UNIVERSITAT DE
BARCELONA

Impacte dels residus plàstics en organismes marins: avaluació de la bioacumulació de plastificants i retardants de flama

Berta Sala Solà

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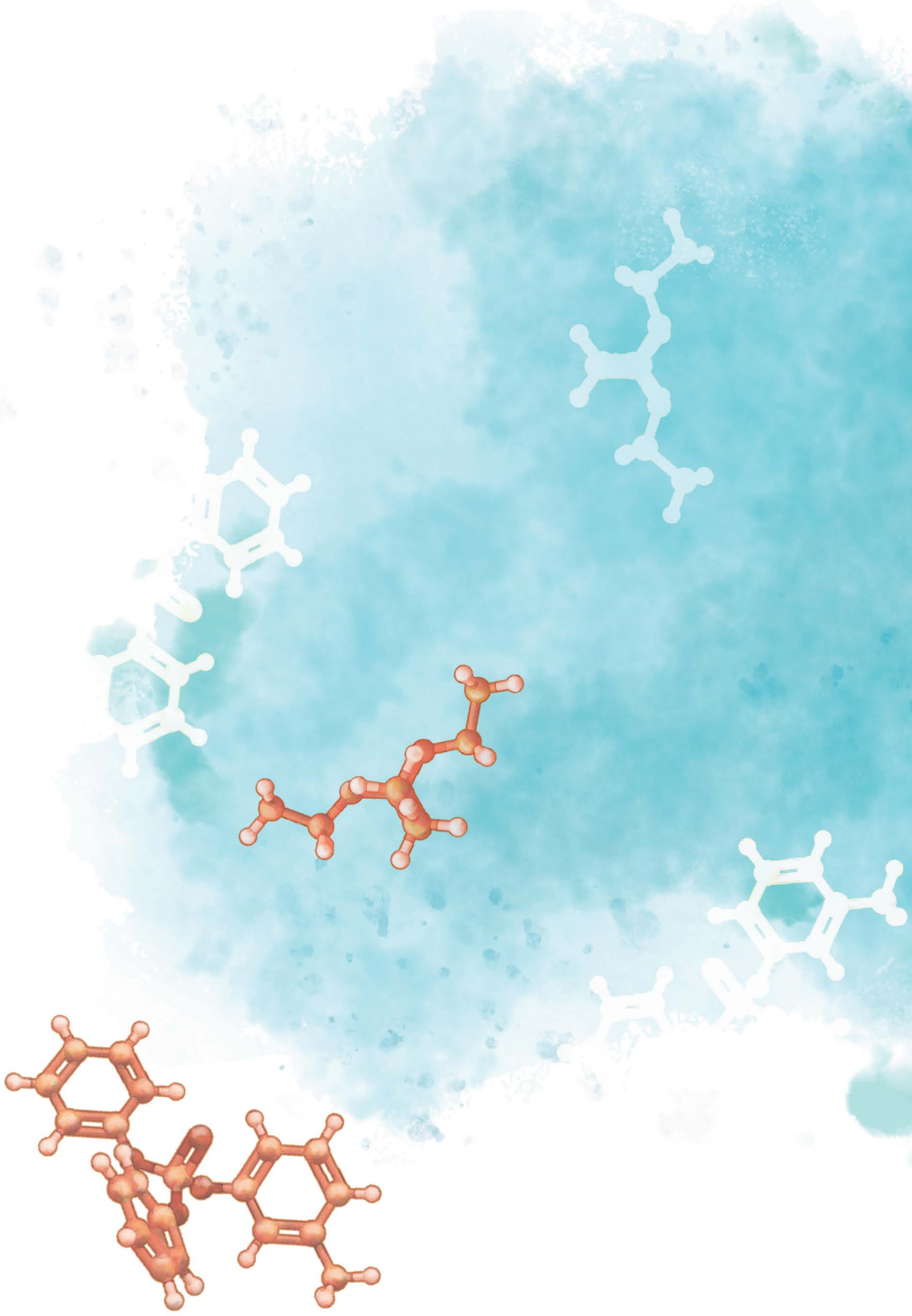

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TESI DOCTORAL

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IMPACTE DELS RESIDUS PLÀSTICS EN ORGANISMES MARINS: AVALUACIÓ DE LA BIOACUMULACIÓ DE PLASTIFICANTS I RETARDANTS DE FLAMA

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Programa de doctorat
"Química Analítica i Medi Ambient"

Impacte dels residus plàstics en organismes marins: avaluació de la bioacumulació de plastificants i retardants de flama

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Memòria presentada en compendi d'articles per optar al títol de Doctorat per la Universitat de Barcelona

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“La salut dels oceans assegura la salut del planeta Terra”

Josefina Castellví

Per tu, avi.

