban and remote areas. This proves that they undergo long-range atmospheric transport over the global oceans toward the Arctic and Antarctica.

- **Indoor Dust**

Because of their vast usage in building materials, furnishings, textiles and electronic equipments, OPEs have been ubiquitously found in the dust from various indoor environments. The occurrence level is likely dependent on the type and amount of furniture, building materials and electronic appliances located in the room and the degree of ventilation (Wei *et al.,* 2015). Once indoors, these compounds are less prone to degradation due to the nature of indoor environment (*e.g.* cool temperatures, less direct sunlight) and thus can persist for longer periods, especially when dust is trapped in carpets (Fan *et al.,* 2014). Indeed, indoor dust represents the most described compartment in the literature so that there is lot of studies dealing with

the analysis of OPEs in indoor and outdoor dust all over the worldwide. In this part we are going to present the most recent ones from different regions in the world (America, Asia, EU) covering the different indoor environments (cars, private homes or offices) as well as the seasonal trend variation.

In 2012, Dodson *et al.* provided data on changing exposure patterns to a broad range of FRs (BFRs and OPEs). For this purpose, dust samples were collected in 16 California homes. Concentration of chlorinated OPEs (including TDCIPP and TCEP), were found up to 100 μg/g in dust. In 75% of the homes, TDBPP was detected knowing that it was banned in children's sleepwear because of carcinogenicity. Staptelon *et al.* (2014) studied the FRs associations between and children's handwipes and house dust from samples collected in North Carolina, USA. TDCIPP, TCEP and TCPP were ubiquitously detected in house dust samples with significant associations between handwipes and house dust. Increasing house dust levels and age were associated with higher levels of FRs in handwipes, and high hand washing frequency (>5 times a day) was associated with lower FR levels in handwipes. The analysis of Canadian dust was illustrated by Kubwabo *et al.* (2016). The samples were collected from 2007 to 2010 under the Canadian House Dust Study protocol. TBEP showed the highest concentrations, from 2.8 to 275 μg/g. The other three major OPEs detected were TCP, TDCPP and TPP with median concentrations 9.3, 5.4 and 3.9 μg/g, respectively. In their previous work, Fan *et al.* (2014) determined 13 OPEs in dust samples collected from 134 urban Canadian homes. TBEP, TPP, TCPP, TCEP, TDCPP, TCP and TnBP were detected in the majority of samples with median concentrations (range in parenthesis) of 22.8 (2.4-236) μg/g and 1.6 μg/g (<MDL-95) for TBEP and TPP, respectively, as being the predominant OPEs.

Cao *et al.* (2014) reported the temporal seasonal trends of PBDEs, novel BFRs (NBFRs) and OPEs in indoor dust collected from three offices in Beijing, China (1 office located in a company, 2 offices belong to domestic corporations). The abundance order for OPFRs was: winter > autumn > summer, with peak values occurring in late winter and early spring. This pattern attributed probably to the sensitivity of these compounds to temperature changes, which could influence the emission of FRs from products, and the partitioning between air and dust. Other factor that may contribute to these temporal variations is ventilation, which could influence the residence of FRs indoor. Generally, in winter, the emission of FRs from sources is usually lower and ventilation is poorer.

Tajima *et al.* (2014) measured the levels of 10 OPEs in indoor floor dust and upper surface dust from 128 Japanese dwellings of families. Again, the floor dust was dominated by TBEP, followed by TCIPP and TPP with median concentrations of 30.9, 0.7 and 0.9 μg/g, respectively. The upper surface dust was dominated by TBEP, TCIPP, TPP, TCEP and TnBP with median concentrations of 26.5, 2.2, 3.1, 1.2 and 0.7 μg/g, respectively. Araki *et al.* (2014) also measured the levels of 11 OPEs in indoor floor dust and multi-surface dust in 182 single-family dwellings in Japan. TBEP and TCIPP were detected in all samples with median values for TBEP

(580-111 µg/g) and for TCIPP (8.69-25.8 µg/g) in floor and multi-surface dusts, respectively. Araki *et al*. have obtained significant relationships between housing characteristics (electronic equipments and ventilation levels) and the level of OPEs in the house dust.

Brandsma *et al.* (2014) detected nine OPEs in 8 samples house and car dust from the Netherlands. House dust was dominated by TBEP (median 22 µg/g), followed by TCPP (1.3 µg/g), TCEP (1.3 µg/g) and TPP (0.82 µg/g). TBEP with a median concentration 27 µg/g was predominant in house dust collected on the electronic equipments. Brandsma *et al.* suggested that the levels in house dust are probably more related to various sources like furniture, floor polish, carpet padding, *etc.* Car dust was dominated by TDCIPP (1.1 µg/g in dust samples collected from the car seats) which is probably due to the use of this compound in polyurethane foam. Langer *et al.* (2016) reported the mass fractions of organophosphates in dust samples (collected in 2008-2009) from 500 bedrooms and 151 daycare centers of children living in Odense, Denmark. Organophosphates with median mass fractions above the limit of detection were: TCEP from homes (6.9 µg/g), and TCEP (16 µg/g), TCPP (5.6 µg/g), TDCIPP (77.1 µg/g), TBEP (26 µg/g), TPP (2 µg/g) and EHDP (2.1 µg g^{-1}) from daycare centers. When present, TBEP was typically the most abundant of the identified OPEs.In addition, Dahlberg *et al.* (2016) gave an insight on concentrations of OPEs in house dust from Swedish homes. All 9 target compounds except TiBP and TBP were present in the analysed samples. TBEP and TCIPP were found in highest concentrations (20 and 19 µg/g, respectively). Fromme *et al.* (2014) found detectable concentrations of commonly used OPEs in indoor air and dust samples collected from 63 daycare centers in Germany. Median values of 225 µg/g for TBEP, 2.7 µg/g for TCPP and 0.5 µg/g for TPhP were found. On the other hand, a significant correlation was found between the dust and air samples in the levels of TnBP, TCEP and TBEP. In 2012, Van den Eede *et al.* analysed indoor home dust samples, three collected from Romania, one collected from Spain and eight collected from Belgium. Highest concentrations were obtained for TnBP (1.5 µg/g) and TBEP (36 µg/g) in samples collected from Belgium homes. Samples of Romanian origin showed highest amounts accounted for TPP (3.7 µg/g) and TBEP (2.7 µg/g). Cristale and Lacorte (2013) investigated the presence of 10 OPEs in dust samples collected at five houses in Spain. The median concentration levels ranged between 0.05 and 5 µg/g with the maximum level recorded for TCPP.

Wong *et al.* (2016) demonstrated the occurrence of OPEs in dust collected from different countries (Australia, United Kingdom, Canada, Sweden and China). TBEP, TCIPP and EHDP were the three OPEs measured at highest concentrations, with medians of 2.6, 8.8 and 2.0 µg/g, respectively.

Comparable OPE concentrations were obtained in private house dust samples from EU (Netherlands, Denmark, Germany and Spain), America (USA (California and North carolina) and Canada) and Asia (China and Japan), in which the predominant compositions were TBEP, TCPP, TnBP, TCEP, TPP and TDCIPP.Particularly, the TBEP level in Japanese home dust samples was significantly higher than that from any other country, which might be explained by the more frequent use of floor polish due to high percentage of wooden floors in Japanese homes. The maximum concentrations found were:
Asia (Japan): TBEP and TCPPin private homes, dominant up to 111 and 26 µg/g, respectively.
North America (Canada): TBEP and TPP in private homes, dominant up to 236 and 95 µg/g, respectively.
EU (Denmark): TDCIPP and TBEP in daycare centers, up to 77 and 26 µg/g, respectively.
(Belgium): TBEP in private homes up to 36 µg/g, respectively.

1.4.3.2. **Contamination levels in biotic compartments**

Compared to the efforts on the examination of OPEs in dust, air, water and sediment, limited information is available on their occurrence in biota samples, including fish and domestic birds and other foodstuffs. Recent usage of OPEs has increased substantially and dietary intake is considered as important human exposure pathway. In this part, we will present the most recent findings related to the analysis of some representatives of environmental contamination in bird and fish species and food, noting that fish can also be considered as food for human.

- **Birds**

As being in the environment, birds might take the OPE contamination from various sources. It is supposed for example, through the inhalation of contaminated dust/ air. These findings are from previous works for example in Canada on eggs from wild birds, raising to studies on China for domestic and free-range birds, etc.

Recently, Greaves *et al.* (2016) reported that herring gull (*Larus argentatus*) eggs collected between 1990-2010 (n=55 pools) from a nesting colony in eastern Lake Huron (Canada) contained quantifiable concentrations of TCPP, TCEP and TBEP, with maximum concentrations of 4.1, 1.4 and 5.0 ng/g fw, respectively. Chen *et al.* (2012) analysed seven non-halogenated, three chlorinated and two brominated OPEs in 13 herring gull eggs from the Channel-shelter island colony in Lake Huron (Canada). TCPP, TCEP and TBEP were detected and quantified at the following levels: TCPP (<MLOQ-4.1 ng/g fw), TCEP (<MLOQ-0.6 ng/g fw) and TBEP (<MLoQ-2.2 ng/g fw), where MLoQs ranged from 0.06 to 0.20 ng/g fw.

Zheng *et al.* (2016) investigated the extent of contamination of OPEs in free-range chicken eggs (n=45) from e-waste recycling area in China. The median values of the total OPEs were 1.6-2.6 ng/g fw. All three chlorinated OPEs (TCEP, TCIPP and TDCPP) were found in eggs, while only EHDP and TPP, out of five non-

chlorinated OPEs were detected. This is probably implying that chlorinated OPEs might be more persistent or bioaccumulative in eggs than non-chlorinated OPEs.

Eulaers *et al.* (2014) investigated the accumulation of 6 OPFRs in plasma and feathers of White-tailed Eagle *Haliaeetus albicilla* nestlings from Trondelag, Norway. All 6 OPFRs were detected in feathers (0.95-3,000 ng/g), while in plasma only 2 OPEs (*i.e.* TCPP and TDCPP) out of 6 targeted compounds could be measured (0.12-0.74 ng/g).

Ma *et al.* (2013a) analysed 14 OPEs in domestic birds, including 6 chickens *(Gallus gallus)* and 6 ducks *(Anas platyrhynchos*) that were collected from the Pearl River Delta region in Southern China. TCEP, TCPP and TBEP were present in all of samples that were analysed, and dominated by TnBP (11.7 to 281 ng/g lw), TCEP (33.7 to 162 ng/g lw) and TBEP (48.1 to 266 ng/g lw).

- **Fish**

Although limited data are available on the presence of OPEs in fish, there exist studies dealing with the occurrence levels in both freshwater and marine ecosystems in different regions in the world. We are going to present data findings mainly from Canada, EU, China and Phillipines. The levels are illustrated in terms of lipid weight (lw) and sometimes the fresh weight (fw) unit is used. It is important to highlight here the difference between the two terms. On one hand, the lipid weight unit reflects the influence of lipophilic content/nature on bioaccumulation of the compounds. On the other hand, the fresh weight unit takes into account the moisture content and is most commonly used in the practices of exposure assessment. The comparison between levels in terms of lw versus fw, is difficult.

In 2014, McGoldrick *et al.* screened body homogenates of Lake Trout (*Salvelinus namaycush*) or Walleye (*Sander vitreus*) collected from Canadian lakes, for 15 OPEs. Six OPEs were detected above quantification limits, with TCEP and TBEP the most frequently quantified at concentrations ranging from <0.07 to 9.8 ng/g fw.

Santin *et al.* (2016) analysed twelve river fish samples collected on the Llobregat River (Spain) for the occurrence of 16 OPEs. As a result, 13 OPEs were detected in barbell, trout and carp samples with maximum concentration found at 2,423 ng/g lw (lipid weight), expressed as the sum of the 16 OPEs analysed. TBEP was the most frequently detected compound.

In their recent work, Malarvannan *et al.* (2015) investigated the profiles of contamination of OPEs in wild European eels (*Anguilla anguilla*) from freshwater bodies in highly populated and industrial Flanders region (Belgium). Yellow eels (n=170) were collected at 26 locations between 2000 and 2009. The order of

contamination was as follows TCPP>TPhP>EHDP>TBEP>TCEP>TDCIPP. The median sum concentrations was 44 ng/g lw (8.4 ng/g fw) and levels ranged between 7 and 330 ng/g lw (3.5 and 45 ng/g fw).

Sundkvist *et al.* (2010) investigated the levels and relative proportions of 11 OPEs in fish and mussels samples from Swedish lakes and coastal areas. Biota samples were collected at locations with known potential sources of OPEs, as well as background locations. Different fish species were analysed in the study, including perch, eelpout, salmon, carp and herring. TCPP and TPP dominated in the profile with levels ranging from 170 to 770 ng/g lw for TCPP and from 21 and 180 ng/g lw for TPP, in perch. However, the marine eelpoul from a contaminated local area (leakage of hydraulic fluids from ships) contained an extremely high OPE levels especially for EHDP with 14,000 ng/g lw.

Brandsma *et al.* (2015) detected 9 OPEs in a pelagic and benthic food web of the Western Scheldt estuary, The Netherlands. The highest concentrations in the benthic food web were found in sculpin, goby and lugworm with median concentrations of 17, 7.4, 4.6 and 2 ng/g fw for TBEP, TiBP, TCIPP and TPP, respectively.

Ma *et al.* (2013a) analysed biological samples *i.e.* fish, including 6 catfish *(Claris fuscus)* and 8 grass carp (*Ctenopharyngodon idellus*) that were collected from the Pearl River Delta region in Southern China. Among the 14 studied OPEs, TnBP, TCEP, TCPP and TBEP were present in all of the biological samples that were analysed, and dominated by TnBP (43.9 to 2,946 ng/g lw), TCEP (82.7 to 4,692 ng/g lw) and TBEP (164 to 8,842 ng/g lw).

Kim *et al.* (2011) worked to elucidate the occurrence and contamination status of OPEs in 58 marine fishes of 20 species collected from Manila Bay, The Philippines. OPEs were detected in most of the samples and found up to µg/g lw, which suggest their ubiquitous presence in the coastal marine environment of the Philippines. Mean concentrations of TEHP (4,600 ng/g lw), TEP (3,600 ng/g lw), TnBP (2,700 ng/g lw) and EHDP (2,100 ng/g g lw) were the highest in all fish samples and were one order of magnitude higher than those of TPrP (110 ng/g lw), TCP (110 ng/g lw) and TBEP (120 ng/g lw). TEHP highest concentration was attributed to the fact that hydrophobic compounds with a higher log K_{ow} have the higher tendency to accumulate in the lipid layers and then magnified through the food chain. However, TEP with the lowest log K_{ow} of 0.80, was also detected in most samples, attributed probably to the extensive usage of this compound in Philippines. Regarding their bioaccumulation pattern, higher levels (>1,000 ng/glw) of total OPEs were determined in yellow striped goatfish, silver sillago, tripletail wrasse and bumpnose trevally indicates either their active uptake from ambient water or lower metabolic capacity of these species. Kim *et al.* have suggested also that the bioaccumulation pattern appeared to be different even within the same fish species, which is due to the differences in the food habits, metabolic capacity, body size or developmental stage.

In terms of OPE loads reported from different regions, the concentrations reported China in freshwater systems were up to 9,000 ng/g lw. This was followed by the levels reported in the Manila Bay, the Philippines, up to 2,000 ng/g lw and then the EU with levels up to 1,000 in Swedish lakes. Obvious differences in the concentrations and profiles of OPEs were found between freshwater fishes and those from locations near known sources were much important. This was illustrated for example in the dominance of EHDP up to 14,000 ng/g lw in eelpout fish from Swedish coastal areas. In Canadian Lakes, the concentrations were up to 10 ng/g fw.

Comparable OPE loads in fish from the background lakes and the marine areas suggested that OPEs can primarily be spread by diffusive sources (Sundkvist *et al.,* 2010).

To conclude, the previous findings in the fish could raise an important question as follows:

With the evidence of metabolism of OPEs as illustrated in the section (Metabolism), and noting that for example, the evidence of metabolism of TDCIPP and TCPP in fish has been illustrated in zebrafish (Wang *et al.,* 2015).

Is it possible then that the OPE levels are linked to metabolic (in the biota) or environmental (in water and sediment) degradation?

To respond to this question, additional investigation of water, sediment and biota concentrations of OPEs would be required to address the relative importance of metabolism and other degradation processes versus the extent of bioaccumulation of OPEs in fish.

- **Foodstuffs**

The presence of OPEs in foodstuffs presumably arose from the diffusion of these substances through the wrapping material used to package food (ATSDR, 2012). From the findings of some available reports, it could be hypothesized that human exposure to OPEs from food is plausible, but the studies on the occurrence of OPEs in foodstuffs are still very limited. The described data will be derived from previous findings from US, UK, EU and China.

Poma *et al.* (2016) analysed 8 OPEs in more than 50 food samples obtained from a recent Swedish market basket study in 2015. The highest levels were measured in cereals, pastries, fats/oils, and sugar/sweets up to 19.1 ng/g fw). EHDP showed the highest measured concentrations among the considered OPEs, up to 10.1 ng/g fw in the pastries food-group.

Still with the recent studies in 2016, Guo *et al.* conducted the analysis of 9 OPEs in 15 milk products from various local supermarkets in China. Only TEP was detected in one brand, with a concentration of 0.4 ng/g fw.

In their work, Zhang *et al. (*2016b) showed that rice ingestion can be considered as a major pathway for human exposure to OPEs in China. The presence of OPEs was investigated in 50 rice samples and 75 commonly consumed foods. The concentrations ranged from 0.004 ng/g to 287 ng/g and the highest levels were found in rice and vegetables.

In December 2006, the US FDA has published a summary of pesticide analytical results in food from the Total Diet Study (TDS) market baskets 1991-3 through 2003-4 collected between September 1991 and October 2003. The analysis included 7 OPEs (EHDP, TBP, TCP, TPP, TBEP, TCEP and TCPP). The maximum mean concentrations were recorded for EHDP, where a value of 2,465 ng/g was found in candy and caramels samples (n=40).

In a study based in the United Kingdom, similar to the US FDA TDS, the most prevalent of the selected OPEs were TnBP and TPP, occurring in meats, cereals, nuts and some vegetables (ATSDR, 2012).

In 1982, the Food and Drug Administration (FDA) found detectable levels of phosphate esters present in food samples during a portion of the annual Pesticide Screening program. The presence of these phosphate esters in foodstuffs presumably arose from the diffusion of these substances through the wrapping material used (Daft, 1982) but it can be also due to another contamination source like the dust. Since 1982, OPEs are regularly tested in various foods by the FDA's Total Diet Study. In these analysed food, the most frequently identified OPE was TPP, which also had the highest reported content. TPP was found in caramels and margarine at approximately 40 ng/g. In baby foods, turkey and vegetables contained the highest level of TPP at approximately 20 ng/g. TnBP was the second most frequently detected phosphate ester, but most levels measured were below 4 ng/g, with baby cereal (prepared with water) and apple sauce being the highest (US FDA, 2006). Additionally, in a study from the United Kingdom, similar to the US FDA TDS, the most prevalent of the selected OPEs were TnBP and TPP, occurring in meats, cereals, nuts, and some vegetables (Gilbert *et al.,* 1986).

As illustrated in details in paragraph 1.4.3, the quantitative human exposure assessment step in our work define the levels of exposure of population through contaminated food chain and then to correlate it with the food consumption habits. It is worth to note that, and as mentioned since the beginning, we were focusing on the food chain as the food chemical safety being our main objective. The difficulties in this part are related to the determination of food habits as well as the occurrence levels which are rare to the pollutants like the re-emergent compounds, OPEs.

Indeed, the studies on the occurrence of OPEs in foodstuffs are very limited. The most significant conclusion was the dominance of EHDP in diet market baskets from Sweden and US. The maximum recoded level for EHDP was up to 2,500 ng/g fw in candy and caramels from US TDS market baskets collected between 1991 through 2003.

The ending question is mostly related to the sources of such contamination which is still unclear, whether to be attributed to the food contact materials added to the packaging material or more to the processing of food itself (contamination during production, storage, transport, etc.).Having response to this question is highly indispensable if one would like to have a well drawn conclusion on the reported contamination.

All the described steps of risk assessment and in particular the quantitave approach will be employed in the last part of Chapter 4 where our objective will be to base on our findings in order to contribute quantitatively to the assessment of human exposure to the dominant OPEs.

As we have seen in this detailed literature review, the data on the occurrence levels of OPEs in biotic compartments, is scarce. This is partly due to the lack of efficient analytical strategies for this purpose. In the next section, we are going to describe the available analytical strategies and which will enables us to orient our choice for next chapters.

1.5. **AVAILABLE STRATEGIES FOR OPES ANALYSIS**

There are different steps for any analytical strategy, which includes sample collection, preparation (extraction and purification) with concentration in order to pemit the subsequent extract analysis. It is worth to note in the analytical chemistry field, the importance of knowledge about target compounds physico-chemical properties prior to implement appropriate analytical strategies. Besides, the development of method for the analysis at trace levels in complex biological samples is always the most challenging issue for the analytical chemists.

The collecting step and storage and preservation of samples represent a critical step during which it is important to ensure that no loss of analytes will occur or to avoid any external contamination phenomenon. Upon arrival of the samples at the lab, a homogenous tissue sample is created and then stored at – 20 ^{o}C.

1.5.1. EXTRACTION TECHNIQUES

Different analytical and clean-up methods have been described by several works to analyse OPEs in various biotic and abiotic matrices (as described in paragraph 1.4.3). Generally, extraction techniques such as Soxhlet (Takigami *et al.,* 2009), Pressurized Liquid Extraction (PLE) (Stapleton *et al.,* 2009) Ultrasound-Assisted Extraction (UAE), Matrix Solid Phase Dispersion (MSPD) and Microwave-Assisted Extraction (MAE) (García

Lopez *et al.,* 2007) have become popular for the analysis of OPEs (van der Veen and de Boer, 2012; Dirtu *et al.,* 2013).

PLE was mainly used for extraction of OPEs from fish, egg and sludge (Sundkvist *et al.,* 2010) while ultrasonication was the most applied technique for dust extraction (Brommer *et al.,* 2012). Extraction of OPEs was usually done with hexane/dichloromethane (Hex/DCM) (1:1, v/v) (Stapleton *et al.,* 2009) or DCM (Marklund *et al.,* 2003; Van den Eede *et al.,* 2011) although more polar extraction solvents such as acetone (Kanazawa *et al.,* 2010) or mixtures containing acetone (Ingerowski *et al.,* 2001) were also used.

Among the most recent works, Santin *et al.* (2016) compared three extraction procedures: shaking, ultrasound and PLE with Hex/acetone 1:1 (v/v) as the extraction mixture. The ultrasound extraction was chosen because of its quickness and allowing a lower amount of interfering compounds in the extracts. Additionally, Guo *et al.* (2016) applied the QuEChERS methodology for the extraction and cleanup in milk powder samples.

Based on our literature review, the mostly employed extraction technique was PLE. The basic set-up of this technique has previously been described in detail. Briefly, it consists of a stainless-steel cell in which the sample is placed and kept at the selected temperature and pressure during the extraction, electronically controlled heaters and pumps for solvent delivery and a vial for the collection of the liquid extract. The factors/variables affecting the PLE process, such as the nature and temperature of the extraction solvent and the extraction time, can be derived from the principle of the technique (Mustafa and Turner, 2011; Gao, 2014; Vazquez-Roig and Pico, 2015). Briefly, the main parameters to be considered are described hereafter:

- **Nature of extraction solvent**

The extraction solvent must be highly selective, with high solvation capacity of the target compound and minimize the co-extraction of other matrix components. The polarity of the solvent should be close to that of the target compound. Generally, mixtures of low- and high- polar solvents provide more efficient extractions of the analytes than single solvent. Non-polar solvents such as n-hexane or a non-polar with medium-polarity solvents, such as cyclohexane/ethyl acetate, have frequently been used in the extraction of apolar and lipophilic compounds, like OPEs. Other important solvent characteristic is its ability to aid in the release of compounds from matrix and helping with the breaking of the interactions matrix-compounds.

- **Extraction temperature**

Temperature is an important parameter and usually the higher yields obtained with PLE in comparison with other extraction techniques are attributed to this parameter. High temperatures decrease the solvent viscosity, helping with its penetration inside the matrix and consequently, improve the extraction process. Furthermore, elevated temperature decreases the surface tension of the solvent, compounds and matrix and therefore enhances the solvent wetting of the matrix. Therefore, lead to a higher contact between the solvent and those compounds inside the matrix. The use of high temperatures increases the diffusion

coefficients, increasing the mass transference rates, furthermore helps to disrupt the compounds–matrix interactions. All these changes improve the contact of the analytes with the solvent and enhance the extraction, which can then be achieved more rapidly and with less solvent consumption compared with classical methods. In a way to get benefit of the use of elevated temperature, but in the same time, maintaining our compounds of interest, the temperature of 100°C is usually used. Higher temperature could cause the decomposition of compounds, especially the ones with low boiling points.

- **Extraction time**

The duration of the static extraction time is important in the extraction efficiency since prolonged contact periods between the matrix and the solvent permits increased swelling with enhanced matrix wetting and increased penetration of solvent into the sample with a greater contact of the solvent with the analyte. Generally, for analytical applications, extraction times between 5 minutes are enough to guarantee the extraction of the most compounds with a high yield. However, the long extraction times prolong the time required and could induce the degradation of the compounds and the matrix. According to Gao *et al.* (2014), who worked on the determination of OPEs in fish using PLE, when the extraction time was longer than 5 min, the extraction efficiency didn't improve further.

- **Pressure**

Pressure is a parameter that does not present an important influence on the yield of extraction process, because liquids are not compressible fluids. Therefore, even under large pressure changes the solvation power of the solvent is not significantly affected. Otherwise, the use of high pressures facilitate the extraction of compounds located inside the matrix pores, due to a pressure increase which forces the solvent to penetrate into places which are normally not reached by the solvent at atmospheric pressure. Depending of the structure of the matrix and the particularities of each process, the use of high pressures could be a positive or negative influence on the extraction process, for instance, at higher pressures, the matrix may be compacted, affecting the flow of the solvent. The high pressure helps to force the solvent into the matrix pores and to keep the solvent in the liquid state at the operating temperature. Pressure was usually set as 100 bars, by referring to previous works as for example Gao *et al.* (2014).

1.5.2. PURIFICATION TECHNIQUES

As a clean-up step, various methods were described in the literatures. The extracts were cleaned up by centrifugation or filtration (Marklund *et al.,* 2003), by SPE using Oasis® HLB sorbent (García-Lopez *et al.*, 2007) on alumina column (Stapleton *et al.,* 2009). Adsorbents such as Florisil®, alumina and primary-secondary amine have also been used for OPEs clean up (van der Veen, 2012; Quintana, 2008). Noting that, gel permeation chromatography (GPC) using glass column containing Biobeads SX-3 was mainly reported for

biota and sludge samples (Sundkvist *et al.,* 2010), while silica-gel columns used for fish samples (Kim *et al.,* 2011) and amino-propyl-silica columns for eggs (Chen *et al.,* 2012). Since biological samples often have high lipid content, and most of the OPEs (particularly the halogenated ones) are lipophilic compounds, the extracts might be accompanied by a considerable amount of lipids (Sundkvist *et al.,* 2010). To avoid the significant influence of these lipid compounds on the performance of chromatographic column and the ion source of the mass spectrometer, a clean-up procedure is highly required (Quintana *et al.,* 2008). In the selection of the most cleanup step, a number of results must be fulfilled (*e.g.,* selectivity to compounds, rapidity, lipid depletion and less consumption of solvents). The compromise to be taken is not always combining all these outcomes. In the next paragraph, we are going to focus on the two most commonly employed purification steps (SPE and GPC).

- **Solid-phase extraction (SPE)**

Solid phase extraction, or SPE, is perhaps the most powerful sample preparation technique in common use today. It is a method that uses a solid phase (sorbent) and a liquid phase (solvent) to isolate analytes from a solution. The general procedure is to load a solution onto the SPE phase, to wash out the interferences and then with the appropriate eluting solvent, analytes are allowed to pass un-retained through the sorbent bed, while the interferences are retained. The interactions between the analyte and the sorbent surface include for example hydrogen bonding between OPEs and the silanol group. The OPEs can then be eluted by using solvent that discrupts this binding mechanism.

As a basic principle of SPE (Figure 1-16), the analytes retain on the selected sorbent while the sample matrix liquid is loaded through the column, then the sorbent is washed to remove undesired interferences, like lipids, and the purified analytes subsequently eluted from the column. However, SPE may also be used to retain interferences, allowing the targeted OPEs to pass unretained through the sorbent.

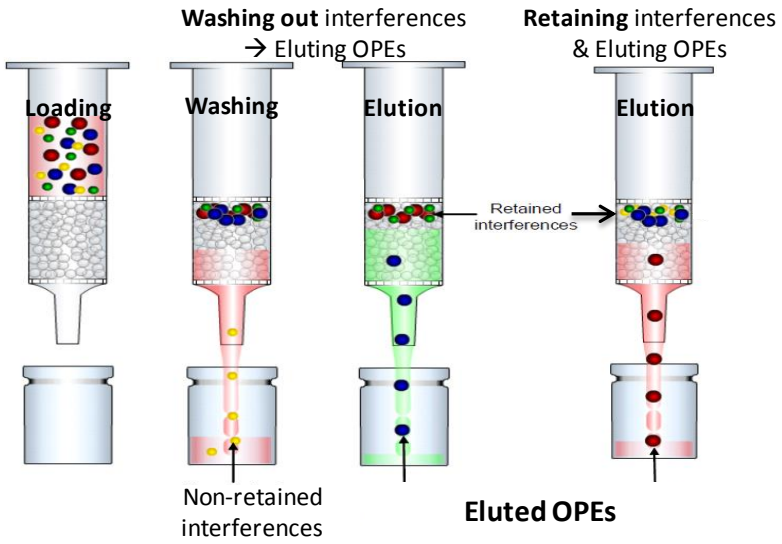


Figure 1-16: Schematic representation of the two main phenomena on SPE columns, enabling the elution of targeted analytes without interferences.

Since OPEs possess a wide range of polarity but generally low polar compounds, their analysis using SPE is preferable under normal phase conditions (*i.e.* low polarity analyte, a mid-to non-polar matrix solvent and a polar stationary phase). A number of solvents has already been described by the previous literature (*i.e.* acetone/ethyl acetate (EtAc) 3:7 (v/v) (Ma *et al.*, 2013a), EtAc (van den Eede *et al.*, 2011), Dichloromethane (DCM) (Kim *et al.,* 2014), EtAc/cyclohexane (*c*-Hex) 5:2 (v/v) (Cristale *et al.,* 2013). The elution is most successfully accomplished with a solvent having the highest eluotropic strength toward the sorbent being used.

The most important inorganic oxide sorbents for SPE are silica gel, alumina, Florisil® (synthetic magnesium silicate) and diatomaceous earth. Adsorbent properties that increase retention are a larger surface area and a high activity, noting that the adsorbent activity can usually be controlled by the intentional addition of water to the dried adsorbent prior to use. Based on literature concerned in the analysis of OPEs, silica gel and Florisil® in their activated or deactivated forms are the mostly used sorbents. In general, silica gels used for SPE have surface areas of about 300–800 m^2/g, pore sizes from 4 to 10 nm, and an apparent pH of 5.5–7.5. Florisil® has a surface area of about 250–300 m^2/g and an apparent pH of about 8.5. According to the literature concerned in the analysis of OPEs, the use of deactivated silica gel is well illustrated with various percentages of H_2O, mainly 3% (Ma *et al.*, 2013), 5% (Kim *et al.*, 2014) and 10% (Möller *et al.*, 2011). The use of Florisil® was also reported but not as frequently as the use of silica gel.

Additionally, other sorbents can be used for the same purpose of lipid depletion. These include on one hand the patented zirconia-coated silica particles (Z-Sep) that can selectively remove more lipid and pigment interferences from sample extracts than traditional cleanup sorbents. Zirconium dioxide has hard Lewis acid sites on its surface. These sites are present because zirconium (IV) has vacant 3d orbitals. As illustrated in

Figure 1-17, lewis acid sites can interact strongly with lewis bases such as R–SO_3^-; R–PO_3^- and R–OO^- creating coordination bonds. Thistlethwaite *et al.* 1996 investigated the adsorption of oleic acid on Z-sep. They concluded that adsorption at pH 3 occurs thanks to electrostatic interaction between oleate anions and the positively-charged zirconium dioxide surface. However, at pH 9, coordination bonds are responsible for adsorption. In the adsorption of carboxylic acid, the main role is played by the carboxylic group yet the presence of a second COO^- group makes the adsorption stronger.

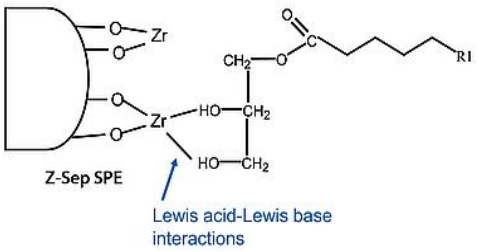


Figure 1-17: Retention mechanisms of fats on Z-Sep sorbents (Supelco).

Enhanced Matrix Removal—Lipid (EMR—Lipid) is another novel sorbent material that selectively removes major lipid classes from the sample extract. The use of EMR consisted of two steps, the first starting with Agilent Bond Elut EMR—Lipid dSPE and the second using EMR—$MgSO_4$ polish pouches. In its principle, the enhanced post sample treatment incorporates anhydrous $MgSO_4$ for phase separation and sample drying. This significantly improves the removal of water and water-dissolved residue without sacrificing the matrix removal of EMR—Lipid cleanup. It worth noting that the use of Z-Sep and EMR was not yet described for the analysis of OPEs.

- **Gel permeation chromatography (GPC)**

GPC is a purification technique that showed previous applications in analysis of OPEs in biological matrices (Ma *et al.,* 2013a). It is a size exclusion chromatography that separates the compounds according to their steric hindrance (Figure 1-18). In principle, the stationary phase pores permit a longer mean path to smaller molecules in comparison to the larger ones which are therefore eluted later than the smaller ones. In most studies, c-Hex/EtAc 1:1 (v/v) solvent mixture was used for Bio-Beads S-X3.

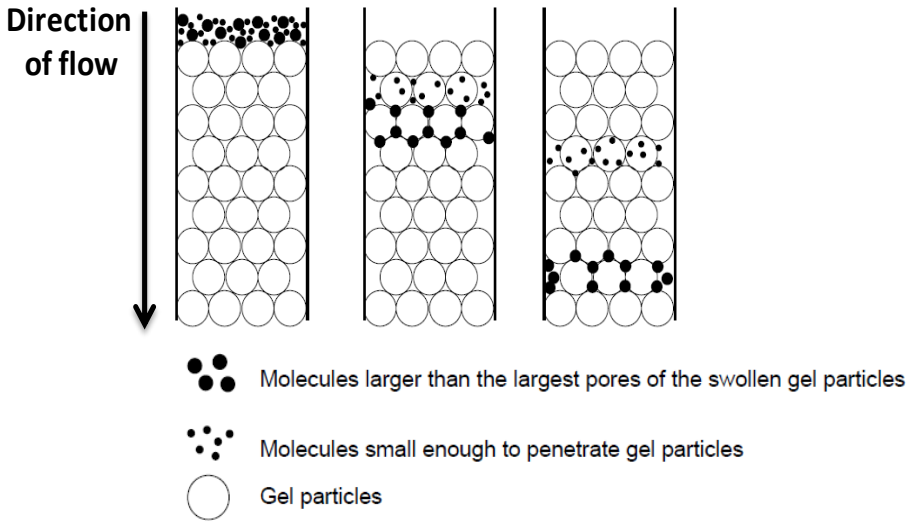


Figure 1-18: Schematic representation of the general principle of GPC.

1.5.3. SEPARATION AND DETECTION INSTRUMENTAL SYSTEMS

The analysis of prepared samples can only be feasible with the analytical techniques that are used to separate, identify and quantify the compounds of interest. For OPEs, GC coupled to nitrogen-phosphorous detection (GC-NPD), MS operating in selected ion monitoring at low or high resolution, and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) are the most reported techniques for the analysis of these compounds in environmental samples (Quintana *et al.,* 2008; Sundkvist *et al.,* 2010; Van den Eede, 2011; Brandsma *et al.,* 2013).

- **LC-MS with electrospray ionisation**

LC-ESI-MS is well known for its capability of analysing both small and large molecules of various polarities in a complex biological sample. Based on the literature dealing with OPEs, different spectrometric conditions were used but ESI mode was the only described mode. In this ionisation mode, there are three important processes that occur in order to transfer sample ions from the LC eluent into the gas phase within the mass spectrometer. These processes are (i) production of charged droplets at the capillary tip, (ii) desolvation of the droplets assisted by the flow of nitrogen (drying gas) and (iii) production of gas phase ions from small / highly charged droplets. Finally, the charged analytes are released from the droplets, some of which pass through a sampling cone or the orifice of a heated capillary (kept in the interface of atmospheric pressure and the high vacuum) into the analyser of the mass spectrometer, which is held under high vacuum.

Generally, the ions derived by ESI process are multiply charged (for large molecules) and the analyte remains unfragmented (appears as an intact molecular ion). In positive ion mode (positive potential), the charging generally occurs *via* protonation and the protonated molecular ion $[M+H]^+$ is detected. In negative ion mode (negative potential), charging occurs *via* deprotonation of the analyte where $[M-H]^-$ ion is detected. A number of previous works for OPEs had demonstrated the use of LC-MS/MS with different spectrometric conditions. The ionisation conditions varied from one work to another, taking examples of Chen *et al.,* 2012 and Guo *et al.,* 2016 who used the following parameters; the ionisation temperature (100°C, 150°C, *etc.*), capillary voltages (4 kv, 0.5 kv, etc.) and cone gas flow (150 L/h and 50 L/h), respectively. ESI-MS is coupled with a liquid chromatography (LC) for molecular fractionation prior to mass spectrometric analysis, which renders the technique more powerful. The use of H_2O, MeOH and ACN as mobile phase was frequently described, with the addition of modifiers as ammonium acetate (10mM) (Kim *et al.* 2011) or formic acid (0.1%) (Chen *et al.,* 2012). Different chromatographic columns were also used, like Asentis express C18 (100 x 2.1 mm, 2.7 µm) (Kim *et al.,* 2011), Waters Xterra phenyl column (2.1 mm x 100 mm, 3.5 µm) (Ghen *et al.,* 2012) and Phenomenox kinetex PFP (50 mm x 3.5 mm, 2.6 µm) (Guo *et al.,* 2016).

- **GC-MS/MS with EI, CI and APCI**

The main ionisation techniques used in GC-MS analysis of OPEs are electron impact (EI), chemical ionisation (CI). On one hand, under EI ionisation, the analyte molecules are directly ionised through collision with a bombarding electron stream resulting in the removal of an electron to form a radical cation species. On the other hand, using CI ionisation, the analyte molecules are charged through reaction processes with charged reagent gas plasma producing either anion or cation species, depending upon the analyte and analyser polarity. Additionally, atmospheric pressure chemical ionisation (APCI) is a powerful ionisation technique that can be coupled to GC nowadays (as shown in Figure 1-19). The ionisation mechanisms occurring in atmospheric pressure ionisation (API) sources are of low-energy (soft), which generate spectral data typically rich in molecular or protonated molecule information, shows less fragmentation and is more sensitive in comparison with EI and CI.

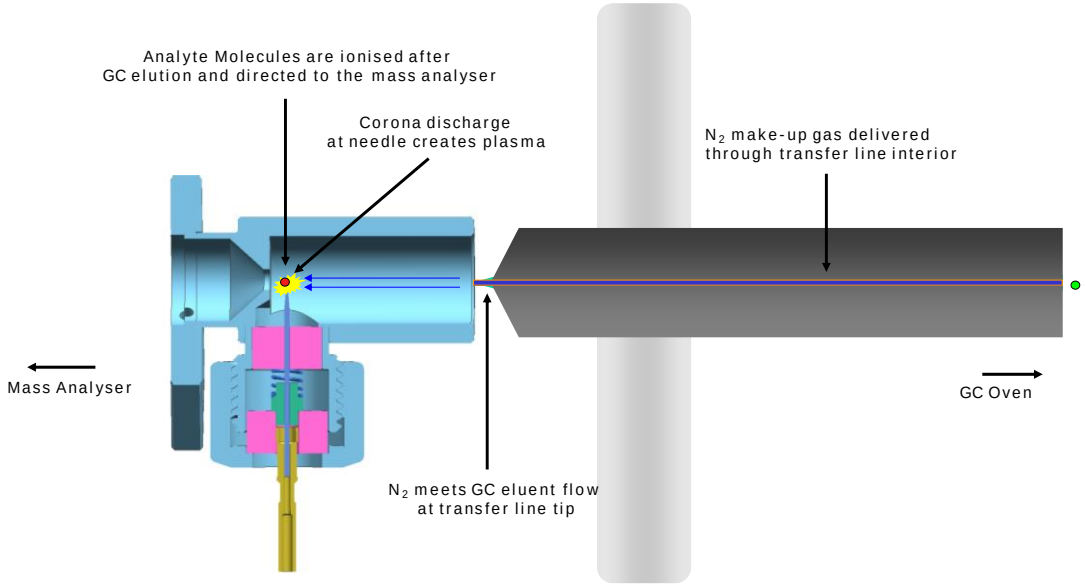


Figure 1-19: Schematic representation of the APCI source coupled to GC.

The APCI-MS instrument can handle higher helium flow rate which could be advantageous because it may reduce analyte degradation in the inlet (faster transfer of the analytes from the inlet to then analytical column during splitless injection) and leads to faster elution of the analytes (shorter GC run time). As described by Figure 1-19, the nitrogen make-up gas is delivered at relatively high flow (400 $mL.min^{-1}$) on the same axis as the GC column through the transfer line interior. This make-up gas meets the GC column flow at the tip of the transfer line. Plasma is created by the corona discharge at the needle which ionises molecules eluting from the GC column either by charge transfer or proton transfer depending on the conditions in the source. The ions created are then transferred to the mass analyser

As illustrated in Figure 1-20, two ionisation processes are observed with the APGC ion source.

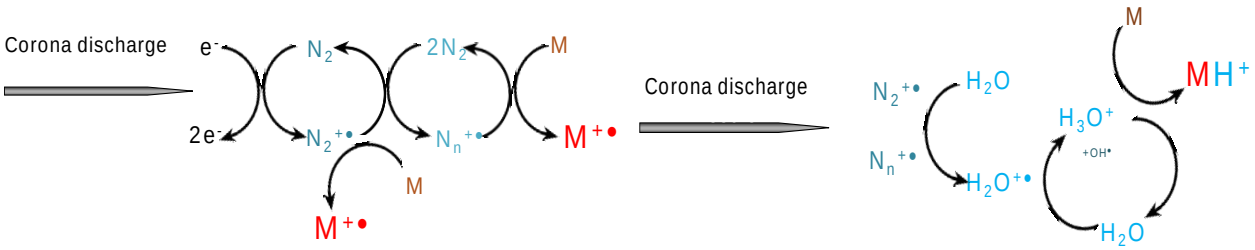


Figure 1-20: The two primary mechanisms in the APCI source (WATERS); charge transfer (left) and protonation (right).

In dry conditions (means no modifier introduced into the ion source) and as observed by Figure 1-20 (to the left), the corona discharge initiated an ion/molecule reaction and lead to a radical positive molecular ion. In more detailed way, the corona discharge emitted electrons which ionised nitrogen molecules into $N_2^{+\bullet}$ and further $N_4^{+\bullet}$. The charge is transferred from the ionised gas to the target analyte ($M^{+\bullet}$).

When a protic donor (modifier) (*e.g.* MeOH, H_2O) is introduced directly into the ion source and as observed by Figure 1-20 (to the right) the analyte can be preferentially protonated. In this case, the corona discharge emitted electrons which ionised nitrogen molecules into $N_2^{+\bullet}$ and further $N_4^{+\bullet}$ and provokes the ionisation of the modifier by charge transfer, and then after ion-molecule interaction the formation of $(modifier+H)^+$. The protonation of the analyte is then observed by proton transfer from the modifier to M, leading to the observation of $[M+H]^+$.

Indeed, the history of GC-APCI-MS dates back to 1970s when Horning et al. gave the first impulse to generate the coupling of GC instrument to an APCI ion source. Since then, a series of papers have been published for the application of this technique in analysis of environmental samples containing certain contaminants. However, it had never become popular until 2005 when McEwen and Schiewek et al. proposed a combination LC/MS: GC/MS ion source (Li *et al.,* 2015). Among the recent publications on the application of this technique in the field of flame retardants, we can mention Portoles *et al.,* 2015 and Bichon *et al.*, 2016 working on the BFRs. In the field of OPEs, we can mention Ballesteros-Gomez et al., 2013 who analysed 7 OPEs (alkyl, aryl and chlorinated) in electronic waste and car interiors.

Some advantages of GC-atmospheric pressure ionisation are already described in the literature (Li et al., 2015) and hence are expected in our work: (i) The use of API enables the combination of GC separation with a wide range of advanced mass spectrometers which initially are specifically developed for combination with LC,(ii) The molecular ion is largely preserved, and (iii) Because of reduced fragmentation, the selection of the precursor ion in MS/MS is no longer a compromise between sensitivity and selectivity.

- **Tandem MS with CID**

With SIM mode, interfering peaks occurred often in samples, although these were not present in standards. This resulted in the need to use the 2^{nd}, 3^{rd} or 4^{th} most abundant peaks for identification and quantification, which again resulted in higher LODs (van der Veen and de Boer, 2012). From here, tandem mass spectrometry has a distinct advantage over the single in that this technology provides the ability to measure with analytical sensitivity and specificity, of multiple analytes in one run. Selected Reaction Monitoring (SRM) is the most common mode of using a triple quadrupole MS/MS for quantitative analysis, allowing enhanced sensitivity and selectivity. Figure 1-21 gives a schematic representation of the distinctive steps of tandem MS. The first quadrupole filters a specific precursor ion of interest. Ions generated in the ion source having a different m/z can not pass Q1. The collision cell is optimized to produce a characteristic product ion by collision of the precursor ion with a neutral collision gas, such as nitrogen. This process is called Collision Induced Dissociation (CID). Generated product ions are transferred into the third quadrupole where only a specific m/z is allowed to pass. All other product ions are filtered out in Q3.

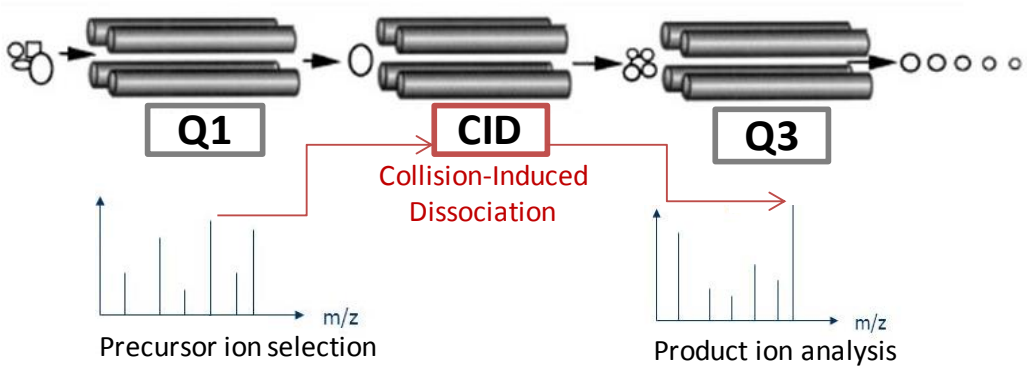


Figure 1-21: Schematic representation of the main steps in triple quadrupole MS/MS , including selection in Q1, fragmentation in CID and analysis in Q3.

1.5.4. DATA GAP AND SCOPE OF THE THESIS

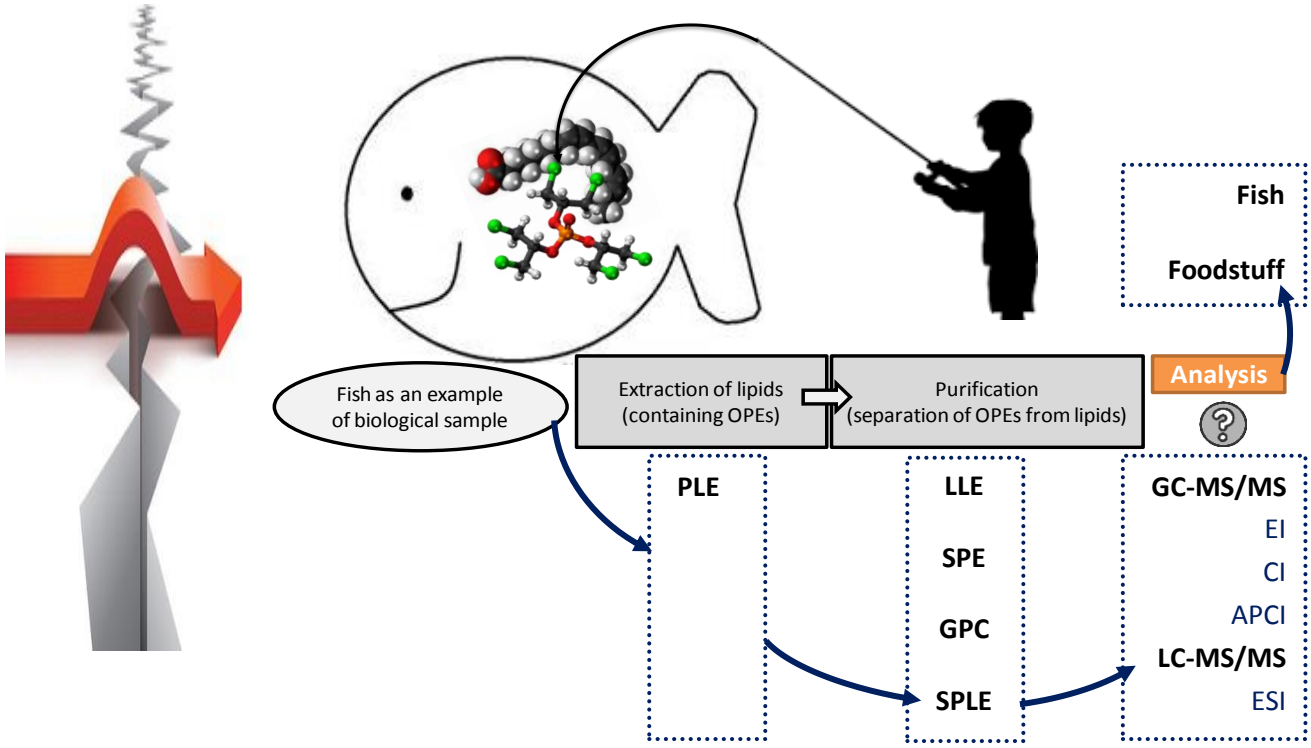


Figure 1-22: Schematic repre sentation of the work flow for developing our analytical strategy.

The main points illustrated from the previous chapter can be summarized as follows:

- OPEs, a subgroup of FRs and plasticisers, present a wide differences in physic-chemical properties and hence in their toxicity and environmental behavior.
- Adverse health effects were addressed to different OPEs. Some chlorinated and brominated OPEs are considered as probable human carcinogens.
- Detailed toxicological studies required for a risk assessment approach, are highly lacked and old, if exist.
- Studies on their occurrence focus on the abiotic compartments, mainly the dust. The levels vary from one country to another. And in the same country, it depends whether it is indoor or outdoor environments and even indoor, it varies between offices and private homes. For example in Canadian houses, OPEs levels ranged from 2.8 to 275 µg/g for TBEP.
- Besides, studies on their occurrence in biotic compartments like fish and birds exist but still very limited. A number of studies have been assessed in different regions in the world and the levels reached up to 9000 ng/g lw for TBEP in Pearl River in China.

Tables I, II and III in the Annex summarises the main detection and extraction techniques mentioned in the literature. After having an overview on the available literature dealing with OPEs, it seems clear that these contaminants may be found in the food chain in general and in fish in particular. However the data on their occurrence levels is still scarce and limited, especially in France. In the same issue, the occurrence and profile of contamination are highly lacked in foodstuffs where food packing materials are used and hence the OPEs might be introduced as plasticizing agents. Along with the gaps in the toxicological data, a complete risk assesmment cannot be performed.

Therefore, the scope of this thesis can be summarized in these two points;

- To evaluate the occurrence of these emerging compounds in fish and other foodstuffs at French level in order to contribute to the dietary human exposure assessment.
- Fish was selected as a biological matrix of interest because … (may be contaminated in the environment and constitute a food item of interest …)
- To ameliorate the possible analysis techniques for large panel of OPEs, through the investigation of sample preparation and detection techniques.