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GRÀCIES

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ABREVIACIONS I ACRÒNIMS

ASE	Extracció amb dissolvent accelerada
BAF	Factor de bioacumulació
BCF	Factor de bioconcentració
BDE-209	Decabromodifenil éter
BFR	Retardant de flama bromat
CI	Ionització química
cm	Centímetres
DBDPE	Decabromodifenil età
DCM	Diclorometà
DDT	Diclorodifeniltricloroetà
Dec 602	Decloran 602
Dec 603	Decloran 603
Dec 604	Decloran 604
decaBDE	Èter difenílic decabromat
DP	Decloran plus
dw	Pes sec
EDAR	Estació depuradora d'aigües residuals
EHDPP	Fosfat 2-etilhexidifenil
ECHA	Agència Europea de Substàncies Químiques
EFR	Retardant de flama emergent
EI	Ionització electrònica
EPS	Poliestirè expandit
ESI	Ionització per electroesprai
FR	Retardant de flama
GC	Cromatografia de gasos
HBB	Hexabromobenzè
HBCD	Hexabromociclododecà
HCB	Hexaclorobenzè
hepta-BDE	Èter difenílic heptabromat
hexa-BDE	Èter difenílic hexabromat
HFR	Retardant de flama halogenat
HN	Norbornè halogenat
HPLC	Cromatografia de líquids d'alta resolució
IDPP	Isodecildifenil fosfat
IPPP	Isopropilfenil fosfat
iLOD	Límit de detecció instrumental
iLOQ	Límit de quantificació instrumental
IMO	Organització Internacional Marítima
IWC	Comissió balenera internacional
Kg	Quilogram
Kow	Coeficient de partició octanol-aigua
Kpw	Coeficient partició polímer-aigua
LC	Cromatografia de líquids
LOD	Límit de detecciót
LOQ	Límit de quantificació
lw	Pes lipídic
mm	Milímetres
mLOD	Límit de detecció del mètode
mLOQ	Límit de quantificació del mètode
MS	Espectrometria de masses
MS/MS	Espectrometria de masses en tàndem
Mt	Milions de tones
NICI	Ionització química d'ió negatiu
nm	Nanòmetres
nona-BDE	Èter difenílic nonabromat
octa-BDE	Èter difenílic octabromat
OPE	Organofosforats èsters
PBDE	Èter difenílic polibromat
PBEB	Pentabromoetilbenzè
PBT	Persistents, bioacumulables i tòxiques
PCB	Bifenils policlorats
PCDD	Dibenzodioxines policlorades o dioxi

ABREVIACIONS I ACRÒNIMS

PCDF	Dibenzofurans policlorats o furans
PCN	Policloronaftalè
penta-BDE	Èter difenílic pentabromat
PLE	Extracció amb líquid pressuritzat
PNUMA	Programa de les nacions unides pel Medi Ambient
POP	Contaminant orgànic persistent
ppb	Part per billion
PVC	Clorur de polivinil
QqLIT	Híbrid triple quadrupol – trampa d'ions lineal
REACH	Registre, avaluació, autorització i restricció de substàncies químiques
RSD	Desviació estàndard relativa
SCCP	Cloroparafines de cadena curta
SPE	Extracció en fase sòlida
SRM	Monitoratge de reaccions seleccionades
SVHC	Substància altament preocupant
T	Tones
TBP	Tributil fosfat
TBBPA	Tetrabromobisfenol-A
TBOEP	Tris (2-butoxietil) fosfat
TCEP	Tris (2-cloroetil) fosfat
TCIPP	Tris (2-clorosopropil) fosfat
TCP	Tricresil fosfat
TDCIPP	Tris (1,3-dicloro-2-propil) fosfat
TEP	Trietil fosfat
TEHP	Tris (2-etilhexil) fosfat
THP	Trihexil fosfat
TMF	Factor de magnificació tròfica
TNBP	Fosfat de tri-n-butil
TPHP	Fosfat de trifenil
TPPO	Òxid de trifenil fosfà
UE	Unió Europea
vPvB	Molt persistent I molt bioacumulable
ww	Pes humit
XPS	Poliestirè extrudit

RESUM

Els organofosforats èsters (OPEs) s'apliquen a una gran varietat de materials tant com a retardants de flama (FRs) i/o com a plastificants. En són un exemple els aparells electrònics, els teixits, mobles i els plàstics en si. Cada vegada hi ha una demanda més gran d'aquests compostos ja que són els principals substituts dels FRs èters difenílics polibromats (PBDEs), prohibits el 2009 pel Conveni d'Estocolm.