In the next chapters, we will describe;

- In Chapter 2, the investigation of different instrumentation tools in order to select the most performant technique.
- In Chapter 3, the investigation of different preparation techniques for the extraction of OPEs from complex matrices and further purification from interferences.
- In Chapter 4, the application of the developed strategy for the analysis of a set of fish samples and another set of foodstuffs in order to release a first survey on the occurrence levels of OPEs at French level. By the end of this chapter, approximate QRA exercises will be conducted.

CHAPTER TWO

ASSESSMENT OF INNOVATIVE DETECTION STRATEGIES

2. ASSESSMENT OF INNOVATIVE DETECTION STRATEGIES

2.1. **INTRODUCTION**

The implementation of mass spectrometry (MS) as a detection technique has become invaluable across a wide range of chemical contaminants in food. MS measures the mass-to-charge ratio (m/z) of ionized atoms or molecules to identify and quantify them. It also adds another degree of separation/selectivity on top of chromatographic separations. With these unique features, MS has increasingly become the method of choice for the detection and identification of trace-level organic chemical contaminants.

The first step in the development of an analytical strategy consists of developing and optimizing a reliable instrumental method. First, GC-MS has become popular for the analysis of volatile and semivolatile compounds. More polar, thermolabile and less volatile analytes (as the case for some OPEs) were difficult to analyse until the more recent introduction of atmospheric ionisation techniques, such as electrospray, for LC-MS.

As illustrated in the previous chapter, OPEs can be analysed by LC or GC coupled to MS. However, no method was yet described in the literature for the analysis of the whole set of OPEs presented in this work. The challenge was to investigate a chromatographic instrumentation (LC and GC) coupled with MS operating in different ionisation modes enabling the separation and analysis of the studied range of OPEs. Additionally, the innovation of this part in particular was the full investigation of APCI as a soft ionisation technique on GC-MS. As described in the Chapter 1 for the literature overview, the previous studies have used SIM of one or two ions without a systematic investigation of the full range mass spectra and without interpreting the mass spectra in terms of fragmentation mechanisms and ion structures.

In this chapter, we will describe the instrumental method and the experiments done in a way for optimizing a method for the analysis of OPEs in complex matrix. In this work, the 18 studied OPEs present a broad molecular weight range from 183 to 1017.3 $g.mol^{-1}$, which means it is not an easy task to find a compromise for an optimal resolution and hence efficient separation for all the 18 compounds in one method. The main ionisation modes tested *via* GC-MS/MS (Scion^{TM} TQ, Bruker) were electron impact (EI), negative and positive chemical ionisation (NCI and PCI) modes. The atmospheric pressure chemical ionisation (APCI) was also investigated *via* GC-MS/MS (Xevo^{TM} TQS, Waters). Electospray ionisation (ESI) mode was investigated *via* LC-MS/MS (Xevo^{TM} TQS, Waters) and HRMS (Exactive, ThermoScientific^{TM}). After investigating in details, the fragmentation patterns of the targeted OPEs *via*

these different modes, the collision-induced dissociation (CID) fragmentation was studied after EI, APCI and ESI. Chromatographic separations in both LC and GC were also presented. The sensitivities of the developed MS methods were then evaluated in terms of the instrumental detection limits (IDLs). The method performances were also investigated in terms of linearity and stability of calibration curves. The results obtained in this chapter have been published in Journal of Mass Spectrometry, focusing on the comparison of APCI ionisation performances compared to EI and CI.

2.2. **LIQUID CHROMATOGRAPHIC MS COUPLINGS**

As presented in the previous chapter, liquid chromatography tandem mass spectrometry (LC–MS/MS) is arguably a promising platform and has been successfully applied for analysis of OPEs in water and gull egg samples (Chen *et al.,* 2012; Li *et al.,* 2014; Woudneh *et al.*, 2015). One of main advantages of LC is the elimination of the inherent problems encountered with GC-MS analysis due to the high temperatures applied resulting in several difficulties in the analysis of some thermolabile compounds.

2.2.1. CHOICE OF IONISATION AND MS PARAMETERS

2.2.1.1. **ESI polarity**

The ionisation in both positive and negative (ESI) tune modes was investigated for a number of OPEs, including 5 alkyl (TEP, TPrP, TnBP, TBEP and TEHP), 2 aryl (TPP and EHDP) and 3 chlorine-containing compounds (TCEP, TCPP and TDCIPP). Not all OPEs were analysed because we had not all the standard solutions at that moment. The mobile phase consisted of acetonitrile and 0.1% acetic acid in water. A mixture of standard solutions was analysed *via* both ESI(+) and ESI(-) on full scan mode over a mass range from 150 to 500 *m/z*.

Our results showed that all the compounds were detected in the positive tune mode but not in the negative one. Solution chemistry for negative ion analysis involves the creation of $[M - H]^-$ ions in solution and the generation of these ions in solution is a function of the sample pKa and the eluent solution pH. The analyte will lose a proton in solution when the eluent pH is increased and become negatively charged. Hence, ESI-MS negative mode sensitivity depends on raising the eluent pH. In our study, the use of acetic acid modifier for both modes could be the reason of low degree of deprotonation, because of lower pH conditions generated. Contrary to the results from the negative tune mode, all the targeted compounds were detected on the positive mode under their $[M + H]^+$ form.

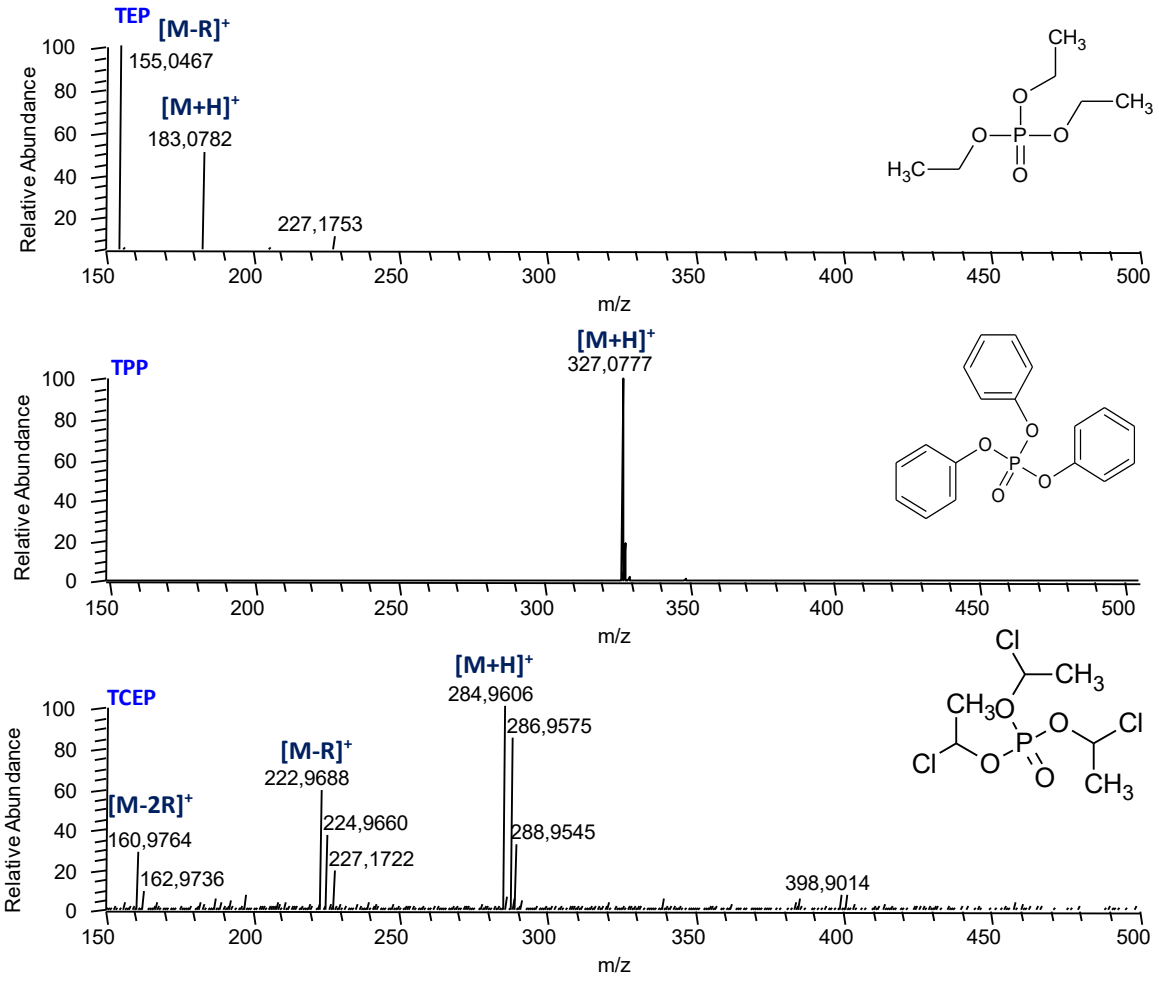


Figure 2-1: Full scan mass spectra obtained for an alkyl (TEP), an aryl (TPP) and a halogenated (TCEP, down) OPEs in ESI (+) (150 to 500 m/z, Exactive).

Figure 2-1 presents the full mass spectra of three OPEs, representative examples for the three subgroups (alkyl, aryl and halogenated) of studied OPEs and analysed on ESI(+) mode, where $[M+H]^+$ was always dominant in their full mass spectra. From here, we selected the positive tune mode over the negative one, for the further investigation of the other factors influencing these mass spectra.

2.2.1.2. **Influence of mobile phase modifier in ESI(+)**

Previous studies have shown that the mobile phase strongly affects the electrospray ionisation. The additives and pH of the mobile phase are critical. After having an overview on the literature, the use in the analysis of OPEs for formic acid (FA) as an additive in the mobile phase was much more frequent than that of acetic acid (AA) (Chen *et al.,* 2012; Li *et al.*, 2014). Additionally, FA is stronger acid than AA, which results in the formation of more efficient hydrogen-bond networks and superior solvation. From here, we replaced the acetic by formic acid and the mobile phase used was composed of ACN+0.1% FA and H_2O+0.1% FA.

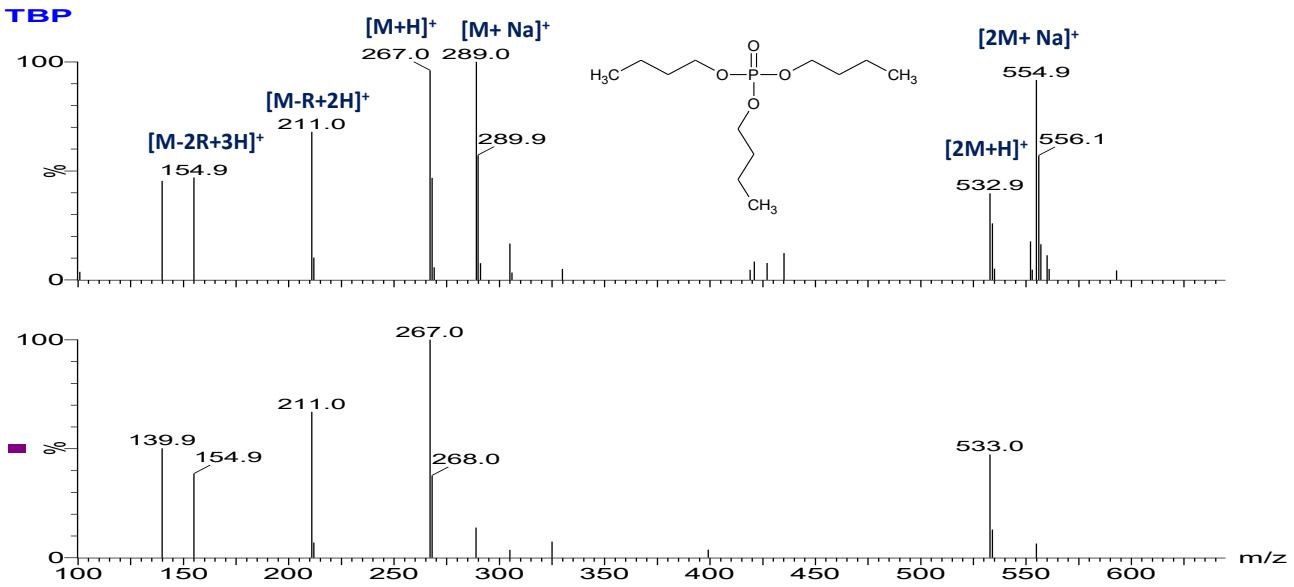


Figure 2-2: Full scan mass spectra obtained for TnBP in ESI(+) with formic acid (0.1%, top) or ammonium acetate (10 mM, down) as modifiers.

Figure 2-2 presents an example of the full mass spectrum of TnBP. Obviously, the spectra contained the $[M+H]^+$ as well as other fragments resulting for example from the loss of one or two side chains. However, adduct ions were also observed. The use of 0.1% FA resulted unfortunately in the formation, of a high abundant sodium adduct ion (i.e. $[M + Na]^+$ and $[2M + Na]^+$).

" When using LC–MS detection for PFR analysis, the formation of stable adducts with metal cations such as Na^+ (e.g. $[M +Na]^+$ and $[2M + Na]^+$) that may be present in the samples, is disadvantageous The relative abundance of these adducts is influenced by the concentration of metal cations and the pH. By adjusting the pH, the adducts formed with metals cations are more efficiently suppressed, …" (van der Veen and de Boer., 2012).

To minimize the adduct intensities; FA was replaced by 10 mM of ammonium acetate in water, noting that ammonium acetate is known to enhance 'protonation' and hence to reduce the intensity of the signals of protonated adduct ions (Figure 2-2). The mobile phase then consists of ACN (A) and 10 mM ammonium acetate in water (B). As illustrated in Figure 2-2, in the full mass spectrum, the fragments resulting from the loss of one and two side chains were observed under both conditions. Ion peaks at m/z 532.9 and 554.9 were suggested to be dimmers representing $[2M + H]^+$ and $[2M + Na]^+$ however no further product scan analysis for these was performed to validate this suggestion. With the use of ammonium acetate as modifier, the Na adduct formation (i.e. $[M + Na]^+$ and $[2M + Na]^+$) was then minimized in an important pattern. This was also observed for most of the other OPEs of interest.

FA is more frequently used in the analysis of OPEs. However, to the best of our knowledge, no previous work has demonstrated yet the influence of modifiers on the adduct formation in the mass spectra of organophosphate esters. Based on our observation, ammonium acetate was selected for further experiments since the adduct formation process is not reproducible and consequently not preferable for SRM transitions. Figure 2-3 illustrates the mass spectra of 4 selected OPEs (alkyl, aryl, chlorinated and brominated). The protonated molecular ion $[M+H]^+$ was always present as a major ion in the full mass spectra. For some OPEs, adduct ions like $[M + CH_3CN + NH_4]^+$ and $[M + NH_4]^+$ as well as dimmers like $[2M + H]^+$ were also present.

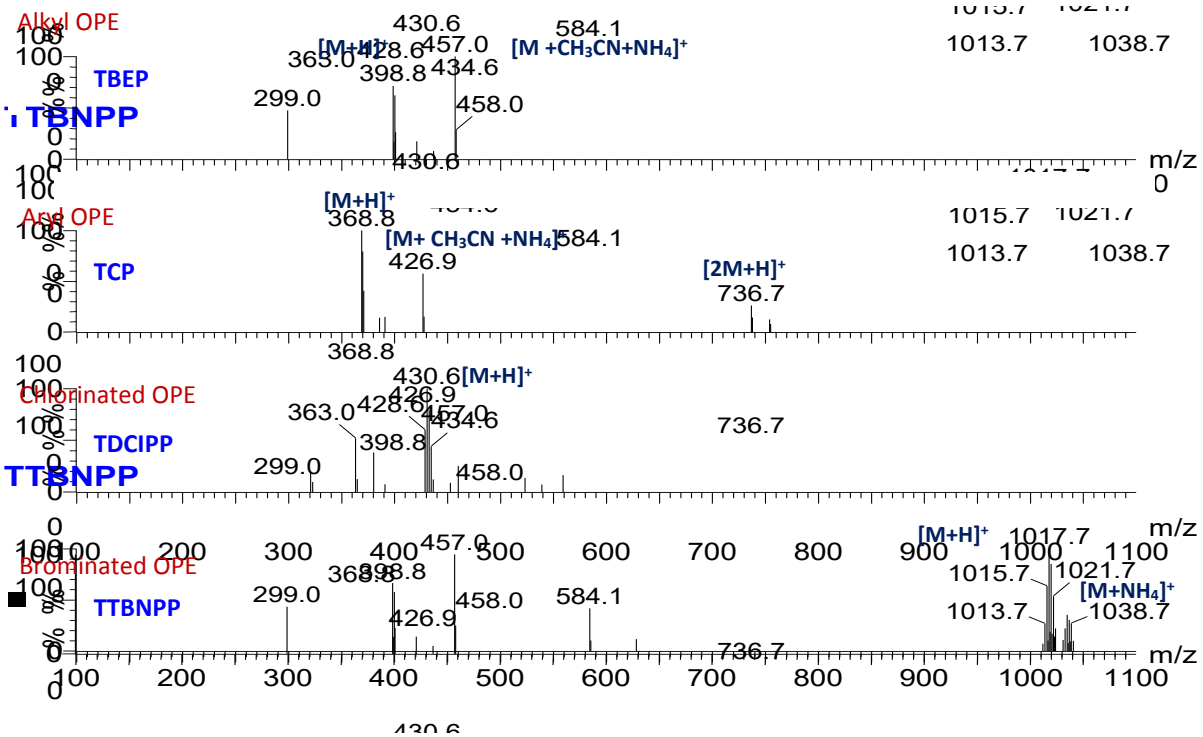


Figure 2-3: Full scan mass spectra (100-1100, m/z) obtained for 4 OPEs *via* ESI(+) ionisation.

For the alkyl OPEs, the fragmentation in the source was not very important, so that the protonated molecular ion $[M + H]^+$ represented the major ion, except for TEHP where the adduct ion $[M+ CH_3CN +NH_4]^+$ at m/z 493 represented the highest abundant ion. Other adduct ions associated with sodium atom $[M + Na]^+$ were produced as well as adduct ions such as dimmers $[2M + H]^+$.

The same observations as for the alkyl compounds were obtained for the 7 aryl OPEs, with the dominance of $[M + H]^+$ in their full mass spectra, except for EHDP, where the major ion resulted from the loss of the ethylhexyl group at m/z 251. The formation of adduct ions was also observed but was much less important than the case of alkyl compounds, which is maybe due to the complexity of the structure of aromatic rings to form adducts.

The halogenated OPEs, in particular the chlorine-containing ones, behaved not necessarily in the same way as do the non halogenated compounds. The isotopic clusters were well-defined for the 3 chlorinated OPEs, with the dominance of $[M + H]^+$ cluster. Finally, the brominated OPEs showed also well-defined isotopic clusters of protonated molecular ion $[M + H]^+$.

As a conclusion, the use of ammonium acetate in water *via* positive tune mode was retained for further optimisation of the MS and chromatographic conditions.

2.2.1.3. **CID fragmentation**

After analysing in full scan mode on LC-ESI(+)-MS, $[M + H]^+$ ion was selected for further product scan analysis during which different collision energies (5 to 25 eV) were tested. As illustrated in Figure 2-4, which shows the product mass spectra of 4 representative OPEs, the CID fragmentation of the compounds of the same group (either alkyl, aryl or halogenated) seems to be quite similar. The figure is presenting the product scan mass spectra at collision energies of 10 eV but 20 eV for the aryl one. The idea out of this was to demonstrate all the detected fragments. For most of these compounds, the fragmentation starts with the loss of first side chain and ends up in the formation of the protonated phosphoric acid $[H_4PO_4]^+$. Optimised transitions were then selected in a selective reaction monitoring (SRM) method.

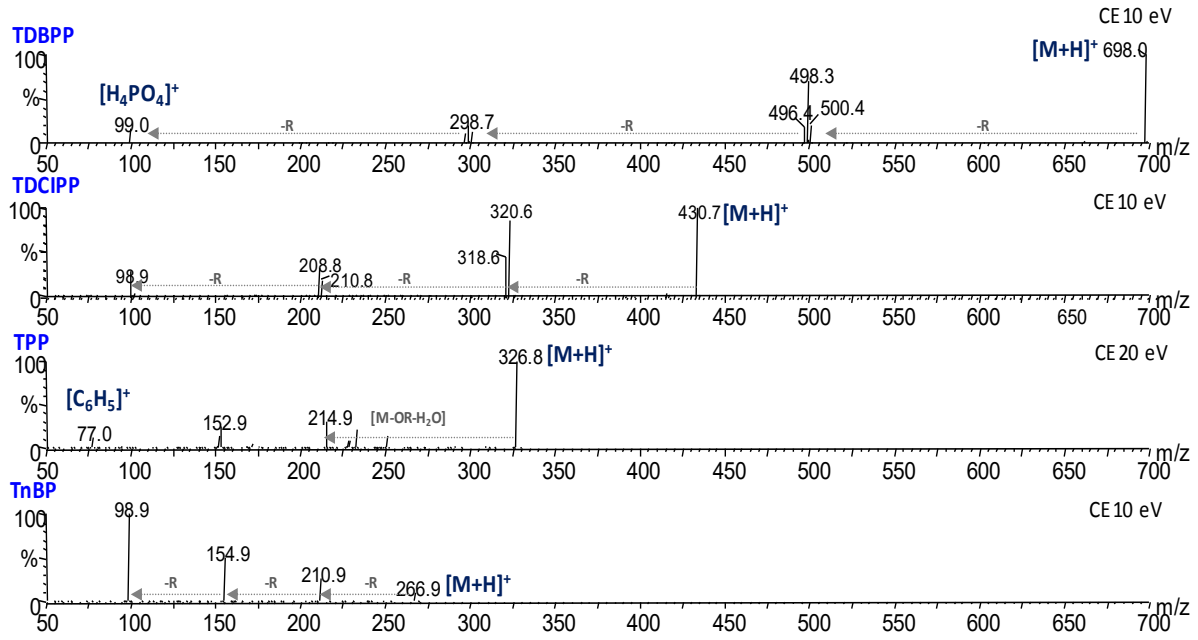


Figure 2-4: Product mass spectra of the precursor ions corresponding to 4 representative OPEs including the alkyl, aryl, chlorinated and brominated compounds, as analysed by LC-ESI(+)-MS.

Being always in the trend of optimising the spectrometric conditions, variable cone voltages (15, 30 and 40 V) were also tested and no important differences were recorded. Cone voltage of 30 V was selected in the final SRM method where optimised transitions were specified in defined acquisition windows. Table 2-1 presents the optimised transitions as well as the corresponding collision energies (in eV) for the 18 OPEs. The table illustrates also the bibliographic findings on the selected transitions from previous studies.

Table 2-1: Optimised transition as well as corresponding collision energies (eV) in SRM method on LC-ESI(+)-MS/MS

Compound	Transition 1 (CE in eV)	Transition 2 (CE in eV)	Literature (Reference)	
TEP	182.9>98.9 (15)	182.9>126.9 (10)	NA	
TPrP	224.9>98.9 (5)	224.9>140.9 (10)	225.3>99 (5)	(Chen *et al.,* 2012)
TiBP, TnBP	266.9>98.9 (15)	266.9>154.9 (10)	267>99 (20)	(Chen *et al.,* 2012)
TBEP	398.9>198.9 (15)	398.9>298.9 (10)	399>199 (15)	(Chen *et al.,* 2012)
TEHP	435>98.9 (15)	435>322.9 (5)	435.3>99 (20)	(Chen *et al.,* 2012)
TPP	326.8>214.8 (25)	326.8>152.9 (25)	327.1>77.1 (40)	(Chen *et al.,* 2012)
DBPhP	268.9>174.9 (15)	286.9>230.9 (5)	NA	
DPhBP	306.9>250.8 (15)	306.9>152.9 (25)	NA	
EHDP	363>250.8 (5)	251>152.9 (15)	363.2>250.8 (10)	(Chen *et al.,* 2012)
o, m, p-TCP	368.8>90.9 (25)	368.8>165.9 (25)	NA	
TCEP	284.7>98.9 (20)	284.7>222.8 (10)	284.4>63 (25)	(Chen *et al.,* 2012)
TCPP	326.8>98.9 (15)	326.8>174.8 (10)	329>99 (20)	(Chen *et al.,* 2012)
TDCPP, TDCIPP	430.7>98.9 (25)	430.7>320.7 (10)	430.9>99 (25)	(Chen *et al.,* 2012)
TDBPP	698>98.9 (20)	698>298.6 (15)	698.6>99 (30)	(Chen *et al.,* 2012)
TTBNPP	1018>144.8 (25)	1018>306.6 (25)	1018>145 (63 V)	(Santin *et al.,* 2016)

2.2.2. LC SEPARATION

2.2.2.1. **Stationary Phase**

For the separation of compounds, two stationary phases were tested: the C_{18} silica based phase which is mostly used in the case of non polar compounds, and the pentafluoro-phenyl bonded (PFP) phase which possesses rigid nature of the aromatic ring so that solute shape can dictate selectivity (how closely the solutes can approach the ring). First, an Accucore^{TM} C_{18} (100 mm x 2.1 mm) with rugged 2.6 µm solid-core particles was tested. On this column, co-elutions were encountered for some target compounds (e.g. TnBP, TiBP, o-, m- and p-TCP). Therefore, we were interested in the investigation of

another type of chromatographic phase that could be more selective for the isomers. From here, Hypersil Gold™ PFP column (100 mm x 2.1 mm, 1.9 µm) was tested. As illustrated in Figure 2-5, the use of PFP phase resulted in less co-elution, which is more interesting for three TCP isomers (o, m and p-TCP) and which can be then separated by approximately 0.1 min. However, this was not the case for the isomers of TBP where co-elution was observed via the two investigated phases. As a result, PFP column was then selected rather than the C18 column, for the further optimisation of the mobile phase gradient composition.

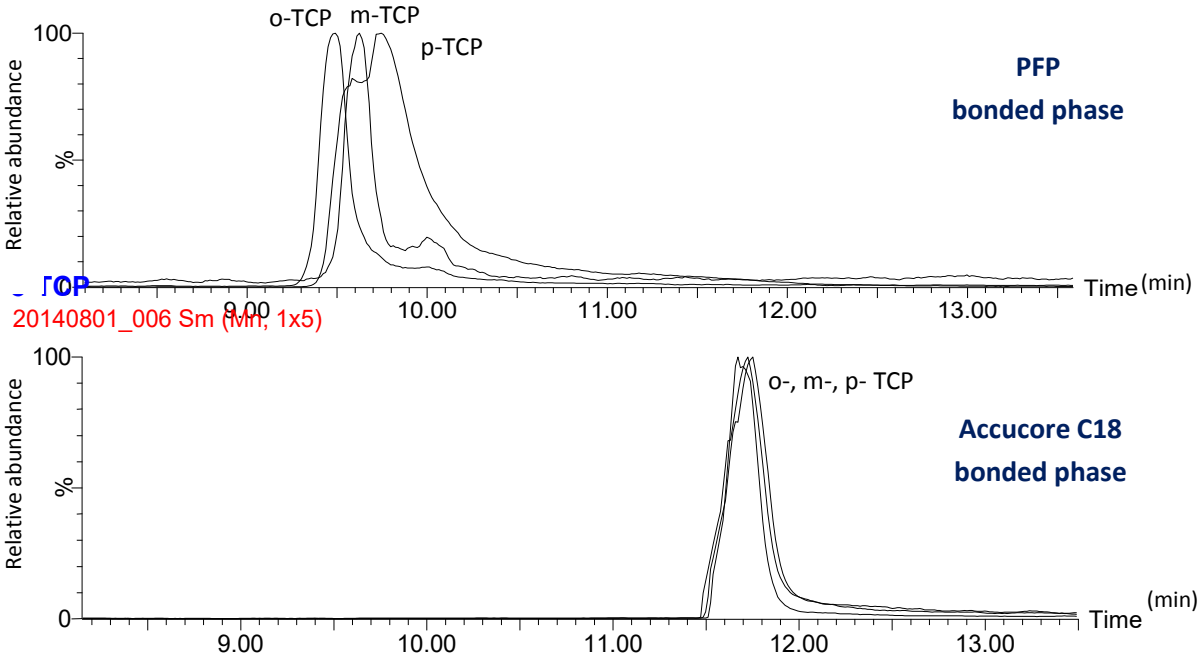


Figure 2-5: The influence of chromatographic stationary phase on the separation of three isomers (o-, m- and p-TCP), flow of 0.4 mL/min.

2.2.2.2. **Mobile phase**

LC Gradient can help overcome some problems like poor resolution or early eluted peaks. For the purpose of optimising the separation of the compounds with good resolution and peak shape, a mixture of the 18 OPEs were prepared in a mixture of ACN/H_2O 1:1 , (v/v) and injected on the PFP column with different gradients. After comparing the separation profile of the targeted OPEs, as obtained from several gradients, we selected the gradient described in Figure 2-6, with a total run time of 28 min. The selection was mainly focused on the co-eluted OPEs. The problem of separting the three TCP isomers remained not totally resolved, noting that the plateau at 40% of ACN served mainly to minimise as much as possible this coelution issue.

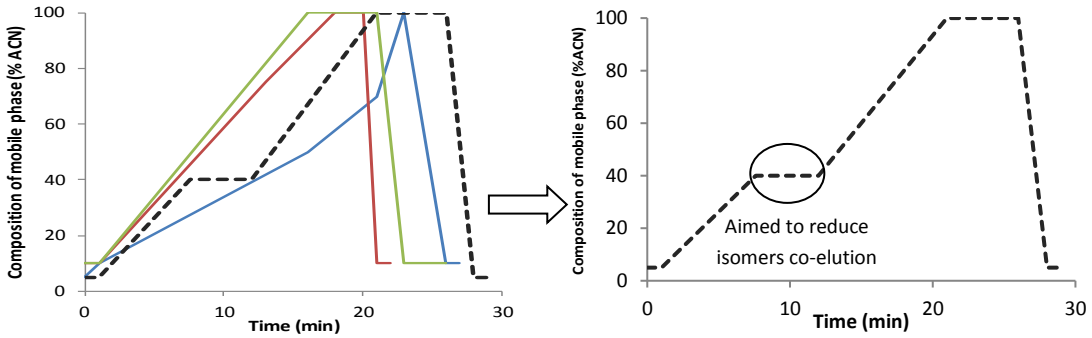


Figure 2-6- Optimisation of the mobile phase gradient on LC (Hypersil Gold PFP column, 0.4 mL/min, ACN and 10mM ammonium acetate in water).

The flow of the mobile phase is another important parameter to be optimized. High flow rates are related to an increase in the electrospray droplet size and a resulting decrease in the effeciency of the droplet charging process. However, we expect that higher flow rate can result sometimes, in better resolution of co-eluting compounds. Using the PFP column, the mobile phase flow rates of 0.4 and 0.6 mL/min were compared. The major difference was the separation between o-TCP and m,p-TCP with 0.8 min in case of flow rate 0.6 mL/min while only 0.4 min time difference in case of flow rate 0.4 mL/min.

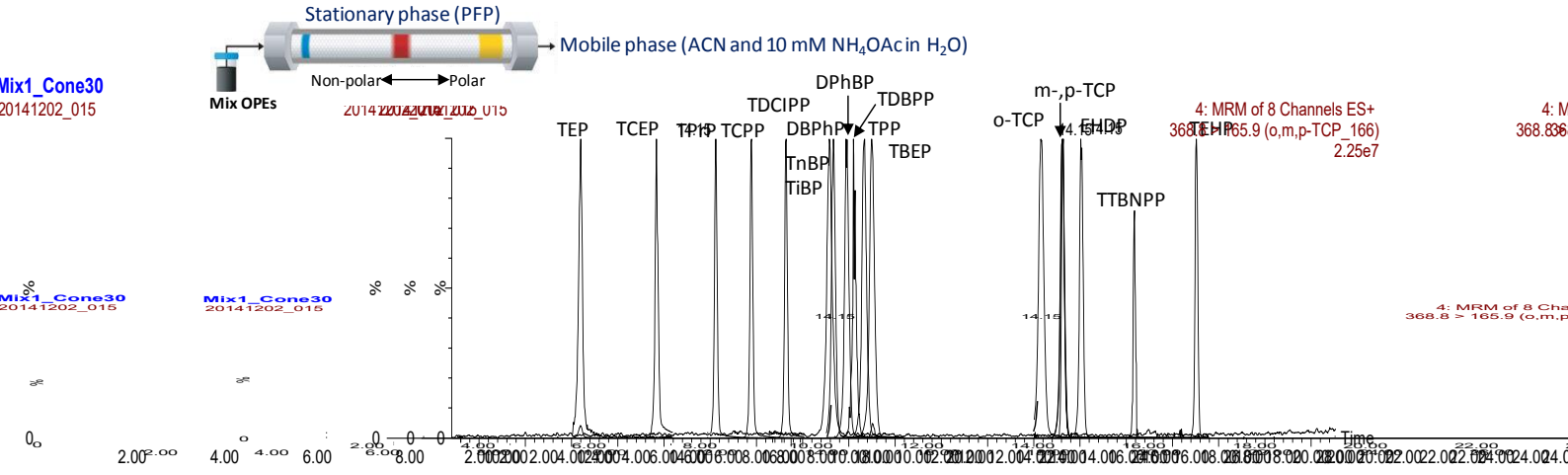


Figure 2-7: Overlaid extracted ion chromatograms obtained with the optimised LC conditions in SRM mode.

The chromatogram for the optimal separation of the 18 OPEs is presented in Figure 2-7 resulting from the extracted ion chromatograms of each compound. The relative chromatographic retention of different OPEs into the chromatographic phase is largely determined by the nature of each compound (non polar OPEs tend to retain more on the PFP bonded phase). As obtained in the Figure 2-7, the co-elution issues were not fixed for the isomer compounds (TiBP and TnBP on one hand, and m- and p-TCP on the other hand).

As a conclusion on the LC –MS coupling,

- **MS conditions**

- ESI(+) *vs* ESI(-) were compared, all the targeted OPEs were dected on the positive mode.
- The influence of the modifier (formic acid HCO_2H *vs* ammonium acetate NH_4OAc) on the adduct formation in the mass spectra, was studied. Minimal Na adduct formation was observed with NH_4OAc so the later was the selected modifier.
- $[M + H]^+$ was dominant in the mass spectra of most targeted OPEs and hence was selected in the development of SRM method.
- Collision energies (in eV) were optimised for each specified transition.

- **LC conditions**

- C-18 *vs* PFP chromatographic stationary phases. PFP showed slightly better resolution of TCP isomers.
- Flow and gradient of mobile phase were optimised, consisting of ACN and 10 mM NH_4OAc in H_2O)

Main limitation encountered with LC – MS was,

- Co-elution issue with some compounds (isomers like o-, m- and p- TCP)

As perspectives,

- LC-MS was not used in the further work.
- GC-MS coupling technique will be investigated in the next part with the illustration of the use of different ionization modes in order to select the most appropriate one in terms of selectivity and/ or sensitivity.

2.3. **GAS CHROMATOGRAPHY MS COUPLINGS**

For the detection of OPEs in extracts of biota and as mentioned in the Chapte 1, GC-MS coupling technique was widely used (Green *et al.,* 2008; Sundkvist *et al.,* 2009; Ma *et al.* 2013a). According to the literature (Li *et al.,* 2015), GC-MS could present several advantages in comparison to LC-MS, namely higher chromatographic resolution and higher peak capacity, fewer issues with solubility, and separations that can be optimized by electronic controls such as temperature programming.

After investigating the behavior of targeted OPEs *via* LC-ESI-MS couplings, we were interested in investigating the GC mass spectral attributes. The main objective of this part was to select the most

suitable ionisation strategy for the specific and sensitive mass spectrometric analysis of the set of target OPEs. For this purpose, the fragmentation patterns and hence the ionisation efficiency were investigated in details *via* EI, CI and APCI modes in GC-MS and then compared with the available literature. After the selection of the ionisation technique, the SRM method was developed and the GC conditions were optimized and finally the methods were evaluated through selected performance parameters.

2.3.1. CHOICE OF IONISATION MODE

2.3.1.1. **Electron Impact (EI)**

Under EI mode, with electron energy of 70 eV, the fragmentation pattern of the studied compounds was observed as highly affected by their category (alkyl, aryl or halogenated) as well as the side chains present in the structure.

Regarding the alkyl OPEs (Figure 1-9), the molecular ion of $[M]^{+\bullet}$ appeared to undergo three successive McLafferty rearrangements. As explained by Ma and Hites, (2013b) and as illustrated in Figure 2-8, the rearrangement in the ion-dipole complex starts with a ϒ-H which initially transfers to the oxygen of the P=O bond. After resonance stabilization, a second ϒ-H transfers to the α-oxygen, resulting in the formation of three ions $[M\text{-}R+2H]^+$, $[M\text{-}2R+3H]^+$, $[M\text{-}3R+4H]^+$ at m/z 99.

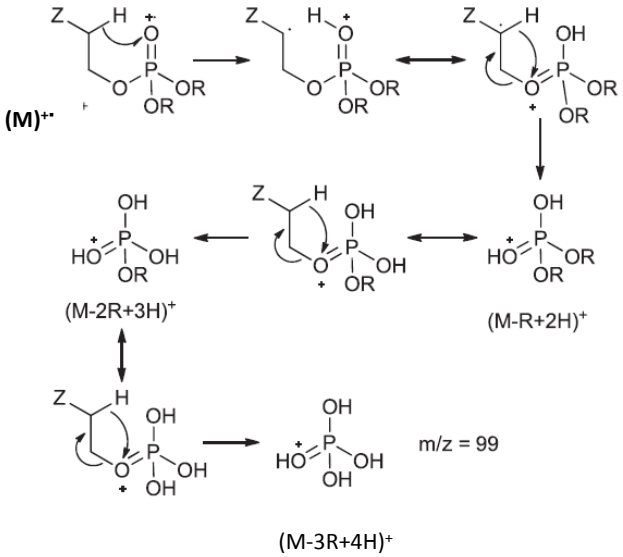


Figure 2-8: Mclafferty rearrangement of the alkyl OPEs (Ma and Hites, 2013b).

For all alkyl OPEs, the base peak was represented by $[H_4PO_4]^+$ at m/z 99. This suggests that this ion peak would be optimal (in terms of intensity) if using EI-MS instrumentation. However, it is not able to give information about the R-groups in the compound of interest and hence is not specific enough for further use as precursor ion in the single reaction monitoring (SRM) transitions. Therefore, other ions

resulting also from the successive McLafferty rearrangements were selected. Two exceptions were observed for TBEP, exhibiting a base peak was at m/z 85, corresponding to $[C_5H_9O]^+$ and for TEHP where the fragmentation did not follow the same pattern since the first ion appears at m/z 113 representing a side chain $[C_8H_{17}]^+$.

Regarding the aryl OPEs, the base peak was not the same for all compounds. The major ions present in the mass spectra corresponded to the quasi-molecular ion $[M]^{+\bullet}$, m/z 170, m/z 94 and m/z 77 for phenyl phenol radical cation $[(C_6H_5)_2O]^{+\bullet}$, phenol radical cation $[C_6H_5OH]^{+\bullet}$ and phenyl cation $[C_6H_5]^+$, respectively. For example, the mass spectrum of TPP was dominated by $[M]^{+\bullet}$, which can be explained by the stability of aromatic rings. It is worth noting here that the choice of the $[M]^{+\bullet}$ can be highly useful for further quantitative measurement purposes. When other alkyl chains (R) were present in the molecule of aryl OPEs, the major ions in their spectra were $[M]^{+\bullet}$ and $[M - R]^+$. For example for EHDP, the base peak was represented by an ion at m/z 251 resulting from the loss of an ethylhexyl side chain. For DBPhP, the only peak present in the spectrum referred to an ion at m/z 175 resulting from the loss of the two butyl chains. For DPhBP, the presence of $[M]^{+\bullet}$ was noticeable. Other fragment ions were present in the spectrum, such as an ion at m/z 251 resulting from the loss of butyl side chain and an ion at m/z 94 for phenol radical cation, corresponds to the base peak. For o, m, p-TCP, the patterns of fragmentation were quite similar with noticeable differences in terms of intensities. This can be explained in terms of the steric effect where the electrophile has higher tendency to attach where there is the least amount of steric hindrance. This can also be explained in terms of steric decompression effect, resulting in more fragmentation in the case of o-TCP, the most sterically hindered compound in comparison with m- and p-TCP. Hence, the molecules with meta and para substituents are more stable than the molecules with ortho substituents. From here, and as confirmed by the mass spectra of these isomers, the base peak of m-TCP and p-TCP corresponds to $[M]^{+\bullet}$ which is much more intense than that of o-TCP. With the same explanation, higher intensities for fragment ions were observed for o-TCP, where the base peak is represented by m/z 165 for $[(C_6H_4)_2CH]^+$ cation.

Regarding the chlorine-containing OPEs, the presence of the electronegative chlorine atoms appeared to change the profile of the mass spectra, so that the base peak resulted sometimes from the loss of one chlorine ion $[M - Cl]^+$. The presence of $[H_4PO_4]^+$ at m/z 99 was always detected for these compounds. The McLafferty rearrangement appeared in the spectra but was generally less abundant maybe due to the presence of chlorine atom on the γ-hydrogen position. For TCEP, for example, the base peak was represented by $[M - Cl]^+$ at m/z 249, other major ion presented at m/z 223 for $[M-R+2H]^+$. For TCPP, the base peak was represented with m/z 125 for $[M-2R-CH_2Cl+2H]^+$, other major ion

was represented by an ion at m/z 201 referring to $[M\text{-}R\text{-}CHCl]^+$. For TDCIPP, the spectrum was dominated by $[M - CH_2Cl]^+$ at m/z 381, $[M\text{-}C_6H_9Cl_4O]^+$ at m/z 191 and m/z 75 for $[C_3H_4Cl]^+$.

Finally, the mass spectra of the two bromine-containing compounds (TDBPP and TTBNPP) were investigated. These compounds are considered as special members of OPEs due to their high molecular masses of 697.6 and 1017.3 $g.mol^{-1}$, along with partitioning coefficient log Kow values of 3.71 and 9.03, respectively. To the best of our knowledge, the available literature on these compounds is scarce, so that no work was yet demonstrated for the detection of TTBNPP, while only two previous works were dealing with TDBPP analysis on GC *via* EI mode (López *et al.*, 2011; Ma and Hites, 2013). Under EI circumstances, and starting with TDBPP, the ion corresponding to $[M]^{+\bullet}$ was not detected. The ion corresponding to the loss of one Br at m/z 616.6 was detectable but with low abundance, as well as an ion at m/z 416.6 resulting from the loss of a side chain and another Br. This ion likely results from a McLafferty type rearrangement from the $[M - Br]^+$ ion. Moreover, the m/z 216.8 fragment ion likely comes from a second McLafferty rearrangement of the ion with m/z 416.6. The loss of HBr resulted in the formation of an ion with m/z 137 (no Br) corresponding to the base peak. The ion at m/z 118.8 corresponds to a side chain with one bromine atom $[C_3H_4Br]^+$. For TTBNPP, the most specific ion resulted from the loss of one side chain at m/z 710.6, showing a six Br isotope pattern. Other abundant ions include for example the ion at m/z 306.7 representing one side chain, and the consequent loss of two HBr leading to the formation of ion at m/z 144.8. The m/z 99 was always present as an indicator ion representing protonated phosphoric acid $[H_4PO_4]^+$.

Table 2-2 presents the chemical formulas of all the targeted OPEs, the major ion source fragmentations (classified in the order as base peak, quantifier and qualifier), observed upon their analysis via full scan mode. Beside each fragment ion, is presented the suggested structure. To have a spectral representation of these profiles, Figure 2-9 presents the full mass spectral profile of 4 representative OPEs; an alkyl (TnBP), aryl (EHDP), chlorinated (TDCIPP) and brominated (TDBPP) compounds.

As a conclusion from the EI mode, the molecule is extensively fragmented during ionization process. The highly diagnostic molecular ion is often absent. For many compounds, characteristic mass spectra are obtained. But in other cases, fragment ions are less specific, or fragmentation is too extensive, which reduces the sensitivity.

As a perspective, the behavior of targeted OPEs will be investigated via chemical ionization (CI) technique. Positive and negative CI are commonly used in GC/MS and are well known to have considerably less fragmentation than EI.

Table 2-2: The fragmentation in the EI source as observed in the full mass spectra of the targeted OPEs

Alkyl OPEs	TEP	$C_6H_{15}O_4P$	(R=R'=R'') $-C_2H_5$	*99,* **155,** 127	$[H_4PO_4]^+$, $[M-R+2H]^+$, $[M-2R+3H]^+$
	TPrP	$C_9H_{15}O_4P$	(R=R'=R'') $-C_3H_7$	*99,* **141,** 183	$[H_4PO_4]^+$, $[M-2R+3H]^+$, $[M-R+2H]^+$
	TiBP	$C_{12}H_{27}O_4P$	(R=R'=R'') $-C_4H_9$	*99,* **155,** 211	$[H_4PO_4]^+$, $[M-2R+3H]^+$, $[M-R+2H]^+$
	TnBP	$C_{12}H_{27}O_4P$	(R=R'=R'') $-C_4H_9$	*99,* **155,** 211	$[H_4PO_4]^+$, $[M-2R+3H]^+$, $[M-R+2H]^+$
	TBEP	$C_{18}H_{39}O_7P$	(R=R'=R'') $-C_2H_4OC_4H_9$	*85,* **125,** 299	$[CH_2OC_5H_9]^+$,
	TEHP	$C_{24}H_{51}O_4P$	(R=R'=R'') $-C_8H_{17}$	*99,* **113**	$[H_4PO_4]^+$, $[R]^+$
Aryl OPEs	TPP	$C_{18}H_{15}O_4P$	(R=R'=R'') $-C_6H_5$	***326,*** 170, 77	$[M]^+$, $[(C_6H_5)_2O]^{+\bullet}$, $[C_6H_5]^+$
	EHDP	$C_{20}H_{27}O_4P$	R=$-C_8H_{17}$ R'=R''=$-C_6H_5$	***251,*** 170, 94	$[M-R]^+$, $[(C_6H_5)_2O]^{+\bullet}$, $[C_6H_5OH]^{+\bullet}$
	DBPhP	$C_{14}H_{23}O_4P$	R=$-C_6H_5$ R'=R''=$-C_4H_9$	***175***	$[M-R'-R''+3H]^+$
	DPhBP	$C_{16}H_{19}O_4P$	R=$-C_4H_9$ R'=R''=$-C_6H_5$	*94,* **251 ,** 306	$[C_6H_5OH]^{+\bullet}$, $[M-R+2H]^+$, $[M]^+$
	o-TCP	$C_{21}H_{21}O_4P$	(R=R'=R'') $-C_6H_4-CH_3$	*165,* **368,** 91	$[(C_6H_4)_2CH]^+$, $[M]^+$, $[(C_6H_4)CH_2]^+$
	m-TCP	$C_{21}H_{21}O_4P$	(R=R'=R'') $-C_6H_4-CH_3$	***368,*** 165, 91	$[M]^+$, $[(C_6H_4)_2CH]^+$, $[(C_6H_4)CH_2]^+$
	p-TCP	$C_{21}H_{21}O_4P$	(R=R'=R'') $-C_6H_4-CH_3$	***368,*** 165, 91	$[M]^+$, $[(C_6H_4)_2CH]^+$, $[(C_6H_4)CH_2]^+$
Chlorinated OPEs	TCEP	$C_6H_{12}Cl_3O_4P$	(R=R'=R'') $-C_2H_4Cl$	***249,*** 223, 99	$[M-Cl]^+$, $[M-R+2H]^+$, $[H_4PO_4]^+$
	TCPP	$C_9H_{18}Cl_3O_4P$	(R=R'=R'') $-C_3H_6Cl$	*125,* **201,** 99	$[M-2R-CH_2Cl+2H]^+$, $[M-R-CHCl]^+$, $[H_4PO_4]^+$
	TDCIPP	$C_9H_{15}Cl_6O_4P$	(R=R'=R'') $-C_3H_6Cl$	*75,* **191,** 99, 381	$[C_3H_4Cl]^+$, $[M-C_6H_9Cl_4O]^+$, $[H_4PO_4]^+$, $[M-CH_2Cl]^+$
Brominated OPEs	TDBPP	$C_{12}H_{21}Br_6O_4P$	(R=R'=R'') $-C_3H_5Br_2$	*137,* **337,** 99, 121, 217	$[C_3H_6PO_4]^+$, $[M-C_3H_5Br_4]^+$, $[H_4PO_4]^+$, $[C_3H_4Br]^+$, $[M-C_6H_8Br_5]^+$
	TTBNPP	$C_{15}H_{24}Br_9O_4P$	(R=R'=R'') $-C_5H_8Br_3$	*145,* **711,** 99, 309	$[R-2HBR]^+$, $[M-R]^+$, $[H_4PO_4]^+$, $[R]^+$

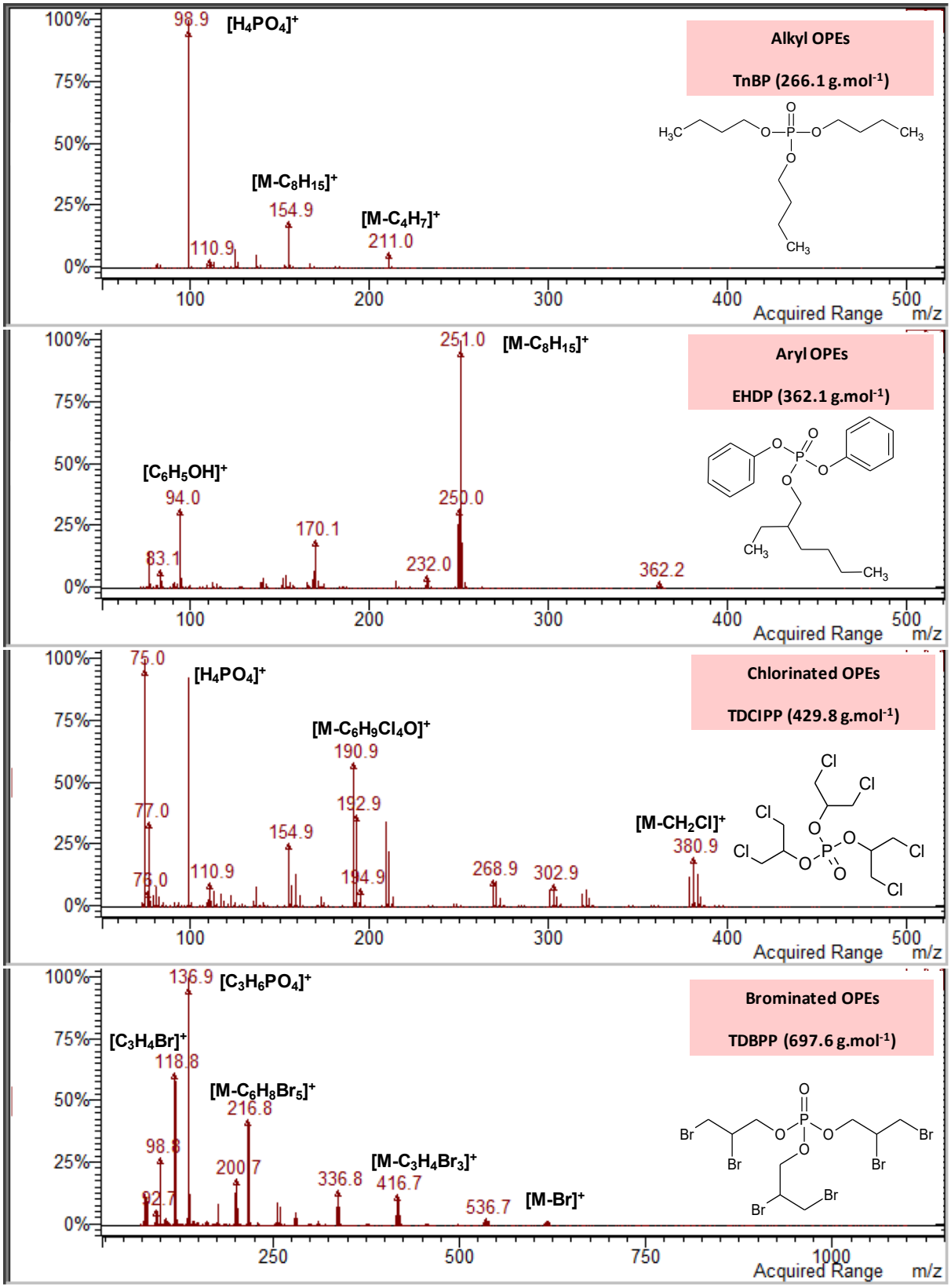


Figure 2-9: Full scan mass spectra obtained in EI mode for alkyl, aryl, chlorinated and brominated OPEs. For the halogenated OPEs, theoretical mass reported for the most abundant isotopologue ion.

2.3.1.2. **Chemical ionisation (CI)**

The analysis was also performed *via* chemical ionisation (CI) technique for the purpose of reducing fragmentation and preserving the molecular ion. Among the targeted OPEs, 9 selected compounds from alkyl, aryl and chlorinated OPEs were studied *via* both positive and negative CI (PCI and NCI) modes while for the brominated OPEs, the analysis was only done *via* NCI mode.

Figure 2-10 presents the full mass spectra of three selected OPEs representing an alkyl (TnBP), an aryl (EHDP) and a halogenated (TDCIPP) as observed along a scan of m/z range 72 to 600 via CI in positive mode.

The mass spectra of alkyl and aryl compounds (*e.g.* TBP, TPrP) were mostly dominated by $[M + H]^+$, except for TEHP which had a first fragment resulting from the loss of 2 side chains and a base peak at m/z 111 corresponding to an ethylhexyl side chain. For TBEP where the base peak was at m/z 399 corresponds to $[M + H]^+$ and for EHDP which had the base peak at m/z 251 resulting from the loss of an ethylhexyl group. This can be explained by the fact that alkyl compounds display charge retention on the side chain whereas aryl compounds give charge retention on the phosphate moiety keeping aryl groups while eliminating the alkyl side chain.

For the 3 chlorinated compounds, the $[M + H]^+$ was present but not always as the base peak. For TCEP, $[M + H]^+$ was the base peak but for TCPP and TDCPP, the base peaks corresponded to ions at m/z 77 and m/z 75 for $[C_3H_6Cl]^+$ and $[C_3H_4Cl]^+$, respectively. The analysis of the 2 brominated compounds was not performed on PCI due to the high electron affinity for the bromine atom of 324 $kJ.mol^{-1}$, favoring the production of stable negative ions through the attachment of an electron *via* the negative CI mode.

As illustrated in the Figure 2-10, the formation of adduct ion $[M+C_3H_5]^+$ was also observed for some compounds.

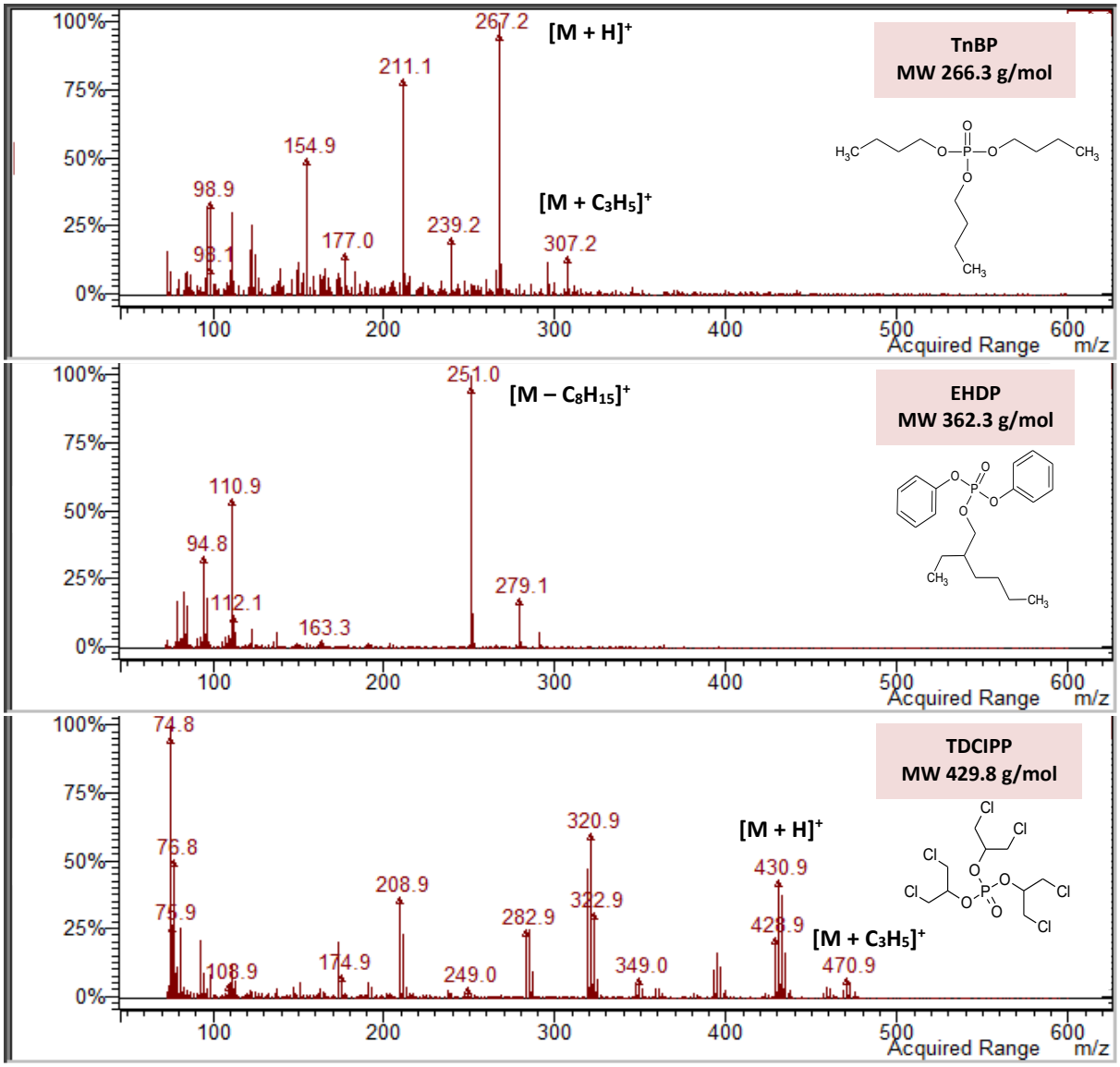


Figure 2-10: Full mass spectra of 3 selected OPEs, as observed over a scan of m/z range 72 to 600 analysed via PCI ionisation mode.

In order to investigate the CI in its other possible mode, the analysis was then performed on NCI mode since it provides a certain degree of selectivity that is not available with other technique. Figure 2-11 presents the full mass spectra of the 3 selected representative OPEs. The mass spectra of the alkyl phosphates, with or without halogen substitution as well as the aryl ones, presented most of the time the ion m/z 127 for $[C_2H_8PO_4]^-$, as well as $[M - R]^-$ ion, but in addition, these compounds also show abundant $[M - H]^-$ ion. For TPP for example, the base peak referred to an ion at m/z 249 for the loss of a phenyl group. In addition, the ion m/z 325 for $[M - H]^-$ was also observed, which as reported by Ma and Hites (2013), may exhibit a cyclic structure due to the elimination of an ortho positioned H and the consequent donation of the electron pair to the oxygen of the P=O bond to form a C-O bond. For EHDP and as illustrated in the Figure 2-11, the base peak resulted from the loss of an attached phenyl group

$[M - C_6H_5]^-$. In the case of TCEP, the major ion was at m/z 221 for the loss of a side chain $[M - C_2H_4Cl]^-$. For TCPP, the base peak resulted from the loss of a side side chain $[M - C_3H_5Cl]^-$ at m/z 249. For TDCIPP, the base peak was represented by an ion at m/z 319 for the loss of a side chain $[M - C_3H_5Cl_2]^-$.

Regarding the two brominated compounds, fewer fragments were observed in their mass spectra compared to EI mode. However, the base peak in CI mass spectra was represented by ions at m/z 79 referring to $[Br]^-$. For both TDBPP and TTBNPP, other ions were observed, *e.g.* $[Br_2]^{-\bullet}$, $[M - Br]^-$ and $[M - R]^-$.

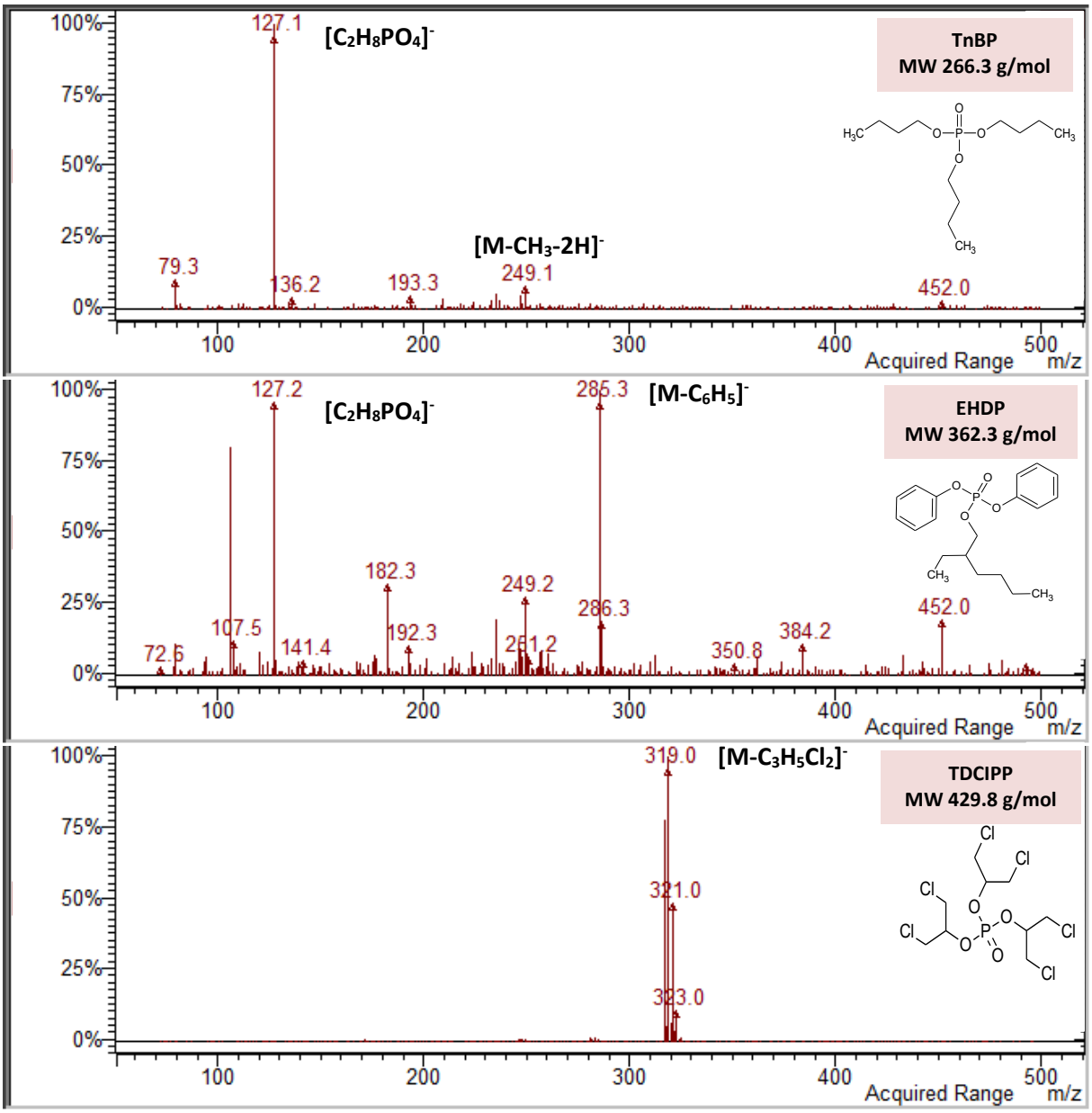


Figure 2-11: Full mass spectra of 3 selected OPEs, as observed over a scan of m/z range 72 to 600 analysed via NCI ionisation mode.

2.3.1.3. **Comparison with the literature on GC-EI/NCI/PCI-MS**

The observations of the study were compared with the available interpretations of previous studies. In their work, Ma and Hites (2013), proposed an overview of the EI, NCI and PCI mass spectra of 13 OPEs (including as common compounds 3 alkyl, 2 aryl, 3 chlorinated and 1 brominated). In comparison to their work, quite similar observations were denoted. The EI mass spectra of these 13 OPEs were dominated by ions such as $[H_4PO_4]^+$, $[M - Cl]^+$, $[M - CH_2Cl]^+$ or $[M]^{+\bullet}$ depending on the chemical structures (*e.g.* side chain, halogen). This was already investigated by the work of Van den Eede *et al.* (2011) who demonstrated the analytical characteristics (identification and quantification ions) acquired for 10 OPEs including 5 alkyl, 2 aryl and 3 chlorinated compounds. The same observations were also reached by Dodson *et al.* (2012) whose list of compounds included in common, 6 alkyl, 2 aryl and 3 chlorinated OPEs. Among the targeted brominated OPEs, only TDBPP was analysed previously via EI mode as described in the works of Lopez *et al.* (2011) and Ma and Hites (2013b) suggesting that ions at m/z 119 and 201 are the most abundant characteristic ions of TDBPP on EI. On the NCI mode and accordingly with the results from Ma and Hites (2013b) the spectra were generally dominated by $[M - R]^-$. TDBPP was analysed on NCI mode in some previous works suggesting that ions and m/z 79, 496.6 and 616.6 are the most abundant characteristic ions. With PCI mode, the spectra were mainly dominated by the quasi- molecular ion $[M + H]^+$, same as previously described by Quintana *et al.,* 2008 and Ma and Hites (2013b). Table IV (in the Annex) proposes a detailed comparison of results observed using different techniques for the 18 compounds in terms of fragment ions on one hand, and the available literature on the other hand, where it is easy to conclude that the work on APCI technique is somehow new in the field of OPEs analysis

As a conclusion and by comparing the detection results for the investigated OPEs *via* the 3 tested modes (EI, PCI, NCI), intensities seemed to be higher in EI mode for all the tested compounds, except for TDCIPP which showed higher intensity *via* NCI mode. For the brominated compounds and in terms of peak response area, NCI showed importantly higher areas than that observed *via* EI, which is attributed to the high abundance of $[Br]^-$ which is in turn not specific enough and not preferred to be used as precursor ion for SRM transitions. As a conclusion, the EI yielded important results in terms of peaks response however the fragmentation for certain compounds was too hard so that this could result in less specificity in the selected ions for the SRM transitions. CI has considerably less fragmentation but with reduced sensitivity in comparison with EI. Therefore, the investigation of other soft ionization techniques for GC is necessary.

As a next perspective, the investigation of atmospheric pressure chemical ionization (APCI) will be illustrated based on the fact that APCI is a soft technique that can help in reducing the in-source fragmentations and showed to be more sensitive in comparison with EI and CI (Li *et al.,* 2015).

2.3.1.4. **Atmospheric pressure chemical ionisation (APCI)**

The results from the comparison of EI and CI under vacuum showed that it was necessary to look for another soft technique in order to as much as possible reduce the 'in-source' fragmentation. For that, atmospheric pressure chemical ionisation (APCI) was applied to investigate its effectiveness assuming that it is the first work to demonstrate the analysis of these OPEs *via* this technique on positive mode.

- **Charge transfer versus protonation mechanisms**

APCI is an ionisation technique using gas-phase ion-molecule reactions at atmospheric pressure, where primary ions are normally produced by corona discharge. It is a method analogous to CI (commonly used in GC-MS). As already described in paragraph 1.7.3 (Figure 1-20), two primary mechanisms can take place using APCI technique. When using N_2 as makeup gas, the nitrogen plasma by the corona discharge needle leads to $N_2^{+\bullet}$ and $N_4^{+\bullet}$. After reacting with water and formation of charged water clusters a proton transfer to the analyte molecule can occur (Li *et al.,* 2015).

To evaluate the effectiveness of APCI technique for our targeted compounds, a mixture of targeted OPEs compounds was analysed on full scan mode under both dry and protic conditions (with the presence of MeOH/H_2O 1:1 (v/v)). Indeed, ionisation via charge or proton transfer could be highly affected by the purity of the gas delivered to the source. Moreover, the source parameters have to be correctly set. In our work, the source parameters (Table 2-3) were set as recommended by Waters, the instrument's manufacturer.

Table 2-3: Applied source parameters for dry and protic mode conditions on a TQ-S, as recommended by Waters.

Source (APGC+)	**Dry conditions**	**Protic conditions**
Corona (µA)	2.0	
Corona (kV)	1.5	
Source temperature (°C)	150	
Makeup gas flow (mL/min)	400	
Cone gas flow (L/hr)	225	170
Auxillary gas flow (mL/min)	50	200

Hereafter, Figure 2-12 illustrates the obtained results in terms of the influence of dry and protic conditions on the full mass spectra.

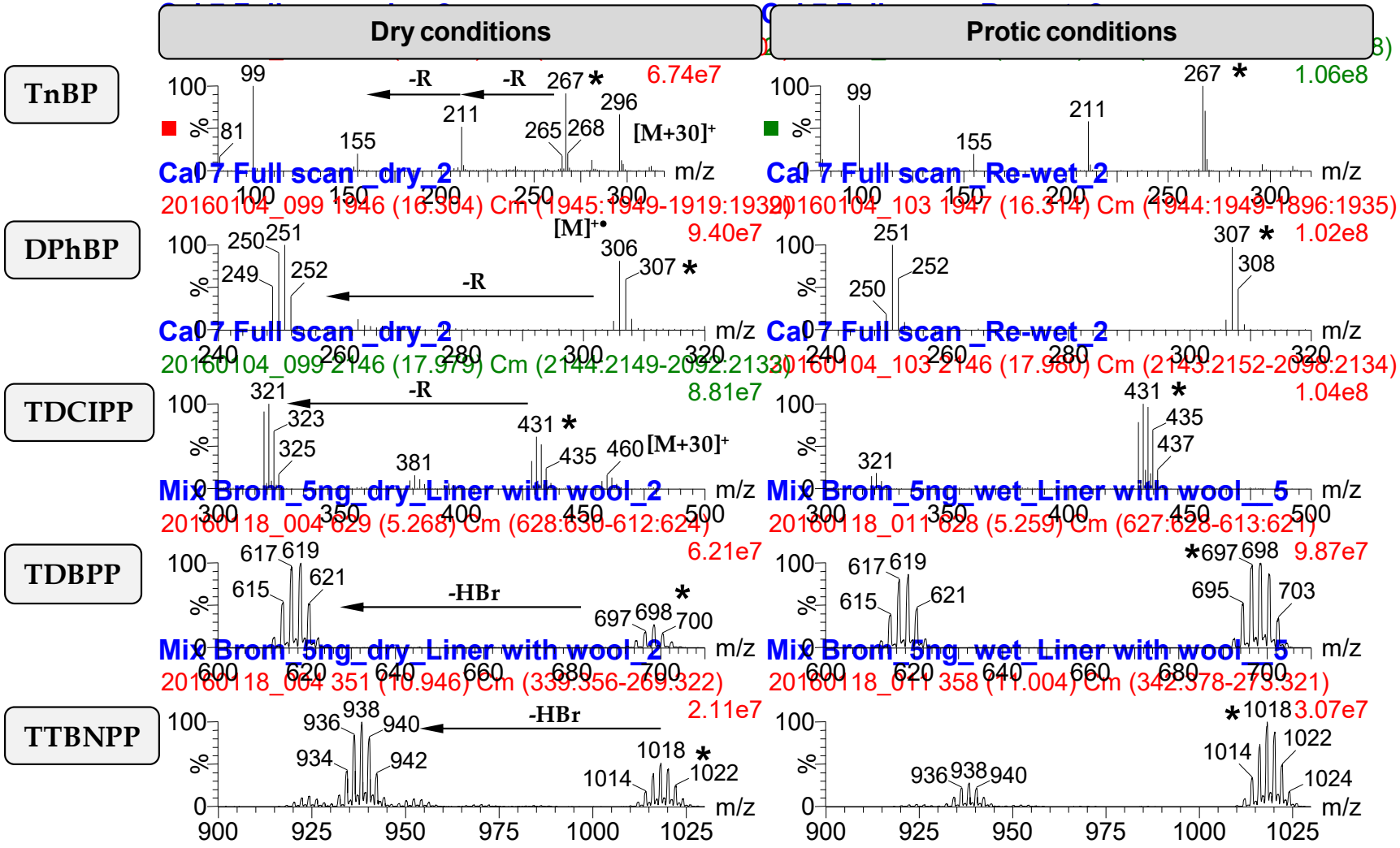


Figure 2-12: **Comparison of full scan obtained in APCI mode in dry (to the left) versus protic (to the right) conditions for alkyl, aryl, chlorinated and brominated OPEs.**

Generally speaking, the $[M + H]^+$ was detected in both investigated conditions but was slightly preserved and intense *via* the protonation mechanisms than the charge transfer mechanisms.

Despite ultrahigh nitrogen quality with efficient nitrogen filters to trap residual water, few analytes showed the presence of $[M]^{+\bullet}$, as illustrated in Figure 2-12. Most of them exhibited fairly intense $[M + H]^+$ ions which was attributed to uncontrolled traces of protic donors. Unexpectedly, $[M + 30]^+$ ion were observed for all and only trialkylated OPEs (except TBEP) at relative intensities to the base peak ranging from 16% for TTBNPP to 66% for TnBP. These ions were hypothesized as $[M + NO]^+$ nitrosyl adducts. The production of NO_x in corona discharges has already been reported and well-studied. According to Sabo and Matejcik, 2013, several processes leading to NO^+ ions in corona discharge APCI source exist, necessitating only traces of O_2 or NO. It was anticipated that uncontrolled variation of such traces could impact adduct formation and then selected signals for quantitative approach. In the same issue, Drazi and Tabrizchi, 2013 evaluated the performance of ionisation *via* NO^+ as reactant ion in the corona discharge ionisation source, mentioning that the major reactant ions in positive mode of operation are NH_4^+, and H_3O^+. NO^+ may also be formed to some extent. Unlike hydronium ion, NO^+ reacts via charge transfer with species having ionisation energy less than that of NO (9.26 eV). Otherwise, the simplest way is the formation of an adduct ion $[M + NO]^+$. There are several instrumental parameters affect the intensity of the NO^+ peak, including the corona voltage, oxygen content of the carrier gas*, etc.*

Since $[M+H]^+$ was observed for most compounds in both dry and protic conditions, protic conditions were then promoted by placing a mixture of MeOH/H_2O 1:1 (v/v) in the source in a vial with a capillary introduced through the septum. Consequently, $[M + 30]^+$ adducts disappeared and the relative abundance of $[M + H]^+$ was increased, confirming the enhancement of proton transfer mechanism. In such APCI mode via favored protonation mechanism, the in-source fragmentation patterns were quite clear for all the compounds.

- **Ionisation efficiency *via* APCI**

In APCI positive mode and *via* protonation mechanism, the fragmentation patterns were quite clear for all the compounds, where $[M + H]^+$ was always preserved. Regarding the alkyl OPEs, the spectra were mainly dominated by $[M + H]^+$ and $[H_4PO_4]^+$ ions and sometimes other ions were present such as $[M - R]^+$, $[M - 2R]^+$. $[M + H]^+$ was the base peak for this group of compounds except for TEP, TPrP and TiBP, where $[H_4PO_4]^+$ was the base peak. Regarding the aryl OPEs, the spectrum was highly dominated by $[M + H]^+$ as the base peak, except for EHDP, DBPhP and DPhBP where the spectra were dominated by $[M - R]^+$ (with the presence of high intense $[M + H]^+$ for DBPhP and DPhBP). Regarding the halogenated OPEs, the spectra were dominated by $[M + H]^+$ as the base peak, $[M - X]^+$ and $[M - RX]^+$.

Figure 2-13 presents the mass spectra of the 18 OPEs of interest characterized using APCI in protic conditions.

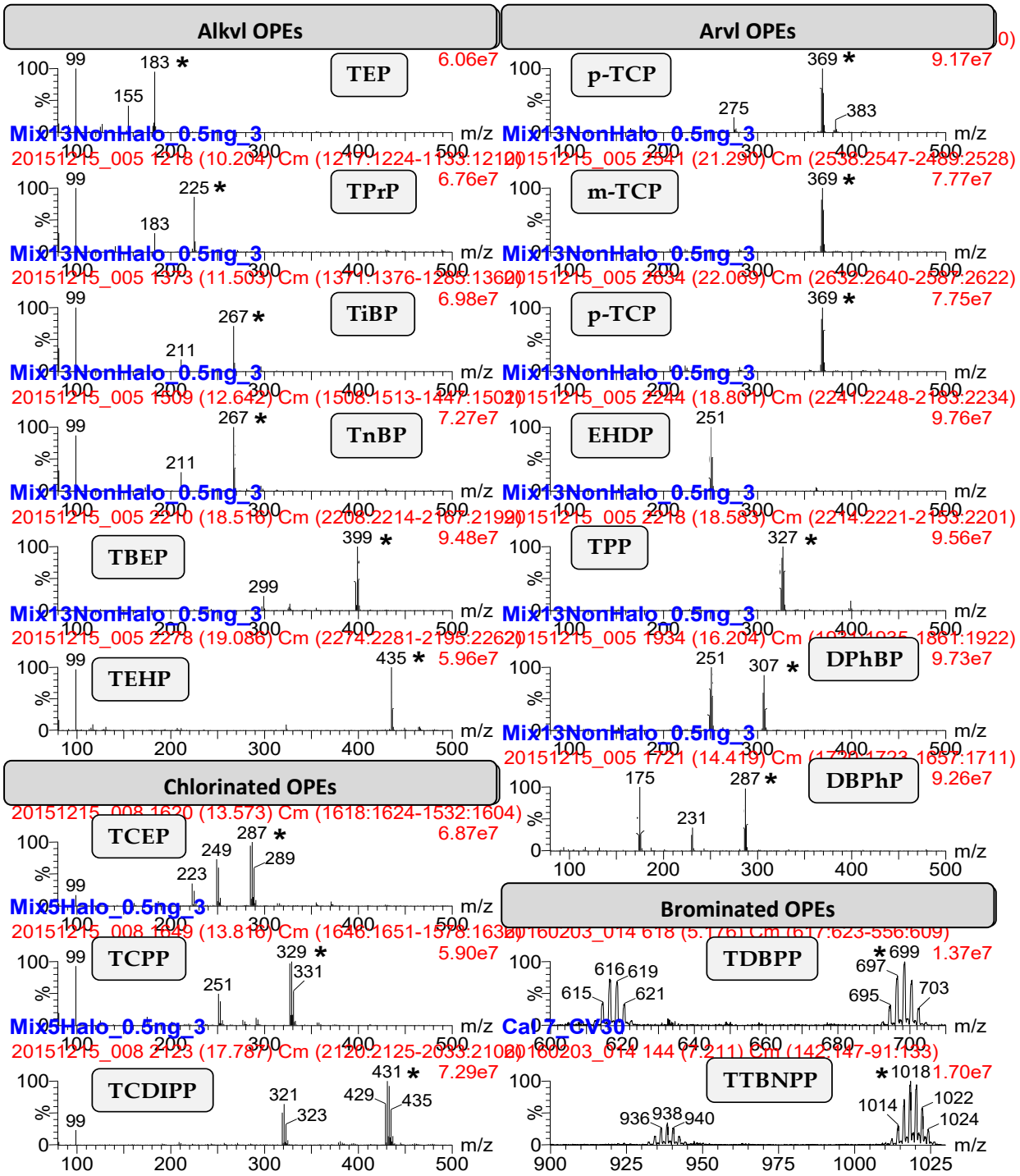


Figure 2-13: Full scan mass spectra obtained in APCI mode of the alkyl, aryl, chlorinated and brominated OPEs. *: $[M + H]^+$ ion. Absolute intensity appears in the upper right of each mass spectrum.

2.3.1.6. **Comparison with the literature on GC-APCI-MS**

In the correlation with the available literature, it is obvious that the applications of APCI using GC-MS/MS are still scarce. The technique was recently used by Bichon *et al.,* 2016 for the simultaneous determination of 16 BFRs in food and feed of animal origin with LOQs down to 1 pg/g fw. Another work was previously demonstrated by Portoles et al., 2015 for the analysis of 14 BFRs and 2 NBFRs. The method LODs were lower than 10 fg and showed to be especially relevant for highly brominated congeners.

For the analysis of OPEs, only one work from Ballesteros-Gomez *et al.* (2013)was assessed but for analysing samples from electronic waste and car interiors. In their work, 7 OPEs were selected (TiBP, TBP, TBEP, TEHP, EHDP and TCEP, TCPP) to be analysed under dry conditions and the achieved detection limits ranged between 0.5 and 25 pg using GC-APCI-HRTOF-MS.

In terms of mass spectral profiles, the main ions of OPEs in APCI positive mode was [M + H]+, except for EHDP ([M - C_8H_{17} + H_2]), while secondary ions with enough sensitivity for confirmation purposes were not observed (except for EHDP, where $[M + H]^+$ was used). It is worth to note that Ballesteros-Gomez *et al.* have applied the technique under dry conditions and as confirmed by our work, $[M + H]^+$ is mostly dominant. All in all, the results are agreed with those obtained from this previous work concerned with OPEs. However, in our work, a wider range of compounds including not only alkyl, aryl and chlorinated OPEs but, for the first time, the brominated compounds were targeted. Besides, much more detailed illustrations on the behaviour of OPEs *via* two ionisation conditions (dry *vs* protic) are given. More and more, the stability and performance of this innovative technique will be investigated.

As a conclusion, the two primary mechanisms of ionisation via APCI were investigated. The dry conditions yielded the production of $[M + H]^+$ as well as the nitrosyl adduct at $[M + 30]^+$. This has encouraged us to favorise the protic conditions with the use of MeOH:H_2O 1:1, (v/v) as a protic modifier mixture.

The next step consists in the complete method optimisation on the retained techniques (i.e. EI and APCI). This would include on one hand the optimisation of spectrometric conditions and the evaluation of method performances, and on the other hand the optimisation of the chromatographic conditions for optimal separation of such wide range of compounds.

2.3.3. CID FRAGMENTATION AFTER EI AND APCI

SRM-MS sensitivity is dependent upon the appropriate tuning of the instrument parameters, mainly the cone voltage and the collision energy. For the purpose of optimizing these spectrometric conditions, pure individual solutions of the 18 OPEs were first analysed on the full scan mode in order to select the precursor ions. Various cone voltages (0 to 40 V) were tested in order to select the one yielding the optimal ionisation/fragmentation for each compound. After that and to characterize and optimize the fragmentation pathways of the selected precursor ions in the collision cell, various collision energies were tested (10 to 45 eV for EI, 5 to 30 eV for APCI) in order to end up by the development of a method in SRM mode. The SRM methods with optimized cone voltages and collision energies are shown in Table 2-4, where the 2 transitions of highest intensities were specified to reach 4 identification points according to EU Commission Decision 2002/657/EC, noting that the first transition (T1) was used for quantification and the second one (T2) was used for confirmation purpose.

Table 2-4: Optimized SRM parameters for the 18 OPEs by GC-MS/MS on both positive EI and APCI modes, along with obtained instrumental detection limits (IDL, in pg). CE: collision energy (in eV); CV: cone voltage (in V).

	GC-APCI(+)-MS/MS						GC-EI(+)-MS/MS				
OPE	**T1**	**CE 1**	**T2**	**CE 2**	**CV**	**IDL**	**T1**	**CE 1**	**T2**	**CE 2**	**IDL**
TEP	183>99	15	183>155	5	20	1	155>99	10	127>99	10	0.4
TPrP	225>99	10	225>183	5	20	0.4	141>99	10	183>99	15	0.4
TnBP	267>99	15	267>155	10	30	0.4	155>99	10	211>99	20	0.4
TiBP	267>99	15	267>155	10	30	0.4	155>99	10	211>99	10	0.4
TEHP	435>99	15	435>323	5	30	0.4	113>57	10	113>95	10	10
TBEP	399>199	15	399>99	25	30	0.4	125>99	10	199>99	10	40
TPP	327>77	25	327>125	25	30	0.4	326>215	20	326>169	20	1
EHDP	251>95	20	363>251	5	40	0.4	251>77	20	251>152	20	1
DBPhP	287>175	15	287>231	5	20	0.4	175>77	15	175>51	10	1
DPhBP	307>251	10	251>153	15	30	0.4	251>152	15	306>251	10	2
o-TCP	369>91	25	369>166	25	40	0.4	368>181	10	165>139	25	2
m-TCP	369>166	25	369>91	25	40	0.4	368>165	25	368>261	10	1
p-TCP	369>166	25	369>91	25	40	0.4	368>108	15	368>198	15	1
TCEP	285>223	10	287>99	15	30	0.4	249>125	10	249>99	10	1
TCPP	329>99	15	327>251	5	20	0.4	125>99	10	201>125	10	1
TDCIPP	431>321	5	321>209	5	30	1	191>75	10	381>159	10	2
TDBPP	698.5>99	25	698.5> 299	15	30	1	336.8> 137	5	216.8>137	5	100
TTBNPP	1018.4> 147	30	1018.4> 307	20	30	10	712.5> 309	15	712.5>145	15	500

2.3.4. GC SEPARATION

The injection on GC system was performed in splitless mode through a liner with glass wool (900 µL, 4 mm id). 1 µl was injected at 295 °C injection temperature. A DB-5MS capillary column (30 m x 0.25 mm i.d., 0.25 µm) was used for the separation of 16 OPEs. The optimized temperature program was as follows: initial temperature at 85 °C for 5 min, ramped to 240 °C with rate 15 °C.min^{-1}, to 255 °C at 3

°C.min^{-1}, then to 300 °C at 20 °C.min^{-1} and finally held for 5 min. The total run time was 27.58 min. The initial temperature and initial hold time were chosen in order to allow the detection of the most volatile compounds such as TEP and TPrP. The ramp of temperatures was optimized in order to obtain a satisfying separation while keeping a good resolution between TPP, TEHP, TBEP and EHDP, which exhibited co-elution issues. The carrier gas flow rate was constant at 1 mL.min^{-1}. The brominated compounds (TDBPP and TTBNPP) with large molecular weights have the tendency to pass a longer time through the column, due to the higher interaction affinity with the non-polar stationary phase, resulting in more band broadening and less efficiency. To resolve the efficiency problem for these two heavy compounds through minimizing their longitudinal diffusion, a shorter capillary column ZB-5HT (15 m x 0.25 mm i.d., 0.10 µm) was used. On GC-APCI-MS/MS, the optimized temperature program was as follows: the initial oven temperature was set as 85 °C for 1 min, ramped to 350 °C at 35 °C.min^{-1} and held for 5 min. The total run time was 16.63 min. The carrier gas flow rate was constant at 3 mL.min^{-1}. The chromatographic conditions used were the same on GC-EI-MS/MS, with some exceptions due to the differences in the two systems and hence the limitations of vacuum source system, so that the final temperature of the oven program was set at 310 °C (limited by the maximum allowed transfer line temperature of 310 °C). The total run time was 12.43 min. The carrier gas flow rate was constant at 1.5 mL.min^{-1} (in order to be compatible with the flow rate requirements). Figure 2-14 shows the ion chromatogram for the optimized chromatographic separation of the 18 OPEs as observed on GC-APCI-MS/MS.

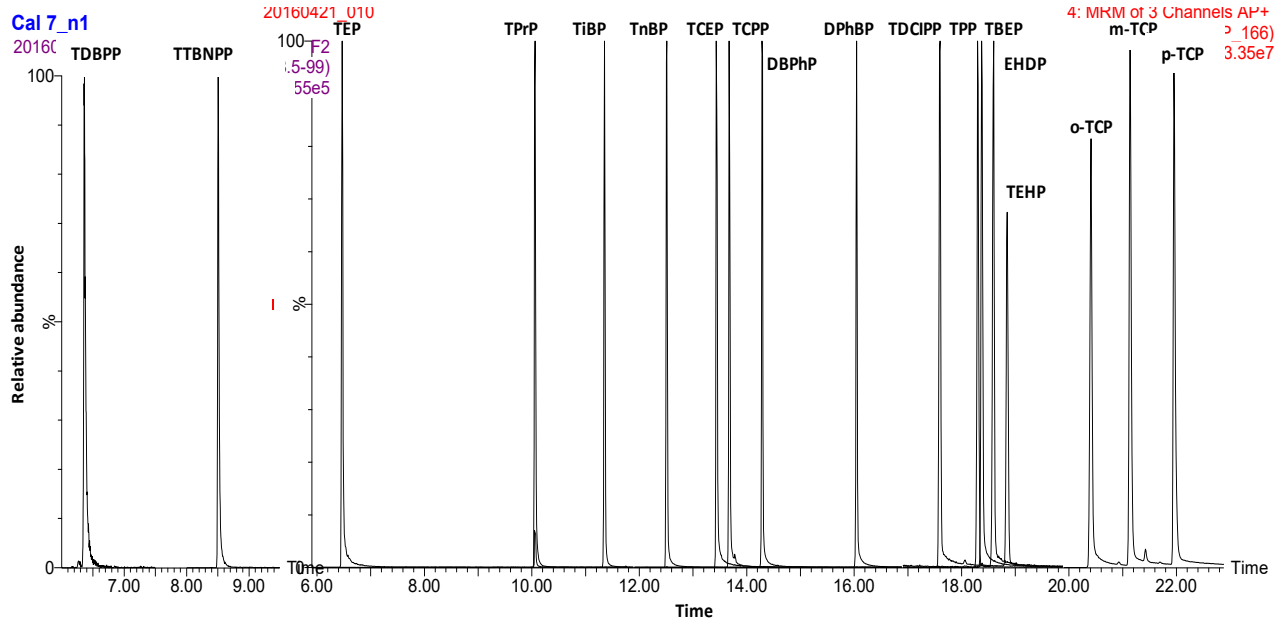


Figure 2-14: Overlaid ion chromatograms obtained for the optimized SRM transitions of the 18 OPEs (GC-APCI-MS/MS).

As a conclusion,

- LC *vs* GC were investigated and GC was retained because of less co-elution issues.
- Two methods were developed on GC-EI(+)-MS/MS and GC-APCI(+)-MS/MS.
  Even though APCI showed more specificity in terms of transitions and hence better sensitivity is expected; the two techniques (EI *vs* APCI) are to be tested and compared on matrix.

The next perspective is to investigate the methods performances by responding to the following three points:

- Stability
- Dynamic range
- Instrumental detection limits (IDLs)

2.3.5. CALIBRATION STANDARDS CURVES

In a way to respond to our raised questions on the performances of the instrumental methods, calibration levels were prepared in order to provide information on the instrumental robustness and the dynamic range. A total of 8 standard calibration curves were injected for each ionisation mode. Each standard calibration curve was composed of 8 target compounds levels including a "zero" point and then increasing exponentially from 2 to 500 ng/mL. Each point also contained the 7 internal standards (deuterium or ^{13}C) at 50 ng/mL and the recovery standard at 50 ng/mL. Fish and food samples were analysed by both GC-EI-MS/MS and GC-APCI-MS/MS techniques through 4 sequences each within 2 months (see Chapter 4). The quantification was based on isotopic dilution method.

2.3.5.1. **Stability**

Firstly, we were interested in investigating the stability of responses of two sets of standards. On one hand, the internal standards which are some times refered to as surrogates and which are usually added prior to the sample preparation and can be helpful to to track the sample preparation process. These include 7 isotopically-labeled OPE standards. On the other hand, the recovery standard added to the sample extracts right before chromatographic analysis and can be contained in the solvent used to reconstitute the sample. These two set of standards are useful for troubleshooting to figure out if something went wrong during analysis. For example, if the recovery of both internal and recovery standards is low, an injection error might have occurred and a reanalysis of the extract should be performed. If the recovery of the recovery standard is within the tolerance range, but the recovery of

the internal standard is low, a problem during sample preparation might have occurred and a new sample aliquot should be prepared and analysed. However, poor precision of the recovery standard points to problems during injection and chromatographic analysis. The two sets of standards can also help to reveal steps in the analytical method that increase the variance of the analytical process.
From here, the response of the recovery standard [^{13}C- tetra chlorinated biphenyl 111 ($^{13}C_{12}$-PCB-111)] was verified *via* both APCI and EI techniques as a measure of the instrumental stability (Figure 2-15).

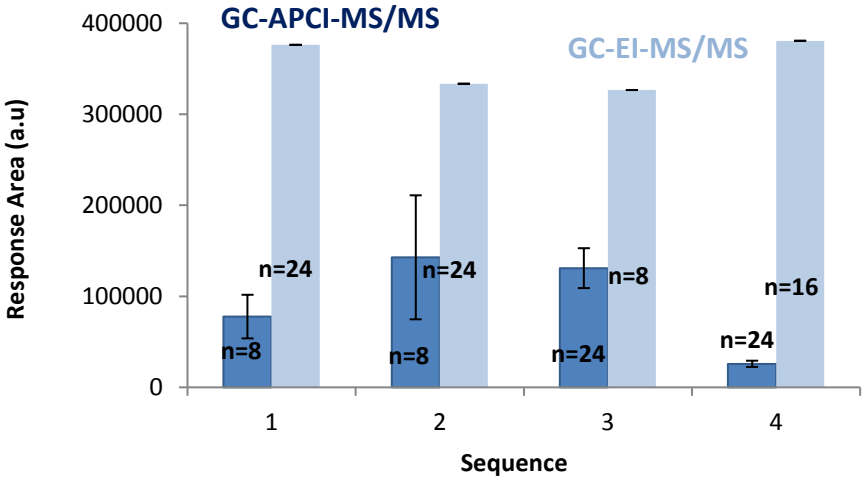


Figure 2-15: Observed response area for the recovery standard in the calibration curves along successive sequences.

The results were illustrated in Figure 2-15 for the average response area from the standard calibration solutions along with the corresponding standard deviation for the specified number of injections. The figure reflects the repeatability of APCI and EI mode, where the latter showed to be much better over different dates as do the variation within the same sequence.

Besides, the results were illustrated in Figure 2-16 for the average response area from the injected samples (Chapter 4) along with the corresponding standard deviation for the specified number of analysed samples. Similar conclusion was drawn from the response of this recovery standard from the analysed fish samples so that the repeatability by EI was better than that of APCI but in the case of samples, standard deviations are higher than those from standard solutions because of the influence of difference in matrix and the matrix itself.

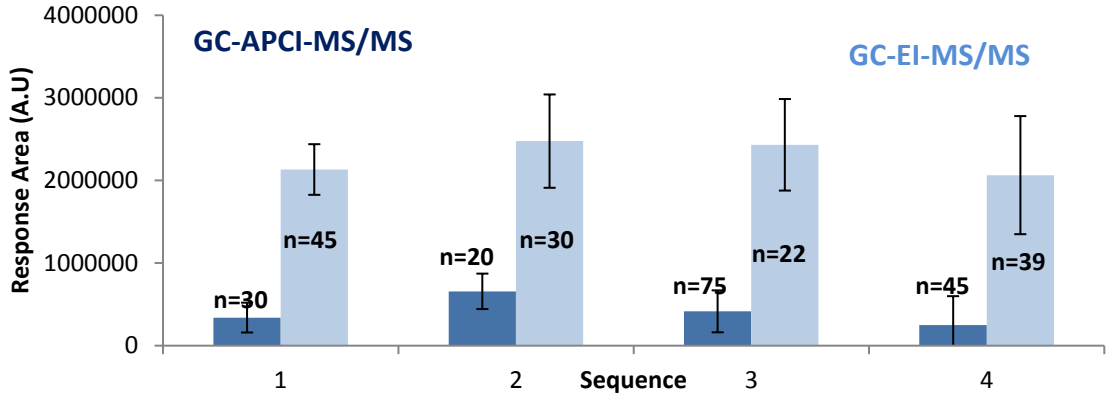


Figure 2-16: Obtained repeatability of the recovery standard in the injected samples (fish and foodstuffs) through the injected sequences and within the same sequence (evaluated by the standard deviation).

After investigating the stability of recovery standard, we were interested in investigating the stability of internal standards via the two investigated techniques. This was done through by the help of calibration curves which were injected during the sequences of analysis. The repeatability of the relative response factor (RRF) of internal standards was an issue of great interest. RRFs express the sensitivity of to a standard substance. It can be expressed as: $\mathbf{RRF} = (\mathbf{Ai}/\mathbf{Ast})/(\mathbf{Qi}/\mathbf{Qst})$, where A: Response Area, Q: Quantity, subscripts i and st refer to the compound and standard, respectively.

The RRF of internal standards were evaluated based on the recovery standard and the results were investigated within the same day as well as between different days. This is illustrated hereafter in Figure 2-17.

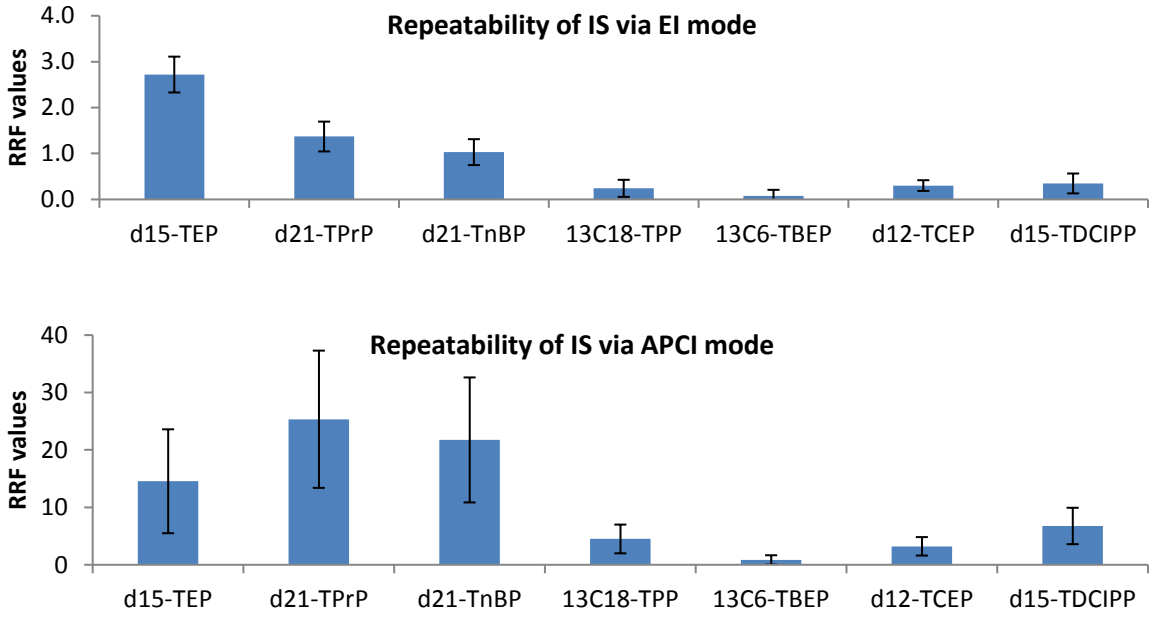


Figure 2-17: Internal standards RRF (n= 56) obtained by EI (top) *versus* APCI (down) in the calibration curves along successive sequences

Figure 2-17 illustrates the repeatability and hence the stability of the internal standards based on the recovery standard. As shown in the mentioned Figure and as agreed with the previous section, the standard deviation (n=56) for most IS compounds was much more important *via* the APCI technique than the EI one.

This might be attributed to the fact that the APCI source still requires some improvements for maintaining the stability of the instrumental analysis.

2.3.5.2. **Dynamic range and RRFs**

The dynamic range is the interval over which the method provides results with an acceptable uncertainty. Dynamic range is where the response function increases linearly with an increase in the amount of the analyte.

In our work, the dynamic range was evaluated through the relative response factor (RRF) by looking into: (i) the intra-day repeatability, (ii) the inter-day repeatability and (iii) the RRF values from the OPEs having their analogues internal standard.

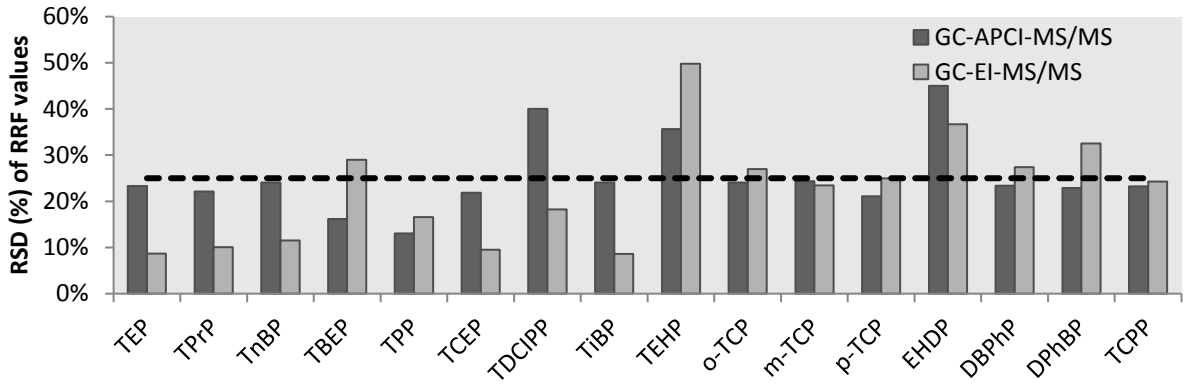


Figure 2-18: RSD of the RRF values obtained for non brominated OPEs by GC-APCI-MS/MS (n=56 data points) and GC-EI-MS/MS (n=49 data points).

In terms of the intraday variation, the RSDs of the RRF values were firstly monitored within the same injection day but also within the same calibration range replicate. The values for most compounds were lower than20 %, which reflects a little intraday variation.

Secondly, the variation was studied from different sequences between days and the results are presented in Figure 2-18. The RSDs of the RRF for most of compounds were below 25%, which approved to be acceptable. Exceptions were for EHDP and TEHP *via* both techniques, TDCIPP *via* APCI and DPhBP *via* EI modes. For EHDP, DPhBP and TDCIPP, it could be attributed to the use of an internal

standard which is not isotopologue of the native compound. For TDCIPP having its isotopologue IS (as d_{15}TDCPP) the low RRF value could be attributed to the fact that the selected precursor ion for the native compound didn't correspond to the one for its isotopogue IS.

For the two brominated OPEs (TDBPP and TTBNPP), EI led to unacceptable RSD values, mainly due to much lower sensitivities. TTBNPP remained a quite difficult compound by APCI with RSD values closer to 30%.

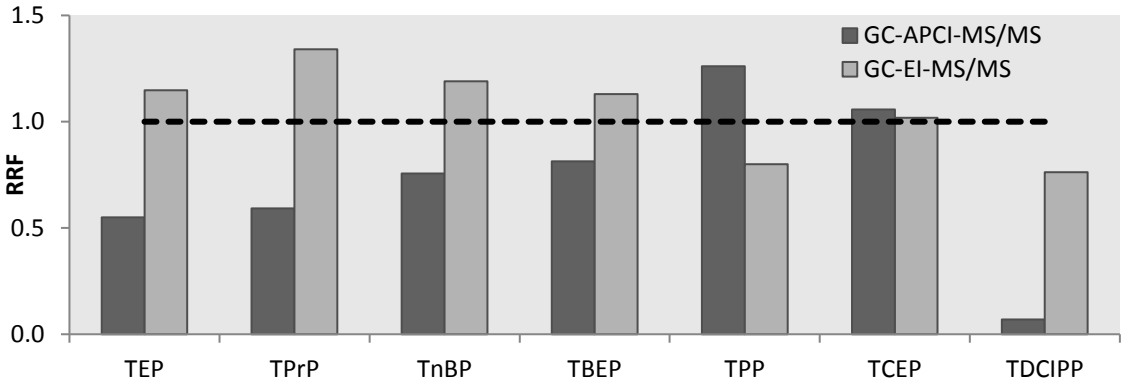


Figure 2-19- The RRF values for the compounds having their analogous isotope-labeled internal standards.

As a third objective in this part, it was interesting to look at the RRF obtained for the 7 target compound having an isotopologue IS (Figure 2-19). By using both EI and APCI, the RRF values for these compounds should be close to 1 (response of internal standard alter exactly the same way as do the native compound). In the case of EI the values were closer to 1, than those observed *via* APCI. An exception was observed for TDCIPP for the same previously explained reaon.

2.3.5.3. **Instrumental detection limits (IDLs)**

A series of low concentration levels were analysed in order to determine the instrumental detection limits (IDLs) for the 18 OPEs (Table 2-4). These IDLs were defined as the lowest detection limit where S/N > 3. By EI, the values ranged from 0.4 to 2 µg/L, except for TEHP, TBEP, TDBPP and TTBNPP with 10, 40, 100 and 500 µg/L, respectively, confirming previous observations. By APCI, the values were slightly better and ranged from 0.4 to 1 µg/L, except for TTBNPP with 10 µg/L. These results suggest that it would be easier to go down to trace level analysis with the APCI technique. However, it should be pointed out that even though from fairly recent and comparable generations, two different instruments from two different constructors were used. Indeed, no available single instrument permits EI and APCI for facilitated comparison. Thus, conclusions in terms of sensitivity should be moderated in the light of this fact.