En aquesta tesi s'ha volgut avaluar l'impacte d'aquests compostos i d'altres FRs ja prohibits en el medi ambient i més concretament, en els organismes marins. Per fer-ho, s'ha estudiat el comportament espai-temporal dels OPEs analitzant 4 espècies diferents de dofins: dofí comú, dofí llistat, dofí d'estuari Indo-Pacífic i dofí mular Indo-Pacífic. Per tal de poder escollir quin teixit dels dofins era el més idoni per poder estudiar la concentració d'OPEs en biota marina, es va dur a terme un anàlisi d'aquests contaminants en diferents teixits del dofins recol·lectats al Mar d'Alboran. L'estudi va demostrar que el múscul era el més adient i per tant, és el teixit que es va mostrejar en els altres dofins.

Les diferents espècies de dofí s'han mostrejat en 3 zones: el Mar Mediterrani per la costa catalana, el Mar d'Alboran i l'Oceà Índic per la costa de Sudàfrica. Al tenir mostres d'anys diferents, l'anàlisi espacial només s'ha realitzat per a les dues zones que disposàvem de mostres d'anys similars: Mar Mediterrani (per la costa catalana) i l'Oceà Índic (per la costa de Sudàfrica). En aquest estudi, es va poder observar que la concentració d'OPEs a la costa de Sudàfrica era 1,5 vegades superior a la del Mediterrani occidental. Tot i així, la diferència de concentracions entre ambdues zones no era significativa. També es va poder estudiar la concentració de PBDEs en les mateixes zones d'estudi, observant que eren molt més baixes que les trobades pels OPEs. Es va destacar que pel cas de la costa de Sudàfrica els OPEs eren significativament superiors als PBDEs, fins a dos ordres de magnitud per sobre. Aquest fet, va fer pensar que la contaminació d'OPEs, a banda de venir del seu ús com a FRs, també hi tenia un gran impacte la seva funció com a plastificant ja que es tracta d'una zona molt contaminada pels residus plàstics. Els perfils d'OPEs trobats a les 3 zones ens mostra la semblança que hi ha entre les dues costes del mateix mar, el Mediterrani, enfront a la del sud-oest de l'Índic. En el cas de la tendència temporal dels OPEs, es va poder veure només en el Mar Mediterrani per la costa catalana ja que era l'únic lloc d'on disposàvem de mostres de més de 10 anys. Els OPEs són uns compostos que s'ha anat augmentant el seu ús al llarg dels anys i en conseqüència, s'esperaria que les concentracions en els organismes marins estudiats augmentessin en el temps. Per contra, s'observa una concentració constant d'aquests contaminants, donant peu a pensar que hi juga un paper clau el metabolisme en els organismes.

A banda, també s'ha volgut estudiar el comportament que tenen aquests contaminants en els organismes marins així com la bioacumulació i la biomagnificació. En aquest cas, s'han pogut analitzar per primera vegada mostres de diferents espècies d'organismes marins de diferents nivells tròfics, com per exemple la medusa i la tortuga babaua, entre d'altres. Es va poder observar que no existien diferències significatives entre els nivells tròfics estudiats, indicant una manca de biomagnificació global dels OPEs.

Tot i així, al disposar de mostres de parelles predador-presa es va poder calcular el BMF i veure quins contaminants tendien a biomagnificar-se de manera individual. En aquest cas, es va observar que compostos que la bibliografia descriu com que tendeixen a metabolitzar-se, com per exemple el TNBP, presenten un potencial de biomagnificació. També es va comprovar que l'aportació dels OPEs als organismes marins pot venir donada tant per la dieta com per la ingesta directa de plàstics (a banda dels altres mecanismes de bioacumulació). Els resultats de les mostres de lluç -peix comestible- ens va permetre calcular la ingesta d'OPEs a través del consum de peix, observant una nul·la amenaça per a la salut pública d'aquests contaminants per aquesta via d'entrada. Tot i així, s'ha de tenir en compte que l'exposició dels humans cap aquests contaminants és més àmplia.