The previous study presents the ability of GC-MS/MS to analyse a wide range of OPEs including alkyl, aryl and halogenated compounds. For the purpose of comparing and determining which ionization technique to be used, we investigated the fragmentation patterns of 18 OPEs *via* different ionization modes (EI, CI and APCI).

EI and APCI provided good sensitivity for the further quantitative measurements of these compounds, but in the case of EI the good sensitivity came at a cost of decreased selectivity due to the abundant presence of m/z 99, especially in the case of alkyl OPEs. APCI is a soft technique that produces abundant $[M+H]^+$ ions for the 18 OPEs, making it possible to generate specific and sensitive SRM transitions. These observations allowed us developing instrumental methods on both GC-EI-MS/MS and GC-APCI-MS/MS through the optimisation of the chromatographic and spectrometric conditions (in positive mode). For almost all the studied compounds, IDLs achieved were 2.5 to 25 times lower in the APCI mode than those in the EI mode, 50 times for TTBNPP and 100 times for TBEP and TDBPP. In the issue of method performances, some practices were illustrated:

- The repeatability of recovery standard ($^{13}C_{12}$-PCB-111) was studied *via* both GC-EI-MS/MS and GC-APCI-MS/MS. Higher stability in response area via EI than APCI mode
- The repeatability of internal standards was also investigated *via* GC-EI-MS/MS and GC-APCI-MS/MS. Lower RSD values (n=56) via EI, in particular for the smallest OPEs (dTEP, dTPrP and dTnBP).
- The dynamic range of compounds was illustrated *via* GC-EI-MS/MS and GC-APCI-MS/MS. RSD values were lower than 25%, except EHDP and TEHP via both techniques, DPhBP via EI and TDCIPP via APCI mode.

To the best of our knowledge, it is the first study to report the analysis of such wide range of OPEs *via* APCI technique. In particular, it is the first work to demonstrate the analysis of TTBNPP on GC system. These results have been published in Journal of Mass Spectrometry (see next page).

As a perspective, even though lower stability was demonstrated with APCI in comparison to EI. However, APCI is interesting as an innovative technique for the analysis of OPEs. Therefore, more comparisons are going to be illustrated on the matrices (*i.e.* matrix effects, limits of quantifications).

Another perspective will be to optimize the sample preparation method for the purpose of comparing the two selected techniques in terms of their sensitivity and their ability to deal with matrix effect issues. In parallel, the other perspective is to analyse these 18 OPEs in fish and other food samples in a chemical food safety context.

Research article

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APCI as an innovative ionization mode compared with EI and CI for the analysis of a large range of organophosphate esters using GC-MS/MS

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Organophosphate esters (OPEs) are chemical compounds incorporated into materials as flame-proof and/or plasticizing agents. In this work, 13 non-halogenated and 5 halogenated OPEs were studied. Their mass spectra were interpreted and compared in terms of fragmentation patterns and dominant ions via various ionization techniques [electron ionization (EI) and chemical ionization (CI) under vacuum and corona discharge atmospheric pressure chemical ionization (APCI)] on gas chromatography coupled to mass spectrometry (GC-MS). The novelty of this paper relies on the investigation of APCI technique for the analysis of OPEs via favored protonation mechanism, where the mass spectra were mostly dominated by the quasi-molecular ion $[M+H]^+$. The EI mass spectra were dominated by ions such as $[H_4PO_4]^+$, $[M–R]^+$, $[M–Cl]^+$, and $[M–Br]^+$, and for some non-halogenated aryl OPEs, $[M]^{+\bullet}$ was also observed. The CI mass spectra in positive mode were dominated by $[M+H]^+$ and sometimes by $[M–R]^+$, while in negative mode, $[M–R]^-$ and more particularly $[X]^-$ and $[X_2]^{-\bullet}$ were mainly observed for the halogenated OPEs. Both EI and APCI techniques showed promising results for further development of instrumental method operating in selective reaction monitoring mode.

Instrumental detection limits by using APCI mode were 2.5 to 25 times lower than using EI mode for the non-brominated OPEs, while they were determined at 50-100 times lower by the APCI mode than by the EI mode, for the two brominated OPEs. The method was applied to fish samples, and monitored transitions by using APCI mode showed higher specificity but lower stability compared with EI mode. The sensitivity in terms of signal-to-noise ratio varying from one compound to another. Copyright © 2016 John Wiley & Sons, Ltd.

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Keywords: organophosphate ester; flame retardant; plasticizer; atmospheric pressure chemical ionization; nitrosyl adduct

Introduction

Organophosphate esters (OPEs) represent a group of chemicals that have been extensively used for several decades for two main purposes, depending greatly on the type of side chain of the phosphate ester. (1) The halogenated compounds are applied as flame retardants (FRs). Examples include, on one hand, tris(2-choroethyl) phosphate (TCEP) that is used in flexible and rigid polyurethane foams, plastics, and textiles[1] and, on the other hand, tris(2,3-dibromopropyl) phosphate (TDBPP) that has been used in polyurethane as well as in polystyrene foams.[2] (2) The non-halogenated compounds are mostly used as plasticizers although they are also used as FRs. Alkyl or aryl phosphates such as tributyl phosphate (TnBP) and triphenyl phosphate (TPP) are predominantly used as plasticizers and lubricants.[3,4] With the gradual discontinuation of the use of some brominated flame retardants (BFRs) due to their proved persistence, bioaccumulation in the environment, and/or toxicity to animals and humans, organophosphorus flame retardants generally and OPEs in particular, which have already been used for over 150 years, are considered as suitable alternatives for BFRs.[5–8] According to statistical data, the global consumption of organophosphorus flame retardants was 500 000 t in 2011 and was expected to reach 680 000 t in 2015.[9]

The investigated OPEs are introduced as additives rather than chemically bonded to the final products. This can result in a simple release via volatilization, abrasion, and/or leaching during their lifetimes including production, usage, disposal, and recycling processes.[4,10] Monitoring studies have reported the presence of these OPEs in various environmental matrices, such as dust, air, water, sediment, soil, and biota samples. This ubiquitous occurrence in the environment may pose a threat to human health through diverse routes, e.g. dermal contact, dust ingestion, inhalation, and dietary intake.[4]

Although there is insufficient knowledge about the toxicity of OPEs, some studies have reported adverse reproductive, endocrine, and systemic effects in animals as a result of long-term

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exposure to these contaminants.[9,11] Alkyl OPEs like TnBP and tris(2-butoxyethyl) (TBEP) have been shown to induce sick house syndrome.[2] Additionally, a significant association was found between the presence of TnBP in floor dust and the prevalence of asthma and allergic rhinitis.[12] Aryl OPEs have been shown to disturb the expression of transcriptional regulators in zebrafish and to cause heart toxicity.[4] Tri-ortho-cresyl phosphate (o-TCP) has been shown to cause peripheral nerve damage and degeneration of the spinal cord upon human exposure.[13] Moreover, chlorinated OPEs, such as TCEP and tris(1,3-dichloro-2-propyl) (TDCIPP), have been proven to be neurotoxic and carcinogenic.[11,14] TDBPP is considered as anticholinesterase compound[15] and is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in animal experiments.[2,16] Tris(tribromoneopentyl) (TTBNPP) was predicted as likely to have a high persistence according to European Food Safety Authority.[16]

Due to their worldwide increasing consumption volumes and adverse effects, OPEs are of increasing concern, and their reliable monitoring in various compartments becomes necessary. Consequently, great efforts are required in order to conduct a comprehensive risk assessment and to evaluate the relative importance of the various exposure routes for the population. From here, several analytical strategies have been described over the past 10 years in environmental samples, such as gas chromatography coupled to flame photometer, nitrogen–phosphorus, atomic emission, single quadrupole or tandem mass spectrometry, and liquid chromatography coupled to tandem mass spectrometry.[6,7,17,18] GC-MS presents several advantages, namely high chromatographic resolution and peak capacity and, compared with LC-MS, fewer issues with solubility.[19] Among the GC-MS ionization techniques, electron ionization (EI) is the most adopted one.[17] However, the fragmentation is sometimes observed as too extensive, disabling thus proper identification of the monitored structures. Soft ionization techniques such as positive and negative chemical ionization (PCI, NCI) induce considerably less fragmentation but exhibit limited sensitivity in comparison with EI.[19]

Since the very first developments dedicated to the hyphenation of GC-MS with soft atmospheric pressure ionization (APCI) in the 1970s, this coupling only recently gained attention from the scientific community, especially during the last decade.[19,20] With APCI, several advantages are attained, mainly the preservation of molecular ion, which renders the selection of a precursor ion for MS/MS no longer a compromise between sensitivity and selectivity.[19] To the best of our knowledge, the use of this ionization technique in the field of FRs was only applied to the analysis of BFRs.[20,21] Hence, the specific analysis of OPEs is not cited yet in the available literature.

The objectives of the present study were (1) to investigate the fragmentation profiles in the mass spectra of 18 OPEs by using different ionization techniques on GC-MS, namely EI, NCI, PCI, and APCI, in dry and protic conditions and then to choose the most selectively relevant techniques; (2) to develop the instrumental methods by the selected ionization techniques (GC-EI-MS/MS and GC-APCI-MS/MS) by optimizing both spectrometric and chromatographic separation; (3) to evaluate the instrumental detection levels (IDLs) of the developed methods in order to reflect the method sensitivities and; (4) to briefly check for applicability on fish sample extracts. It is worth noting here that the background contamination is a major problem in the analysis of OPEs,[7] so that some precautions need to be taken prior to or during their analysis.

Experimental

Chemicals

Eighteen compounds were studied, among them six alkyl phosphates [triethyl (TEP), tri-*n*-propyl (TPrP), tri-*n*-butyl (TnBP), tri-*i*-butyl (TiBP), TBEP, tri(2-ethylhexyl) (TEHP)], seven aryl phosphates [triphenyl (TPP), 2-ethylhexyl diphenyl (EHDP), dibutyl phenyl (DBPhP), diphenyl butyl (DPhBP), tri-o,m,p-cresyl (o-TCP, m-TCP, p-TCP)], three chlorine-containing phosphates [tris(2-chloroethyl) (TCEP), TDCIPP, tri(chloropropyl) (TCPP)], and two bromine-containing phosphates [tris(2,3-dibromopropyl) (TDBPP) and TTBNPP]. Seven compounds were used as internal standards (ISs) for the quantification purpose according to the isotopic dilution analysis [d_{15}-triethyl (dTEP), d_{21}-tri-*n*-propyl (dTPrP), d_{21}-tri-*n*-butyl (dTnBP), tris(2-butoxy-[$^{13}C_2$]-ethyl) (MTBEP), $^{13}C_{18}$-triphenyl (MTPP), d_{12}-tris(2-chloroethyl) (dTCEP), and d_{15}-tris(1,3-dichloro-2-propyl) (dTDCPP)]. TEP, TPrP, TnBP, TiBP, TBEP, TEHP, TPP, EHDPP, TCEP, TCPP, TDCIPP, dTEP, dTPrP, dTBP, MTBEP, MTPP, dTCEP, and dTDCPP were obtained from Wellington Laboratories (Guelph, Ontario, Canada). DBPhP, DPhBP, o-TCP, m-TCP, p-TCP, and TDBPP were purchased from Chiron Laboratories (Trondheim, Norway) and TTBNPP from AccuStandard Inc. (New Haven, CT, USA). Table S1 in supporting information contains the list of these 18 OPEs, by classifying them into four groups (A for alkyl, B for aryl, C for chlorinated, and D for brominated compounds), along with the seven IS compounds as well as some of their physicochemical properties. For OPEs without isotopologue IS, the one from the same subgroup and with the closest retention time was attributed. Working solutions were prepared in toluene (Picograde®, LGC standards GmbH, Wesel, Germany). From individual solutions prepared at 10 $\mu g\,l^{-1}$, three mixtures (halogenated, non-halogenated, and IS) were prepared at 1 $\mu g\,l^{-1}$. All the solutions were stored at 4 °C until further use.

A set of serially diluted standard solutions of target compounds (from 500 down to 2 $\mu g\,l^{-1}$) and a fixed concentration of ISs (50 $\mu g\,l^{-1}$) were used to test the linearity on the selected techniques. IDLs were defined as the lowest detection concentration level where the signal to noise (peak to peak) was ≥3.

Instrumentation and analytical methods

Two GC systems were employed, a Scion™ 436-GC from Bruker (Billerica, MA, USA) and a 7890-A from Agilent (Santa Clara, CA, USA), on which Helium (>99.99%) was used as the carrier gas. Injection was performed in splitless mode, with 1 µl as injection volume and 295 °C as injection temperature. Two capillary columns were used: DB-5MS (Agilent) and ZB-5HT (Phenomenex, Torrance, CA, USA).

Two coupled mass spectrometers were used: a Scion TQ-MS from Bruker operating in the EI, PCI, and NCI modes with methane (99.995%, Messer Group GmbH, Germany) as the reagent gas and a Xevo TQ-S from Waters (Milford, MA, USA) operating in APCI mode on which nitrogen (>99.999%) was used as sheath gas and auxiliary gas. The temperature of the source was set at 250 and 150 °C and that of the transfer line at 300 and 350 °C, via the EI/CI and the APCI (for the analysis of brominated OPEs, transfer line at 310 and 380 °C), respectively. Electron energy was set at 70 eV by EI mode and corona current at 2 µA by APCI mode.

Glassware treatment

Several authors reported procedural blank contamination as an important issue concerning OPE analysis at trace levels because

these compounds are ubiquitous contaminants in indoor environment and may be present in dust.[7] Glassware (tubes, vials, beakers, and pipettes) was preferred over plastic materials, allowing for removal of adsorbed trace OPEs by baking at 400 °C for 4 h prior to use, excepted for plastic pipette tips that were previously checked.

Results and discussions

In-source fragmentation patterns

The main objective of the work was to select the most suitable ionization strategy for the specific and sensitive mass spectrometric analysis of a set of target OPEs ($n = 18$). For this purpose, the fragmentation patterns and hence the ionization efficiency were investigated in details by GC-MS via EI(+), PCI, and NCI modes compared with the available literature, as well as via APCI(+) mode.

Electron ionization mass spectra

Under EI mode, the fragmentation pattern of the studied compounds was observed as highly affected by their category (alkyl, aryl, or halogenated) as well as the side chains. In almost all cases, extensive fragmentation was observed, leading to intense ions at low *m/z* and dispersion of the signal. Figure S1 illustrates these patterns for four OPEs, representatives of the four studied subgroups (A, B, C, and D).

Regarding the seven alkyl phosphates (group A) and in accordance with previous work,[22,24,25] these undergo three successive McLafferty rearrangements, the base peak being mostly represented by the protonated phosphoric acid $[H_4PO_4]^+$ at *m/z* 99. Exceptions were observed with TBEP, exhibiting a base peak at *m/z* 85 for $[C_5H_9O]^+$, due to the presence of an ether group creating a β cleavage and with TEHP for which the first ion appeared at *m/z* 113 for $[C_8H_{17}]^+$ from the side chain. However, these ions were not considered specific enough to be further used in selected reaction monitoring (SRM) mode. Therefore, ions of higher *m/z* but lower intensities were selected (e.g. $[M–R + 2H]^+$, $[M–2R + 3H]^+$).

Regarding the seven aryl phosphates (group B) and in accordance with the previous work on four compounds,[22,24,25] the base peak was not always the same, in some case quasi-molecular ion $[M]^{+\bullet}$ (TPP, m-TCP, and p-TCP), $[M–R]^+$ (EHDP), $[M–2R]^+$ (DBPhP), the phenol radical cation $[C_6H_5OH]^{+\bullet}$ (DPhBP), or $[(C_6H_4)_2CH]^+$ (o-TCP).

Regarding the three chlorinated phosphates (group C) and in accordance with the previous work,[22,24,25] the presence of electronegative atoms appeared to change the profile of the mass spectra. The base peaks were $[M–Cl]^+$ for TCEP, $[M–2R–CH_2Cl + 2H]^+$ for TCPP, or $[C_3H_4Cl]^+$ for TCDIPP.

Regarding the two brominated phosphates (group D) and in accordance with the previous work available for TDBPP only,[22,23] $[M]^{+\bullet}$ was not detected. These compounds are considered as special members of OPEs due to their high molecular masses (697.6 and 1017.3 g mol^{-1}) along with log partitioning coefficient values (k_{ow}) of 3.71 and 9.03, respectively. Extensive fragmentation was observed, leading to base peaks at *m/z* 137 for $[C_3H_6PO_4]^+$ and *m/z* 145 for $[C_5H_6Br]^+$, for TDBPP and TTBNPP, respectively. The protonated phosphoric acid at *m/z* 99 was present for both compounds. For TTBNPP, the most specific ion resulted from the loss of one side chain at *m/z* 712.6, showing a six Br isotope pattern. Other abundant ions included for example the ion at *m/z* 308.7 representing one side chain.

Chemical ionization mass spectra

Chemical ionization was also investigated for the purpose of reducing fragmentation and preserving the molecular ion. Nine OPEs representative from groups A, B, and C were selected for both PCI and NCI. Observations were in accordance with previous work from Ma and Hites[22] for both modes as well as with Quintana *et al.*[26] for PCI. Additionally, the two brominated OPEs from group D were subjected to NCI.

Using PCI mode, the mass spectra of selected alkyl and aryl OPEs were mostly dominated by $[M + H]^+$. Exceptions occurred for TEHP and EHDP with base peaks at *m/z* 111 and *m/z* 251 corresponding to an ethylhexyl and the loss of an ethylhexyl side chain, respectively. For the three chlorinated OPEs, the $[M + H]^+$ ion was observed but present as the base peak only for TCEP. Indeed, regarding TCPP and TDCPP, the base peaks corresponded to relatively unspecific ions at *m/z* 77 and *m/z* 75 for $[C_3H_6Cl]^+$ and $[C_3H_4Cl]^+$, respectively. The analysis of the two brominated OPEs was not performed on PCI due to the high electron affinity for the bromine atom of 324 kJ mol^{-1}, favoring the production of stable negative ions through the attachment of an electron via the negative CI mode.

Using NCI mode, the mass spectra of alkyl and aryl OPEs presented most of the time the ion *m/z* 127 for $[C_2H_8PO_4]^-$, as well as $[M–R]^-$ ion, but in addition, these compounds also showed abundant $[M–H]^-$ ion. Regarding the three chlorinated OPEs, the base peaks resulted from the loss of a side chain $[M–R]^-$. It is expected that $[Cl]^-$ halogen ions, which were out of scan range, were also intense. Indeed, regarding the two brominated OPEs, $[Br]^-$ was the base peak. Fewer fragments were observed in their mass spectra compared with EI mode (e.g. $[Br_2]^{-\bullet}$, $[M–Br]^-$, and $[M–R]^-$), but similarly to chlorinated OPEs, quasi-molecular ion was not observed.

By comparing groups A, B, and C via the three tested modes (EI, PCI, NCI) on the same instrument, intensities seemed to be higher in EI mode for all the tested compounds, except for TDCIPP that showed higher intensity via NCI mode. For group D and in terms of peak response area, NCI showed importantly higher areas than that observed via EI, which is attributed to the high abundance of $[Br]^-$ that is, in turn, not specific enough and not preferred to be used as precursor ion in SRM transitions.

Atmospheric pressure chemical ionization mass spectra

The results from the comparison of EI and CI under vacuum showed that it was necessary to look for another soft technique. For that, APCI was applied to investigate its effectiveness assuming that it is the first work to report the analysis of such a wide range of OPEs via this technique on positive mode.

The two primary mechanisms of APCI were investigated, i.e. (1) the charge transfer, initiated by corona discharge ionization of the present nitrogen to generate nitrogen cations that then undergo charge transfer with the target molecules, and (2) the proton transfer that can be favored by the addition of a protic modifier (e.g. water, methanol).

Charge transfer was investigated under dry conditions. Despite ultrahigh nitrogen quality with efficient nitrogen filters to trap residual water, few analytes showed the presence of $[M]^{+\bullet}$, as illustrated in Fig. 1. Most of them exhibited fairly intense $[M + H]^+$ ions that was attributed to uncontrolled traces of protic donors. Unexpectedly, $[M + 30]^+$ ion were observed for all and only trialkylated OPEs (groups A, C, and D, except TBEP) at relative intensities to the base peak ranging from 16% for TTBNPP to 66% for TnBP. These ions were hypothesized as $[M + NO]^+$ nitrosyl adducts. According to

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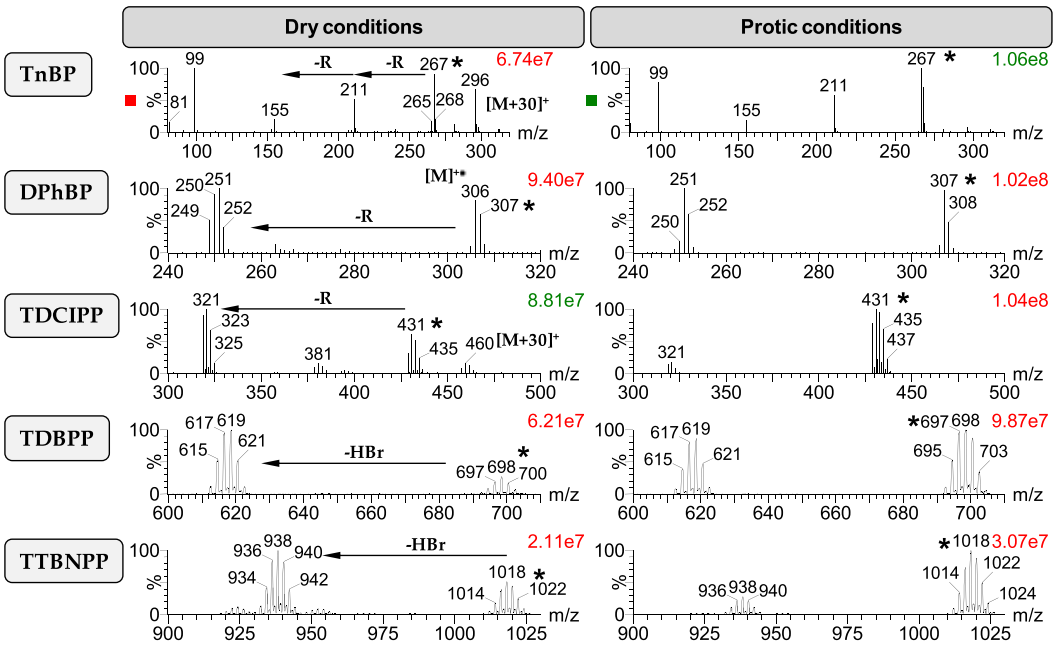

Figure 1. Full-scan mass spectra obtained by positive atmospheric pressure chemical ionization in dry *versus* protic conditions for selected alkyl (TnBP), aryl (DPhBP), chlorinated (TCDIPP), and brominated (TDBPP, TTBNPP) organophosphate esters. The asterisk indicates $[M+H]^+$ ion.

Sabo and Matejcik,[27] several processes leading to NO^+ ions in corona discharge APCI source exist, necessitating only traces of O_2 or NO. It was anticipated that uncontrolled variation of such traces could impact adduct formation and then selected signals for quantitative approach.

Protic conditions were then promoted by placing a mixture of MeOH/H_2O 1:1 (v/v) in the source in a vial with a capillary introduced through the septum.[21] Consequently, $[M+30]^+$ adducts disappeared, and the relative abundance of $[M+H]^+$ was increased, confirming the enhancement of proton transfer mechanism. In such APCI mode via favored protonation mechanism, the in-source fragmentation patterns were quite clear for all the compounds. The $[M+H]^+$ quasi-molecular ion was always preserved (excepted for EHDP) and highly intense if not the base peak (Fig. 2). Regarding the six alkyl OPEs, the mass spectra also exhibited intense $[H_4PO_4]^+$ ions (expected for TBEP) and minor losses of alkyl chains such as $[M–R]^+$ and $[M–2R]^+$ ions. Regarding the aryl OPEs, the $[M+H]^+$ was the only observed ion for TCP isomers and TPP. For EHDP, DBPhP, and DPhBP, the spectra also exhibited dominating losses of butyl or ethylhexyl alkyl chains, the $[M–R]^+$ being the only observed ion for EHDP. Regarding the five halogenated OPEs, the spectra were dominated by $[M+H]^+$ as the base peak. Other observed specific ions were $[M–R]^+$ for chlorinated and $[M–Br]^+$ for brominated OPEs.

Selection of ionization technique

The choice of the ionization technique depends on different factors that are related, on one hand, to the nature of the compound itself (e.g. polarity, molecular mass, thermal stability, etc.) and, on the other hand, the applied technique (e.g. fragmentation pattern, ionization efficiency). Table S1 summarizes the results observed from the four tested techniques and compared between the four different studied groups as well as to the available literature. Clearly, under EI conditions, the highly specific molecular ion is often absent due to the extensive fragmentation resulting in the formation of less specific fragment ions. PCI and NCI have considerably fewer fragmentations but with reduced sensitivity in comparison with EI (in terms of peak response area), except for TDCIPP (group C) that responded well by using NCI and for group D (TDBPP and TTBNPP) where the presence of bromine atoms favors the detection of high abundant $[Br]^{-\bullet}$ via NCI mode. In the opposite, with positive APCI under protic conditions, the quasi-molecular ions were largely preserved, and by using them as precursor ions, the derived SRM method is expected to exhibit a higher specificity. Despite that APCI is a soft ionization technique, still a number of fragments also observed with EI were obtained (e.g. $[M–R]^+$ for alkylated OPEs, $[M–X]^+$ for brominated OPEs). On one hand, EI is the mostly used ionization source for GC-MS analysis, and on the other hand, compared with EI where the precursor ion is most often a fragment ion, APCI offers the advantage of possible use of the protonated molecule as a precursor ion for the SRM transitions. Hence, it appeared interesting to select the two techniques for further development and optimization of spectrometric and chromatographic conditions on GC-MS/MS. The final selection between these two techniques will subsequently depend mainly on their comparison in terms of sensitivity upon the trace analysis of OPEs in biological matrices, noting that better results are expected via APCI mode due to higher expected selectivity of the selected transitions.

Optimized instrumental methods on GC-(EI/APCI)-MS/MS

Optimized GC separation

In this work, the 18 studied OPEs present a broad mass range from 183 to 1018 g mol^{-1}, which means that it is not an easy task to find a compromise for an efficient separation in one method. Figure 3 shows the ion chromatograms obtained by GC-APCI-MS/MS for the 18 OPEs with the optimized chromatographic separation.

For the separation of the 16 OPEs from groups A, B, and C, a DB-5MS capillary column (30 m × 0.25 mm, 0.25 μm) was used. The optimized temperature program was as follows: initial temperature

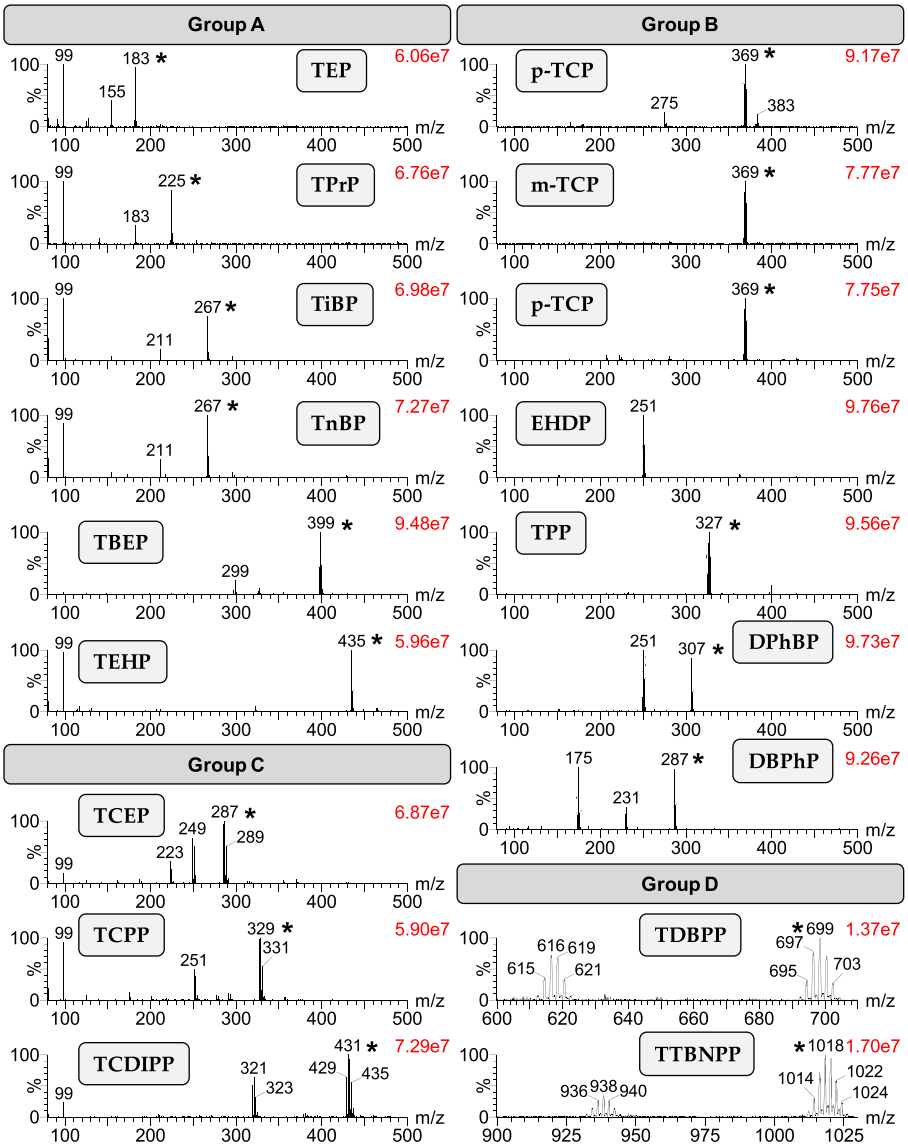

Figure 2. Full-scan mass spectra obtained by positive atmospheric pressure chemical ionization for alkyl (A), aryl (B), chlorinated (C), and brominated (D) organophosphate esters. The asterisk indicates $[M+H]^+$ ion.

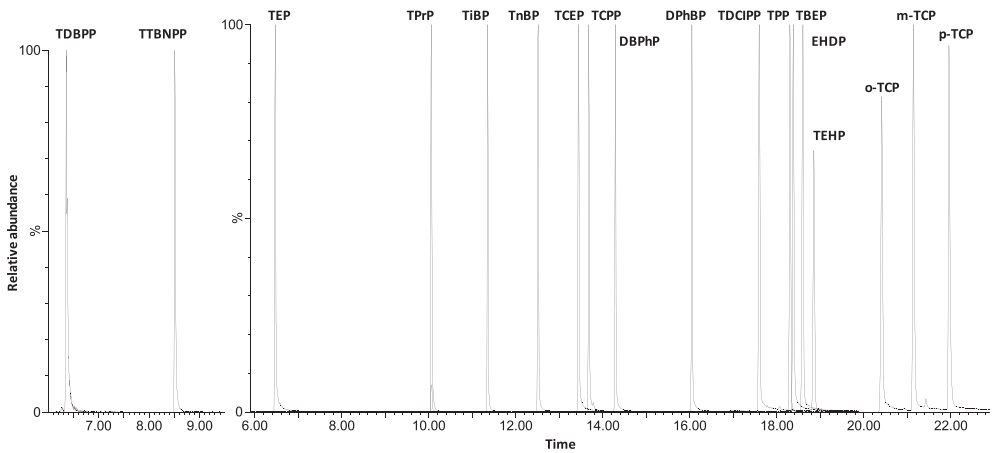

Figure 3. Overlaid ion chromatograms obtained by GC-APCI-MS/MS for the 18 organophosphate esters on the 15 m (left) and 30 m (right) long columns.

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at 85 °C for 5 min, ramped to 240 °C with a rate of 15 °C min^{-1}, to 255 °C at 3 °C min^{-1}, then to 300 °C at 20 °C min^{-1}, and finally held for 5 min. The total run time was 27.58 min. The initial temperature and initial hold time were chosen in order to allow the detection of the most volatile compounds such as TEP and TPrP. It is worth noting that a tailing was always obtained for the first eluting compound (TEP), which can be explained by inappropriate film thickness for such a relatively volatile compound or a polarity mismatch with the stationary phase. The further ramp of temperature was optimized in order to obtain a satisfying separation while keeping a good separation between TPP, TEHP, TBEP, and EHDP, which showed co-elution issues. The carrier gas flow rate was constant at 3 ml min^{-1} by APCI and 1 ml min^{-1} by EI in order to remain compatible with the flow rate requirements due to limitations of vacuum source system.

Brominated OPEs (group D), with large molecular weights and low volatility, have the tendency to pass a longer time through the column, due to their high boiling point, resulting in more band broadening. To resolve the efficiency problem for these two particular OPEs through minimizing their longitudinal diffusion, a shorter ZB-5HT capillary column (15 m × 0.25 mm, 0.10 μm) was used. The optimized temperature program was as follows: The initial oven temperature was set as 85 °C for 1 min, ramped to the final temperature at 35 °C min^{-1}, and held for 5 min. The final temperature was set at 350 °C by APCI but only 310 °C by EI due to transfer line limitation to 310 °C on the selected instrument. The total run times were 12.43 and 16.63 min by APCI and EI, respectively. The carrier gas flow rate was constant at 3 ml min^{-1} by APCI and 1.5 ml min^{-1} by EI for similar reasons as previously explained.

Optimization of selected reaction monitoring conditions using electron ionization and atmospheric pressure chemical ionization

Selected reaction monitoring MS/MS sensitivity is dependent upon the appropriate tuning of the instrument parameters, mainly the cone voltage and the collision energy. For the purpose of optimizing these spectrometric conditions, pure individual solutions of the 18 OPEs were first analyzed on the full scan mode in order to select the precursor ions. Various cone voltages (0 to 40 V) were tested in order to select the one yielding the optimal ionization/fragmentation for each compound. After that and to characterize and optimize the fragmentation pathways of the selected precursor ions in the collision cell, various collision energies were tested (10 to 45 eV on EI, 5 to 30 eV on APCI) in order to end up with the optimized SRM method displayed in Table 1. Two transitions of highest intensities were specified to reach four identification points according to EU guidelines for organic environmental contaminants[28,29] noting that the first transition (T1) was used for quantification and the second one (T2) for confirmation purpose.

Evaluation of instrumental performances

A series of seven calibration curves were analyzed on different days both by APCI and EI modes, in order to evaluate stability and linearity of the developed SRM methods. Each curve included six points, ISs being at 50 μg l^{-1} and native compounds from 2 to 500 μg l^{-1}. Relative standard deviations were calculated for the relative response factors of each curve and compared. For most compounds, relative standard deviation (RSD) values were below 24% and mean RSD below 16%. EI exhibited slightly better RSD values for most compounds, compared with APCI. This could be due to lower stability of source conditions at atmospheric pressure. Regarding TPrP, TiBP, and TnBP, APCI RSD values were about two times higher compared with EI, but remained below 22%. Inversely, regarding TEHP, TBEP, TDBPP, and TTBNPP, EI led to unacceptable RSD values, mainly due to much lower sensitivities. TTBNPP remained a quite difficult compound by APCI with RSD values close to 30%. Then, APCI appeared as a suitable compromise for the analysis of all the targeted OPEs in a single run.

Table 1. Optimized selected reaction monitoring parameters for the 18 0rganophosphate esters by GC-MS/MS on both positive electron ionization and atmospheric pressure chemical ionization modes, along with obtained instrumental detection limits

		GC-APCI(+)-MS/MS						GC-EI(+)-MS/MS				
Group	Compound	T1	CE 1	T2	CE 2	CV	IDL	T1	CE	T2	CE 2	IDL
A	TEP	183>99	15	183>155	5	20	1	155>99	10	127>99	10	0.4
	TPrP	225>99	10	225>183	5	20	0.4	141>99	10	183>99	15	0.4
	TnBP	267>99	15	267>155	10	30	0.4	155>99	10	211>99	20	0.4
	TiBP	267>99	15	267>155	10	30	0.4	155>99	10	211>99	10	0.4
	TEHP	435>99	15	435>323	5	30	0.4	113>57	10	113>95	10	10
	TBEP	399>199	15	399>99	25	30	0.4	125>99	10	199>99	10	40
B	TPP	327>77	25	327>125	25	30	0.4	326>215	20	326>169	20	1
	EHDP	251>95	20	363>251	5	40	0.4	251>77	20	251>152	20	1
	DBPhP	287>175	15	287>231	5	20	0.4	175>77	15	175>51	10	1
	DPhBP	307>251	10	251>153	15	30	0.4	251>152	15	306>251	10	2
	o-TCP	369>91	25	369>166	25	40	0.4	368>181	10	165>139	25	2
	m-TCP	369>166	25	369>91	25	40	0.4	368>165	25	368>261	10	1
	p-TCP	369>166	25	369>91	25	40	0.4	368>108	15	368>198	15	1
C	TCEP	285>223	10	287>99	15	30	0.4	249>125	10	249>99	10	1
	TCPP	329>99	15	327>251	5	20	0.4	125>99	10	201>125	10	1
	TDCIPP	431>321	5	321>209	5	30	1	191>75	10	381>159	10	2
D	TDBPP	698.5>99	25	698.5>299	15	30	1	336.8>137	5	216.8>137	5	100
	TTBNPP	1018.4>147	30	1018.4>307	20	30	10	712.5>309	15	712.5>145	15	500

ILD, instrumental detection limits (in pg); CE, collision energy (in eV); CV, cone voltage (in V).

A series of low concentration levels were analyzed in order to determine the IDLs for the 18 OPEs (Table 1), which were defined as the lowest detection limit where signal-to-noise is >3. By EI, the values ranged from 0.4 to 2 $\mu g\,l^{-1}$, except for TEHP, TBEP, TDBPP, and TTBNPP with 10, 40, 100, and 500 $\mu g\,l^{-1}$, respectively, confirming previous observations. By APCI, the values were slightly better and ranged from 0.4 to 1 $\mu g\,l^{-1}$, except for TTBNPP with 10 $\mu g\,l^{-1}$. These results suggest that it would be easier to go down to trace level analysis with the APCI technique. However, it should be pointed out that even though being from fairly recent and comparable generations, two different instruments from two different constructors were used. Indeed, no available single instrument permits EI and APCI for facilitated comparison. Thus, conclusions in terms of sensitivity should be moderated in the light of this fact.

The two methods were then applied to real fish samples in order to verify the absence/presence of detrimental matrix effects. Twenty replicates of 'in-house' fish pool were prepared by spiking the native compounds at 11.5 $ng\,g^{-1}$ fresh weight, extracted by pressurized liquid extraction and purified by gel permeation chromatography. As expected, TBEP, TDBPP, and TTBNPP did not show interpretable results by EI. For the other compounds, by EI and APCI, identification was confirmed by ion ratios of the selected transitions (qualifier/quantifier) being within the established tolerance intervals. The sensitivity was then compared in terms of signal-to-noise ratios, but this seemed to vary from one compound to another. RSD values ($n = 20$) via EI were ≤14%, except for TEP with 29%, and via APCI were ≤22%, except for TCEP, TCPP, TDBPP, and TTBNPP with 27, 27, 32, and 43%, respectively.

Conclusion

The following study presents the ability of GC-MS/MS to analyze a wide range of OPEs including alkyl, aryl, and halogenated compounds. For the purpose of comparing and determining which ionization technique to be used, we investigated the fragmentation patterns of 18 OPEs via different ionization modes (EI, CI, and APCI). EI and APCI provided good sensitivities for the further quantitative measurements of these compounds, but in the case of EI, the good sensitivity came at a cost of decreased selectivity due to the abundant presence of $[H_4PO_4]^+$ at *m/z* 99, especially in the case of alkyl OPEs. APCI is a soft ionization technique that produces abundant $[M+H]^+$ ions for the 18 OPEs, making it possible to generate specific and sensitive SRM transitions. These observations allowed us to develop instrumental methods via both GC-EI-MS/MS and GC-APCI-MS/MS through the optimization of the chromatographic and spectrometric conditions (in positive mode). For almost all the studied compounds, IDLs achieved were lower in APCI mode than in EI mode, up to 50 times for TTBNPP and 100 times for TBEP and TDBPP. The application of the method to a number of fish sample replicates showed that the transitions via APCI mode were more specific than those via EI mode. However, the comparison of method sensitivity for each compound seemed to depend on the compound itself, and repeatability was generally lower by using APCI. To the best of our knowledge, it is the first study to report the analysis of these OPEs via APCI technique and, more particularly, the first work to demonstrate the analysis of TTBNPP on GC system. As a perspective, the next objective will be to optimize the sample preparation method for the purpose of comparing the two selected techniques in terms of their sensitivity and their ability to deal with matrix effect issues.

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Supporting information

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APCI as an innovative ionization mode compared to EI and CI for the analysis of a large range of organophosphate esters using GC-MS/MS

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Table S1: List of the 18 studied OPEs, classified in 4 groups, along with some of their basic physicochemical properties [3,5] as well as comparison of different ionization modes in terms of observed fragment ions (m/z) in the present study and the available literature. IS: internal standard; MW: molecular weight; Bp: boiling point at 760 mm Hg; Kow: octanol-water partitioning coefficient

Figure S1. Full scan mass spectra obtained in EI mode for alkyl (A), aryl (B), chlorinated (C) and brominated (D) example OPEs. *: theoretical mass of the most abundant ion in the molecular isotopic cluster.

Table S1: List of the 18 studied OPEs, classified in 4 groups, along with some of their basic physicochemical properties [3,5] as well as comparison of different ionization modes in terms of observed fragment ions (m/z) in the present study and the available literature. IS: internal standard; MW: molecular weight; Bp: boiling point at 760 mm Hg; K_{OW}: octanol-water partitioning coefficient.

Group	Compound	IS	MW ($g.mol^{-1}$)	Bp (°C)	Log K_{OW}	EI mode		NCI mode		PCI mode		APCI mode	
						Base peak, **Quantifier**, Qualifier ions	**Literature (Quantifier**, Qualifier ions)	*Base peak*, **Quantifier**, Qualifier ions	**Literature (Quantifier**, Qualifier ions)	*Base peak*, **Quantifier**, Qualifier ions	**Literature**	*Base peak*, **Quantifier**, Qualifier ions	**Literature**
A	TEP	dTEP	182.1	216	1.08	*99,* **155**, 127	**155**, 99 [24,25]	-	-	-	-	*99*, **183**	-
	TPrP	dTPrP	224.1	254	1.87	*99,* **141**, 183	**183**,99 [24,25]	127	-	*225,* 183, 99	-	*99*, **225**	-
	TnBP	dTnBP	266.1	289	4	*99,* **155**, 211	**211**,155 [22,24-25]	*127,* 249	209 [22]	*267,* 211	267 [22,26]	*99*, **267**	-
	TiBP		266.1	264	3.60	*99,* **155**, 211	**211**, 155 [24,25]	-	-	-	267 [26]	*99*, **267**	-
	TEHP		434.3	220	4.22	*99,* **113**	**99** [22]	*127,* 321	321, 305 [22]	*111,* 435, 211	435 [22,26]	*99*, **435**	-
	TBEP	MTBEP	398.4	414	3.75	*85*, **125**, 299, 199	**299**, 199 [22,24,25]	*127,* 235	297, 291 [22]	*399,* 299	399 [22,26]	***399***, 299	-
B	TPP	MTPP	326.1	370	4.59	***326***, 169, 77	**326**, 325 [22,24,25]	*249,* 325		*327,* 95	327 [22,26]	***327***	-
	EHDP		362.1	421	6.64	***251***, 169, 94	**251**, 250 [25]	*285,* 127	-	*251,* 363, 111	251 [26]	***251***	-
	DBPhP		286.3	333	4.08	***175***	-	-	-	-	-	*175*, **287**	-
	DPhBP		306.2	368	4.41	*94,* **251**, 306	-	-	-	-	-	*251*, **307**	-
	o-TCP		368.3	410	5.48	*165*, **368**, 91	**368**, 367 [24,25]	-	277 [22]	-	369 [22,26]	***369***	-
	m-TCP		368.3	442	6.34	***368,*** 165, 91	**368**, 367 [24,25]	-	-	-	-	***369***	-
	p-TCP		368.3	439	5.11	***368,*** 165, 91	**368**, 367 [24,25]	-	277 [22]	-	369 [22]	***369***	-
C	TCEP	dTCEP	285.9	351	1.47	***249,*** 143, 99	**249**, 251 [22,24,25]	*221,* 127	221 [22]	*285,* 249	285 [22]	***287,*** 249, 99	-
	TCPP		327.9	359	2.59	***125,*** 201, 99	**277**, 279 [22,24,25]	*249,* 127	249 [22]	*327,* 251	327 [22]	*99*, **329**, 251	-
	TDCIPP	dTDCPP	429.8	457	3.27	*75,* **191**, 99, 381	**381**, 379 [22,24,25]	*317*	317 [22]	*321,* 431, 75	429 [22]	***431***, 321, 99	-
D	TDBPP	dTDCPP	697.6	544	3.71	*137,* **337**, 99, 121, 217	**201**, 119 [22,23]	*79,* 496.7, 616.7	**487**, 79, 617, [22,24-25]	-	83 [22]	**698.5**, 616.5	-
	TTBNPP		1017.3	595	7.55	*145,* **711**, 99, 309	-	*79,* 160, 710.7, 938.8	-	-	-	**1018**, 938	-

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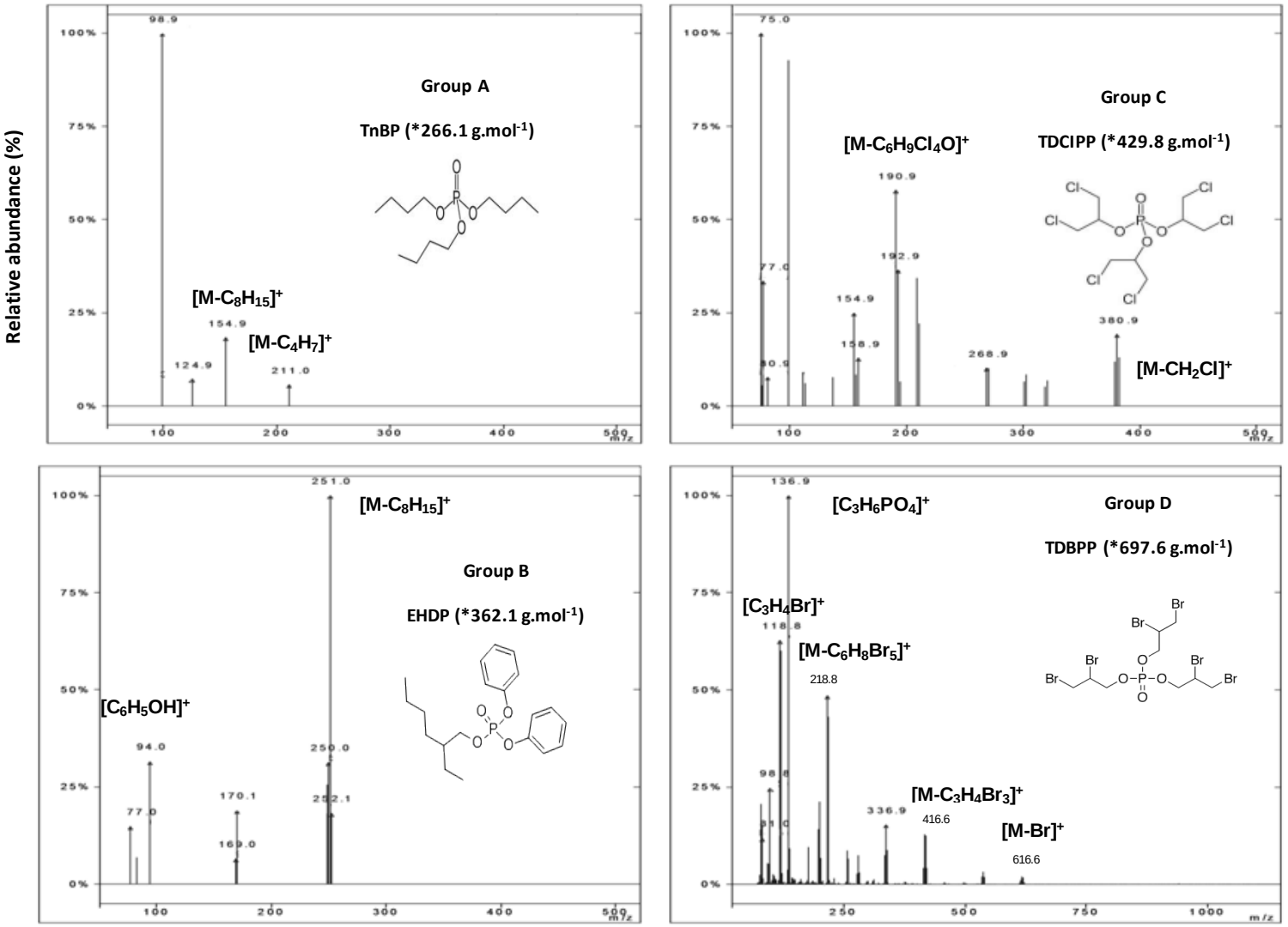


Figure S1: Full scan mass spectra obtained in EI mode for alkyl (A), aryl (B), chlorinated (C) and brominated (D) example OPEs. *: theoretical mass of the most abundant ion in the molecular isotopic cluster.

CHAPTER THREE

SAMPLE HANDLING STRATEGY

3. SAMPLE HANDLING STRATEGY

3.1. **INTRODUCTION**

The sample preparation is considered as very important step and often referred as bottleneck of chemical analysis. It has a great effect on the performances and the reliability of the analytical method.

As stated in the general introduction, one of our objectives was to develop an analytical strategy dedicated to the extraction of OPEs prior to their analysis *via* the investigated and developed instrumental methods described in Chapter 2. The development of the analytical strategy was performed on standard solutions as a first step and then on fish matrix in which we are interested as being a major contamination source to human through the ingestion route.

From here, as other researchers, we are interested in extracting the targeted contaminants with the lowest possible amount of interferences. Indeed, interferences from lipids for measuring trace residues in such fatty and complex biological matrices can have negative influence on the analyte sensitivity as well as the robustness of the obtained results. These interferences can also have negative impacts on the instrument (*e.g.* decreasing the life time of the analytical column and the lipid deposition on the MS source). To achieve required performance characteristics, cleanup techniques are commonly employed for their removal. Therefore, the analysis protocol involves two main stages: The isolation of the OPEs from the matrix and the analytical method for their determination. Sample handling, which involves both the extraction of the OPEs and the purification of the sample extract obtained, still remains as the bottleneck of the entire procedure, despite much progress on automation has been accomplished. The need for lipid removal through purification step(s) is well understood but unfortunately the methods often sacrifice analyte recovery.

The following chapter describes first the challenge in the investigation of different purification techniques allowing maximal lipid elimination while retaining the targeted OPEs. As a pre-treatment step of fish samples, we decided to perform freeze-drying, a technique highly applied in the laboratory, noticeably for the solid matrices in order to have access to the dry matter. This technique enhances the stability and the conservation of samples, ensures a better homogeneity of samples and improves the extractability of analytes because of the increased surface area of the samples as well as the enhanced solvation by the extraction solvent.

Various purification techniques were tested first on standard solutions and then applied to fish. The second part sheds the light on the chosen extraction strategy, the Pressurized Liquid Extraction (PLE). The novelty of this part of the work was the exploration of in-cell PLE or selective PLE (SPLE) procedure for the simultaneous extraction and lipid purification, using Florisil® as lipid removal adsorbent. It is worth noting that the analysis of results was performed using the developed and retained methods by GC *via* EI and APCI modes as described in Chapter 2.

Finally, by the end of this chapter, we will be able to conclude on the retained purification technique to follow the extraction step, but also on the workflow and the overall developed protocol for the OPEs extraction and lipids purification from biological matrices like fish.

3.2. **INVESTIGATED PURIFICATION STEPS**

The purification step, as mentioned before, is a fundamental step in the analysis. It is the challenge to remove the undesirable compounds while searching for our targeted ones at trace levels. As illustrated in Chapter 1, a number of purification techniques were reported while analysing OPEs in biotic compartments like fish. The investigated techniques included Liquid Liquid Partitioning (LLE), Solid Phase Extraction (SPE) and Gel Permeation Chromatography (GPC). These are the mostly applied techniques and appeared to be the most relevant to our objectives. Therefore, we decided to investigate these three strategies.

3.2.1. LIQUID LIQUID PARTITIONING

As being a simple and rapid technique of preparation, liquid-liquid partitioning between acetonitrile (ACN) and *n*-hexane (*n*-Hex) was investigated on OPE standard solutions. This solvent mixture was selected, expecting that the *n*-Hex layer would contain the lipids while the OPEs would be partitioned into the ACN layer. For this purpose, a mixture of the 18 OPEs (50 ng each) was added to a mixture of 10 mL ACN/n-Hex 1:1, (v/v). After equilibration, solvent layers were separated, reconstituted in toluene and analysed by GC-EI-MS/MS. The results are displayed in Figure 3-1, where the recoveries were calculated based on pure ACN reference solution (n=3) not partitioned with *n*-Hex solvent.

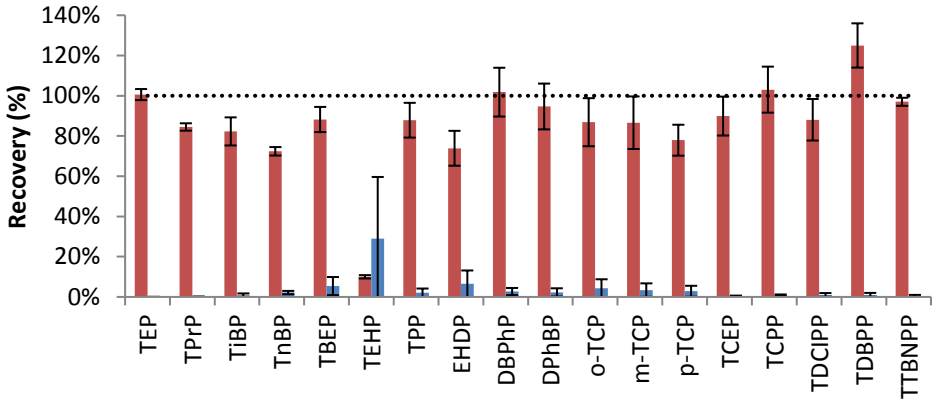


Figure 3-1: Recoveries (%) obtained for 18 OPEs after LLE between ACN (red) and n-Hex (blue) (n=3) as analysed by GC-EI-MS/MS.

As shown in Figure 3-1, the recoveries for most OPEs were satisfactory and the values were in the range 70-125% with RSD (n=2) ≤13%, except for TEHP, with a recovery lower than 20%, which might be due to the high log Kow value of 10 for this compound

As a conclusion, the use of LLE showed good recoveries for most targeted OPEs **except for TEHP**. Since LLE was not commonly for the analysis of OPEs, we were interested then, in investigateingother techniques mentioned in the literature (*e.g.* SPE, GPC,...) that might be suitable for all the targeted OPEs and powerful for lipid removal in the same time.

3.2.2. SOLID PHASE EXTRACTION

3.2.2.1. **Behaviour of pure analytical standards**

According to the literature overview as described in Chapter 1, silica gel and Florisil® in their activated and deactivated forms were the most employed. These adsorbents were investigated as well as other form (acidification). For each assay, the general preparation protocol involved an amount of 6 g of adsorbent phase to be tested packed between glass wool and ~1 g of sodium sulphate (added to the bottom and the top layers of the adsorbent) as dehydrating agent in a glass column of dimensions 300 mm length x 10 mm internal.diameter,. For each condition, 3 columns were always prepared, two of them being considered as replicates and one as solvent blank. Conditioning of the column was obtained using 50 mL of n-Hex. Then, a mixture of native OPEs (50 ng each, except the brominated OPEs which were not available at that moment) and a mixture of internal standards (50 ng each) were loaded on the column. For elution, 50 mL of different solvents were employed in order to select the one yielding the best results in terms of compounds elution. In parallel, a standard reference tube

containing 50 ng of each OPE along with 50 ng of each internal standards was always introduced into the series of samples for the purpose of calculating the recoveries. The samples were then reconstituted in ~50 µL of toluene prior to the analysis.

➢ Silica gel

Silica gel can be used as a very successful adsorbing agent, as it does not swell or strain, has good mechanical strength and can undergo heat treatment. The surface of silica particles is heterogeneous, with a variety of different types of silanol groups. The described protocol was applied on silica gel column with which four different elution solvents were tested: *n*-Hex, dichloromethane (DCM), ethyl acetate (EtAc) and a mixture of *n*-Hex/DCM 1:1 (v/v). These solvents were selected according to the literature related to the analysis of OPEs in biota as well as the values reflecting the elutropic strength on silica (0.01 for n-Hex, 0.30 for DCM and 0.48 for EtAc). After eluting fractions from the different solvents, the analysis was performed by GC-EI-MS/MS.

Results were expressed in terms of compounds' recoveries (%) based on the standard reference sample (as described in the beginning of this section). As can be observed in Figure 3-2, EtAc provided the best elution pattern for all of the compounds. The recoveries obtained for the 16 selected compounds (excluding the two brominated ones) ranged between 50 and 117% with RSDs ≤20 % (n=2 replicates). Two exceptions were observed for TBEP (2% recovery) and for TCEP (45%RSD). Besides, only TPP appeared to be eluted with all types of solvents except *n*-Hex. With *n*-Hex or *n*-Hex/DCM 1:1 (v/v), no compound was eluted except for TPP with a recovery of 118% however with RSD of 58%. In the same trend, some compounds could be eluted with DCM, however with recoveries ranging between 8-76% and RSD ≤ 32%.

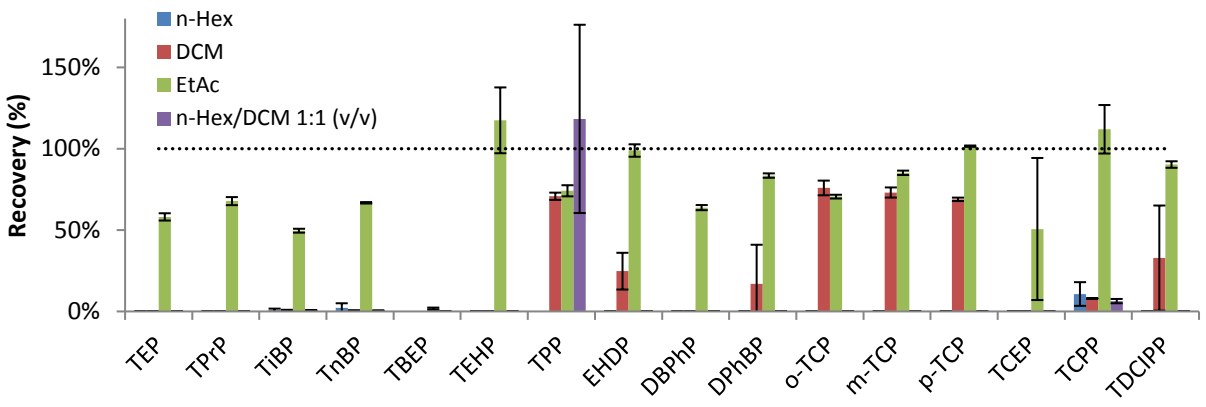


Figure 3-2: Recoveries (%) obtained for 16 OPEs on activated silica gel using various elution solvents (n=2) as analysed by GC-EI-MS/MS.

As a conclusion, EtAc was selected as the elution solvent with silica gel as lipid sorbent, yielding best compounds recoveries compared to other tested solvents. However, this conclusion is not to be generalised for the other sorbent types so that the elutropic strength of solvent can differ from one sorbent to another.

- **Acidified silica gel**

The use of sulfuric acid on silica gel in certain fields showed to be highly effective for lipids removal (Hovander *et al.,* 2000). Based on the results from the previous experiment on activated silica gel, both *n*-Hex and *n*-Hex/DCM 1:1 (v/v) were excluded of the list of solvents to be investigated. EtAc and DCM solvents were retained since they were the only solvents to show good elution for all (EtAc) and for some OPEs (DCM). In addition, we were interested to test a mixture of EtAc/acetone 1:1 (v/v) in order to investigate the influence of the presence of a more polar solvent on the elution of compounds. Hence, the tested solvents in this experiment (22% acidified silica gel) were DCM, EtAc and a mixture of EtAc/acetone 1:1 (v/v).

The use of EtAc/acetone mixture on acidified silica gel columns was followed by the direct appearance of dark yellow color resulting maybe from the chemical reaction between acetone and sulfuric acid. Indeed, in the presence of sulfuric acid, acetone could undergo aldol condensation resulting in the formation of mesitylene, a colorless compound tending to be yellow in excess amount of acid. Regarding EtAc, the evaporation of the fraction was much longer than expected, probably due to the formation of acetic acid (boiling point 118 ^{o}C in comparison to 77 ^{o}C for EtAc) as a result of the reaction of EtAc with the sulfuric acid. These fractions were then discarded. Therefore, only the fractions eluted by DCM solvent were analysed, the elution pattern of selected OPEs was compared to the pattern obtained in the previous experiment with the use of activated silica. The recoveries were similar. As a conclusion, using acidified silica gel columns, no elution solvent was found to be efficient for eluting the OPEs, and therefore the choice of using this sorbent was abandoned.

- **3% H_2O deactivated silica gel**

Based on the literature and especially the previous works focusing on the analysis of OPEs in biota, the use of SPE as a purification step is often accompanied with 3% H_2O deactivated silica gel as the adsorbent of choice. The deactivation of silica and Florisil is widely used in the field of solid phase extraction, as an action of controlling the activity of adsorbent. The same elution solvents as in the previous experiment were tested with the deactivated silica gel. Additionally, and based on the

literature, toluene is sometimes employed. Hence, the tested solvents were toluene, DCM, EtAc and EtAc/acetone 1:1 (v/v).

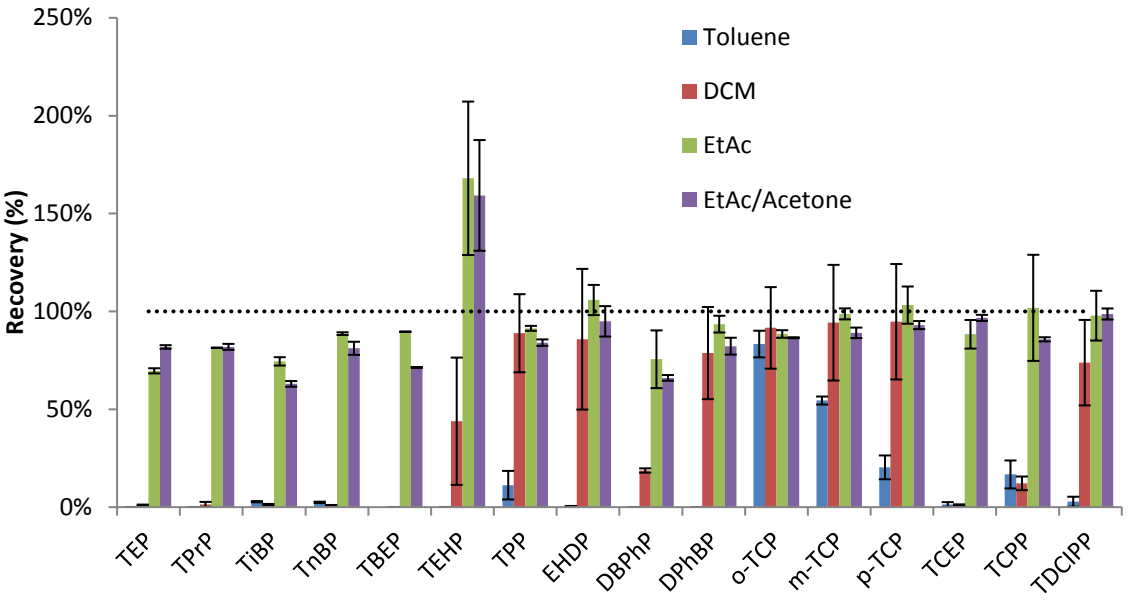


Figure 3-3: Recoveries (%) obtained for the 16 OPEs on 3% H_2O deactivated silica gel, using various elution solvents (n=2) as analysed by GC-EI-MS/MS.

In the trend of comparison and as observed in Figure 3-3, EtAc and EtAc/acetone were the solvents yielding the best results in terms of compounds recoveries (between 66 and 103%) and RSD (≤27%), except for TEHP (168% ± 39%). The recoveries obtained for most compounds on 3% H_2O deactivated silica columns using EtAc as elution solvent were quite similar to those obtained on activated silica column. The silica gels in activated and H_2O deactivated forms were retained for further comparison with Florisil® in activated and H_2O deactivated forms in order to select the adsorbent with best compromise in terms of recoveries and interferences removal.

- **Florisil®**

The elution behavior of targeted OPEs was then tested on Florisil column. According to the literature, the elution solvents chosen in this experiment were n-Hex, toluene and EtAc.

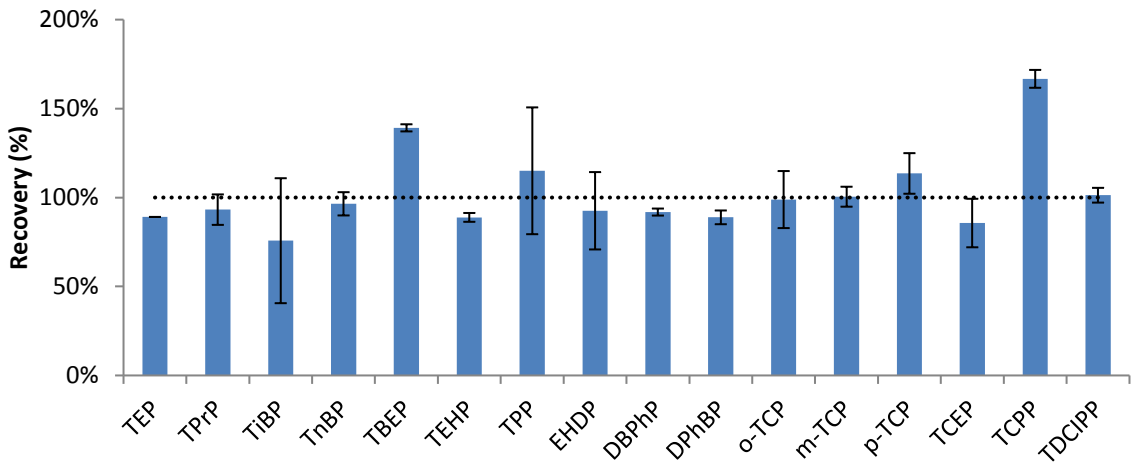


Figure 3-4: Recoveries (%) obtained for the 16 OPEs on Florisil® column, using EtAc as elution solvent (n=2) as analysed by GC-EI-MS/MS.

As illustrated in Figure 3-4, EtAc eluted all the compounds with good recoveries, between 76 and 115% (RSD≤22%). The TEP was lost in one out of the two replicates (which could be during the evaportation step), so that the recovery was reported from only one replicate and that's why the RSD value is not shown on the mentioned Figure. The exceptions in RSD include also TiBP and TPP with 35 and 36%. Other exceptions were TBEP and TCPP (139% ± 2 and 167% ± 5%, respectively). This could be attributed to their presence in the blank sample.

On the contrary, no compound was eluted with n-Hex or with toluene. This can be explained by the eluotropic values of these solvents. Unfortunately, these values are not available yet on Florisil however on silica and alumina, they correspond to 0.01 and 0.2 for n-Hex and toluene, respectively (Synder, 1968)

- **3% H_2O deactivated Florisil®**

The deactivation of Florisil® was also investigated with the addition of 3% H_2O but based on previous results observed with activated Florisil®, only EtAc was tested for the elution of compounds. Again, good recoveries were obtained (76-112%, RSD ≤27%). Exceptions were for TBEP and TCPP (due to their ubiquitous presence in the blank).

To make a clear conclusion on the use of Florisil®, activated and deactivated forms were compared in terms of compounds recoveries with the use of EtAc as elution solvent. No important difference was observed between the results. However recoveries with the use of 3% deactivated Florisil® column appeared to be slightly higher than those observed on activated Florisil column.

➢ Comparison between adsorbents standards with EtAc

By referring to the results obtained from the experiments done with several solvents, it was obvious that EtAc was the best eluting solvent for all compounds of interest yielding the highest recoveries whatever the adsorbent used. This can be interpreted by its important elutropic strength in comparison with other solvents. DCM has also high elutropic strength but it didn't show good recoveries for all the compounds, maybe due to polarity reasons.

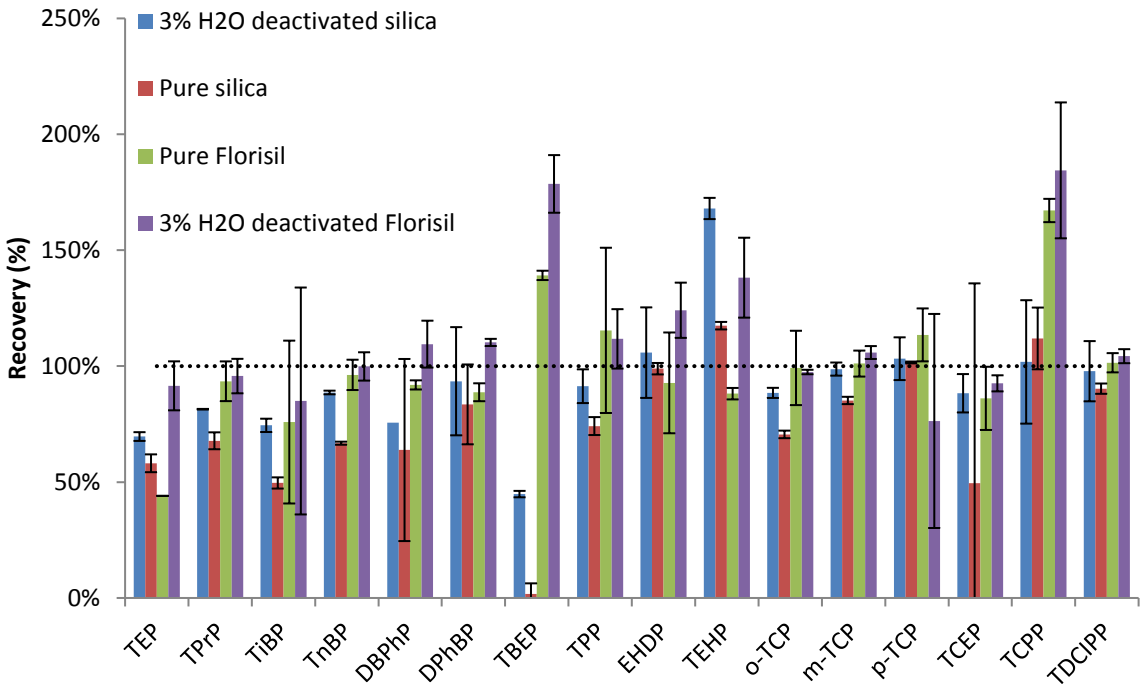


Figure 3-5: Comparison of the recoveries (%) of the 16 OPEs on the four tested sorbents, using EtAc as elution solvent (n=2) as analysed by GC-EI-MS/MS.

Figure 3-5 represents a concluding comparison for the recoveries of 16 OPEs using EtAc as eluting solvent on the investigated sorbents. In general manner, the four investigated sorbents yielded good recoveries. For most targeted OPEs, the use of Florisil® and silica in their deactivated forms (with H_2O) yielded better recoveries than the use of activated forms, except for TPP and p-TCP but which can be attributed in the case of p-TCP to the high RSD value of 35% (n=2).

As a summary on the SPE experiments perfomed on standard OPEs solutions:

- Several elution solvents were tested: Toluene, n-Hex, DCM, EtAc, n-Hex/DCM 1:1 (v/v) and EtAc/Acetone 1:1 (v/v).
- Silica and Florisil® were investigated in different forms; activated, H_2O deactivated and H2SO4 acidifed.
- EtAc exhibited the highest elution strength on all investigated sorbents.
- Acidification of silica yielded poor recoveries for almost all OPEs.
- Silica and Florisil in their deactivated forms (3% H_2O) yielded slightly higher recoveries for most compounds than those obtained with the activated forms.
- Inconvenience of the method was the procedural blank contamination obtained with certain compounds, mainly TCPP and TBEP. It is worth to note here the paper of Brandsma *et al.* in 2013 for the interlaboratory worldwide study, illustrating the TBEP contamination from plastic and rubber materials and which might be used in SPE. TCPP was also previously reported in blank samples using SPE as purification technique (Lu *et al.,* 2014).

In comparison to the literature:

- A number of previous works used deactivated silica gel with 3% H_2O (Ma *et al.*, 2013a) or 5% H_2O (Kim *et al.*, 2011) for cleanup of biological samples or even 10% H_2O (Möller *et al.*, 2011) for cleanup of air samples.
- Besides, some works used Florisil® cartridges for the cleanup of dust samples (Van den Eede *et al.*, 2011; Cristale and Lacorte, 2013).
- Deactivation of Florisil was also described in the literature (Liu *et al.*, 2015) but not as frequently deactivated silica gel.

In comparison to the results from LLE experiments, for the partitioning behavior between n-Hex and ACN, good recoveries could be obtained for most compounds except for TEHP with very low recovery (20%). This issue was surpassed using SPE.

As a first decision at this stage, we retained the SPE but not the LLE for further investigation of the technique. Indeed, the experiments on pure standard solutions are always considered as a first step, allowing us to have an idea about the behavior of targeted compounds by the investigated technique. However, the challenge of lipid removal cannot be investigated without the introduction of real lipids in our experiments and the further investigation of the behavior of OPEs *vs.* fish lipids. This will be our next perspective, noting that fish will be our main matrix of interest on one hand and a good representative for biological on the other hand.

3.2.2.2. **Behaviour of fish lipids**

EtAc has provided high recoveries over a wide polarity range of OPE standards with different physical-chemical properties. However, at the same time we can anticipate that when working with biological matrices, many matrix components can be co-extracted. Food matrices are notoriously complicated because they contain components such as carbohydrates, lipids and proteins, making it even more important that the technique chosen is efficient in extracting the targeted compounds rather than the unwanted ones.

As mentioned in the general introduction as well as in the precedent concluding section from SPE experiments, our objective now is to investigate the behaviour of lipids present in fish but also to maximise as much as possible (nearest to 100%) the isolation of the target OPEs from potential co-extractives like lipids.

- **Washing out the lipids**

According to the literature, diethyl ether (DEE) is sometimes employed in the extraction of lipids (Leonards, 2011). The use of a mixture of DEE/*n*-Hex 15:85 (v/v) was introduced in a washing step prior to the elution step. In our work, 100 mg of fish lipids in hexane (extracted on a Pressurised Liquid Extractor (PLE) system) were loaded on columns containing silica gel in activated or 3% H_2O deactivated forms (n=3) replicates for each assay. The columns were conditioned using 50 mL of n-Hex. This was followed by a step of washing either by n-Hex or by DEE/n-Hex 15:85 (v/v), during which 5 fractions (of 10 mL) were collected. The elution was then accomplished using 50 mL of EtAc.

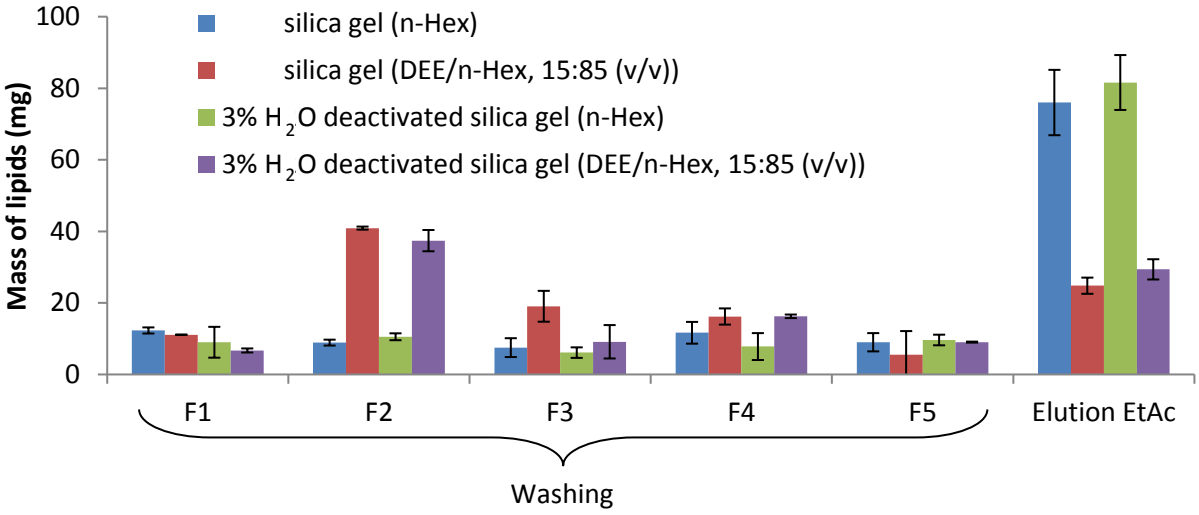


Figure 3-6: Lipid recoveries observed in successive fractions eluted from activated/3% H_2O deactived silica gel with/without DEE in the washing step (n=3).

The objective out of this experiment was to estimate the influence of adding DEE to *n*-Hex in the washing step, on the elimination of lipid. The amounts of lipid were measured on an analytical balance. As illustrated by Figure 3-6, with the use of n-Hex (15% DEE) on both activated silica and 3% H_2O deactivated silica columns, an amount of 87 and 75 mg of lipids, respectively, could be recovered in the total of 5 fractions, while the rest amount was recovered in the elution fraction. The use of activated n-Hex on both activated silica and 3% H_2O deactivated silica columns resulted in the recovery of 43 and 49 mg of lipids, respectively, in the sum of 5 fractions, while the rest amount was recovered in the EtAc fraction.

As a conclusion for this experiment, it is true to say that the use of DEE helped in removing up to 87% of the lipids on 3% H_2O deactivated silica column. However, this might be not sufficient while analysing more fatty matrices which might be the case with highly fatty fish samples, for example Mackerel containing up to 14% total fat content (based on the US FDA Database).

- **Retaining the lipids**

Still in the same course of lipid elimination, but in the manner of retaining lipids while eluting the OPEs, sorbents like Florisil® are well known to be able to retain non-polar lipids. Starting with 100 mg of fish fat (the same as used in the previous experiment) and in both cases of Florisil® and 3% H_2O deactivated Florisil® as sorbents in SPE column for clean up step (n=3). Results showed that in both cases (*i.e.* activated and deactivated forms), only about 55% of initial fat content were retained by the Florisil®, which was not as satisfactory as expected. We concluded that Florisil® sorbent was not highly effective for retaining the lipids.

As a conclusion regarding SPE evaluation as a purification strategy, the experiments performed on standard solutions reflected good results for most targeted OPEs in terms of their elution behaviour with EtAc as elution solvent on SPE columns of silica gel and Florisil®, especially in the deactivated form of these sorbents. Unfortunately, two negative issues were encountered;

- Procedural blank issues for certain OPEs (mainly TCPP and TBEP).
- Results from experiments performed on fish lipids were not highly satisfactory in terms of the lipid removal efficiency.

These results encouraged us to search for other purification techniques like Gel Permeation Chromatography (GPC) and which might be more efficient for lipid elimination.

3.2.3. GEL PERMEATION CHROMATOGRAPHY

Gel permeation chromatography (GPC) is recommended for the elimination of high-molecular weight compounds such as lipids and dispersed and is appropriate for both polar and non-polar analytes (Zuloaga *et al.*, 2012). In our particular field of OPEs, GPC was typically used as a cleanup step in the determination of few OPEs in biological samples and it was already described in several previous related works (Sundkvist et al., 2010; Ma *et al.*, 2013; Kim *et al.*, 2014).

From here, the objective in our work was on one hand to separate our target OPEs from lipids and other low volatile larger non-polar co-extractives in order to finally build up our GPC method. In our work, a high capacity chromatographic column (58 cm length, 24.4 mm diameter) was used. The column was packed with S-X3 polymer Bio-Beads (200-400 mesh) that have been soaked in a mixture of cyclohexane c-Hex/EtAc 1:1 (v/v) overnight. This mixture also constituted the mobile phase with a flow rate set at 5 mL/min.

3.2.3.1. **Behaviour of standards**

Once the GPC column was conditioned, the standard mixture solution of target OPEs was added. A 10-mL fraction was collected every 2-minute up to 70 minutes, evaporated under nitrogen flux (N_2), reconstituted with toluene. For comparison, a fish fat sample (150 mg) extracted by PLE was also injected noting that a maximum of 200 mg can be allowed to be loaded in order to avoid column blocking. The analysis was done by GC-EI-MS/MS.

Figure 3-7 represents the elution profile of targeted OPEs (alkyl, aryl and halogenated OPEs) *vs.* the fish fat. As shown in this mentioned figure, OPEs eluted between 12 and 30 min and the lipids between 8 and 18 min. As illustrated, the alkyl OPEs were excluded in terms of their size from the largest (TEHP) to the smallest (TEP). It was not exactly the same conclusion for the aryl OPEs where the last eluted compound was TPP rather than DBPhP. Interestingly, the brominated OPEs possessing the highest molecular weight were not the compounds exhibiting the highest steric hindrance. Indeed, TPP was the last eluted OPE. Therefore, the end of the collection window was set at 30 min. In order to maximize the lipid removal, the beginning of the collection window was then set at 18 min. However, this choice comes at a cost of major losses for TEHP and some of TBEP.

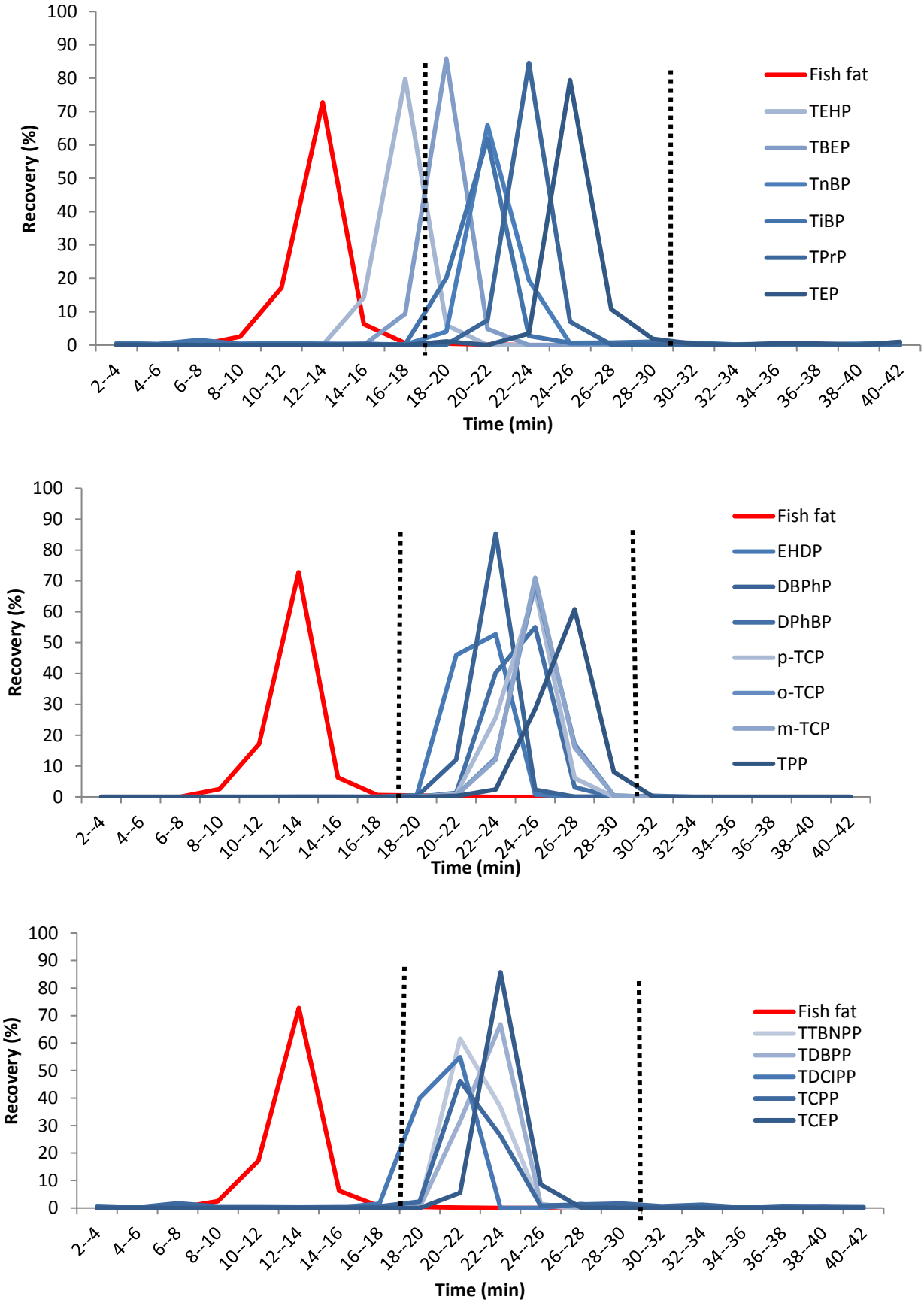


Figure 3-7: Elution pattern obtained by GPC for alkyl (top), aryl (middle) and halogenated (down) OPEs as well as for a fish fat. Slashed lines: selected collection time window.

3.2.3.2. **Selected time window**

In order to evaluate the efficiency of the method developed by GPC and hence to make sure of the defined time window, a mixture of OPEs was loaded with 150 mg of sunflower oil, as representative for lipid interference. Indeed, the calibration procedure in the US EPA Method 3640A on GPC, recommends the use of the corn oil. We used instead the sunflower oil, since the later is more easily found and used in France. In the collected fraction (18-30 min), only ~1 mg of the sunflower oil was recovered. The recoveries were calculated for each OPE of interest and the results are presented in Figure 3-8. According to the hypothesis formulated in the previous paragraph, TEHP was lost and all the other OPEs showed satisfactory recoveries (near to 100%).

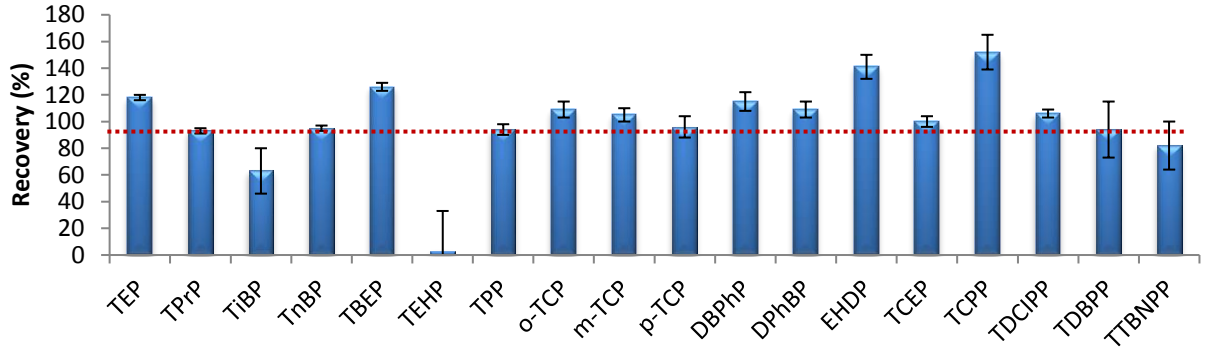


Figure 3-8: Recoveries (%) obtained for the 18 OPEs after GPC purification in the 18 to 30 min fraction (n=3) as analysed *via* GC-EI-MS/MS.

As a conclusion regarding GPC relevance in our research work, the basic aim behind this part was fulfilled by selecting the optimal time window containing all our targeted OPEs, well separated from the possible accompanied lipids in biological matrices like fish. Unfortunately, in our developed method, we had scarified the elution of TEHP (exclusion started too early at 12 min). However and as shown in Chapter 2, this compound has already showed problematic issues in terms of sensitivity and linearity of calibration curve. So, we prioritize eliminating the maximum of lipids (8→ 18 min) over collecting the TEHP.

As an overall conclusion, SPE showed unsatisfactory efficiency for lipid elimination and blank issues mainly with TCPP and TBEP. LLE showed good recoveries but was not tested for lipid depletion efficiency. After investigating different purification techniques, GPC showed to be the most relevant in terms of lipid depletion which is mandatory for the analysis of complex matrices. After doing the method optimisation on fish lipid and sunflower oil, our next perspective would be to evaluate our developed GPC method with selected time window on real fish samples. This will require first to develop and optimize the extraction technique enabling us to obtain the extract to be purified.

3.3. **EXTRACTION TECHNIQUE**

The choice of sample treatment applied depends heavily on the complexity of the matrix. As described in Chapter 1, several extraction techniques were applied for the analysis of OPEs. Based on this literature review and the previously related works, we were interested in the investigation of the most suitable technique for destroying the OPE-matrix interactions and hence their extraction at trace levels.

The main objective (s) can be summarised in three points:

- To investigate the efficiency of QuEChERS simple technique in the extraction of OPEs and the purification of extracts using different lipid sorbents in the dispersive SPE step.
- To investigate of the efficiency of PLE to breakdown the interaction of OPEs with the matrix. In the same issue, to study the influence of solvent nature on the extraction process.
- To investigate the combination of cleanup and extraction step in a selective PLE procedure by introducing the Florisil® into the extraction cell.

3.3.1. QUECHERS-BASED APPROACH

The use of the quick, easy, cheap, effective, rugged and safe (QuEChERS) method for sample preparation was not so frequently mentioned in the literature for the analysis of OPEs. However, we were interested in investigating this technique, expecting advantages especially in terms of less contamination since no extraction system is used. For this purpose, three sorbents for the dispersive (d)-SPE step were tested (n=3): Primary Secondary Amine (PSA), zirconia-based (Z-Sep) sorbent and Enhanced Matrix Removal-Lipid (EMR). The use of PSA was described by Guo *et al.*2016, however the use of Z-Sep and EMR is the first time described in our work. The objective was to investigate and to compare the effectiveness of these lipid sorbents.

The followed protocol consisted of weighing 1 g of lyophilised fish sample into a 50 mL centrifuge tube and spiking with the a mixture of OPEs (50 ng each). This is followed by the addition of 10 mL of acetonitrile (ACN) as extraction solvent as well as the content of QuEAcetate (Ac) tube containing 6 g of $MgSO_4$ and 1.5 g of NaAc. The supernatant layer obtained from centrifugation (3000 rpm, 5 min) was then transferred into a centrifuge tube containing PSA, Z-Sep or EMR-Lipid. Each tube was then centrifuged and the supernatant layer was taken and in the case of EMR-Lipid sorbent, another step was performed with EMR-final polish product. The tubes were then evaporated to be reconstituted in the injection solvent.

The samples were analysed by GC-APCI-MS/MS and were interpreted in terms of recoveries of compounds (Figure 3-9).

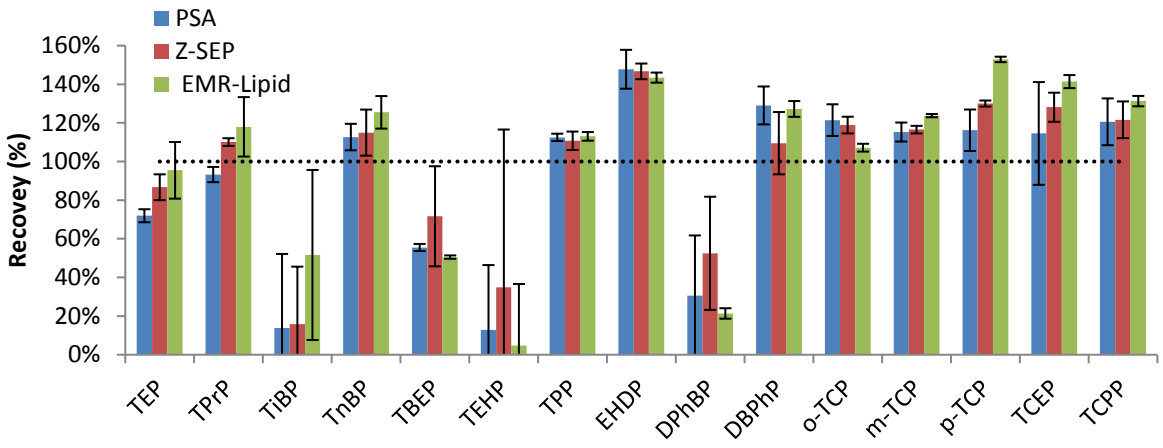


Figure 3-9: Recoveries (%) obtained for selected OPEs after using different purification sorbents.

The analysis was not performed for the two brominated compounds so that we wanted to have an idea for the other wider range of OPEs and to decide later if it would be interesting to see results for the two brominated ones. The Figure presented hereby also excluded the TDCIPP which showed an overestimated recovery issue. Generally speaking, comparable recoveries (near to 100%) were obtained from the use of three sorbents. The exceptions were attributed to the same compounds (TiBP, TBEP, TEHP and DPhBP). In terms of lipid removal efficiency, the best results were obtained with the use of EMR- Lipid sorbent with the capability to remove up to 69 ± 3%, followed by Z-sep sorbent with lipid removal percentage of 53±14% and then the PSA with 48±4%. From here, the results were not highly satisfactory.

As a conclusion regarding the Quechers approach,

- The recoveries were good for most OPEs except for TiBP, TBEP, DPhBP, TEHP and TDCIPP.
- The maximum achieved lipid depletion was 69% upon the use of EMR lipid sorbent, which is still not enough for purification technique in case of high complex and fatty matrices.

The results were compared to the available literature. Guo *et al.* 2016 used QuEChERS with 3 different cleanup sorbent (PSA, GCB and C_{18}). PSA revealed acceptable recoveries between 70.3% and 105.7%.

As a perspective, we were then interested in extending our work to test the PLE as a very well known extraction technique especially for the analysis of OPEs in biological samples. And the novelty added at this time to the technique will be described in the next section.

3.3.2. PRESSURIZED LIQUID EXTRACTION

PLE is the technique mostly employed for the extraction of OPEs from biological matrices like fish (Sundkvist *et al.,* 2010; Gao *et al.*, 2014; Kim *et al.,* 2014) Therefore, this strategy was also tested in the present study. The extraction was performed on a PLE system from Büchi (Flawil, Switzerland), allowing the extraction of 4 samples simultaneously. About 1 g of lyophilised fish sample (whitefish) was loaded in the 40 mL stainless steel extraction cell, spiked with a mixture of 18 OPEs (50 ng each, corresponding to ~12.5 ng/g fw) and capped with two filtration end fittings, which are tightly fitted for high-pressure closure.

Main parameters that influence the extraction efficiency are temperature, extraction time, number of extraction cycles, sorbent type and solvents. The selection of most of these parameters was based on literature (Sundkvist *et al.*, 2010; Gao *et al.*, 2014; Kim *et al.*, 2014a). The temperature was set at 100 ^{o}C in order to get a compromise between the benefit of elevated temperature and the stability of target compounds. The extraction time was set at 5 min static time and 100% dynamic flush. A final nitrogen purge (180 s) was included in order to guarantee the complete removal of the solvent from the PLE system. Pressure was set at 100 bars. Only a parameter was optimised, which is the nature of the solvent so that mixtures were tested and compared in terms of their efficiency to extract maximum compounds but with minimum lipids.

3.3.2.1. **Extraction solvent**

Generally speaking, mixtures of low and highly polar solvents provide more efficient extractions of analytes than single solvents. This can be explained in a way that on one hand the non-polar solvents (*e.g. n*-Hex, DCM) are efficient in fat extraction in which environmental contaminants are also expected to be present and on the other hand, the addition of a percentage of polar solvent can increase the efficiency for target compounds as in the case of OPEs.

Three mixtures were compared in terms of the mass of lipid extracted from fish samples: *n*-Hex, *n*-Hex/acetone 1:1 (v/v) and *c*-Hex/EtAc 1:1 (v/v) (n=3). Results showed that with *n*-Hex/acetone more lipids were extracted (127 ± 30 mg) than in the cases of *c*-Hex/EtAc (71 ± 12 mg) than in the case of pure n-Hex (44 ± 0.6 mg). This could be attributed to the ability of acetone to retain polar lipids (phospholipids and glycolipids), extensively present in fish.

It would be correct to say that in the precedent sections about the investigation of purification techniques, we were interested in the maximal elimination of lipids with maximal retaining of our OPEs of interest. However, we should bear in mind that extracting more lipids means extracting more OPEs.

The fact that fewer lipids are extracted could reflect that less OPEs is extracted. It sounds more logic that the compounds will be more efficiently extracted where the mass of lipid is higher. Only *n*-Hex/acetone and *c*-Hex/EtAc were then retained since they were able to extract higher amount of fatty matter that will carry the OPEs. The comparison between these two solvents mixtures was then done in terms of compounds recoveries. Figure 3-10 shows also the results of comparison, noting that the compounds with analytical contamination issues were excluded (*i.e.* TPP, EHDP and DPhBP). The recoveries for most compounds were satisfactory using the two solvent mixtures, except fot p-TCP which was not well extracted with *n*-Hex/acetone. As a conclusion, the mixture *c*-Hex/EtAc was selected since it showed the ability to extract lower amount of lipids than in the case of *n*-Hex/acetone, while extracting efficiently all the OPEs.

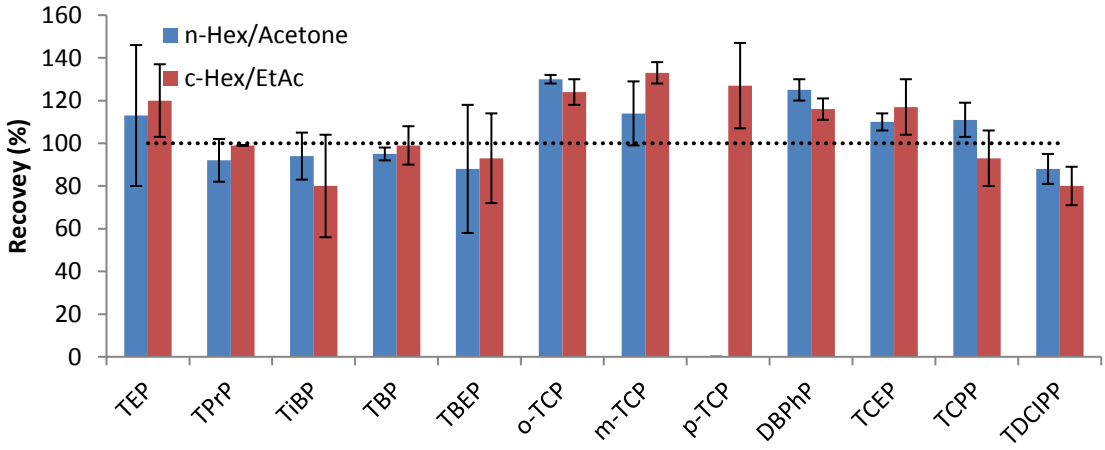


Figure 3-10: Recoveries (%) obtained for 12 OPEs by PLE using two solvent mixtures (n=3).

3.3.2.2. **Selective PLE**

The use of sorbent can serve as in-cell clean-up procedure, known as selective pressurized liquid extraction (SPLE). In our expectation, the addition of in-cell clean up step improves the elimination of interfering substances from the sample extract, which is the key to attain a low limit of quantification and to protect the chromatographic system. Typically, the adsorbent is loaded into the sample cell first (outlet end) and the sample is loaded on top. This way, the flow of solvent during the extraction is such that extracted unwanted compounds are trapped by the adsorbent.

A previous experiment (Vazquez-Roig and Pico, 2015) has illustrated the comparison of the different sorbents for in-cell clean-up in the extraction of persistent pesticides. The selectivity in decreasing order was: Florisil® > acidic alumina > neutral alumina > silica gel > basic alumina > graphitized carbon black. According to the US EPA method 3620C in 2000, Florisil® has been widely employed for in-cell cleanup in OPEs analysis. The main disadvantages that might be considered is that Florisil® might

contribute to the blank contamination. In our work, lipid removal by in-cell introduction (or not) of 15 g of Florisil® was investigated. The presence of Florisil was able to decrease the extracted lipids from 213.8 ± 6.8 mg down to 88.5 ± 19 mg, *i.e*. approximately 60% depletion. We considered this as a good first cleanup step but the addition of further cleanup seems to be mandatory to complete the lipid removal. Compounds recoveries were assessed after additional purification by GPC of the samples along with a reference standard (Figure 3-11).

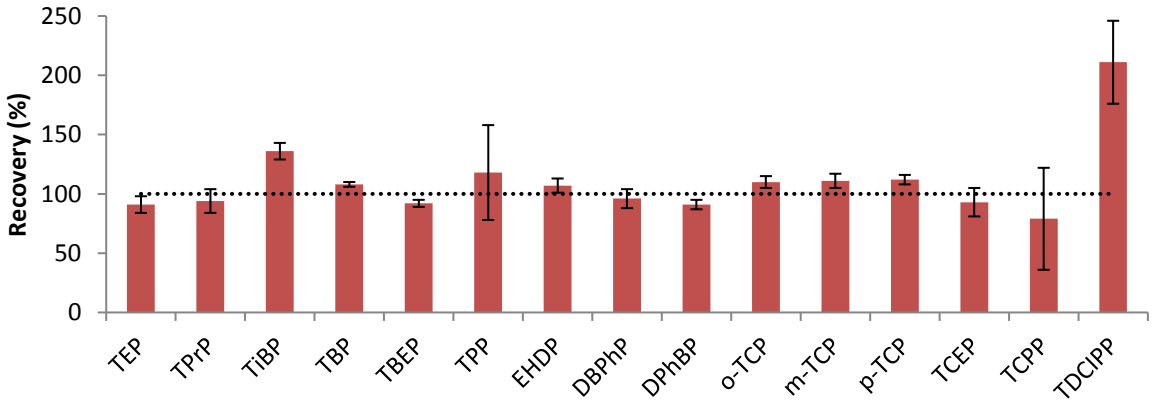


Figure 3-11: Obtained recoveries (%) of OPEs (n=3) based on the reference standard injected on GPC.

Obtained results ranged between 80 and 139% except for compounds suffering procedural contamination and/or being naturally present in the sample (mainly TDCIPP). The RSD values (n=3) were ≤12% (except for TPP, TCPP and TDCIPP).

Finally, the following protocol involving selective Pressurized-Liquid Extraction technique was developed as follows:

- 1 g of freeze-dried sample was introduced into the PLE stainless steel cells.
- 15 g.Florisil® was used as lipid sorbent.
- Some conditions like T °C, duration and number of cycles were set as based to the literature.
- Extraction Solvent constituted of EtAc/c-Hex 1:1, (v/v).

Results showed good recoveries for most OPEs, along with lipid depletion up to 60%

As perspective, the SPLE is to be followed with further cleanup step in order to maximize the purification while investigating the efficiency of the whole strategy.

3.4. **COMPLETE PROCEDURE SELECTION**

After investigating each technique (extraction and purification) separately, we have observed the following main conclusions:

- LLE showed good recoveries for targeted OPEs except TEHP. However, in terms of lipid depletion, it was not illustrated and this will be investigateded in next section
- GPC showed good recoveries for targeted OPEs except TEHP. Besides, it showed high efficacity of more than 98% lipid depletion.

The objective of this section is to investigate in details the overall sample preparation, combining the SPLE to these two purification techniques.

3.4.1. CHOICE BETWEEN LLE AND GPC

In order to compare different purification techniques to be followed after the developed SPLE technique, experiments were done as described in the Figure 3-12. Into PLE cells, 15 g of Florisil were introduced and undergo two rinsing cycles with EtAc/*c*-Hex 1:1 (v/v) mixture solvent. After that, 1 g of lyophilized fish sample was added and spiked with internal standard mixture (50 ng each). The samples were extracted on PLE system with the previously described conditions (See 3.3.2). After extraction, two procedures were performed on (i) liquid liquid partitioning between *n*-Hex and ACN 1:1 (v/v) and (ii) GPC with EtAc/cHex being used as elution solvent. The purified extracts were evaporated till dryness in order to measure the lipid amount. These were then evaporated and reconstituted in 100 µL of toluene for analysis by GC-APCI-MS/MS.