Finalment, al disposar de les mostres de rorqual comú i fetus, es va poder fer el primer estudi de transferència materna d'OPEs i també es va poder mirar la transferència de les SCCPs i el DBDPE. Els resultats van demostrar concentracions d'aquests tres compostos en els fetus, demostrant així la transferència placentària.

CAPÍTOL I

INTRODUCCIÓ GENERAL

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1.1 Convivint amb el plàstic

Para un moment, mira el teu voltant i conta ràpidament quants objectes contenen plàstic, masses, veritat? El plàstic és un material molt utilitzat en la majoria d'indústries, ja sigui pels seus diferents usos, com per la seva versatilitat i capacitat d'oferir diferents solucions a les necessitats requerides de nombrosos productes. Durant l'any 2019, la producció de plàstic va arribar aproximadament a les 370 milions de tones (Mt), de les quals 62 milions es van produir només a Europa (Martín-Lara et al., 2021). Així doncs, estem parlant d'un material gairebé indispensable per a la societat moderna actual.

L'ús excessiu de materials d'un sol ús ha generat una gran quantitat de producció d'aquest material no biodegradable, provocant un augment descontrolat de residus plàstics a nivell mundial. A més, a molts països els hi manquen moltes infraestructures necessàries per prevenir la contaminació per plàstics, així com: abocadors, incineradores; espais amb capacitat de reciclatge i infraestructures d'economia circular; sistemes de gestió i eliminació de residus adequats. Tot això condueix a les anomenades "fuites de plàstic" cap als rius i oceans. El comerç mundial legal i il·legal de residus plàstics també pot ser perjudicial pels ecosistemes, on els sistemes de gestió de residus no són suficients per contenir tots aquests residus.

Entendre el camí i el destí d'aquests tipus de residus és fonamental per tal de poder gestionar i reduir la contaminació que generen. En general, es suposa que la major part dels residus plàstics procedeixen de fonts terrestres, principalment de zones continentals densament poblades, tot i que alguns estudis suggereixen que les fonts marines també hi tenen un paper molt important (Bergmann et al., 2017a; Lebreton et al., 2018). No obstant, existeix un gran desajust entre les estimacions de la quantitat de plàstic generat que entra a les aigües dels mars i oceans (5-12 Mt/any (Jambeck et al., 2015)) i la quantitat total de plàstic que trobem flotant al mar (menys de 0,3 Mt (Cózar et al., 2014; Eriksen et al., 2014; Van Sebille et al., 2015)). De la mateixa manera que també es discuteix si la quantitat de plàstic mesurada en el mar en les últimes dècades ha seguit el ritme del creixement de la producció mundial de plàstic (Lebreton et al., 2019; Ostle et al., 2019; Wilcox et al., 2020). Així doncs, podem pensar que la manera que tenim d'entendre els fluxos de plàstic (vies de transport i destí) estan incompletes. Els processos físics que pateixen els plàstics una vegada al mar, són clau per explicar la discrepància entre les estimacions de les aportacions de plàstic al mar i les reserves de plàstic flotant, ja que hi ha proves substancials de partícules de plàstic que es troben a la columna d'aigua i en els sediments marins (Bergmann et al., 2017b; Song et al., 2018). Tenint en compte que els organismes marins són més abundants en la columna d'aigua que en els 20 cm superiors, la interacció, així com la ingesta, la transferència tròfica i la bioincrustació, entre microplàstics i la biota és major a la columna d'aigua que a la superfície (Song et al., 2018).

A grans trets, els residus plàstics els trobem a diferents capes de la columna d'aigua, depenent principalment de la seva densitat i forma. Hi poden ser presents en forma de macro plàstics (>2,5 cm), els quals es degraden gradualment fins a transformar-se en microplàstics (<5 mm) o nanoplàstics (entre 1 i 1.000 nm). També hi podem trobar directament microplàstics primaris, procedents majoritàriament de pols de pneumàtics (53%), teixits (33%), microperles dels cosmètics (12%) i els pellets de producció (2%) (Boucher et al., 2020). Donat que els microplàstics ocupen la mateixa fracció de mida que els sediments i alguns organismes del plàncton, són potencialment biodisponibles per una amplia gama d'organismes. Per això, els microplàstics, degut a la seva mida reduïda, poden ser ingerits per varies espècies marines de totes les regions oceàniques, provocant danys físics directes i potencials efectes de toxicitat. A més, també poden adsorbir[1] en la seva superfície contaminants orgànics que es troben en suspensió a l'aigua degut a la seva hidrofobicitat (Singla et al., 2020).