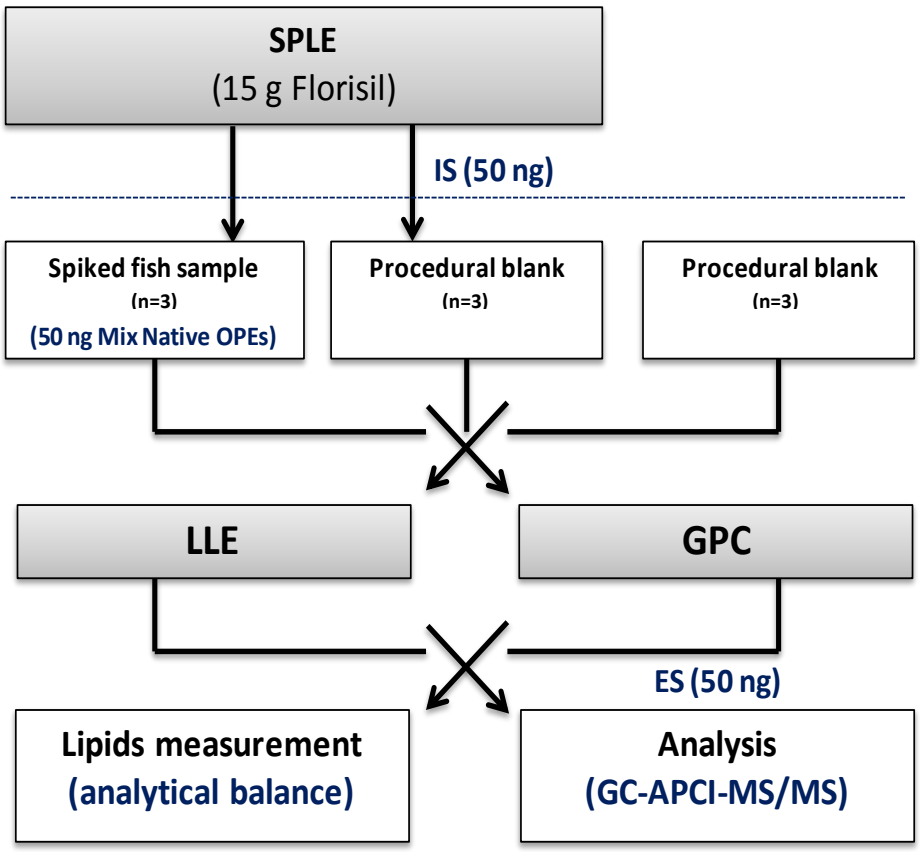


Figure 3-12: Combined preparation procedure starting with SPLE technique for the extraction and 1st cleanup and continued with either LLE or GPC as 2nd cleanup step.

The two strategies were firstly compared in terms of lipid depletion efficiency. The use of GPC as further purification step enabled to remove up to 97% of the lipids in the final extract. However, the use of LLE enabled to remove only 79% of the lipids. Indeed, ACN and *n*-Hex are immiscible above a saturation point of 15% *n*-Hex in ACN. From the point of lipid elimination, it was clear that the GPC was much more efficient.

Figure 3-13 presents the recoveries obtained from the two investigated techniques. With the use of GPC as purification technique, the recoveries for most compounds were satisfactory in the range between 88 and 140%. Besides, with the use of LLE as purification technique, the recoveries ranged between 66 and 125%. In both cases, the RSD (n=3) values were ≤25%. The exceptions were for TPrP EHDP and TDCIPP. For TPrP, the low recovery of 40% might be cause by the high RSDs up to 70%. Indeed, EHDP and TDCIPP were present in the blank samples and/or as contamination in the sample and this was taken into consideration in our calculations. However, the recoveries were up to 220 and 170% for EHDP and TDCIPP, respectively. This might be explained and as described in Chapter 2 (paragraph 2.3.4.2) by the fact that these 2 compounds showed low repeatability of RRFs *via* APCI mode and very low RRF value. for TDCIPP in particular.

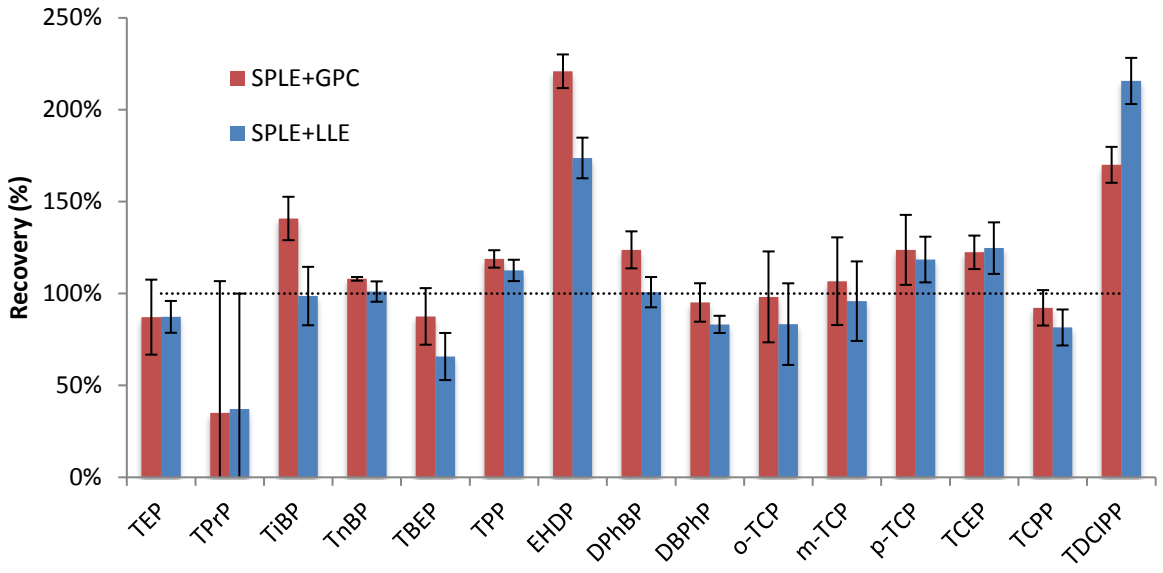


Figure 3-13: Obtained recoveries (%) from the two investigated procedures (SPLE>GPC *vs* SPLE>LLE), as analysed by APCI mode.

As a conclusion, both GPC and LLE showed comparable efficiencies in terms of compounds recoveries but not in terms of lipid depletion where GPC showed much higher capability. From here, SPLE followed by GPC was retained for the finalised protocol which is well illustrated in Figure 3-14.

3.4.2. FINALISED PROTOCOL

As described in Figure 3-14, the retained protocol consists in the first step of freeze drying the collected samples. SPLE is used as a combined extraction/purification step, by adding 15 g of Florisil® (already heated overnight at 600 °C) into the stainless steel PLE cell. To eliminate the maximal possible contamination from the Florisil as well as the whole PLE system, a pre-washing step with 2 cycles is performed. The sample to be extracted is transferred into this PLE cell containing Florisil® and the extraction is started. The Florisil aided to eliminate a percentage of lipids but the extracts might still contain the targeted OPEs as well as other extracted interferences. Therefore, GPC was employed to further purify these extracts. EtAc/c-Hex 1:1 (v/v) was used as pre-washing, extraction and elution solvent on PLE and GPC systems. All the collected fractions were reconstituted in toluene prior to analysis. ^{13}C-PCB-111 was added as RS and analysis was performed using the SRM methods developed with EI and APCI techniques by GC-MS/MS.

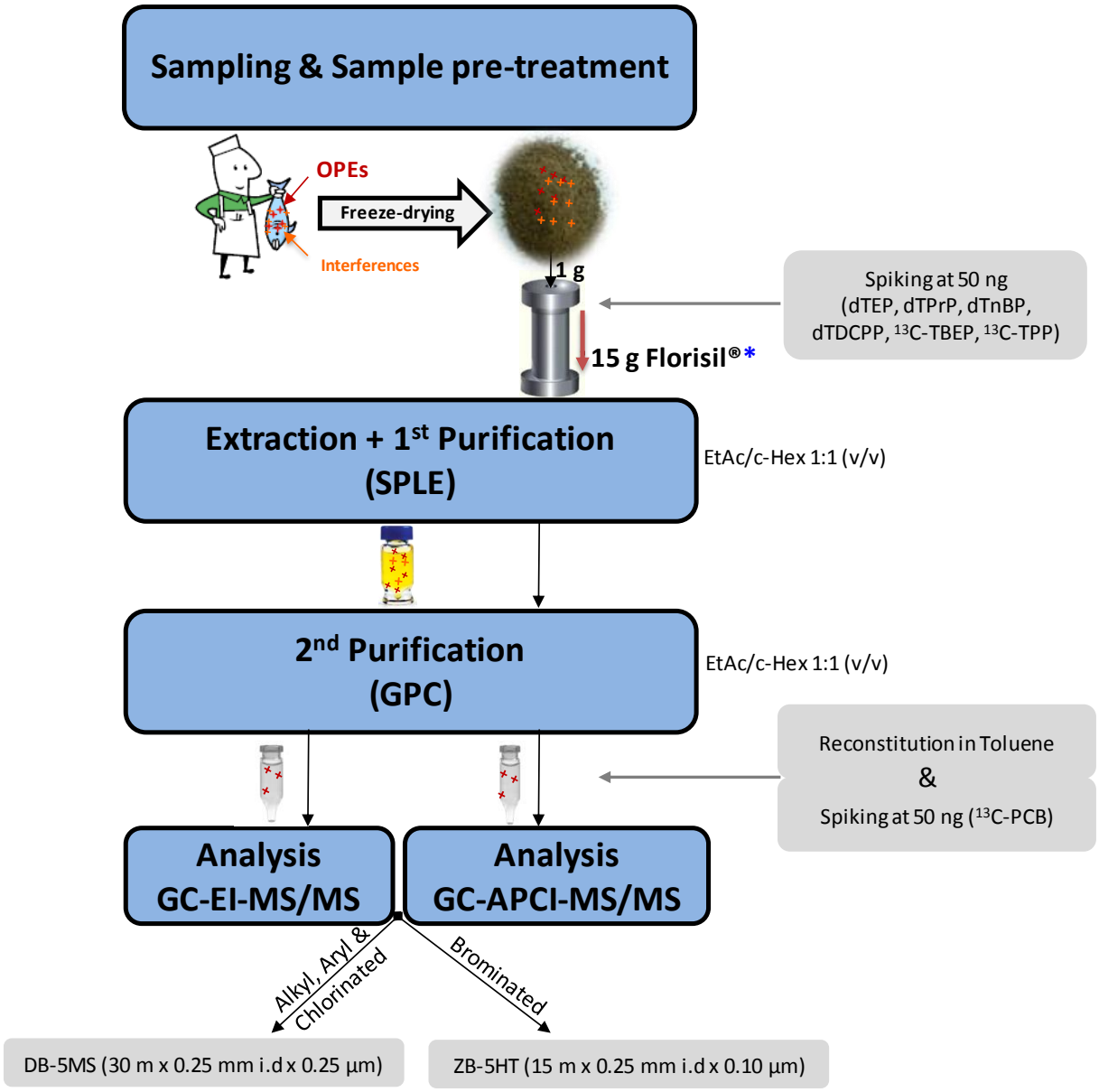


Figure 3-14: Finalised protocol of the retained analytical method dedicated to OPEs in fish muscle. Florisil® undergo 2 pre-washing cycles *via* PLE prior to the introduction of the matrix to be extracted.

3.6. **PROCEDURAL CONTAMINATION ISSUE**

Procedural background contamination is a well-known critical factor in the determination of environmental contaminants in biological matrices with low contamination levels and in particular when dealing with OPEs. It has already been reported as a major issue in the worldwide interlaboratory study on OPEs (Brandsma *et al.,* 2013). Their wide use in experimental materials results in blank interference, which influences the accuracy of analytical results (Liang *et al.,* 2015). In their work, Liang et al. evaluated the presence of blank contamination of sample pretreament procedures and reported majorly TEP, TiBP and TnBP. This was also previously illustrated in the first worldwide interlaboratory study on OPE analysis where different blank contamination was well reported in the participated laboratories with different patterns and concentrations. This is probably due to different sources in the laboratories. Overall, TBP, TiBP, TBEP and TCPP were the most predominant OPEs reported in the blanks. TDCIPP, TCEP and EHDP were also reported in some laboratories.. A number of general precautions were proposed to be taken, of which working in a clean room is highly recommended (Brandsma *et al.,* 2013) noting that all the experiments in this work were always accomplished in the clean room of the laboratory.

The results from our previously described experiment illustrated the contribution of certain compounds in the procedural blank contamination issue. To examine in more details, procedural blanks (n=3) containing no matrix were always taken into considered and hence were prepared and extracted in the same way as the analysed sample. Another procedural blanks (n=3) were prepared without the extraction step in order to evaluate the influence of the extraction system on the contribution to contamination.

According to the environmental protection agency (EPA), the "primary purpose of blanks is to trace sources of artificially introduced contamination". The use of various types of blanks represented in Table 3-1 enables the assessment of how much of the measured signal is from the sample and how much from other causes. They can be used then to correct such unavoidable contamination if relatively constant. In our work, different types of blanks were used in order to track down the possible sources of contamination which include for example the glassware, reagents and instruments used during the sample preparation and the sample analysis steps. Besides, the procedural blank samples were tracked along the overall method in order to estimate the mean contamination level to be used for correcting the levels in the analysed samples.

Table 3-1: Blank types useful for tracking down the possible procedural contamination sources.

Blank type	Purpose	Process
1. **System or analytical instrument blank**	Establishes the baseline of an instrument in the absence of sample	Analytical instrument is run with solvents only
2. **Solvent blank**	To measure the amount of the analytical signal that arises from the solvents	Applying the entire method on solvents only, with volumes V and 2 V
3. **Method blank**	To detect contamination from entire/or each step of the preparation procedure	A blank is taken through each/or entire preparation procedure

3.6.1. SYSTEM OR ANALYTICAL INSTRUMENT BLANK

The analytical instrument contamination sources were was investigated through the frequent acquisition of toluene at the beginning, in between and at the end of every sequence of samples. The instrument blank was also used for two purposes. On one hand, it was used to identify the contamination caused by different sources in the system (adsorption on the insert, septum, gases, etc...). On the other hand, it was used to identify the effects or carryover from previous samples, especially when a low-concentration sample was analysed immediately after a high-concentration sample. This source of contamination was not taken into consideration because by our observations, these blanks generally contained no measurable signals of OPEs.

3.6.2. SOLVENT BLANK

A solvent blank was investigated by checking the solvents which were used in large amounts (EtAc and c-Hex) during sample extraction and cleanup steps, noting that all the solvents were of high purity quality. The contamination of solvents may result from the occurrence of such compounds during the manufacturing, storage and transport processes.

The procedure consisted first in rinsing all the glassware to be needed by the solvent to be tested. Into two sets of glassware with the same surfaces, two solvent volumes (V≈80 mL for the volume used in the finalised method and 2V≈160 mL) were tested. Internal standards were introduced for quantification purpose. A factor of 2 between the two results would indicate that the contamination is mainly due to the solvent and not to any other cause.

Table 3-2: Quantified levels (ng) of OPEs in solvent blank assays (n=3)

Amount (ng)	Ethylacetate				Cyclohexane			
	V		2V		V		2V	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TiBP	0.10	0.05	0.11	0.03	0.15	0.05	0.25	0.01
TBP	0.13	0.04	0.13	0.03	0.17	0.03	0.21	0.01
TDCIPP	0.36	0.12	0.63	0.24	1.93	1.21	1.53	1.00
TCPP	0.74	0.26	0.82	0.09	0.77	0.47	0.99	0.24
EHDP	8.13	11.75	6.02	8.59	0.22	0.02	0.23	0.03
TPP	15.04	20.22	10.75	14.52	0.24	0.17	0.12	0.03

As shown in Table 3-2, 6 OPEs (2 alkyl, 2 aryl and 2 chlorinated) were observed in these assays. In terms of the level, lower contamination was observed in cyclohexane compared to ethylacetate where EHDP and TPP dominated with mean amounts of 8 and 15 ng, respectively. The profile and composition of the compounds seems to differ between the two solvents. The variation between replicates (n=3) was very high and especially for the 2 dominant compounds in ethylacetate (EHDP and TPP). This high variation is not surprising in such case of controlling blank samples containing only solvents and passing into different steps of evaporation. However, the repeatability of the responses of the added internal standards was less than or equal to 25%, which in turns confirms the repeatability of work. The factor of 2 was checked between the amounts observed from 2V/V. The values were different from 2 and ranged between 0.5 and 1.8 in both cases. For the 2 considerably detected compounds (EHDP and TPP) in ethylacetate, the factor was 0.7. This can justify that the solvent is not the main contributor to the contamination, but any other source.

3.6.3. METHOD BLANK

3.6.3.1. **Extraction method**

The PLE system contains lot of parts that can contribute to the issue of procedural blank contamination. This can be attributed to the solvent tubing, the sorbent in the cell, etc. The contamination sources in extraction method blank were investigated in the light of two main parameters: the pre- rinsing of the stainless steel PLE cell and the presence of the Florisil. A mixture *n*-Hex/acetone 1:1 (v/v) was used as the extraction solvent. The results are shown in Figure 3-15. Without pre-rinsing step and Florisil, the main contaminants were EHDP, TPP, TBEP, TDCIPP, TEP, TiBP and TBP in the descending order.

Regarding the influence of the pre-rinsing step prior to the extraction, it is clear that it removes an important part of the contamination particularly of EHDP, TPP and TBEP, the major ones. Regarding

the presence of Florisil®, the results showed that the amount of contaminants increases for the same three compounds. Unfortunately, the impact of pre-rinsing on Florisil was not as efficient as expected.

For the other compounds, the two investigated factors did not seem to show a major impact and no clear conclusion was made regarding their sources. From this experiment, we decided to perform the pre-rinsing step prior to the extraction in order to reduce as much as possible the contamination that can arise from the Florisil on one hand and the entire system on the other hand. It is worth noting also that the Florisil was heated at 600 °C prior to any use.

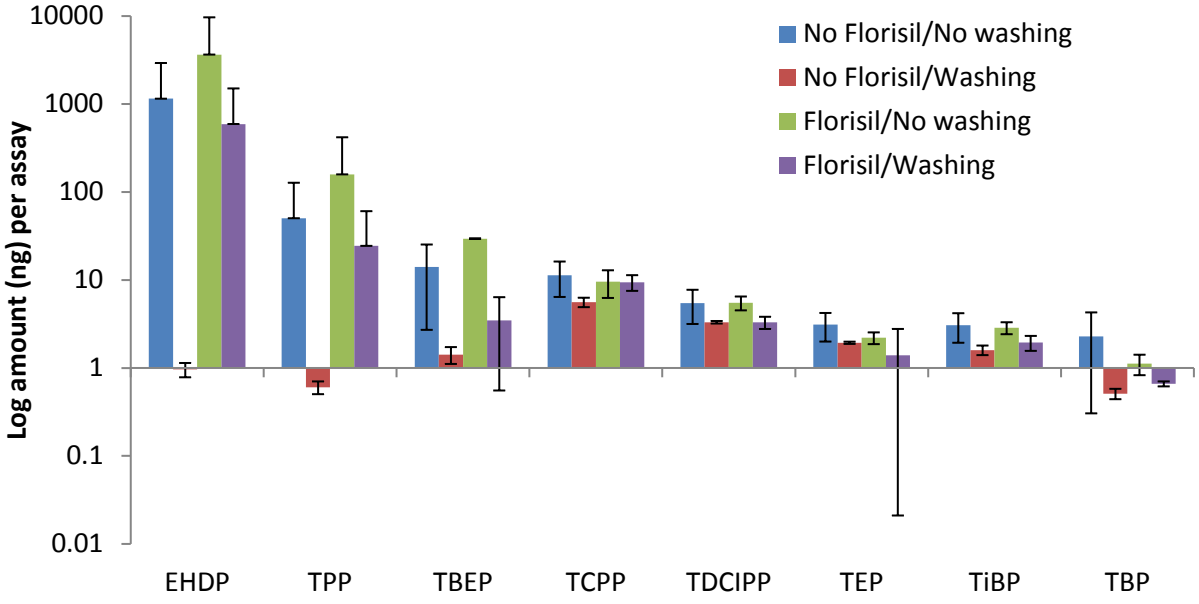


Figure 3-15- Quantity (ng) of OPEs observed in extraction method blank assays, with/without pre-rinsing and/or Florisil (n=4).

After selection of EtAc/c-Hex 1:1 (v/v) as extraction mixture in the final methods, we compared the procedural blank contamination from SPLE system, with the Hex/acetone in a new and similar experiment except that SPLE was followed by GPC. According to the obtained results (Figure 3-16), we concluded that lower contamination levels were generally observed in EtAc/c-Hex, especially for the two aryl compounds EHDP and TPP, designating n-Hex/acetone mixture as a major contamination source.

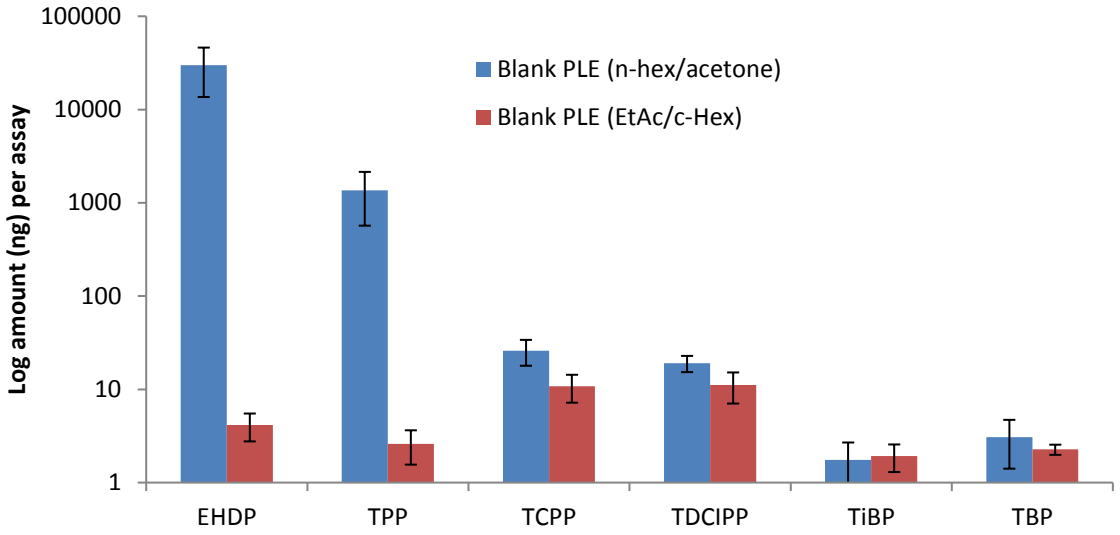


Figure 3-16: Influence of the nature of extraction solvent on the contamination level from procedural blank samples (n=3) as analysed by GC-EI-MS/MS.

3.6.3.2. **Overall method blank**

Finally, we investigated GPC blanks and compared it to the blanks from the complete procedure. As described in the Figure 3-12, blank samples were monitored in order to evaluate the procedural contamination from the whole procedure and from the extraction technique in particular. As observed in Figure 3-17, the preliminary results showed contamination issues when using SPLE followed by GPC mainly with TDCIPP, TCPP and EHDP. Other compounds showed to be more controlled thanks to the precautions taken (*e.g.* pre-washing steps, pre-heating of Florisil®, etc).

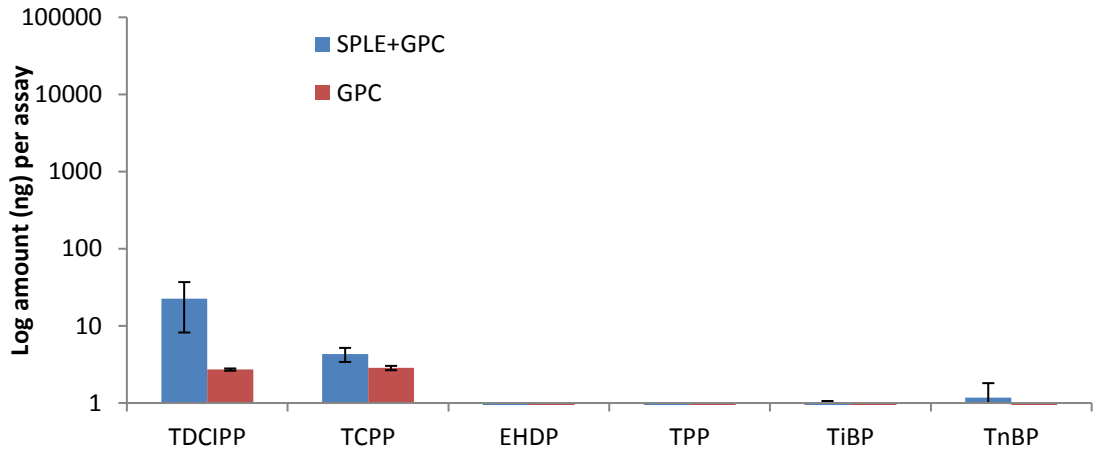


Figure 3-17: Contamination levels in terms of amounts (ng) in the investigated blanks (n=3) as analysed by GC-APCI-MS/MS.

3.6.3.3. **Limits of Reporting**

Despite the huge efforts to investigate the procedural contamination source, control and minimise it, residual levels were still present in blanks. Such a situation is frequent for the analysis of trace levels of environmental contaminants in complex matrices. Thus, the performance limit of the method is no longer its sensitivity but a limit of reporting (LoR), *i.e.* a level above which the sample is statistically different from the procedural contamination.

The objective out of this part was then to determine these LoR values. The distribution of blank sample levels was then used to set these limits at the mean value plus twice the standard deviation, in a way of increasing the margin of trueness of the reported level.

$$Limit\ of\ reporting\ (LoR) = Average\ quantity\ in\ blank\ samples + 2 * standard\ deviation$$

A number of procedural blanks (n=18) were prepared and treated the same way as the analysed samples (see Chapter 4). Results (Table 3-3) reported procedural contamination for 8 OPEs, of which three were chlorinated (TCEP, TCPP and TDCIPP) and two aryl (TPP and EHDP) and three alkyl (TnBP, TiBP and TPrP) and were dominated by the chlorinated OPEs, with a maximum LoR of around 6 ng for TDCIPP.

Interestingly, the results from two techniques (EI and APCI) showed similar profile of contamination distributions and LoR. Mean values will be used then for correcting the quantification in the naturally contaminated samples above LoR.

Table 3-3: Procedural blank contamination as analysed by GC-EI-MS/MS and GC-APCI-MS/MS (as specified in Chapter 2, only EHDP and TDCIPP are analysed by EI).

Amount (ng)	TPrP	TiBP	TnBP	TBEP	TPP	EHDP	TCEP	TCPP	TDCIPP
Mean	0.38	0.70	0.64	1.10	0.68	1.65	1.38	2.03	3.75
SD	0.28	0.29	0.31	0.81	0.31	0.59	0.99	0.78	1.06
LoR	0.93	1.28	1.26	2.71	1.30	2.82	3.36	3.60	5.86

3.7. **METHOD PERFORMANCES**

3.7.1. QUALITY CONTROL

Quality control samples (n=20) were prepared from a pool of fish samples to be analysed. This pool was then spiked at 11.5 ng/g fw with the 18 native compounds and treated exactly the same as the analysed samples, in different series all along the sequences. Then, quantification results can be plotted on a control chart, which is a visual tool used to see if the analytical process is working properly.

Figure 3-18 represents an example for the control chart of TPrP as analysed by GC-APCI-MS/MS and GC-EI-MS/MS. As observed in the mentioned figure, the horizontal dotted lines are drawn at ±2 standard deviations (±2 SD) of the standard value. These lines indicate the Upper and Lower Warning Limits. When standard results approach these values, it is an early warning signal that there may be a problem with the test. Other dashed lines are drawn at ±3 standard deviations (±3 SD) of the standard value. These lines indicate the Upper and Lower Control Limits.

As illustrated in the Figure 3-18, all the points lie within the warning limit range window, except one point, coinciding with the upper warning limit on GC-APCI-MS/MS, noting that the upper action limit is located nearby the target limit of 11.5 ng/g fw. Besides, the repeatability was also estimated in terms of RSD values of these QC samples (n=20); value on APCI was 18% while it was only 5% via EI mode.

Thus, it is worth noting that various situations might indicate a lack of control: (i) the occurrence of a deviating value outside the action limits, (ii) the result in 2 of 3 successive values outside the warning limits, (iii) whenever at least 2 out of 3 successive values fall on the same side of the centerline and more than 2SD units away from the centerline, (iv) the occurrence of 7 successive values on one side of the central line, (v) the occurrence of 7 successive increasing or decreasing values.

For more detailed information, Table 3-4 represents all the obtained results for 17 OPEs in QC samples as analysed by EI and APCI techniques. Using EI, the RSD values ranged between 5 and 14 %, except for TEP with 29 %. Besides and by using APCI mode, the RSD values ranged between 8 and 27 %. For all the 15 OPEs other than the brominated ones, the RSD values were lower by EI than those obtained by APCI, except for TEP.

Table 3-4: Summary of the QC results obtained via both EI and APCI modes, in terms of average values, standard deviation and upper and lower warning and action limits.

"In-house" QC- pooled samples	OPEs	TEP	TPrP	TiBP	TnBP	TBEP	TPP	EHDP	DBPhP	DPhBP	o-TCP	m-TCP	p-TCP	TCEP	TCPP	TDCIPP	TDBPP	TTBNPP
GC-EI-MS/MS	Mean (µg/Kg fw)	28.5	11.9	9.4	11.2	16.0	11.4	26.1	12.5	10.4	11.7	16.6	15.1	12.3	12.3	14.2		
	SD	8.34	0.65	0.74	0.51	1.63	0.63	2.54	0.83	0.57	1.01	1.23	1.04	1.68	1.68	0.68		
	RSD (%)	29	5	8	5	10	5	10	7	5	9	7	7	14	14	5		
GC-APCI-MS/MS	Mean (µg/Kg fw)	26.5	8.7	13.3	10.1	8.2	11.3	36.4	9.0	18.9	10.5	12.3	13.5	11.9	11.9	12.2	12.7	12.4
	SD	4.83	1.55	2.9	2.26	1.36	0.87	5.23	0.93	3.44	1.15	1.16	1.75	3.21	3.21	1.44	2.1	3.7
	RSD (%)	18	18	22	22	16	8	14	10	18	11	9	13	27	27	12	17	30

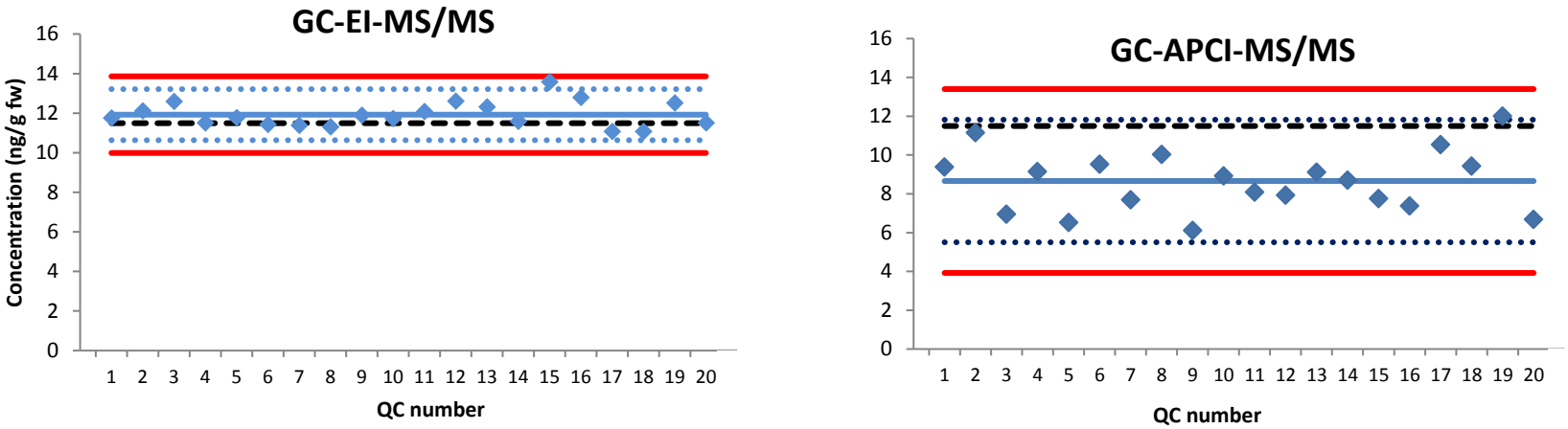


Figure 3-18: QC chart example (TPrP) obtained in EI and APCI. Dashed line: spike level; blue line: average measured level; dotted lines: warning limits; red lines: control limits.

3.7.2. EVALUATION OF LIMITS OF QUANTIFICATIONS

Several methods were described for calculating the limit of quantification (LOQ) but the definition is the same. LOQ is the lowest concentration or amount at which the target analyte can not only be reliably quantified. In most practical works, it is defined as the concentration or mass flow when the signal is ten times higher than the noise amplitude. In our study and to attain this purpose, a pool containing all the fish samples to be analysed was prepared. Three OPE spike levels were prepared: 0, 0.2 and 0.4 ng/g fw. The objective of spiking was to increase the measurement signal in order to improve the S/N ratio. Results showed that S/N ratios for some compounds were equal or near to 10 in the 0 spike level (reflecting endogeneous contamination) but this was not the case for all. For each compound, the concentrations (ng/g fw) were quantified as follows: $\boldsymbol{LOQ} = [\mathbf{measured}] * \mathbf{10}\left(\frac{\mathbf{S}}{\mathbf{N}}\right)$ Additionally, the sensitivity of the method was described in terms of LOQs but also the LoR (See paragraph 3.6.3.3). In this part, we converted these limits which were given in terms of quantity in ng into equivalent concentrations by assuming a fresh weight of 4g. It is just used to illustrate both the quantification and reporting issues in the same time. As illustrated in paragraph 3.6.3.3, LoR were calculated from the analysed blank samples (n= 18) by the mean value plus twice the standard deviation. $\boldsymbol{LoR(ng) = [Mean\ quantity](ng)blank + 2 * SDblank}$

Then, the equivalent LoR values were calculated as follows: $\boldsymbol{LoR\left(\frac{ng}{g}fw\right) = \frac{LoR(ng)}{4(g)}}$

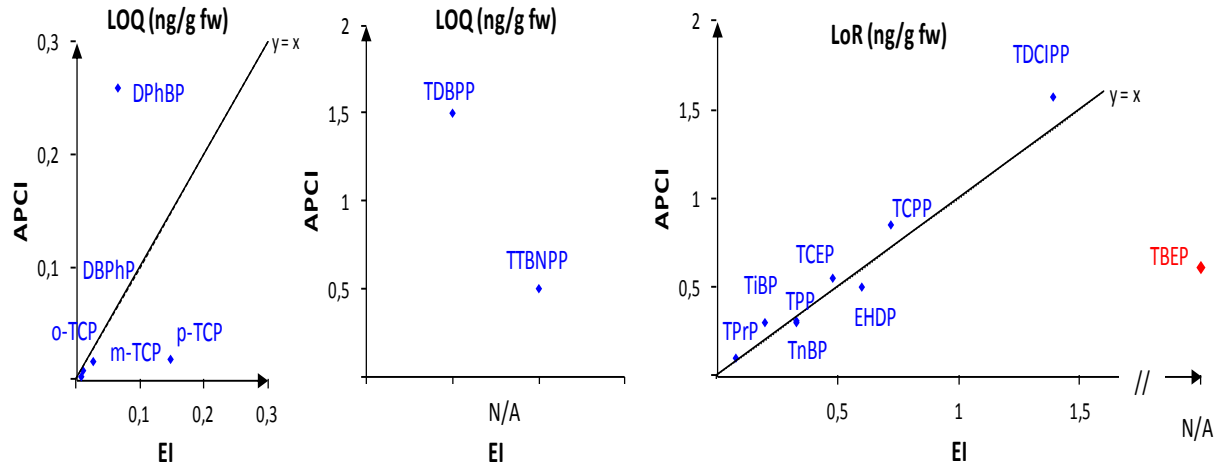


Figure 3-19: Specified limits for the method sensitivity in analysis (ng/g fw, assuming 4 g fw of sample size). LOQs for 5 alkyl OPEs (to the right), LOQs for the 2 brominated OPEs (in the middle) and the LoRs of OPEs present in the procedural blanks (to the left). LOQ: limit of quantification; LoR: Equivalent limit of reporting.

Figure 3-19 presents the estimated LOQ or LoR for each compound. As a reminder from Chapter 3 (paragraph 3.6.3.3), 8 OPEs (TPrP, TiBP, TnBP, EHDP, TCEP, TCPP and TDCIPP) shown to be present in the procedural blanks. For these compounds, the LoR values as analysed *via* the two techniques were highly consistent which means that both EI and APCI are relevant in terms of sensitivity. However, there is compounds which already showed better sensitivity under APCI (i.e. TBEP, TDBPP and TTBNPP) and hence only their limits via APCI are illustrated in this paragraph. It is worth to remind here that TEHP is not included anymore after the development of GPC method (as illustrated in Chapter two).

The main conclusions drawn here regarding the method sensitivity can be summarized in two main points:

- For the compounds present in the blank, the LoRs were comparable from APCI to EI modes.
- For the compounds which are not existing in the blank (*i.e*. o-, m-, p- TCP, DBPhP, DPhBP), the LOQs were compared from the two techniques. With APCI, LOQs values for o-, m-, p- TCP, DBPhP were 1.5-8 times lower than those from EI. With EI, LOQ for DPhBP were 4 times lower than APCI.
- The brominated compounds as well as TBEP showed always better results in APCI and their sensitivity limits were specified by this technique.
- In terms of quantification, the limits were lower than 1 ng/g fw for the majority of compounds including o-TCP, m-TCP, p-TCP, DPhBP, DBPhP and TTBNPP. For OPEs posing procedural blank contamination issue, their reporting limits were lower than 1 ng/g fw for TPrP, TiBP, TnBP, TBEP, TPP, EHDP, TCEP and TCPP. The two halogenated compounds (i.e. TDCIPP and TDBPP) showed up to 2 ng/g fw for reporting and quantification limits, respectively. The exception was for TEP which was highly reported in the blanks with high variation.

From here, it was obvious we can't absolutely select one technique for the whole range of compounds, but rather to select the more suitable one for each compound. After several experiments on standard solutions and fish, it appeared interesting to evaluate the matrix effect via the investigated techniques.

As a perspective, the results will then be interpreted and evaluated in comparison with the method specified limits (i.e. LOQs and LoR). For OPEs present in procedural blank samples, the mean values will be used for correcting the quantification in the naturally contaminated samples above LoR.

3.7.3. MATRIX EFFECT

Matrix effects stand for a variety of effects the extract may have on the chromatography, and/ or ionisation efficiency impacting the quantitative results. It describes the difference between the

response of a target analyte in a neat standard solution and a sample matrix. Matrix effects can be best revealed if a certified reference material is analysed. Unfortunately, certified reference materials are not always available. "True" matrix effects were evaluated by a post-purification spike experiment. The sample matrix (n=3) was extracted and purified and then immediately prior to GC analysis, a known amount of a mixture of standards (50 ng each) was added. As controls, non spiked extracts (n=3) were included. Additionally, pure standard solutions (n=3) of the target analyte in the same concentration were analysed. The average response values from the replicated was used in the further evaluation of the matrix effect (ME) which was then calculated as follows:

$$\mathbf{ME}\ [\%] = \left(\left(\frac{\mathbf{Signal\ spiked\ extract} - \mathbf{Signal\ non-spiked\ extract}}{\mathbf{Signal\ Standard}}\right) - \mathbf{1}\right)\mathbf{x\ 100}.$$

For interpretation, ME=0% indicates no matrix effect, negative ME indicates ion suppression and positive ME indicates ion enhancement. Signal enhancement can be caused by co-eluting matrix components, resulting in a larger peak. Furthermore, active sites in the chromatographic system can be masked by adsorption of nonvolatile matrix components (matrix material deactivating surfaces). As a result, adsorption of the analytes, *e.g*., in the liner, is reduced and subsequently signals enhancement of the analytes is observed. Signal suppression can originate from contaminations of the liner or column head with non-volatile matrix components resulting in the adsorption or decomposition of the analytes, or quenching of the detector signal.

As shown in Figure 3-20, the matrix effect in fish samples was analysed via both EI and APCI modes. Under APCI conditions, significant signal enhancement was observed for most of the compounds contrary to the EI results where signal suppression was observed. EHDP under APCI showed enormous signal enhancement. The same was observed for TDBPP and TBEP which were included to the right of Figure 3-20. However and by using EI mode, the matrix effects (%) ranged from -53% for TEP to -13% for DPhBP. This could be induced by a contamination of the system by non-evaporating by-products, decreasing the peak areas through hindering solute evaporation: the "reducing matrix effects."

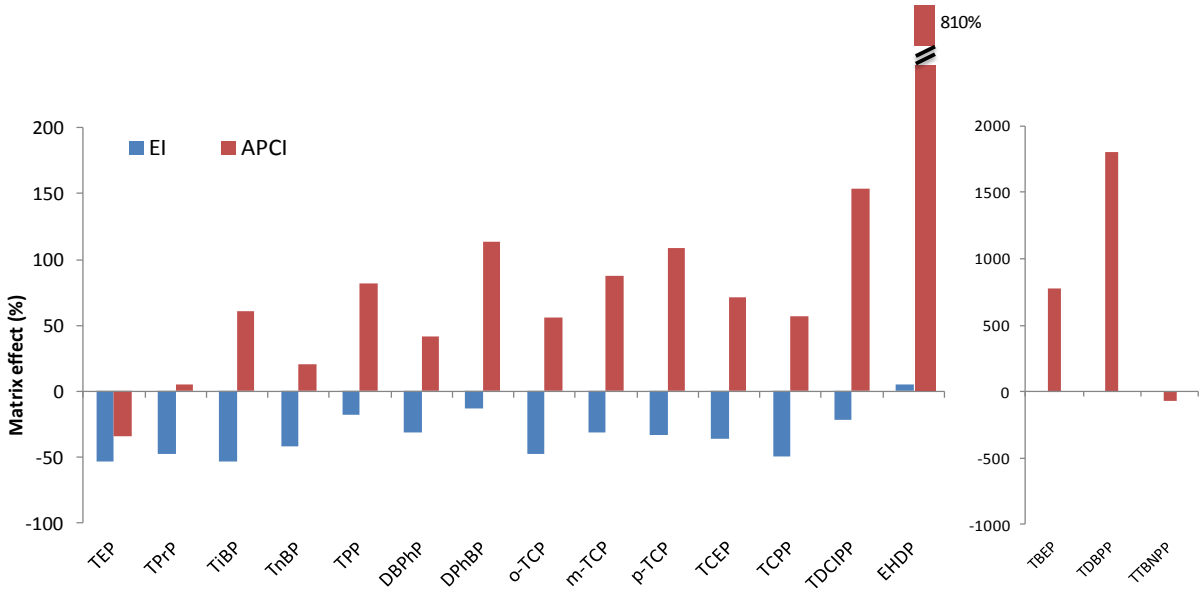


Figure 3-20- Matrix effect (%) in fish samples, for most of studied OPEs, as analysed *via* EI and APCI mode.

Based on our preliminary results in Chapter 2:

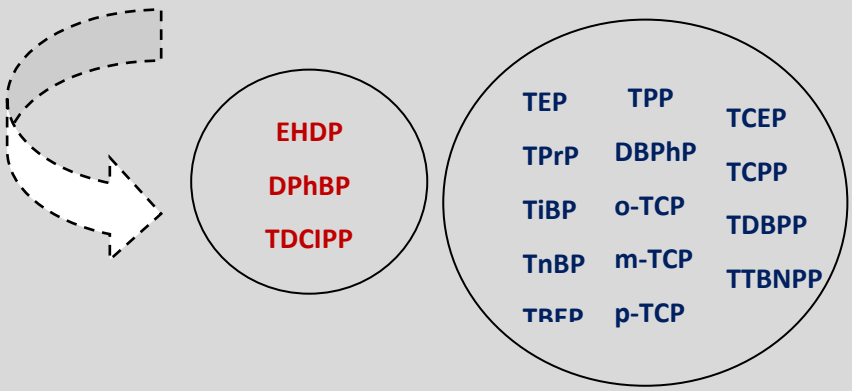
- Lower IDLs on APCI for most compounds.
- Additionally, the analysis ofTBEP, TDBPP and TTBNPP was more sensitive *via* APCI than EI mode.
- EI was better for EHDP and TDCIPP in terms of RRFs variation. The same conclusion for EHDP was also observed from experiment on matrix effects.

Based on the method sensitivity on matrix (LOQs):

- With APCI, LOQs values for o-, m-, p- TCP, DBPhP were 1.5-8 times lower than those from EI.
- With EI, LOQ for DPhBP were 4 times lower than APCI.
- TBEP, TDBPP and TTBNPP showed better results by APCI and their sensitivity limits were specified using this technique

Among targeted OPEs, EI mode was selected for 3 OPEs (EHDP, DPhBP and TDCIPP) while APCI mode was selected for the other 14 OPEs (TEP, TPrP, TnBP, TiBP, TBEP, TPP, DBPhP, o-TCP, m-TCP, p-TCP, TCEP, TCPP, TDBPP and TTBNPP), reminding that TEHP and as illustrated in Chapter 3 was eliminated from the list of targeted compounds.

Performances	EI	APCI
Sensitivity	++	+++
Specificity	+	+++
Quality Control (RSD)	+++	++
RRF	+++	++
Matrix effect	↘	↗ ↗



CHAPTER FOUR

APPLICATION OF THE METHOD TO THE CHARACTERISATION OF FOOD SAMPLES

4. APPLICATION OF THE METHOD TO THE CHARACTERISATION OF FOOD SAMPLES

4.1. **INTRODUCTION**

Chemical contaminants may occur in our food from various sources. The analysis of relevant chemical contaminants is an essential part of food safety testing programs in order to ensure the compliance with regulatory limits when existing and above all, the consumer health. Regarding OPEs and as we have seen from the literature overview in Chapter 1, it is urgent to generate original occurrence data for understanding the fate and risk of this class of emerging pollutants. The investigations performed in Chapter 2 and 3 related to the instrumental as well as the sample preparation method enabled answering one of the two main objectives of the present research work through the development of a robust analytical approach for the trace levels analysis of targeted OPEs. The purpose was then to respond to the second objective for the method implementation in the analysis of fish and foodstuffs.

In this chapter, we will focus on the feasibility of the developed method for determining OPEs at trace levels in fish and food samples under the optimised conditions. As OPEs are mainly used for two purposes (*i.e*. flame retardants and plasticizers), the samples were selected where one can expect to find these contaminants. Indeed, many studies have discussed the occurrence of these OPEs, being used as flame retardants and plasticizers, as environmental contaminants in various compartments.

As mentioned in our general introduction, fish is a major food item for exposure to POP-like substances from environmental contamination. Therefore, it is potentially an important dietary source of OPEs for consumers. Freshwater and marine fish have been analysed for restricted number of OPEs in some studies all over the world but not in France. Therefore, a set combining marine and fresh water fish samples collected in different regions in France was analysed in order to produce the first data at French level for a large number (n=17) of OPEs.

In parallel, a collection of food samples packaged in plastic food contact materials was selected from local retailers in order to produce a second original data set. The purpose was to explore another source of particular potential exposure, resulting from the possible transfer of these OPEs into food, from plastic coatings treated with these plasticizing agents. Here, it is worth to mention the Commission Regulation No 10/2011 on plastic materials and articles intended to come into contact with food. This regulation allows the use of EHDP in the manufacturing process but with a specific migration limit (SML) of 2400 ng/g of food.

For all the analysed samples, not only the contamination level, but also the profiles of OPE compounds will be described. Finally, exposure values will be discussed with regard to available literature.

As mentioned since the beginning of the present manuscript, our ultimate objective was to include a first survey data collection, reflecting the potential exposure of the French population to these substances. Although the two sets of samples are relatively small and therefore limited in terms of representativeness, this has represented an opportunity to present an interpretation exercise of the risk assessment based on our obtained results. However, we know the complexity of this area and do not have all the mastery of the rules, tools and procedures used therein, nor even all material (data) necessary allowing rigorous analysis of this type. In this last part, our aim was to exploit our few data to compare the calculated exposure levels to acceptable daily intake (ADI) values for these compounds. ADIs considered were either already reported ones or, when not available, such values were calculated in the present work as an attempt. We will adopt the methodology from the main principles of quantitative risk assess (QRA), on which we will base our application performance achieved under our thesis project.

The main objectives of the chapter can be summarised in two points:

- To quantify OPEs in a set of fish samples as well as other foodstuffs.
- To exploit the results to determine the contribution of these food items in OPEs diet exposure in a risk assessment perspective. In this context, we will refer to the scientific opinions delivered by the European Food Safety Authority (EFSA).

4.2. **APPLICATION TO FISH CHARACTERISATION**

4.2.1. SELECTED SAMPLES

Fish and fishery products have valuable nutritional qualities which make them an especially sound food choice. However, due to environmental impact, the foods made from fish and fishery products may be contaminated by chemicals and then can be potentially an important dietary source of these OPEs for consumers. Freshwater and marine fish have been already analysed for OPEs from different regions in the world, but not in France. At this step, our objective was to produce and analyse the presence of OPEs in fish and food intended for human consumption as well as other fish types. Therefore, a set of 77 fish samples was selected from both freshwater (n=44) and marine systems (n=33). The selected samples were previously characterized for other classes of environmental contaminants such as polychlorodibenzo-p-dioxins PCDD and polychlorodibenzofurans PCDF (PCDD/Fs), BFRs or PCBs.

Wels catfish (genus *Silurus*) from fresh water systems were collected in the Gironde drainage basin including 21 and 23 samples from 5 and 4 sites located downstream the Dordogne and the Garonne rivers, respectively. These two rivers merge into the Gironde above Bordeaux (Figure 4-1). The samples were collected between March and November 2014 and received to the laboratory on January 2015 to be analysed for PCDD/Fs and/or PCBs.

Fish samples from marine system were collected from both pelagic and benthic zones. All the samples were collected between 2014 and 2015. The selected samples included different fish species from different regions in the world, exported to the French market and intended for human consumption in French market. Among them, n=1 mackerel sample was collected from the Atlantic ocean, n=2 tuna samples from the Maldives in the Indian ocean, n=9 salmon samples from the Baltic sea, n=1 goatfish, n=2 sole and n=1 red mullet samples from Senegal (probably at a point of Atlantic ocean),; n=2 hake fish samples from Canada (probably at a point of Atlantic ocean), n=1 turbot, n=1 sole and n=1 porgy samples from Mauritania, n=1 rail sample from USA as well as n=1 grenadier, n=2 bar samples and n=8 other fish samples of unspecified species from different regions like India and Indonesia.

It is true to say that the lipid content differs from one species to another but also from a geographical area to another. Additionally, the trophic position is another factor that can influence the contamination levels and/or profiles. Most of the selected fish species occupied high trophic levels (between 2 and 5) on the food web.

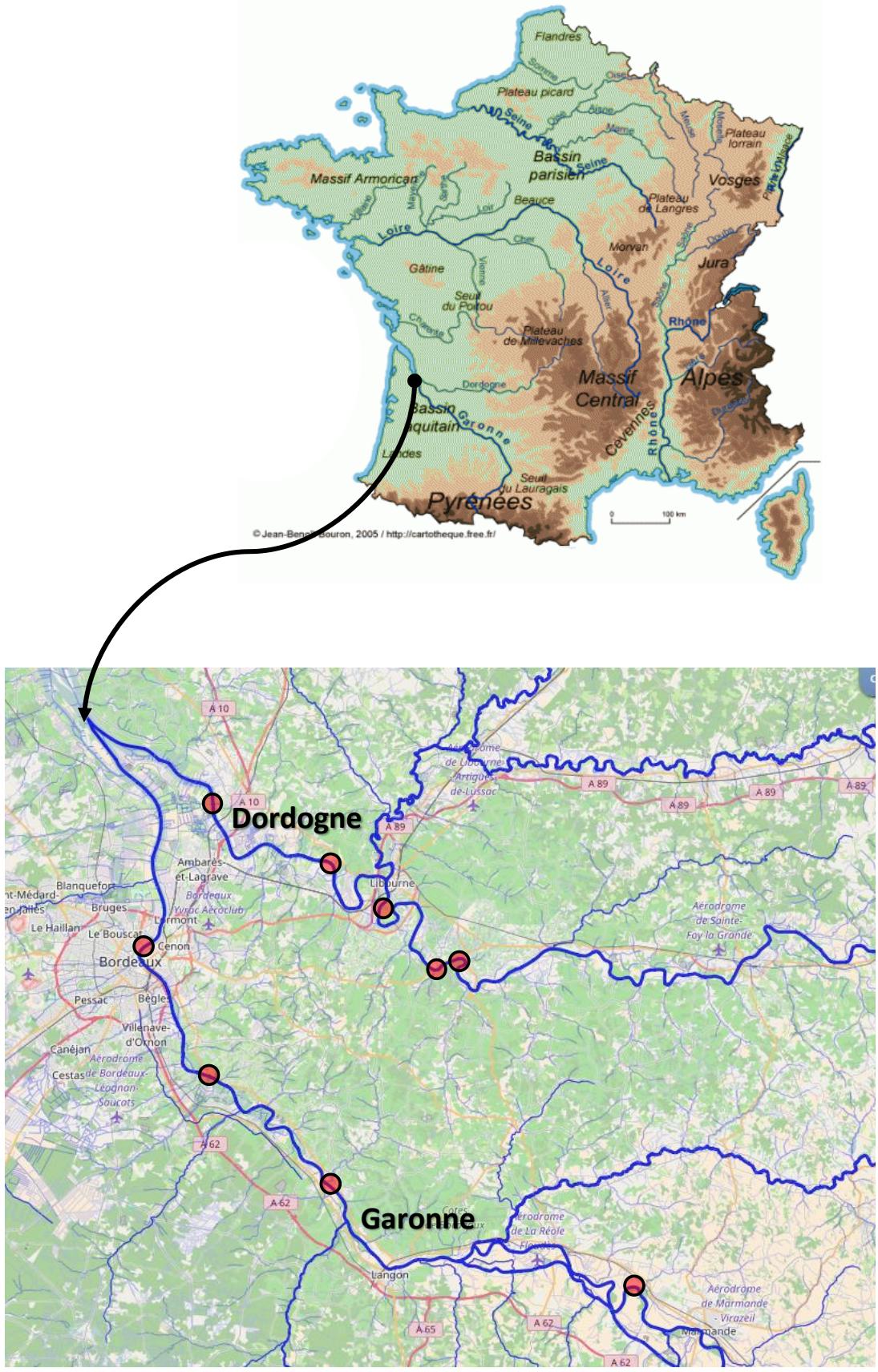


Figure 4-1: Location of the 9 sampling sites in France for the 44 Silurus fish samples on the Dordogne and the Garonne rivers (http://cartographie.nature33.fr/visualiseur/?idlyr=11519**)**

4.2.3. RESULTS AND DISCUSSIONS

By using each of the investigated techniques and before starting the quantifications, we have followed certain identification criteria. Firstly, the response from the recovery standard and that from the internal standards were checked to verify any losses during the preparation and/or the injection. The signals of targeted compounds were then verified for the relative ion intensities between the specified transitions (described in Chapter 2). This was based on the Commission Decision 2002/657 concerning the performance of analytical methods and the interpretation of results. In this Decision, the EU have laid down the maximum permitted tolerance for the relative ion intensities using a range of mass spectrometric techniques including EI and CI by GC-MS/MS. The concentrations were then calculated in terms of equivalent fresh weight of each lyophilised sample.

- These concentrations were only reported if the values were higher than the specified limits (LoRs or LOQs) of the corresponding compound on the chosen ionisation technique. Otherwise, the level would be reported as lower than these limits. In such case and when we are talking about LOQs, the middle bound (MB) concentration was used as the half of the LOQ value.
- For the compounds present at higher than the LoRs, and as mentioned previously in Chapter 3, the quantified amounts were corrected by subtracting the mean amounts found in the procedural blank samples. If the levels are lower than the LoRs, half the blank mean concentration was used rather than the LoR in order to avoid overestimation of the contamination.

4.2.3.1. **Fresh water fish samples**

- **EI vs APCI**

As APCI was selected for the majority of compounds in order to exploit the advantage of lower LOQs in comparison to EI, except for TDCIPP, EHDP and DPhBP, we verified that the important matrix effects for certain compounds didn't have an influence on the reliability of the results to be reported. This was performed through the comparison of concentration levels observed from APCI to those observed from EI mode. Figure 4-2 shows the results obtained for the total quantifiable OPE concentrations (ng/g fw) found in *Silurus* fish samples as analysed by EI and APCI modes.

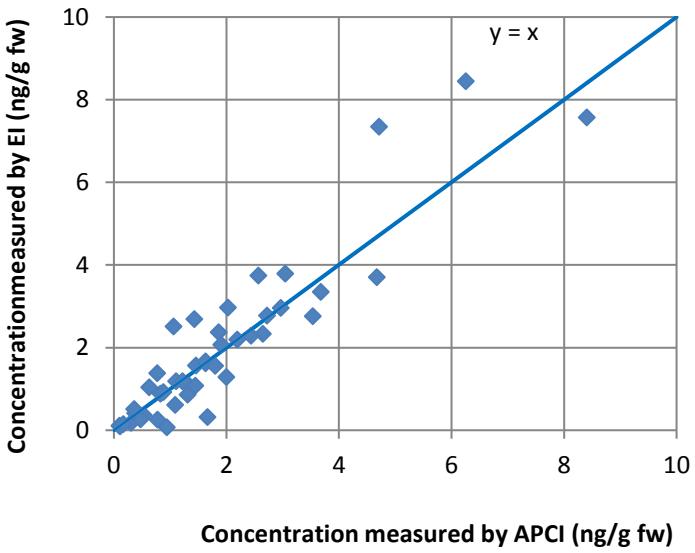


Figure 4-2: Total OPEs concentrations obtained by EI *vs* APCI modes in the 44 *Silurus* fish samples

The concentrations were already corrected from the blank level as described earlier (Chapter.3). The correlation of the results from APCI and EI were illustrated by the deviation to the linear x=y curve, and which was calculated as: $\left|(\mathrm{EI}-\mathrm{APCI})/(\mathrm{EI}+\mathrm{APCI})\right|$

The deviation of the 44 points ranged between 0.002 and 0.3 except for two points at deviations of 0.6 and 0.8. The mean deviation was 0.05, which reflects a satisfactory linearity of results. This was confirmed by the correlation coefficient of 0.9, reflecting the reliability of obtained results and the ability to rely on either of the two techniques for reporting purposes.

Based on our previous illustrations, EI was selected for 3 OPEs (EHDP, TDCIPP and DPhBP). In this part we can reliably use the two techniques as previously specified. The other advantage for analysing the samples *via* the two techniques, other than the sensitivity issue, was the ability to surpass some injection problems that may occur on any of the instruments.

- **Sampling sites total concentrations**

As mentioned before, the samples were collected from different sites along the two rivers (Dordogne and Garonne). The objective in this part was to investigate the trend of contamination along the rivers stream in terms of total concentrations. Figure 4-3 presents the sum of the reported concentrations at these different sites. All the reported concentrations were lower than 10 ng/g fw. The highest levels were reported in samples collected from Branne, Arveyres and Tressac on Dordogne and Cambes on the Garonne River. However, it is worth noting that the number of samples is not equivalent in all the sampling sites, which makes it difficult to compare between the different sites. Highest values were

found at around 9 ng/g fw in Branne and at around 7 ng/g fw in Cambes. As an illustration, Figure 4-4 presents an example for the total ion chromatograms (TIC) for a *Silurus* sample collected in Cambes at the Garonne River, analysed by GC-EI-MS/MS and GC-APCI-MS/MS. The sum of the reported concentrations in this sample was 3.4 ng/g fw. The main reported compounds were TiBP, TnBP, TPP, TCEP, TCPP and TDCIPP. For each of these compounds, the extracted ion chromatograms are presented on the corresponding TIC (i.e. TDCIPP from GC-EI-MS/MS and the others from GC-APCI-MS/MS).

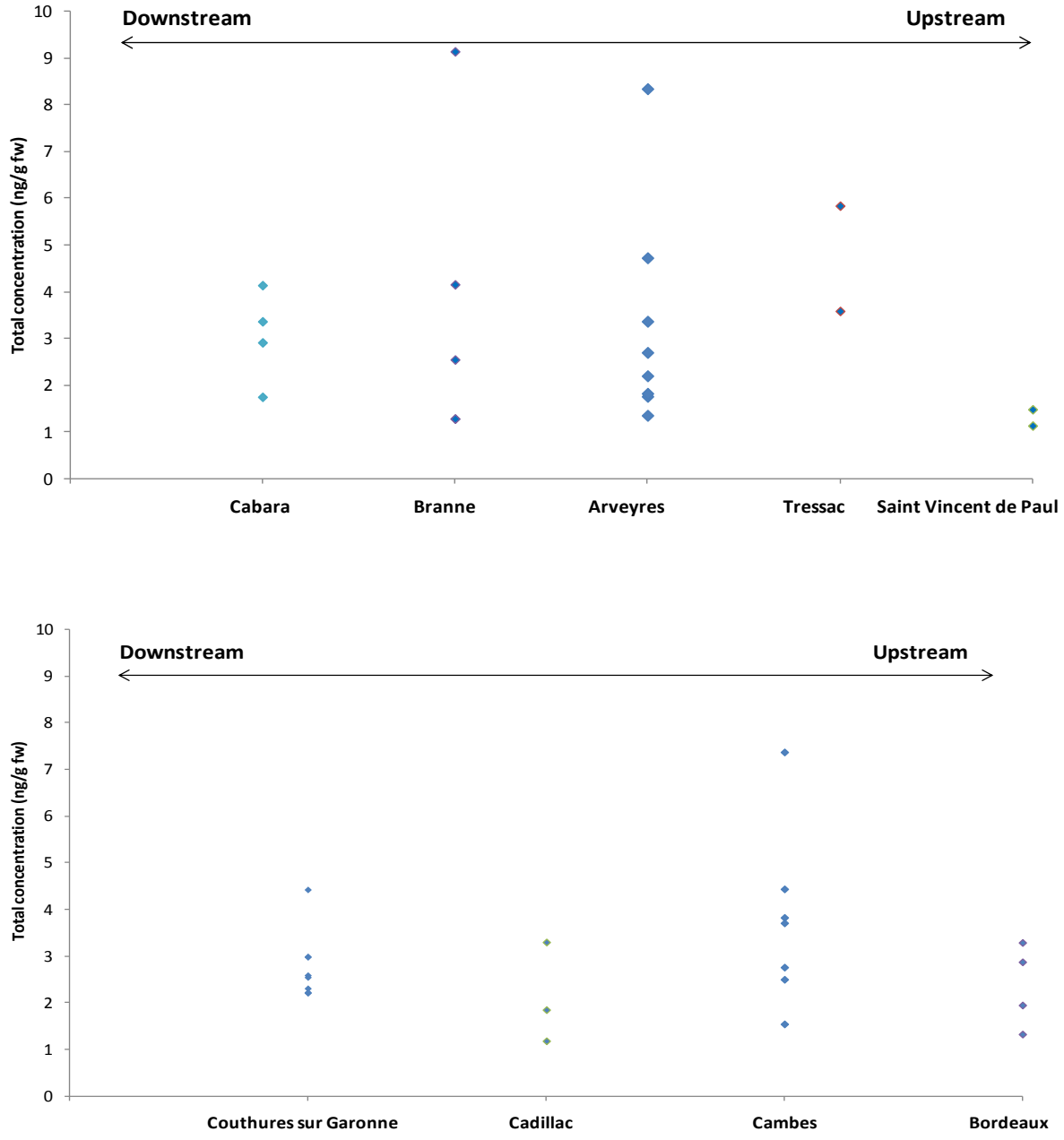


Figure 4-3: Total OPEs concentrations obtained from the different sampling site on the Dordogne (top) and the Garonne (down) covering the flow of watercourse along the two rivers.

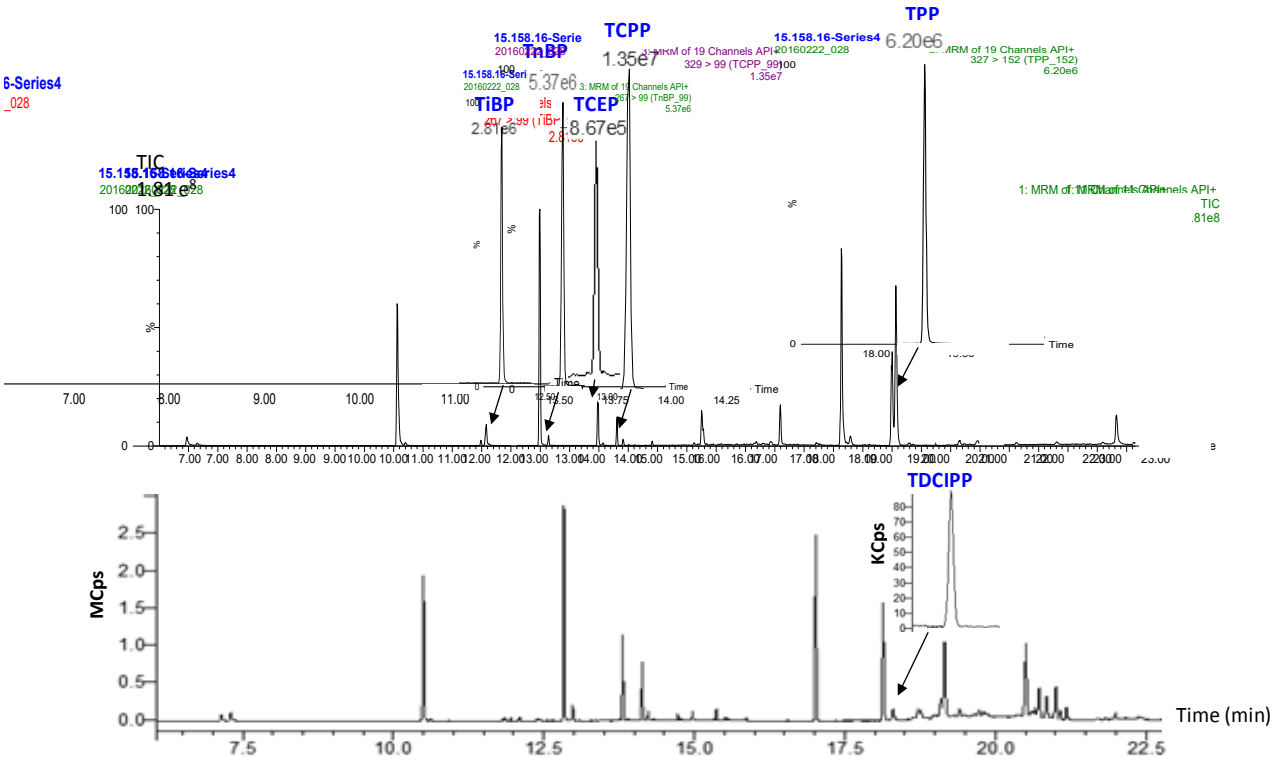


Figure 4-4: Total ion chromatogram (TIC) of a *Silurus* sample collected from Garonne River at Cambes sampling site; along with extracted ion chromatograms (EIC) of the reportable compounds, as analysed by GC-MS/MS *via* APCI (top) and EI (down) modes.

Moreover and in order to compare between the levels reported in the 2 rivers (n=21 samples from Dordogne and n=23 samples from Garonne), we have illustrated this using the t-test, in order to see if any statistical significance exists in our set of observations. The null hypothesis (H0) proposed that there is no difference between the levels found in the two rivers. The statistical t value was -0.7 and lied between ± t critical (two-tail) of absolute value 2.03. The observed difference between the mean values (2.8 and 3.2 ng/g fw) is not convincing enough to say that the levels in samples from the two rivers differ significantly.

All in all, even though the levels were not importantly different between Dordogne and Garonne, however, the distribution of the contamination had no specific trend along the two river streams (neither upstream nor downstream). As a conclusion, the results showed that the level contamination had no specific trend upstream or downstream to the river. But, the next question would be related to the profile of this contamination and if there is a specific trend in this issue.

➢ Detection frequencies

The detection frequencies of the compounds in the analysed *Silurus* fish samples from the two rivers (n=44 samples) ranged between 0 and 80 % (Figure 4-5). Some compounds, namely o-, m-, p-TCP, TPrP and the two brominated compounds (TDBPP and TTBNPP) were not quantified in almost of the samples. The result for the brominated compounds was not surprising because no previous literature has mentioned their presence in fish. However, this maybe also attributed to our relatively high limits of detection for these compounds. Besides, six OPEs were detected in at least 30% of the analysed samples and represented by TiBP, TnBP, EHDP, DBPhP, TCPP, and TDCIPP. For instance, TCPP which is one of the most previously reported OPEs, was quantified in about 50% of the samples analysed from different sites with a mean concentration of 0.45 ng/g fw.

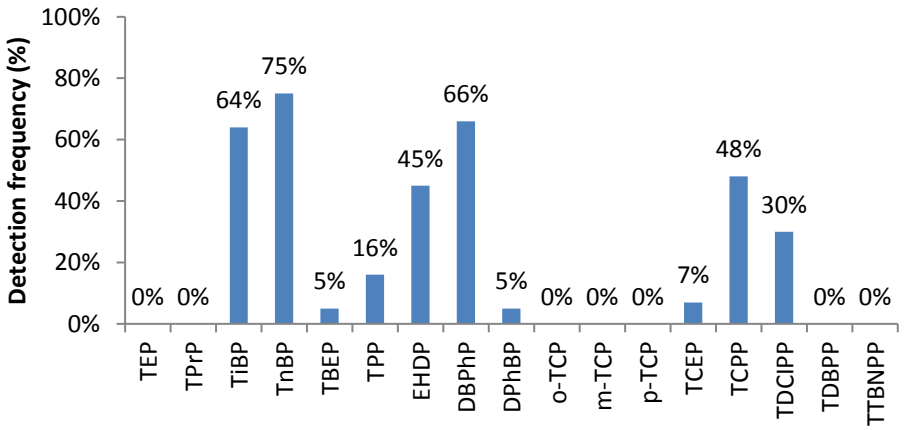


Figure 4-5: Detection frequencies of the studied OPEs in the analysed *Silurus* fish samples (n=44 samples).

➢ Profile of contamination

Then, we were interested to have a look over the profile of contamination per compounds percentages between the two rivers, between the different sampling sites from each river and finally, within the sampling site when possible.

▪ Differences in contamination between Garonne and Dordogne

Even though the Garonne and Dordogne rivers meet at the Gironde estuary, the hydrodynamic conditions can be highly variable from one river to another which may result in different contamination profiles. From here, we were interested in investigating the variation of OPEs levels and profile in the two rivers.

As shown in Figure 4-6, which presents the contamination profile in terms of compounds contribution, it is clear that even though the mean total contamination values are similar (3 ng/g fw) between the two rivers, it can be observed that the individual contributions of each OPEs differs from one river to another except for TDCIPP, TCPP, TnBP. These three compounds showed to be dominant and showed comparable percentile profile in the two rivers.

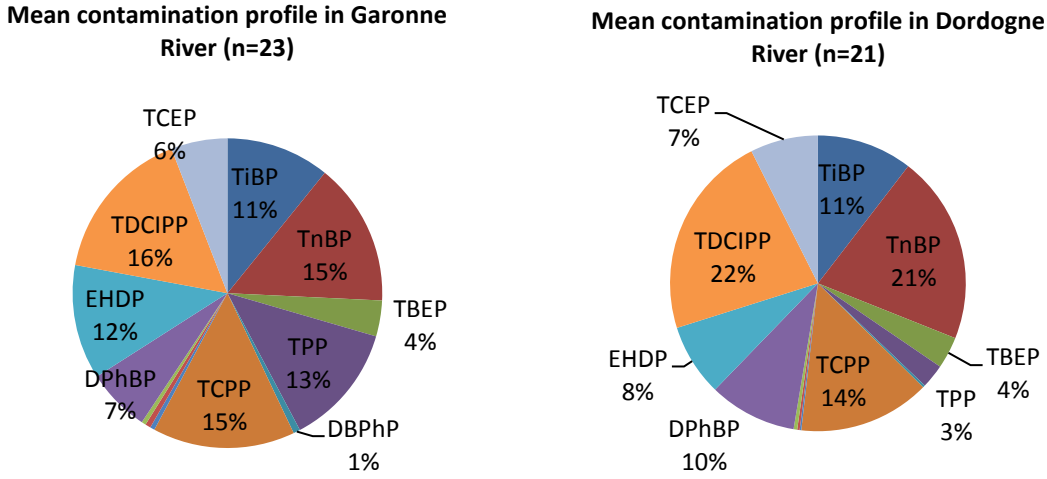


Figure 4-6: Contamination profiles in terms of the mean contamination for Dordogne (left) and the Garonne (right) rivers with ∑Mean=3 ng/g fw in each river.

In order to have a more profounded overview on the variation of contamination levels and profiles from the two rivers, and because each sample is characterized by several individual OPEs concentrations that can be considered as variables, we conducted a Primary Component Analysis (PCA). Such statisitical appoach enables multivariate analysis in order to convert our set of observation data of possibly correlated variables (OPEs) into a set of values of linearly uncorrelated variable combinations called principal components. We tried to employ this exploratory data analysis tool to predict about the possible correlations or variations among observations from Garonne to Dordogne Rivers. Figure 4-7 presents the PCA scatterplot of reported OPE levels in 44 fish samples (21 from Garonne and 23 from Dordogne). The Figure shows the score plot in a first part, which is a projection of data from the two rivers onto subspace. In parallel, the Loadings Plot shows the relationship between the variables (OPEs) and subspace dimensions. The colored clusters refering to the two rivers and each dot in this plot represents one sample. As can be observed, PCA could not highlight any distinguishable variations in the OPEs levels in *silurus* fish collected from the two rivers.

Only 17% of the dataset variability was explained on the two dimensions and this is mainly due to three samples being as outliers (1 from Dordogne and 2 from Garonne) and this was attributed to certain dominated OPEs in these particular samples.

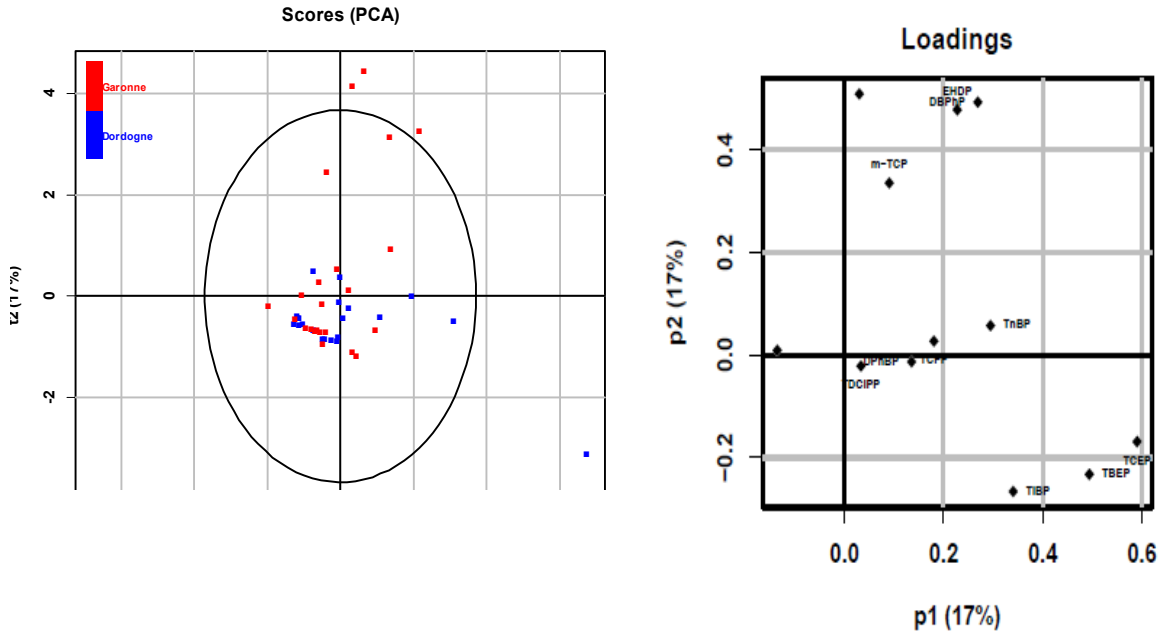


Figure 4-7: PCA summary plots for the 1st and 2nd principal components. Score panel plot (left) and Loadings panel plot (right).

- **Differences in contamination between different sampling sites from the two rivers**

We were then interested in investigating the profile variation between different sampling sites on the same river. The same conclusion was derived here as already seen in the previous section for the global contamination level. However, we should always bear in mind that the number of analysed samples was limited and not the same from one site to another. Remarkable concentrations were found up to 6 ng/g fw for TPP in a sample collected from Cambes site at the Garonne River. Also, a concentration of 5 ng/g fw was found for TDCIPP in a sample collected from Branne site at the Dordogne River. From here, we were interested to investigate the profile of compounds distribution in the contamination found in different sites.

For better illustration, Figure 4-8 presents example for the profile of contamination in three different sampling sites along the Dordogne River. It is obvious that even though comparable loads of total OPEs were observed, however the percentile composition per compound is not consistent and differs from one site to another.

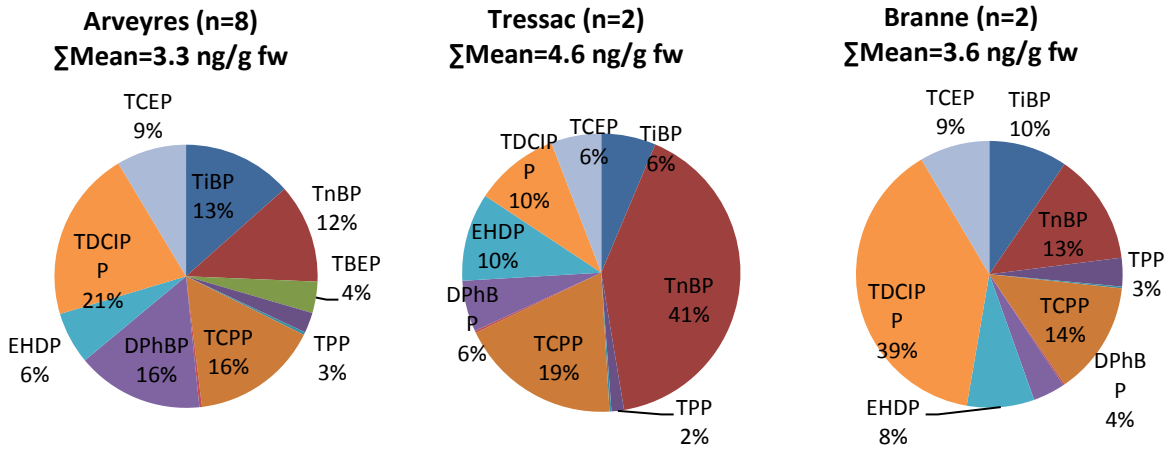


Figure 4-8: Percentile composition of the contamination reported in different sampling sites along the Dordogne River.

▪ **<u>Differences in contamination within the sampling site</u>**

In the same trend, we were interested to investigate the variation within the same site. Figure 4-9 presents the percentile profile of contamination per compound in three samples collected from the same site at Arveyres. We can see that the profiles are not consistent between the samples which do not support the idea that a particular site can be associated to a particular profile of contamination.

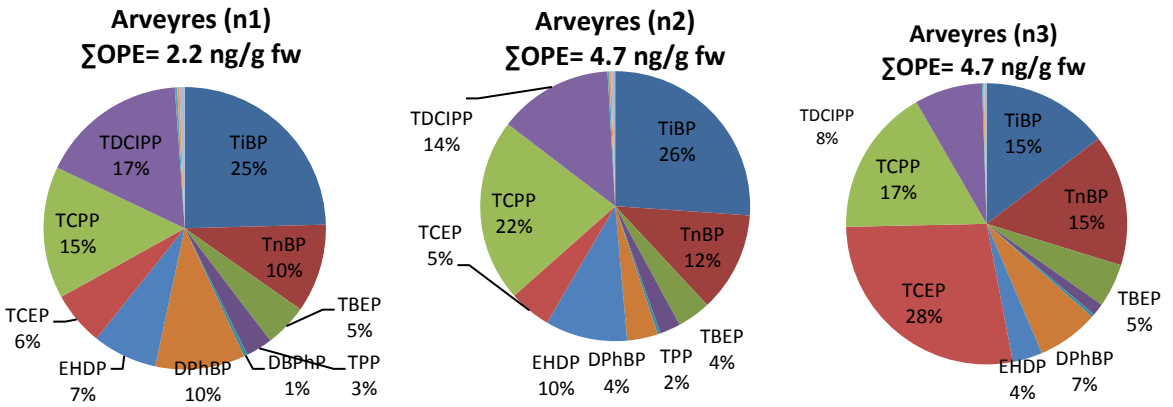


Figure 4-9: The profile of contamination in terms of compounds percentages in three samples collected at the same sampling site (Arveyres) on the Dordogne River.

Table V in the Annex shows the obtained results for each of these compounds, including the measured concentration ranges as well as some descriptive statistics.

As a conclusion from the freshwater fish set analysis:

- Detection Frequencies (higher than LoR or LOQ) ranged between 0 and 80%;
- Sampling sites total concentrations were lower than 10 ng/g fw.

Highest total concentrations were found up to 9 ng/g fw at:

- **Dordogne:** Branne, Arveyres, Tressac;
- **Garonne:** Cambes.

Profile of contamination:

- Between the different sampling sites; similar average contamination values based on the sum concentrations, same dominant OPEs, but not same contribution of individual compounds;
- Within the same site, the contamination levels and profile differ.

As a perspective:

The next step consists of analysing fish samples from the marine system for further interpretation

4.2.3.2. **Seawater fish samples**

- **Detection frequencies**

The detection frequencies for targeted OPEs in the set of 33 analysed samples from the marine system were estimated. As observed in Figure 4-10 and as illustrated from the freshwater samples, 7 OPEs (TEP, TPrP, o-TCP, m-TCP, p-TCP, TDBPP and TTBNPP) were not detected in any sample. For the other detected compounds, the detection frequency is much less important than what observed in river samples and ranged between 3% for TCEP to 49% for DPhBP.

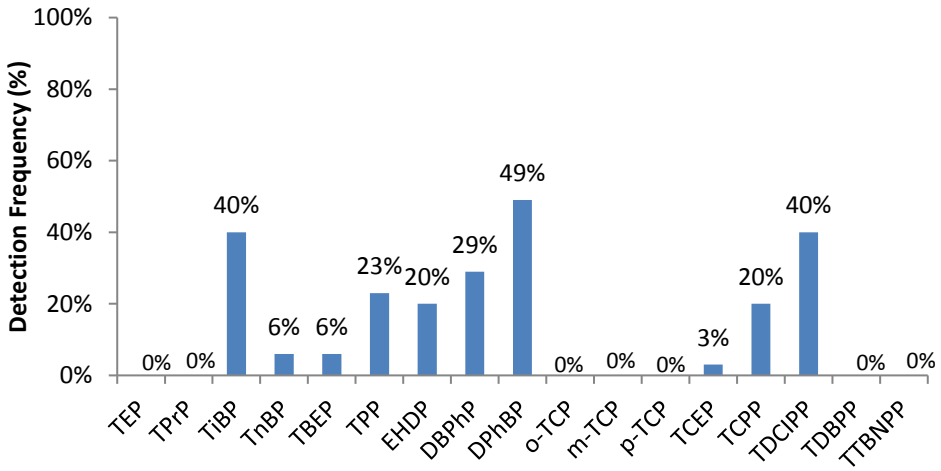


Figure 4-10: Detection frequencies (%) of studied OPEs in the analysed fish samples from the marine system.

➢ Total contamination level

The total contamination level was estimated by the addition of all the reported concentrations of targeted OPEs. The sum of contamination ranged from 1.5 to 9 ng/g fw. Figure 4-11 shows the total concentrations reported for each analysed sample from different species. For both pelagic and benthic species, the concentrations were ordered from the highest to lowest levels, which were always lower than 10 ng/g fw.

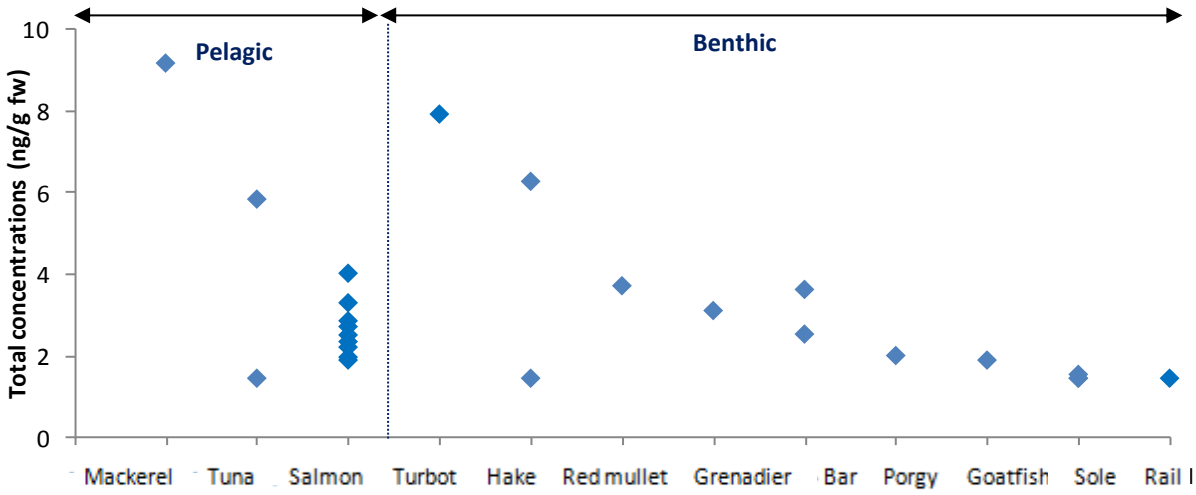


Figure 4-11: Total OPEs concentrations (ng/g fw) obtained for different seawater fish species (n=33).

➢ Pelagic *vs.* Benthic species

The interpretation of results was made by taking into account the living zone of different species. As proposed by Sundkvist *et al.* (2010), the benthic fish like turbot may be more exposed than other fish because OPEs are generally bound to particles and thus are likely to be more abundant in the sediment than in the pelagic zone. Figure 4-11 presents the total concentrations reported by classifying the species into pelagic and benthic ones. All the levels were below 10 ng/g fw and comparable loads were observed except that the highest concentrations were found in mackerel (from the pelagic zone) at around 9 ng/g fw and followed by the turbot (from the benthic zone) at around 8 ng/g fw. However, it is important to note that only one sample was analysed from these species so it is difficult to compare with other species of which we have analysed a higher number of samples. Besides, the samples are collected from different places which can be affected differently by contamination sources. The profile of contamination is represented in another manner in Figure 4-13. The sum of the average concentrations was found at 5 ng/g fw in the pelagic fishes and in the benthic fishes at 3 ng/g fw.

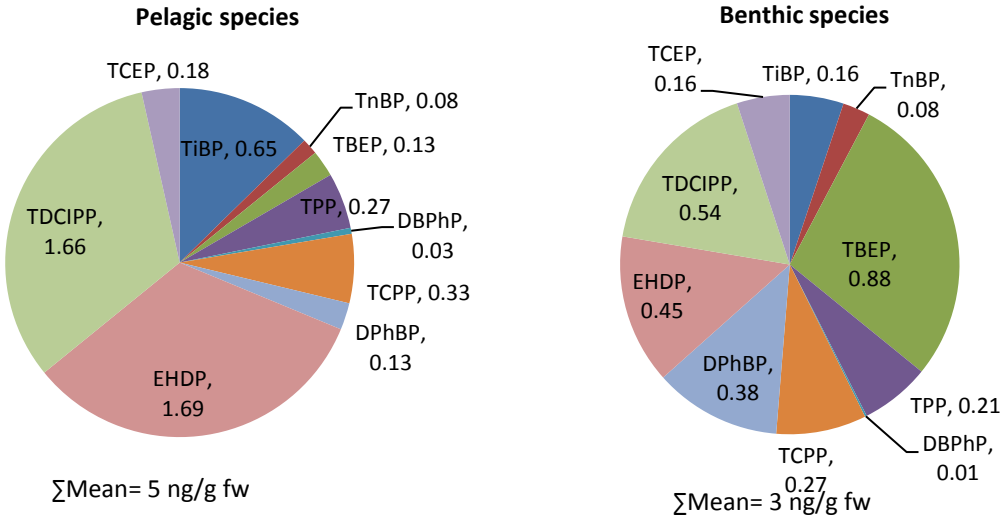


Figure 4-12: The profile of mean MB OPEs concentrations (ng/g fw) reported for fish from pelagic (left) and benthic (right) zones.

➢ **Contamination Profiles**

As mentioned in the beginning of this section and as illustrated by Figure 4-10, TiBP, EHDP, DBPhP, DPhBP, TPP, TCPP and TDCIPP were the compounds with highest detection frequencies. Besides, TiBP, TDCIPP and EHDP were dominated in mackerel sample (n=1) with concentrations at 1.7, 3 and 3.6 ng/g fw. TDCIPP dominated also in tuna (n=2), hake fish (n=2) and salmon (n=9) at mean concentrations of 1.0, 1.4 and 1.0 ng/g fw. EHDP has also been dominated in tuna, hake fish and red mullet (n=1) with mean concentrations of 1.3, 1.2 and 1.1 ng/g fw. TPP dominated in red mullet with concentration of 1.1 ng/g fw. TBEP was found in turbot sample (n=1) at 6.5 ng/g fw.

The correlation between the contamination and the lipid contained in the fish species was not investigated because it was already investigated by previous works and showed no correlation, suggesting that the accumulation of OPEs is not associated with lipids (Malavannan *et al.,* 2015).

4.2.3.3. **Sea versus fresh water fish samples**

Although the set of analysed samples included fish of different species which were collected from different locations, we were interested in performing a comparison between the sea water and river systems. In terms of total concentrations, a similar range from 1 to 9 ng/g fw was reported for all the analysed samples. Besides, Figure 4-13 presents the composition of reported contamination in terms of mean concentrations for river fish samples as well as the sea water fish of pelagic species. The dominating compounds were represented by TDCIPP, EHDP, TCPP, TnBP and TiBP. However, the percentages and hence the distribution per compound differ from the two systems but even between the different sites or species within the same system.

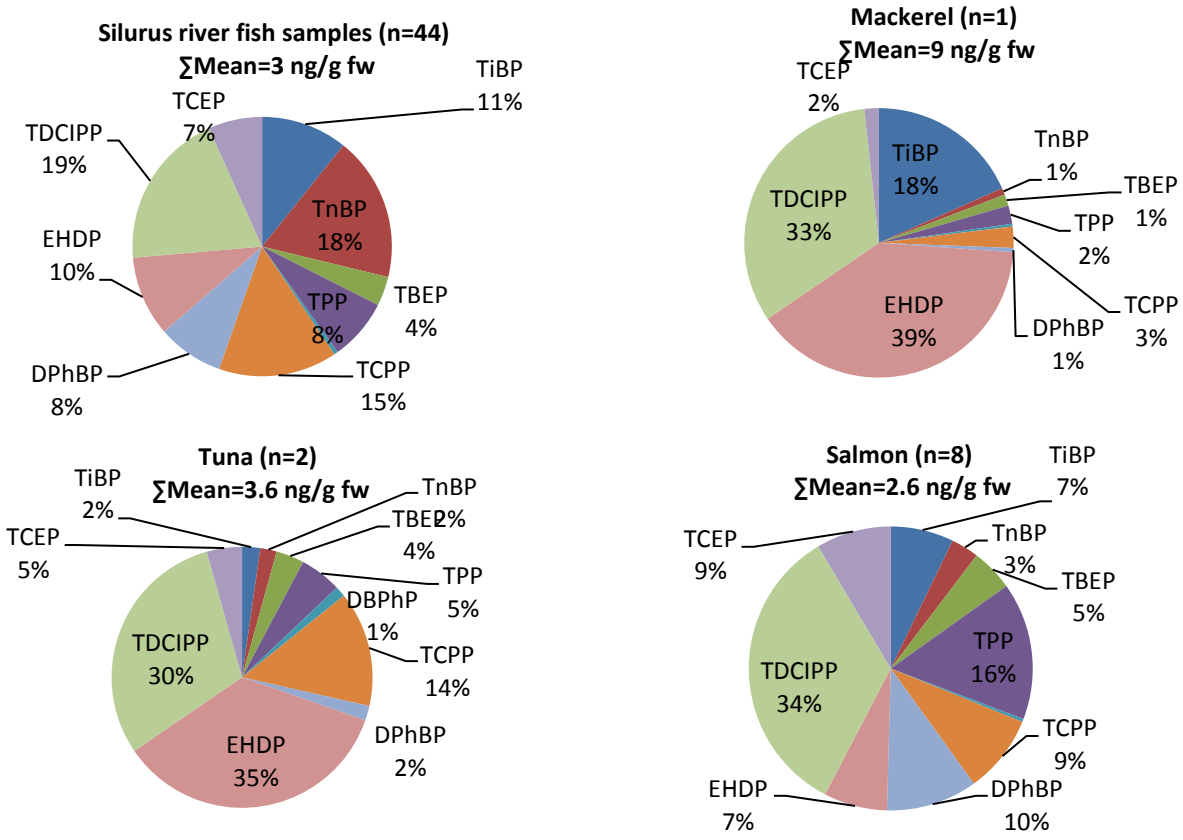


Figure 4-13: Comparison of the contamination profiles in terms of mean MB concentrations (ng/g fw) in river and seawater fish samples

These results of occurrence were the first released data at the national level. To conclude, TCPP, TiBP, TnBP, TCEP, TDCIPP, TPP, EHDP were the major contaminants in all the analysed fish samples. Some of the other compounds were detected but at a lower levels.

In the present context and to investigate further the data set, we also tested the PCA in order to examine the interrelation between results obtained from the freshwater to those from the marine system and hence to make a hypothesis about data distribution. The same plots are used as described in Figure 4-7 but here we have added the observations from marine system. As illustrated in the Figure 4-14, the discrimination between the two sets was not feasible and no great difference could be reported. Only 18% of the dataset variability was explained on first dimension and 13% on the second dimension. The illustration was also extracted for the combination of the first and third dimensions (as described in the two bottom plots in the Figure 4-14). Again here, the discrimination was not very important.

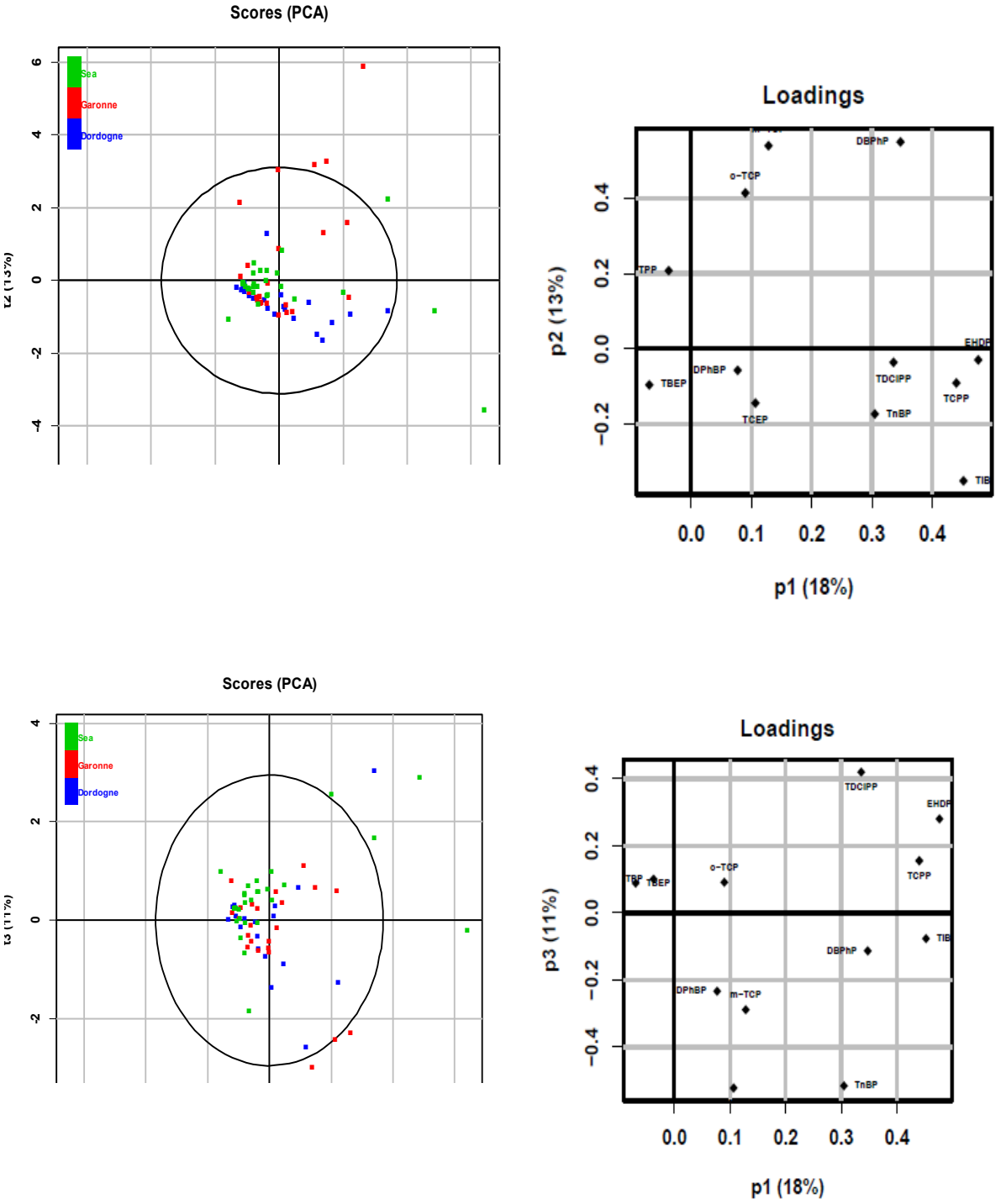


Figure 4-14: PCA summary plots for the comparison of sea to freshwater fish samples (score panel to the left and loading panel to the right). Top plot for the 1st and 2nd principal components and Bottom for the 1st and 3rd principal components.

By looking back to the literature review, it is obvious that this is a first study conducted at the French level. Overall, the OPEs loads (total concentrations lower than 10 ng/g fw) reported in our study showed to be comparable at both freshwater and marine systems.

If we were to compare with previous studies and more particularly at the European level, comparable OPEs loads were observed. Malarvannan *et al.* (2015) found levels ranging between 3.5 and 45 ng/g fw in wild European eels from freshwater systems in highly populated and industrial Flanders region (Belgium).

The possible explanation of low OPE concentrations suggest that such OPE levels can be due to the metabolic degradation, which is already verified in Zebrafish embryos (Malarvannan *et al.,* 2015).

As a perspective, comprehensive investigations on the bioaccumulation and biomagnification of these compounds in the food web are required to clarify their species-specific accumulation and risk assessment.

4.4. **FOOD SAMPLE SET**

4.4.1. DIET AS EXPOSURE SOURCE

Most foods have been reported to contain trace amounts of OPEs due to their wide use in plastics. Being used as plasticizers, these compounds are used in the synthesis of such packaging materials and hence amounts of OPEs can be present in the final product ready to be consumed by Human. This can represent a source of direct and indirect significant source of human exposure to these substances. In this part, we expect to find the non-halogenated (alkyl and aryl) compounds since these are mostly used as plasticizers.

It is worth to mention here the commission regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. This regulation has set some specific migration limits (SML) for TCEP, TEP and EHDP which are allowed to be used additive or polymer production aid and in the case of TEP it is allowed to be used as monomer or other starting substance. For TCEP and TEP, the SML is indicated as ND so that these substances shall not migrate in detectable quantities, in practice 0.01 mg/kg (equivalent to 10 ng/g fw). For EHDP, the SML was set as 2.4 mg/kg (equivalent to 2400 ng/g fw).

There is very little data on the transfer of organophosphate esters from food packaging in the food itself. Obviously, there is an important lack of data in particular in what concerns the presence of OPEs in foodstuffs.

4.4.2. SELECTED SAMPLES

The objective of this part was to characterize and prevent chemical risks by considering the manufacturing processes and packaging of foodstuffs. It was of great concern to evaluate this possible source of human dietary exposure to OPEs. We have analysed foods that are in contact with various plastic packaging materials. The matrices analysed in our study were principally foodstuffs widely and regularly consumed by Human (*e.g.* croissants, cakes, rice). All the selected samples (n=20) were packaged in plastic food contact materials and were obtained from a local retailer, in 2010 and 2015. Different nutritional categories were included: dairy products, meat, pastries and sweets and other snacks. The initial lipid content was not determined since the extraction included already a first purification step using Florsil®. Based on the extracted lipid mass and assuming similar lipid depletion efficiency of Florisil® along all extractions, the samples could be classified to be varied from high fatty ones like pine nuts and marble cakes to low fatty ones like beets.

4.4.3. RESULTS AND DISCUSSION

First of all and as we've done for fish samples, the procedural blank contamination levels were subtracted from the observed levels. For these food matrices, the limits of quantification were not calculated. Therefore, the same LOQs as for the fish samples were used.

The main analytical difficulty encountered in this part was the highly variable lipid amount from one sample to another. As usual, an amount of 1 g of lyophilized sample was extracted but whenever the amount of extracted lipid is very high like in the case of pine nuts and mascarpone cheese, then an aliquot of 200 mg was taken to be injected on GPC.

The total concentrations observed and quantified in foodstuffs ranged from 1.1 to 5000 ng/g fw and were classified in three groups. The first group includes the marble cake sample with the highest contamination and in which the ∑OPEs reached 5000 ng/g fw, containing mainly EHDP (> 99%). Other reported compounds contributed to a sum of 43 ng/g fw. The second group includes the samples ranging from 10 to 100 ng/g fw. This includes chocolate muffins, madeleines, croissants, milk rice and chips. The third group includes the other samples with total concentrations below 10 ng/g fw. This group is represented by pine nuts, cheddar processed cheese, cooked rice, provence and tomato pizza sauce, microwaveable beef meat, provencal pizza, beef steak (5 and 15% fat), mascarpone cheese, cooked red beets, chicken macaroni, microwaveable lamb with green vegetables and full-cream milk in descending order.

Among the 17 OPEs, 6 compounds were not detected (TEP, TPrP, o-, m-, p-TCP, TDBPP and TTBNPP) while 10 remaining compounds (TiBP, TnBP, TBEP, TPP, EHDP, DBPhP, DPhBP, TCEP, TCPP and TDCIPP) were detected reported in different frequencies in the analysed samples (Figure 4-15).

After having a look over the contamination levels in the different foodstuffs, we were interested to investigate the profile of contamination in terms of compounds composition. The Figure 4-16 represents the profile of contamination in the different food items.

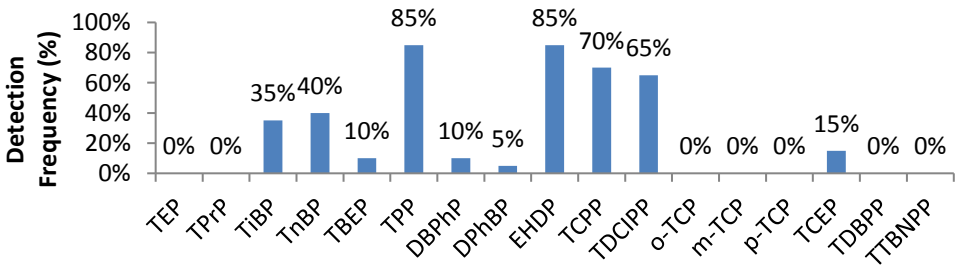


Figure 4-15: Detection frequencies (%) of 17OPEs in analysed foodstuffs (n=20).