Cal tenir en compte que tots els plàstics estan formats mitjançant l'escalfament del petroli, entre d'altre productes, que es descomponen en molècules més petites anomenades monòmers químics, els quals es combinen per arribar a formar polímers sintètics (Worm et al., 2017).

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[1] adsorbir / absorbir
Cal no confondre 'adsorbir', que és adherir els àtoms o les molècules d'un material damunt la superfície d'un altre, amb 'absorbir', que és penetrar una substància en el si d'una altra al nivell molecular.

Molts d'aquests monòmers, com l'estirè o el clorur de vinil són tòxics i la seva presència pot ser perillosa. A més, per tal de millorar les propietats dels plàstics i aconseguir les funcionalitats desitjades, se'ls hi afegeixen els anomenats additius, que poden ser antioxidants, plastificants, retardants de flama (FRs, de l'anglès *flame retardants*), UV i estabilitzadors tèrmics, colorants, lubricants, etc, on els plastificants i els FRs representen el 47% dels additius presents als plàstics (Cheng et al., 2020). Degut a l'ampli ús dels plàstics i dels seus additius, aquests suposen un seguit de riscos potencials per a la salut humana i el medi ambient (Halden, 2010). L'abocament anual d'additius procedents dels plàstics en el medi marí s'estima que son entre 35 i 917 T, de les quals la majoria provenen del clorur de polivinil (PVC, de l'anglès *polyvinyl chloride*) (Suhrhoff and Scholz-Böttcher, 2016). En el cas dels FRs, són additius que també s'afegeixen a altres objectes com ara mobles, material de construcció, vehicles i aparells electrònics (Alaee et al., 2003). Moltes d'aquests substàncies químiques, com per exemple els FR bromats (BFRs, de l'anglès *brominated flame retardants*) i les cloroparafines de cadena curta (SCCPs, de l'anglès *short-chain clorinated paraffins*), són considerades substàncies PBT (persistents, bioacumulables i tòxiques), moltes de les quals es troben dins del Conveni d'Estocolm per ser considerats contaminants orgànics persistents (POPs, de l'anglès *persistent orgànic pollutants*) (Bouwmeester et al., 2015; Hahladakis et al., 2018). Amb la prohibició d'aquests grups de contaminants es van buscar compostos que els poguessin substituir, aquests són els coneguts com a FR emergents (EFR, de l'anglès *emerging flame retardants*).

Molts dels additius plàstics trobats en entorns costaners i en ambients de mar obert són disruptors endocrins i poden arribar als organismes vius a través de la ingesta de plàstics, l'assimilació per contacte de substàncies químiques dissoltes en l'aigua o a través de la xarxa tròfica. Totes aquestes són substàncies que no estan unides químicament al polímer i el seu alliberament a l'aigua es veu afectada directament per la partició polímer-aigua (K_{pw}) (Fauvelle et al., 2021).

Els plastificants i FR acostumen a ser poc solubles en aigua, aquesta característica fa que una vegada es troben en un medi aquós es refugiïn en els sediments i es bioacumulin en els teixits grassos dels animals. Igual que la solubilitat en l'aigua, el K_{ow} (coeficient de partició octanol-aigua) és una propietat decisiva per determinar el potencial de bioacumulació del compostos en els diferents teixits de la biota.

En aquesta tesi ens hem centrat a estudiar principalment un dels grups d'additius més utilitzats actualment, els organofosforats èsters (OPEs, de l'anglès *organophosphates esters*). Aquest grup de contaminants està format tant per compostos classificats com a FRs com per a plastificants. Són coneguts per ser els principals substituents dels BFRs èters difenílics polibromats (PBDEs, de l'anglès *polybrominated diphenyl ethers*), prohibits des del 2009 pel Conveni d'Estocolm. A més, també s'han estudiat els PBDEs, l'hexabromociclododecà (HBCD), alguns EFRs com l'hexabromobenzè (HBB), el pentabromoetilbenzè (PBEB), el decabromodifenil età (DBDPE), els declorans i les SCCPs.

Així doncs, la preocupació ambiental no només està directament relacionada amb l'ús del plàstic sinó també amb la "fuita" de plàstics i/o additius químics potencialment tòxics pel medi ambient durant la producció, el transport, l'ús i la gestió final de la seva vida útil.