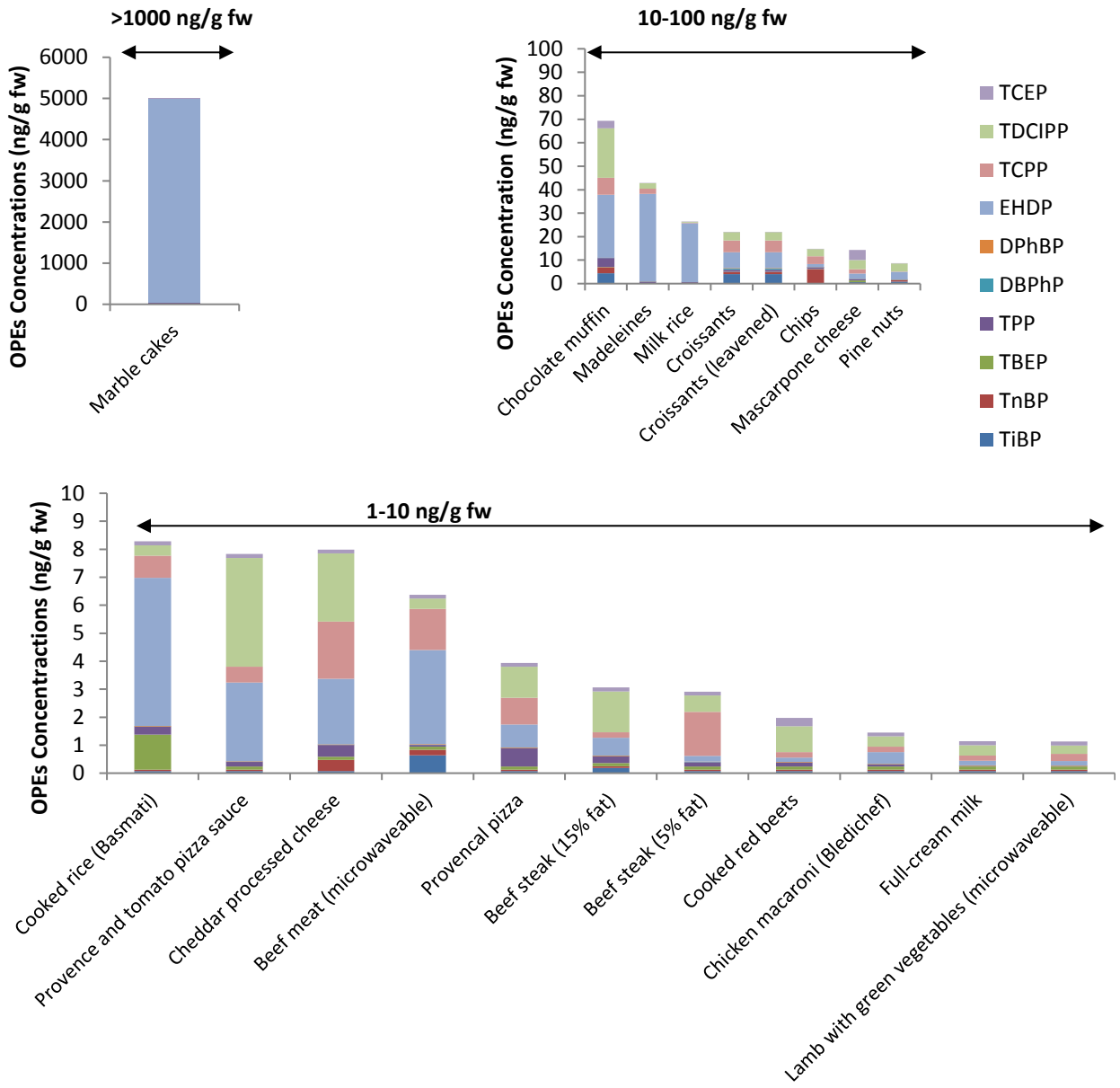


Figure 4-15: Total OPE concentration reported in the food samples, as analysed using GC-MS/MS through the developed SRM methods *via* EI and APCI modes.

As shown in the Figure 4-16, the contamination is attributed majorly to the same compounds (*i.e*. EHDP, TPP, TiBP, TnBP, TBEP, TCEP, TCPP and TDCIPP). In terms of distribution profile of compounds, highest variation was found for marble cakes, chocolate muffins, madeleine and croissants; that was because of the dominance of EHDP (and of TPP in case of the marble cakes) over the other OPEs.

As we have mentioned in the introduction of this chapter, EHDP, TEP and TCEP are allowed to be use in packaging material. However, TEP and TCEP must not show any migration above 10 ng/g while EHDP have an SML of 2400 ng/g. The corrected concentrations of EHDP in marble cake wasfound at 4963 ng/g, while in other samples the contamination order found was as follows; madeleines (37.4 ng/g fw) > chocolate muffins (26.9 ng/g fw) > milk rice (25.1 ng/g fw) > leavened croissants (6.7 ng/g fw). For

TCEP, the range of concentration was from 0.02 to 3.2 ng/g fw with the maximum highest levels were reported for chocolate muffin, croissants (leavened). For TEP, all the levels were lower than the limit of reporting (Below 100 ng/g). These first results showed that the compounds (TCEP and EHDP) are present in the samples and in the case of EHDP at levels higher than the SML. Such observation indicates either that the food item has been in contaminated at various stages of the industrial process or that a particular food contact material was non-compliant. Additionally, we should always bear in mind that our main objective is the food safety rather than the material conformity and unfortunately no tolerable limits are yet lied down for OPEs in food.

Then, we are going to shed the light on the other targeted OPEs, which are not cited in the regulation to be allowed for use in the packaging materials. Regarding the alkyl OPEs, the major contaminated samples with TiBP were reported for chocolate muffin and leavened croissants (4.5 and 4.0 ng/g fw, respectively). The reported contamination ranged from 0.1 to 4.5 ng/g fw. For TnBP, the 4 highest corrected concentrations were reported for chips (6.0 ng/g fw) > chocolate muffins (2.5 ng/g fw) > croissant (2.0 ng/g fw). The reported range was from 0.01 to 6 ng/g fw.

For the aryl OPEs, the highest contamination was found for marble cake which showed very high intensity in comparison to other samples. The corrected concentrations in marble cake were found up to 37.7 ng/g fw for TPP. For the other samples, the 4 highest concentrations of TPP were found for chocolate muffins (3.8 ng/g fw), croissants (1.0 ng/g fw) > chips (0.7 ng/g fw) > madeleines (0.6 ng/g fw). The whole reported range was 0.01 to 37.7 ng/g fw. The total ion chromatogram (TIC) of the marble cake sample is illustrated in Figure 4-17 along with the extracted ion chromatograms (EIC) of the main reportable compounds, as analysed by GC-MS/MS *via* EI (for EHDP, TDCIPP and DPhBP) and APCI (for other OPEs).

Regarding the chlorinated OPEs and more particularly for TCPP, the concentrations ranged from 0.06 to 7.3 ng/g. The maximum levels were reported for chocolate muffins, croissants, chips and Madeleine. For TDCIPP, the concentrations were in the range 0.01 to 21 ng/g with the maximum level reported for chocolate muffin.

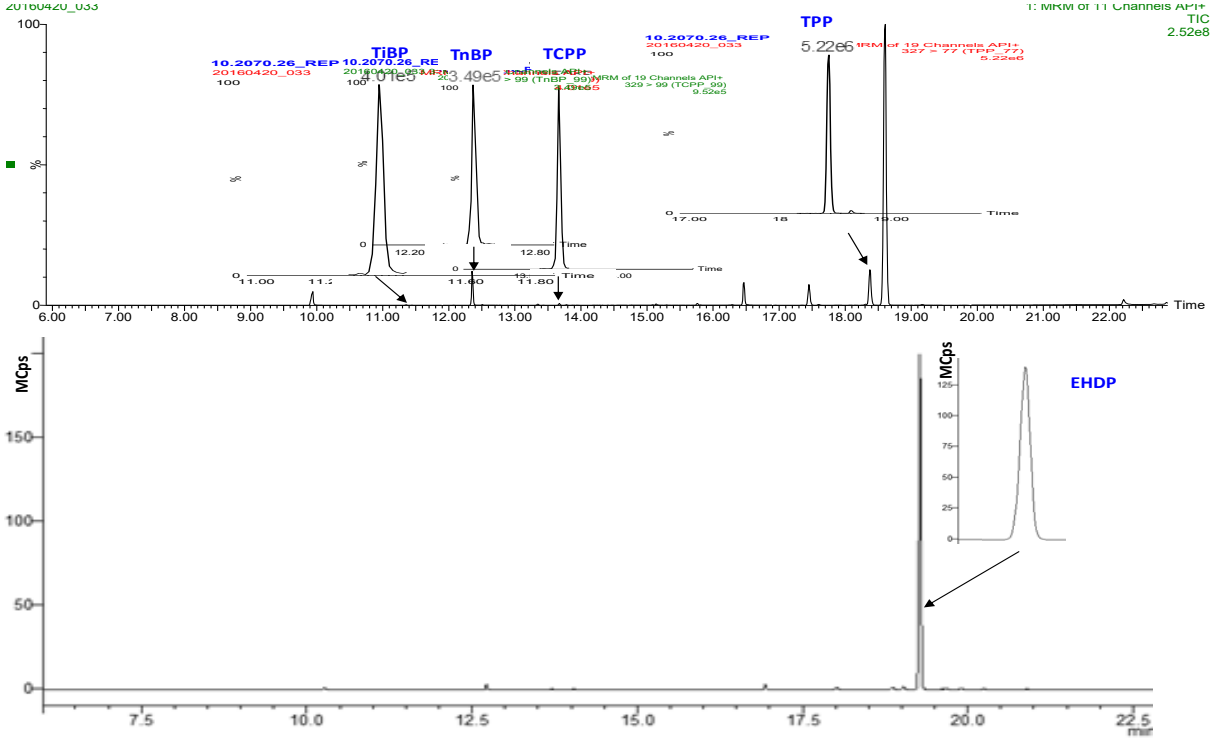


Figure 4-16: Total ion chromatogram (TIC) of marble cake sample; along with the extracted ion chromatograms (EIC) of the main reportable compounds, as analysed by GC-MS/MS *via* APCI (top) and EI (down) modes.

As a conclusion on the foodstuffs analysis, all of the studied set of samples presented OPEs contamination and this to different extent, with total concentrations ranging from 1 to 100 ng/g fw, except for the marble cake. Such contamination could be attributable to any source during the preparation, or transport of product.

4.5. **RISK CHARACTERISATION EXERCISE**

After implementing the developed strategies for the analysis of sets of fish as well as other foodstuffs, we were interested in conducting a first interpretation of our data with regard to consumer exposure and possible health implications. In particular, we intended to provide original data on the contribution of characterized food items to the exposure through diet to studied OPEs. As previously described in Chapter 1, the QRA is the use of measurable, objective data to determine asset value and associated risk(s). It is characterised by assigning a numerical value to the risk, in contrast with qualitative risk analysis, which is typified by risk ranking or separation into descriptive categories of risk. The implementation of the QRA approach would require the selection of toxicological reference value to be compared with the estimated exposure in order to contribute finally to the risk assessment.

4.5.2. TOXICOLOGICAL REFERENCE VALUE

As already explained in Chapter 1, the information from toxicological studies on the dietary chronic exposure was only conducted, if available, for animals. The toxicological reference values (TRVs) are calculated from the toxicological data type NOAEL and/or LOAEL. For human oral exposure, the TRV is called ADI (Acceptable Daily Intake) and is determined by applying an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) to the highest dose in animal studies which has been demonstrated not to cause toxicity (NOAEL commonly used). It is expressed in amount (mg or µg or ng) per kilogram of body weight (bw) per day, and hence calculated as follow:

$$ADI\ ((\text{human dose in mg/kg bw})/\text{day}) = \text{NOAEL}((\text{experimental dose}))/\ \text{Safety Factor(s)}.$$

Indeed, TRVs or ADIs are established by the authorities' bodies such as the World Health Organization (WHO), JECFA (Joint FAO/WHO Expert Committee on Food Additives), ATSDR (Agency for Toxic Substances and Disease Registry), US EPA (Environmental Protection Agency), ANSES (French Agency for Food, Environmental and Occupational Health & Safety) or EFSA at the EU level. However and as illustrated in Table 1-2, these data are not available yet for all OPEs. Therefore, in an attempt to calculate ADIs to be used in the present exercise, we've exploited the available ones but for the other compounds. In this context, we've calculated the ADIs from the available NOAELs (by applying an uncertainty factor of 100, as explained in the previous paragraph). The ATSDR (USA) have derived these values for certain OPEs, but based purely on results from animal studies since no reliable studies were located on health effects in humans exposed orally to these compounds. The Table 4-1 hereafter illustrates the retained ADI values corresponding only to the quantifiable OPEs, to be employed in the further steps of risk assessment exercise.

Table 4-1: ADI values (in mg/kg bw/day) as established by ATSDR and EPA for TnBP and TCEP, and as calculated by our study for the others, for the Human oral exposure*.

OPE	**TnBP**	**TCEP**	**TBEP**	**TPP**	**EHDP**	**TDCIPP**	**TCPP**
ADI in mg/kg bw/day (Endpoint)	0.08 (urinary bladder hyperplasia)	0.2 (renal tubule lesions). 0.007 (kidney effects)	0.15 (Hematological and clinical effects)	6.9 (Fertility) 7.1 (Immune & nervous toxicity)	1.65 (increases in kidney, teste and brain weight)	0.14 (Hematological and clinical effects)	0.08, 0.8 (Histopathologic effects for male and female, resp.)
Exposure Duration	Chronic		Perchronic or Subchronic				
Reference	(ATSDR, 2012)	(ATSDR, 2012) (US EPA, 2015)	(WHO-EHC218, 2000)	(OECD-UNEP, 2002)	(TOXNET, 2016)	(TOXNET, 2016)	(OECD-UNEP, 2002)

- Values extrapolated in the present work from available literature data

4.5.3. EXPOSURE ESTIMATION

Based on literature overview in Chapter 1, ingesting contaminated food is considered a primary source of exposure for humans to OPEs. Most foods have been found to contain trace amounts of OPEs due to their wide use in plastics and presence in the environment. Other route is represented by food contact materials treated with these compounds. We mainly focused, in this exercise, on fish, as the main source of exposure for humans. In this step, we are interested in the calculations of exposed daily intake (EDI) in g/kg bw/day. The difficulties in this part are related to the determination of food habits as well as the occurrence levels which are still rare to the pollutants like OPEs.

As mentioned in this Chapter, two series on fish samples were analysed and contained samples from river and samples from the sea. In these samples, the major contamination was attributed to 7 compounds (TiBP, TnBP, TPP, EHDP, TCEP, TCPP and TDCIPP). So, we intended to focus on these compounds and to estimate population exposure through the river and sea fish samples by taken the mean of the reported concentration levels.

For the illustration of food habits, we have referred to the EFSA comprehensive European food consumption database from the Individual and National Study on Food (INCA2) survey which was conducted over 11 months from 2006 to 2007 and included 2276 adults aged 18 and over and 1444 children (adolescence and other children) aged 3 to 17. Table 4-2 presents the necessary statistics resulted from the INCA2 data survey on the chronic food intake for adults and children. Table 4-3 then illustrates the procedure followed to assess qualitatively, the human risk based on the exposure data based on the selected ADI values presented in Table 4-1. The exposed dietary intake (EDI) to these reported OPEs, was then estimated as follows:

$$\mathbf{EDI}\left(\frac{\mathbf{ng}}{\frac{\mathbf{kg\ bw}}{\mathbf{day}}}\right) = \mathbf{Exposure}\left(\frac{\mathbf{ng}}{\mathbf{g}}\ \mathbf{fw}\right)\mathbf{x}\ \mathbf{Food\ habits}\left(\frac{\mathbf{g}}{\frac{\mathbf{kg\ bw}}{\mathbf{day}}}\right).$$

The occurrence or exposure levels (in ng/g fw) were calculated as the mean of concentrations from river and marine fish samples. The food habits were extracted from the EFSA Database on the INCA 2 dietary survey in France, as presented in Table 4-2.

Table 4-2: Chronic food consumption statistics (g/kg bw/ day) from INCA2, as delivered by EFSA from french dietary survey (INCA2).

Population class	Food group	Number of subjects	Number of consumers	Mean consumption (g/ kg bw/day)	95th percentile of consumption (g/ kg bw/day)
Adult (18 years and over)	**Fish meat**	2276	1716	0.32	1.03

We were interested in evaluating the exposure of adult population through two scenarios of fish meat consumption (in g/kg bw per day). The first scenario represented by normal consumption and is delivered from the mean consumption. The second scenario included those with maximal fish consumption (5% of the studied consumers), and is delivered from the P95 percentile of consumption as given by the survey. From here, the mean contamination level was assigned for the two scenarios and the daily dose of exposure (EDI) was estimated..

4.5.4. APPROXIMATE RISK RATIOS

It is the estimation of the incidence of health effects under the various conditions of human exposure. This step in risk assessment includes the calculation of risk ratio (RR) for the studied populations. This area is complex for us especially with our few data. Our aim was therefore to exploit our data so as to bring the exposure levels thus calculated compared to existing permissible doses for these compounds. The RRs were evaluated by dividing the exposure (the calculated EDI) by the defined TRV. All the values (Table 4-3) obtained were found lower than 1 (down to 10^{-8}) which 'likely' shows no potential of adverse effects.

Table 4-3: Exploitation of information (for chronic-oral exposure to OPEs) required for approximate QRA exercise in adults.

		TnBP	TPP	EHDP	TCEP	TCPP	TDCIPP
Approximate occurrence value in river Fish (ng/g fw)		0.5	0.2	0.3	0.3	0.5	0.6
Exposed daily intake- (ng/Kg bw/day)	Scenario 1	0.16	0.06	0.09	0.09	0.16	0.19
	Scenario 2	0.51	0.2	0.3	0.3	0.51	0.61
Risk ratio- (EDI/ADI)	Scenario 1	Down to 10^{-6}	Down to 10^{-9}	Down to 10^{-8}	Down to 10^{-7}	Down to 10^{-7}	Down to 10^{-6}
	Scenario 2	Down to 10^{-6}	Down to 10^{-8}	Down to 10^{-7}	Down to 10^{-6}	Down to 10^{-7}	Down to 10^{-5}

As a conclusion and despite that the first results showed no risk on human. However, it is worth to note that the exercise only focused on dietary exposure *via* fish and didn't take into consideration the other foodstuff which might be much more consumed by the population and much more contaminated (500 times for marble cakes) as well as other exposure sources. For further exposure assessments, it is important to consider OPEs concentrations from all possible sources and the personal habits of different population categories (*e.g.,* infants).

4.6. **CONCLUSION**

The dietary exposure of OPEs *via* food ingestion is a major concern for the general population as these substances have been classified as potential endocrine disrupters. In this chapter, fishes from rivers and marine areas were investigated for their contribution to human exposure to OPEs. Moreover, foodstuffs consumed by humans can be contaminated with OPEs due to the use of OPE containing packaging plastics. We have evaluated also the occurrence of these OPEs in various foodstuffs and hence the residual transfer of these compounds from material to the packaged food we have investigated. This was feasible through the implementation of the developed analytical strategy for the analysis of these sets of samples.

On one hand, the OPEs compounds were quantified at concentrations of up to a 10 ng/g in river fish and sea fish samples. The analysed set of foodstuffs seem to contain more OPEs so that some samples contained less than 10 ng/g w and other samples contained levels between 10 and 100 ng/g fw and what's surprising was the very high contamination by EHDP in an analysed marble cake sample up to almost 5000 ng/g fw. Based on the commission regulation (EU) No 10/2011, this compound is allowed to be used as additive in food contact materials but with SML of 2400 ng/g. This might reflect the

nonconformity of used packaging material however it might also be attributed to dispersed contamination sources during industrial processes.

Finally, we conducted a first interpretation exercise to determine the contribution of fish to the diet exposure to OPEs for the French population based on their fish consumption habits (from EFSA comprehensive Database). The purpose was to estimate and compare the chronic dietary exposure (EDI) to the ADI, already specified or calculated from the available toxicology studies. Risk ratios reflecting the human health risk *via* dietary intake of OPEs have resulted to be low.

This might suggest that human exposure to OPEs through eating fish is of minor importance in relation to other potential exposure pathways, such as via ingestion and inhalation of indoor dust. However, it is worth to note that the ADI values were calculated from the NOAELs available for certain but not all OPEs. It should be noted that these values are based on relatively old toxicological studies. It is therefore possible that new toxicological data on OPEs may reduce the margin of safety and thus the subsequent ADI.

By the end, it is worth noting that these data provide the first nationally available results and represent a pilot study to provide answers to questions that land on the exposure of the French population in these re-emerging compounds.

GENERAL CONCLUSION AND PERSPECTIVES

Being applied as Flame Retardants (FRs) and plasticisers, Organophosphate Esters (OPEs) are industrially used in high volumes and are incorporated in various products in our daily lives (furniture, electronics, textiles or packaging...). These OPEs are inevitably released into the environment throughout the products' entire lifetimes. They may be then transferred throughout the environment and the food chain related to humans. An issue has been raised in recent years about their potential adverse effects on human health, which may result from exposure to these re-emerging pollutants. This gives rise to a growing interest from the international scientific community and urges the development and implementation of efficient analytical approaches to accurately monitor their occurrence.

Although previous efforts have been dedicated to the analysis of these re-emerging compounds in different environmental compartments (*e.g.* dust and sediments), little information is available on their occurrence in biota samples, which might be partly due to the lack of efficient analytical strategies. The aim of this thesis was then to develop an efficient analytical strategy for the analysis of a large range of 18 OPEs, selected as representative of alkyl, aryl and halogenated compounds exhibiting wide variety of physical chemical properties. With the developed strategy, the research work aimed at contributing to the Human exposure assessment at the French level to these targeted OPEs. In the context of chemical food safety, the thesis focused on the dietary exposure through the consumption of fish, as being an essential dietary source for many people as well as other foodstuffs.

The major conclusions that can be drawn from the studies presented in this thesis can be summarised as follows:

1) In terms of instrumental approaches and using LC-MS/MS, the positive ESI mode was much more relevant than the negative mode for the analysis of OPEs. However, with the use of LC instrumentation, isomers co-elution issues were highly encountered and not finally resolved. This posed a major limitation for the further use of this technique in the study.
2) Still in the trend of instrumental approaches, GC-MS/MS was extensively investigated with the use of the main ionisation modes (EI, NCI and PCI) as well as the innovative APCI mode. To the best of our knowledge, it is the first work to demonstrate in details on the use of APCI for such large range of OPE compounds. Among the investigated ionisation techniques, EI and APCI were retained, SRM acquisition methods were developed and the spectrometric conditions were optimised. In comparison to the GC-EI-MS/MS, the SRM method on GC-APCI-MS/MS showed to be very advantageous in terms of detection's selectivity.
3) GC chromatographic separation of 16 OPEs can be achieved in less than 25 minutes using a GC capillary column (30 m x 0.25 mm, 0.25 µm). In parallel, the GC chromatographic separation

of the 2 brominated OPEs can be achieved in less than 10 min using short GC capillary column (15 m x 0.25 mm, 0.10 µm).

4) Various purification (LLE, SPE, GPC and SPLE) and extraction (QuEChERS and PLE) techniques were investigated and compared in terms of efficiency to deplete lipids while maintaininig the good extractability of targeted compounds.
5) Based on the comparison of different investigated techniques, a complete analytical strategy was developed starting with selective pressurised liquid extraction (SPLE) as an extraction and a preliminary purification step. This was followed by gel permeation chromatography (GPC) as a second purification step. The overall method showed to be highly efficient for the optimal extraction of OPEs with maximal separation (> 98%) from the lipids and other interfering substances in complex matrices like fish.
6) Using some validation parameters, the performances were evaluated with the SRM methods developed *via* both GC-EI-MS/MS and GC-APCI-MS/MS and showed that each technique owns positive and negative sides. For most compounds but with 2 exceptions, the sensitivity *via* APCI was either comparable or better than that using EI mode. In terms of selectivity, APCI was much more relevant than EI where extensive fragmentations were encountered. In terms of stability, the responses were generally more stable with EI than APCI. Based on the detailed performance evaluation of each technique, EI was selected for 3 compounds while APCI was selected for the others.
7) The scarce knowledge on the occurrence of these OPEs in food is very clearly felt, especially in France. Therefore, the finalised protocol for the developed analytical strategy was applied, for the first time, to several sets of samples (fish and other foodstuffs) intended for human consumption at the French level.
8) Overall and in a summarized way, the fish samples contained OPEs at levels lower than 10 ng/g fw. In parallel, the analysed set of foodstuffs appeared to contain more OPEs so that some sample contained less than 10 ng/g w while other samples contained levels between 10 and 100 ng/g fw.
9) EHDP was present importantly in an analysed marble cake sample up to 5000 ng/g fw. Based on the commission regulation (EU) No 10/2011, this compound is allowed to be used as additive in food contact materials but with a specific migration limit of 2400 ng/g. This might reflect the nonconformity of used packaging material however; it might also be attributed to a cumulative contamination through different sources during manufacturing, transport, storage, *etc.*
10) The contribution of fish to the human dietary exposure to OPEs was finally investigated. Results showed that the exposed dietary intake (EDI) may be considered as several orders of

magnitude lower than the acceptable daily intake (ADI). This would suggest that human exposure to OPEs through eating fish is of minor importance in relation to other potential exposure pathways, such as *via* ingestion and inhalation of indoor dust. However, it is worth to note that the ADI values were only available from ATSDR for few OPEs and based on old toxicological studies. It is therefore possible that new toxicological data on OPEs may reduce the margin of safety and thus the ADI.

11) Those data are the first results available at French level contributing to the risk assessment of these re-emerging compounds for the French population; this reinforces the interest of nationwide measures for better exposure characterisation.

A number of perspectives can be highlighted as follows:

1) The full validation of the analytical method according to recognised guidelines in the field of chemical analysis at trace levels in complex biological matrices.
2) The application of the developed analytical method on a larger scale appears as a necessity in order to achieve a higher degree of representativeness.
3) The communication of the generated contamination data to agencies in charge of chemical risk assessment of food (Anses, EFSA, ...). Such data may indeed be considered of interest for assessing the OPEs risk for the general population but also for specific populations such as high fish consumers for instance.
4) The application of the analytical strategy, as a next step of this international joint thesis, to a selection of Lebanese food items in order to provide a very first occurrence set of data in Lebanon. Furthermore, it appears relevant to monitor these re-emerging contaminants in this country, so that the transfer of the analytical process could be studied.
5) The investigation of the extent to which the proposed analytical workflow could encompass other compounds to provide a high throughput multi-residue method for the simultaneous determination of a larger panel of contaminants of different families of compounds.
6) Finally, with the high evidence on the metabolism in fish of organophosphate triesters into diesters; it is possible then, that the low OPE levels measured in the present study are due to metabolic (in the fish) or other environmental degradation processes. An important perspective could be represented the extension of work to the investigation of OPEs body burden in Human through the analysis of major OPEs metabolites which constitute a great concern issue for assessing the associated risks to human. Such internal exposure data would then be fully complementary to external exposure data for robust OPEs risk assessment.

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ANNEXES

Table I: Descrribed detection methods based on gas chromatographic techniques, used for the analysis of OPEs in different matrices. *V_{inj} and T_{inj} : injection volume and temperature, respectively; EI refers to Electron Impact; Q refers to Quadrupole; TQ refers to Triple Quadrupole; LODs and LOQs refer to limits of detection and quantification, respectively).

Matrix	Number of target OPEs	Chromatographic conditions		Spectrometric conditions		Method performances		Reference
		Injection mode	Column	Ionization mode	Mass filter	Recovery (%)	LODs and/or LOQs	
Dust	10	**Pulsed pressure** (200 KPa for 1.5 min) Pulsed **splitless** Carrier gas flow 1.3 mL/min	BPX5 (25 mm x 0.25 mm, 0.25 µm) **Oven program:** 110 °C (3 min) to 190 °C at 15 °C/min, then to 310 °C at 10 °C/min (4 min)	EI (230 °C)	Q (150 °C)	82-112 % (except for TBEP, TEHP and TCP)	LODs 0.2-29 ng/g fw	**Brandsma et al. 2014**
	10	**Splitless** Carrier gas flow 1.5 mL/min V_{inj} 2 µL	DB-5MS (15 m x 0.25 mm, 0.10 µm) **Oven program:** 60 °C to 220 °C at 10 °C/min, then to 315 °C at 15 °C/min (8 min)	EI	TQ	60-138 % (except for TBEP 48%) RSD <15% (except for TCPP in sediment (53%)	LODs 1.9-60 ng/g (sediment) 28-575 ng/g (sludge) 3.8-288 ng/g (dust)	**Cristale et al. 2013**
	8	**Programmed Temperature Vaporizer (PTV)** program: 90 °C (0.03 min), ramp 700 °C/min to 290 °C **Pulsed pressure** 14.3 psi until 1.25 min and purge flow of 50 ml/min after 1.25 min. Carrier gas flow 1 mL/min	HT-8 (25 m x 0.22 mm x 0.25 µm) **Oven program:** 90 °C (1.25 min) to 240 °C at 10 °C/min, then to 310 °C at 20 °C/min (16 min)	EI (230 °C)	Q (150 °C)	80-110 % (except TEP and TPrP) Between day precision < 24% except TEP, TiBP and TBEP	LOQs 10– 370 ng/g	**Van den Eede et al. 2012**
Air	8	**Splitless** V_{inj} 1 µL Carrier gas flow 1.3 mL/min **PTV program**: 60°C (0.1 min), increased at 500°C/min to 300°C	HP-5MS (30 m x 0.25 mm, 0.25 µm) **Oven program:** 40 °C (4 min), 5 °C/min to 170 °C (5 min), 10 °C/min to 230 °C (5min), 5 °C/min to 250 °C then 10 °C/min to 300 °C.	EI Transfer line (280 °C)	Q (230 °C)		LODs 1 - 94 pg/m^3	**Moller et al. 2011**

Biological samples	14	**Splitless** Tinj 280°C V_{inj} 1 µL Carrier gas flow 0.8 mL/min	DB-5MS (30 m x 0.25 mm, 0.25 µm) **Oven program:** 80 °C (1 min), increased to 180 °C with 10 °C/min (1 min), 2 °C/min to 300 °C (10 min)	EI (230 °C) Transfer line (290 °C)	Q (150 °C)	70.3-111% except TMP (38.9-55.6%) RSD<14%	LODs 0.006-0.02 ng/g lw	**Ma et al. 2013**
Breast milk and biota	11	**Splitless** Tinj 250°C Vinj 1µL Carrier gas flow 1.3 mL/min	DB-5 (30 m x 0.25 mm x 0.25 µm) **Oven program:** 80 °C (4 min), increased to 190 °C with 15 °C/min, then at 10 °C/min to 310 °C (4 min)	EI at 36.5 eV	HRMS		LODs In milk, 0.02–3.7 ng/g In biota, 0.05 -23 ng/g lw	**Sundkvist et al. 2010**

Table II: Described detection methods based on liquid chromatographic techniques, used for the analysis of OPEs in different matrices.

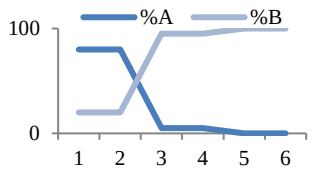
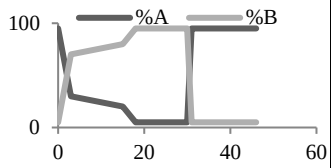
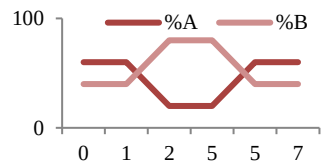
Matrix	Number of target OPEs	Chromatographic conditions		Spectrometric conditions		Method performances		Reference
		Mobile Phase	Column	Ionization mode	Mass filter	Recovery (%)	LODs and/or LOQs	
Fish	9	(A): H_2O (+ 0.1 % Formic acid) (B): MeOH +10mM ammonium acetate 	Asentis express C_{18} (100 x 2.1 mm, 2.7 µm)	ESI (+)	TQ	Rec: 58.1-114% RSD (n=7) < 16%	LODs 0.001-0.014 ng/g	**Kim et al. 2011**
Herring gull eggs	12	(A): H_2O (+0.1% F.A) (B): MeOH (+0.1% F.A) 	Waters Xterra ® phenyl column (2.1 mm x 100 mm, 3.5 µm) T : 40 °C	ESI (+) (100 °C) Capillary voltage 4 kv Cone gas flow 150 L/h	TQ	Mean Rec: 89-104 %, except TDBPP and TBMPP (67 and 72%) RSD (n=6) < 16%	IDLs (0.01-0.12 ng/ml) LOQs 0.06-0.20 ng/g fw	**Chen et al. 2012**
Foodstuffs (milk powder)	9	(A): H_2O (+0.1% F.A) (B): ACN 	Phenomenox kinetex PFP (50 mm x 3.5 mm, 2.6 µm) T : 30 °C	ESI (+) (150 °C) Capillary voltage 0.5 kv Cone gas flow 50 L/h	TQ	Rec: 73.5% and 110.2%. RSD (n=6) < 11%	LODs 0.1–0.25 ng/g,	**Guo et al. 2016**
Breast milk	10	As described in **Kim, (2011)**	As described in **Kim, (2011)**	ESI(+)	TQ	Rec: 70-115% RSD ≤16%	LODs (n=7): 0.01 (2.7%) - 0.08 (7.9%) ng/g lw	**Kim et al. 2014**

Table III: Descibed extraction and clean up techniques for the OPEs analysis in different matrices.

Number of analytes, matrices,…	Extraction technique- Description	IS or ES included	Purification technique- Description	Contamination issue (blank)	Reference
10 Sediment, sewage sludge and dust	**US** 1.5, 0.1 and 0.1 g of sediment, sludge and dust. US (10 min) with **EtAc/cyclohexane** (5:2, v/v), followed by centrifugation (10 min at 3000 rpm). Repeated twice and concentration under N_2 in a Turbovap.	TBP-d_{27} and TPP-d_{15} used as IS	**Florisil cartridges** (10, 10 and 5 g for sediment, sludge and dust) with **EtAc/cyclohexane** (5:2, v/v) for elution (60, 60 and 30 mL for sediment, sludge and dust).	**Glassware** Backed overnight at 350 °C. **Glass materials** Solvent rinsed before use. **Plastic materials** avoided. Samples and glassware covered with aluminum foil whenever possible, to avoid dust particles deposition.	**Cristale et al. 2013**
10 Indoor dust, car dust	**US** 50 mg of dust was extracted in two steps with **acetone and toluene**. Each extraction was performed by 1 min of vortex mixing followed by 15 min of **ultrasonication**. The combined supernatant was filtered over dried sodium sulfate and evaporated under nitrogen.	TBP-d_{27} and TPP-d_{15} used as IS			**Brandsma et al. 2014**
8 Indoor dust	**US** 75 mg dust extracted using US (5 min) with **HEX/ Acetone** (3:1, v/v) and vortex (1 min) repeated three times. This followed by centrifugation at 3500 rpm for 2 min and further evaporation till dryness.	TPP-d_{15} used as IS	Fractionation step: Florisil **SPE** cartridges (500 mg, 3 mL) by using 10 mL **EtAc** for elution.	High and variable contribution of TiBP	**Van den Eede et al. 2012**
10 Indoor dust	**US** 75 mg dust extracted using US (5 min) and vortex (1 min) with **DCM**. This repeated twice	TAP and TPP-d_{15} used as IS	**Florisil SPE cartridges** (500 mg, 3 mL) by using 10 mL **EtAc** for elution.	High and variable contribution of TiBP	**Van den Eede et al. 2011**

	and followed by centrifugation and evaporation until dryness.				
8 **Air**	**Soxhlet** Extraction was based on soxhlet for 16 h using **DCM** and followed by rotary evaporation step.	TBP-d_{27} and TPP-d_{15} used as IS ^{13}C-HCB as ES	**SPE** using 2.5 g of **10% H_2O deactivated silica** gel column and anhydrous sodium sulfate, with 30 mL **DCM/acetone** (1:1, v/v) for elution	All air columns were pre-cleaned with solvents of different polarity. All used glass ware was baked at 250°C for 10 h and rinsed with acetone and silica gel was cleaned with acetone for 12 h and baked at 450°C for 12 h prior to usage.	**Moller et al. 2011**
14 **Biological samples**	**MAE** 1 g of muscle samples was transferred to the extraction cylinders and kept overnight at room temperature in darkness. The extraction was then performed at 1200 W using ramp-to-temperature mode (ramp time: 10 min). Extraction was done at 100 °C for 30 min with **HEX/Acetone**. This was followed by filtration and concentration under N_2.	TBP-d_{27} used as IS	1 **GPC** (300 mm x 10 mm i.d) packed with 5 g of 200-400 mesh Bio-Beads S-X3 using 5 mL DCM/HEX. 2 **SPE** (4 g of **3% H_2O deactivated silica gel** packed in glass column (300 mm x 10 mm i.d) using 30 mL **Acetone/EtAc** (3:7, v/v).	Laboratory glassware was soaked overnight in a K_2CrO_7/H_2SO_4 solution, washed with tap water and redistilled water, baked at 300◦C for 12 h, and then rinsed with Ace, DCM and Hex. Silica gel was cleaned using DCM, MeOH and HEX using Soxhlet for 72 h, activated at 180°C for 12 h and then deactivated.	**Ma et al. 2013**
12 **Herring gull eggs**	**PLE** The sample was subjected to accelerated solvent extraction (ASE 200) with 50:50 (**DCM/HEX**) at 100 ◦C and 1500 psi.	TBP-d_{27} used as IS	The extract was cleaned on a **1 g ISOLUTE aminopropyl silica gel SPE** column packed into a 6 mL SupelcleanTM glass cartridge. Elution using 4 mL 20:80 **DCM/HEX**, followed by 8 mL **DCM**.	The SPE column was prewashed with 15 mL 50:50 DCM: methanol, 15 mL DCM and 20 mL HEX to clean the silica gel absorbent.	**Chen et al. 2012**
9 **Fishes**	**PLE** 5 g of fish muscle tissues were freeze-dried for 72 h and homogenized with anhydrous	TBP-d_{27} used as IS TPP-d_{15} used as ES	**SPE** using glass column (200 x 10 mm i.d) packed with 4 g of **5% H_2O deactivated silica gel**, layered with 1 g of Na2SO4 and		**Kim et al. 2011**

	Na_2SO_4. **HEX/ Acetone** (1:1,v/v) used for extraction on SE-100 system (30°C, 10 mL/min for 30 min). This was followed by rotary evaporation.		conditioned with 25 mL of hexane. Elution was obtained with 100 mL of **DCM**.		
	ASE- 350 250 mg of lipids was extracted with ASE 350. **DCM/ Acetone** (1:1, v/v) used as extraction solvent.	TMP-d_9, TBP-d_{27}, TPP-d_{15} and TAP were used as IS.	**Silica gel** cleanup was used. A fraction containing cyclic PFRs was collected using **15% DEE in hexane.** The aliphatic PFRs were collected using **acetone**.		**Green, (2010) (Screening program)**
12 **Biota**	**ASE-200** (100°C, 1500 psi, flush volume 60%) using **cyclohexane/ diethyl ether** (9:1).	TBP-d_{27} used as an internal standard	**GPC** 15 x 445 mm glass column containing 30 g of Biobeads. Elution was obtained using **cyclohexane-EtAc** (3:1) at flow rate 2 mL/min.		**Sundkvist, (2010)**
12 **Breast milk**	**LLE** 50- 100 g of milk samples was extracted. Prior to extraction, samples were mixed vigorously in separatory funnel with 100 mL ethanol and 50 mL of sodium oxalate- saturated ethanol. Extraction was done using 150 mL **DEE/ Hexane** (7:10, v/v)	TBP-d_{27} used as an internal standard	**Partitioning** Between hexane and hexane- saturated-acetonitrile. This was followed by centrifugation (10 min, 4 800 rpm). The ACN phase was recovered and H_3PO_4 was added. The analytes were then extracted 3 times with MTBE and filtered on sodium sulfate column. **GPC** Same as described above (Sundkvist, 2010)		**Sundkvist, (2010)**
10 **Breast milk**	**SE- 100** 10 mL of freeze dried human breast milk was extracted using SE-100 at 25 °C at 10 mL/min	TEP-d_{15}, TMPP-d_{21}, TCEP-d_{12},	1- **GPC** (Bio-Beads S-X3, 2 cm id, 50 cm length). Elution was obtained using a mixture of cyclohexane/ethyl		**Kim, (2014)**

	for 30 min. Mixture of **HEX/ Acetone** (1:1, v/v) was used.	TnBP-d_{27} and TPP-d_{15}	acetate (3:1) at flow rate of 4 mL/min. 2- **SPE** on glass column (200, 10 mm i.d) packed with 4 g of 5% H2O deactivated silica gel and conditioned with 25 mL oh hexane. Elution was obtained using 100 mL of **DCM**.		

Table IV: List of the 18 studied OPEs, classified in 4 groups, along with some of their basic physicochemical properties as well as comparison of different ionization modes in terms of observed fragment ions (m/z) in the present study and the available literature. IS: internal standard; MW: molecular weight; Bp: boiling point at 760 mm Hg; Kow: octanol-water partitioning coefficient.

					EI mode		NCI mode		PCI mode		APCI mode	
Compound	**IS used**	**MW(g.mol^{-1})**	**Bp (°C)**	**Log Kow**	*Base peak,* **Quantifier,** Qualifier ions	**Literature (Quantifier,** Qualifier ions**)**	*Base peak,* **Quantifier,** Qualifier ions	**Literature (Quantifier,** Qualifier ions**)**	*Base peak,* **Quantifier,** Qualifier ions	**Literature**	*Base peak,* **Quantifier,** Qualifier ions	**Literature**
TEP	dTEP	182.1	216	1.08	*99,* **155**, 127	**155**, 99 (Dodson *et al.*, 2012; Van Den Eede *et al.*, 2012)	-	-	-	-	*99*, **183**	-
TPrP	dTPrP	224.1	254	1.87	*99,* **141**, 183	**183**,99 (Dodson *et al.*, 2012; Van Den Eede *et al.*, 2012)	127	-	*225,* 183, 99	-	*99*, **225**	-
TnBP		266.1	289	4	*99,* **155**, 211	**211**,155 (Dodson *et al.*, 2012; Van Den Eede *et al.*, 2012; Ma and Hites, 2013)	*127,* 249	209 (Ma and Hites, 2013)	*267,* 211	267 (Quintana *et al.*, 2007; Ma and Hites, 2013)	*99*, **267**	-
TiBP		266.1	264	3.60	*99,* **155**, 211	**211**, 155 (Dodson *et al.*, 2012; Van Den Eede *et al.*, 2012)	-	-	-	267 (Quintana *et al.*, 2007)	*99*, **267**	-
TEHP	dTnBP	434.3	220	4.22	*99*, **113**	**99** (Ma and Hites, 2013)	*127,* 321	321, 305 (Ma and Hites, 2013)	*111,* 435, 211	435 (Quintana *et al.*, 2007; Ma and Hites, 2013)	*99*, **435**	-
TBEP	MTBEP	398.4	414	3.75	*85*, **125**, 299, 199	**299**, 199 (Dodson *et al.*, 2012; Van Den Eede *et al.*, 2012; Ma and Hites, 2013)	*127,* 235	297, 291 (Ma and Hites, 2013)	*399,* 299	399 (Quintana *et al.*, 2007; Ma and Hites, 2013)	***399***, 299	-
TPP		326.1	370	4.59	***326***, 169, 77	**326**, 325 (Dodson *et al.*, 2012; Van Den Eede *et al.*, 2012; Ma and Hites, 2013)	*249,* 325		*327,* 95	327 (Ma and Hites, 2013)***;*** (Quintana *et al.*, 2007)	***327***	-
EHDP		362.1	421	6.64	***251***, 169, 94	**251**, 250 (Dodson *et al.*, 2012)	*285,* 127	-	*251,* 363, 111	251 (Quintana *et al.*, 2007)	***251***	-
DBPhP		286.3	333	4.08	***175***	-	-	-	-	-	*175*, **287**	-
DPhBP		306.2	368	4.41	*94,* **251**, 306	-	-	-	-	-	*251*, **307**	-
o-TCP	MTPP	368.3	410	5.48	*165*, **368**, 91	**368**, 367 (Dodson *et al.*, 2012; Van Den Eede *et al.*, 2012)	-	277 (Ma and Hites, 2013)	-	369 (Ma and Hites, 2013)***;*** (Quintana *et al.*, 2007)	***369***	-

m-TCP		368.3	442	6.34	***368***, 165, 91	**368**, 367 (Dodson *et al.*, 2012; Van Den Eede *et al.*, 2012)	-	-	-	-	***369***	-
p-TCP		368.3	439	5.11	***368***, 165, 91	**368**, 367 (Dodson *et al.*, 2012; Van Den Eede *et al.*, 2012)	-	277 (Ma and Hites, 2013)	-	369 (Ma and Hites, 2013)	***369***	-
TCEP		285.9	351	1.47	***249***, 143, 99	**249**, 251 (Dodson *et al.*, 2012; Van Den Eede *et al.*, 2012; Ma and Hites, 2013)	*221,* 127	221 (Ma and Hites, 2013)	*285,* 249	285 (Ma and Hites, 2013)	***287***, 249, 99	-
TCPP	dTCEP	327.9	359	2.59	***125***, 201, 99	**277**, 279 (Dodson *et al.*, 2012; Van Den Eede *et al.*, 2012; Ma and Hites, 2013)	*249,* 127	249 (Ma and Hites, 2013)	*327,* 251	327 (Ma and Hites, 2013)	*99*, **329**, 251	-
TDCIPP		429.8	457	3.27	*75,* **191**, 99, 381	**381**, 379 (Dodson *et al.*, 2012; Van Den Eede *et al.*, 2012; Ma and Hites, 2013)	*317*	317 (Ma and Hites, 2013)	*321,* 431, 75	429 (Ma and Hites, 2013)	***431***, 321, 99	-
TDBPP		697.6	544	3.71	*137,* **337**, 99, 121, 217	**201**, 119 (López *et al.*, 2011; Ma and Hites, 2013)	*79,* 496.7, 616.7	**487**, 79, 617, (Dodson *et al.*, 2012; Van Den Eede *et al.*, 2012; Ma and Hites, 2013)	-	83 (Ma and Hites, 2013)	**698.5**, 616.5	-
TTBNPP	dTDCPP	1017.3	595	7.55	*145,* **711**, 99, 309	-	*79,* 160, 710.7, 938.8	-	-	-	**1018**, 938	-

Table V: List of descriptive parameters from all reported results in fish and other food samples (EI for EHDP, DPhBP and TDCIPP and APCI for the other compounds).

The calculations, as described in paragraph 4.2.2) were based on middle bound (MB) concentrations (LOQ/2) or Mean (Blank value)/2 and always based on the equivalent fresh weight (fw)

Descriptive parameters		TEP	TPrP	TiBP	TnBP	TBEP	TPP	EHDP	DBPhP	DPhBP	o-TCP	m-TCP	p-TCP	TCEP	TCPP	TDCIPP	TDBPP	TTBNPP
LOQ or *LoR* (µg/kg)		*99*	*0.1*	*0.3*	*0.3*	*0.6*	*0.3*	*0.6*	0.01	0.07	0.002	0.02	0.02	*0.5*	*0.9*	*1.5*	1.5	0.5
River samples (n=44)	Detection frequency	0%	0%	64%	75%	5%	16%	45%	66%	5%	0%	0%	0%	7%	45%	30%	0%	0%
	Maximum concentration (µg/kg)	<LoR	<LoR	1.1	3.4	<LoR	6.1	0.9	0.09	3.2	<LOQ	<LOQ	<LOQ	1.31	1.8	5.4	<LOQ	<LOQ
	Minimum concentration (µg/kg)	<LoR	<LoR	<LoR	<LoR	<LoR	<LoR	<LoR	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LoR	<LoR	<LoR	<LOQ	<LOQ
	Median (µg/kg)	<LoR	<LoR	0.3	0.3	<LoR	<LoR	<LoR	<LOQ	0.1	<LOQ	<LOQ	<LOQ	<LoR	<LoR	<LoR	<LOQ	<LOQ
	Mean (µg/kg)	<LoR	<LoR	0.3	0.6	<LoR	<LoR	<LoR	0.01	0.3	<LOQ	<LOQ	<LOQ	<LoR	<LoR	<LoR	<LOQ	<LOQ
	Standard deviation	-	-	0.3	0.7	-	-	-	0.02	0.5	-	-	-	-	-	-	-	-
Sea samples (n=33)	Detection frequency	0%	0%	40%	6%	6%	23%	20%	29%	49%	0%	0%	0%	3%	20%	40%	0%	0%
	Maximum concentration (µg/kg)	<LoR	<LoR	3.3	1.8	6.5	1.7	3.6	0.09	1.7	<LOQ	<LOQ	<LOQ	1.7	1.6	3.0	<LOQ	<LOQ
	Minimum concentration (µg/kg)	<LoR	<LoR	<LoR	<LoR	<LoR	<LoR	<LoR	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LoR	<LoR	<LoR	<LOQ	<LOQ
	Median (µg/kg)	<LoR	<LoR	<LoR	<LoR	<LoR	<LoR	<LoR	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LoR	<LoR	<LoR	<LOQ	<LOQ
	Mean (µg/kg)	<LoR	<LoR	0.3	<LoR	<LoR	<LoR	0.6	0.01	0.2	<LOQ	<LOQ	<LOQ	<LoR	<LoR	<LoR	<LOQ	<LOQ
	Standard deviation	-	-	0.6	-	-	-	0.9	0.02	0.3	-	-	-	-	-	-	-	-
Food samples (n=20)	Detection frequency	0%	0%	35%	40%	15%	85%	85%	10%	5%	0%	0%	0%	15%	70%	65%	0%	0%
	Maximum concentration (µg/kg)	<LoR	<LoR	4.5	6.0	2.7	37.7	4963.4	0.5	0.2	<LOQ	<LOQ	<LOQ	4.3	7.3	63.9	<LOQ	<LOQ
	Minimum concentration (µg/kg)	<LoR	<LoR	<LoR	<LoR	<LoR	<LoR	<LoR	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LoR	<LoR	<LoR	<LOQ	<LOQ
	Median (µg/kg)	<LoR	<LoR	<LoR	<LoR	<LoR	0.3	2.6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LoR	1.2	<LoR	<LOQ	<LOQ
	Mean (µg/kg)	<LoR	<LoR	0.6	0.8	<LoR	2.5	254.6	0.03	<LOQ	<LOQ	<LOQ	<LOQ	<LoR	1.6	5.7	<LOQ	<LOQ
	Standard deviation	-	-	1.3	1.7	-	8.3	1108.4	0.1	-	-	-	-	-	1.8	14.4	-	-

UNIVERSITE BRETAGNE LOIRE

Thèse de Doctorat

ÉCOLE NATIONALE VÉTÉRINAIRE, AGROALIMENTAIRE ET DE L'ALIMENTATION
SANTÉ ET ALIMENTATION AU CŒUR DE LA VIE

Université Libanaise
École Doctorale Sciences et Technologies

Wafaa HALLOUM

Développement d'une stratégie analytique dédiée aux esters organophosphorus. Contribution à l'évaluation de l'exposition de l'homme à ces contaminants ré-émergents *via* l'alimentation.

Development of an analytical strategy dedicated to organophosphorus esters. Contribution to the human dietary exposure assessment to these re-emerging contaminants.

Résumé

Alors que de récentes études ont souligné le potentiel de perturbation endocrinienne des esters organophosphorés (OPE), il convient de conduire une évaluation approfondie des risques associés à ces composés dont l'utilisation globale en tant que retardateurs de flamme et plastifiants est en constante augmentation.
En dépit d'efforts consacrés à l'analyse de ces contaminants ré-émergents dans divers compartiments environnementaux abiotiques, peu d'informations étaient disponibles pour le biote, en partie en raison de l'absence de stratégie analytique efficace. La présente thèse ambitionnait donc de développer une stratégie analytique robuste dédiée à la caractérisation d'une large gamme d'OPE à l'état de traces dans le poisson et d'autres denrées alimentaires. L'approche retenue est basée sur l'extraction liquide sélective sous pression et la chromatographie par perméation de gel. La détection est réalisée par chromatographie en phase gazeuse couplée à la spectrométrie de masse en tandem, avec ionisation par impact électronique ou chimique à pression atmosphérique. Le second objectif visait à produire des données de prévalence utiles à l'évaluation de l'exposition du consommateur en France. L'analyse de poissons et denrées emballées a permis de mesurer des niveaux totaux inférieurs à 10 et 100 ng/g pf, respectivement. Une première exploitation des données sur poissons en termes d'évaluation quantitative de risque pour l'Homme a dévoilé des ratios de risques faibles au regard de données toxicologiques disponibles. Néanmoins, des données complémentaires sur l'exposition et la toxicologie seront indispensables pour conclure quant aux implications en santé publique.

Mots clés
Retardateur de flamme, Plastifiant, Stratégie d'analyse, Evaluation des risques, Sécurité chimique des aliments

Abstract

As recent studies highlighted that several organophosphate esters (OPEs) exhibit potential endocrine disrupting effects, in-depth risk assessment is required, when their global use as flame retardants and plasticizers is considerably increased. Despite previous efforts in the analysis and exposure assessment of these re-emerging contaminants in various abiotic environmental compartments, still limited information is available in biota samples, partly due to the lack of efficient analytical strategies. The thesis aimed first at developing a robust analytical strategy dedicated to the determination of a wide range of OPEs at trace levels in fish and other foodstuffs. The developed strategy involved selective pressurized liquid extraction with Florisil® as lipid sorbent, followed by further purification step by gel permeation chromatography. The analysis was then performed by gas chromatography coupled to tandem mass spectrometry fitted with electron impact or atmospheric pressure chemical ionisation mode, the latter being a more specific and innovative approach. The second aim was to apply the developed strategy to produce original occurrence data that can be useful for exposure assessment at the French level. Fish sample sets exhibited levels below 10 ng/g fw while packaged foodstuffs presented levels up to 100 ng/g fw. A first interpretation of these data in terms of dietary human quantitative risk assessment through fish consumption tended to show low risk ratios in connection with available toxicological data. However, additional exposure and toxicological data is required before any conclusions regarding public health implication can be drawn.

Key Words
Flame retardant, Plasticizer, Analytical strategy, Risk assessment, Chemical food safety

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