1.2 Retardants de flama (FRs) i plastificants, definició i classificació

Els avenços en la ciència dels polímers en els darrers 50 anys, ha portat a la introducció d'un gran nombre de polímers amb diferents propietats i aplicacions. Com a resultat, estem envoltats d'una gran varietat d'aquests compostos des de la roba, els mobles, fins a l'electrònica, els vehicles i els ordinadors. De fet, els cotxes moderns contenen més de 100 Kg de diferents polímers, la majoria dels quals estan basats en el petroli i, per tant, són inflamables. Per tal de complir les normes de seguretat contra incendis, s'apliquen FRs a materials combustibles com els plàstics, la fusta, el paper i els teixits per augmentar la seva resistència al foc (EHC-192, 1997). La funció dels FRs és inhibir una de les quatre etapes del procés de combustió (preescalfament, volatilització o descomposició, combustió i propagació), essent més efectius els que actuen a una de les etapes més avançades del procés. Els FRs poden tenir diferents composicions, poden contenir halògens (brom i clor), fòsfor, nitrogen, metalls, minerals (en base d'alumini i magnesi), o també composicions basades en bòrax, triòxid d'antimoni, molibdè o fins i tot poden ser nanocompostos (EFRA, 2007) (Figura 1). Trobem dues classes principals de FR, segons com s'incorporen en el polímer, els de tipus reactiu que s'incorporen a la pròpia formulació del polímer i els de tipus additiu que s'incorporen al polímer. Aquests últims tendeixen a tenir més facilitat per alliberar-se al medi ambient ja que la seva unió amb el polímer és més dèbil (Hutzinger and Thoma, 1987).

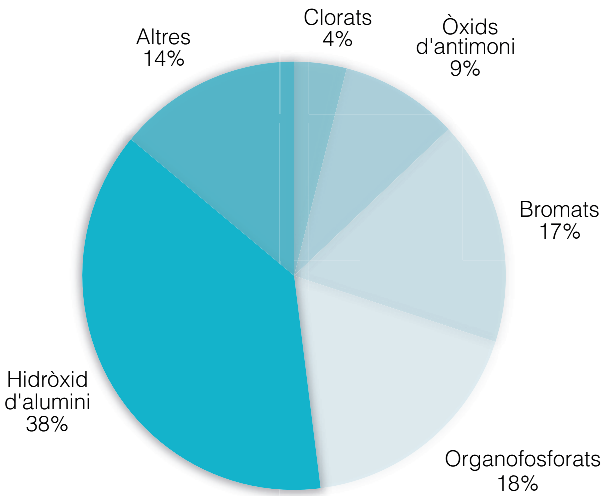


Figura 1. Consum mundial de retardants de flama per tipus, 2019. Adaptació de IHS Consulting, 2020; Flameretardants-online (2019)

Dins dels FRs, un dels grups més populars són els BFRs, amb 75 tipus diferents de mescles comercials, cadascuna amb propietats i comportaments toxicològics específics. L'única característica en comú és que contenen brom i actuen en la fase de vapor mitjançant un mecanisme de trampa de radicals. Es poden presentar en diverses formes i poden ser líquids, en pols o en grànuls. Els BFRs més importants són els PBDEs. Degut a les restriccions en l'ús d'aquests, els OPEs han guanyat importància en els últims anys (Quintana et al., 2007; Rauert et al., 2018). Aquests darrers, són un grup de compostos amb la mateixa unitat de base de fosfat –un fosfat molecular central– amb substituents heterogenis (Greaves and Letcher, 2017; Yang et al., 2018). Els OPEs poden ser utilitzats com a FRs (OPEs clorats) i com a plastificants (OPEs no halogenats) (Andresen et al., 2004). En els darrers anys les circumstàncies de consum dels FRs han canviat considerablement. Pel que fa a la dels OPEs, ha passat de l'13,8% a l'any 2005, al 18% al 2019, mentre que pels mateixos anys, el consum dels BFRs ha passat del 21 al 17% (Flameretardants-online, 2019; Ou and Lang, 2010). Segons un recent estudi de mercat de IHS Consulting (2020), a l'any 2019 els tipus de producte ignífug més importants van ser els hidròxids d'alumini (38% del mercat), seguits dels organofosforats (18% del mercat) i en tercer lloc els bromats (17%). L'ús dels FRs en cada regió està molt condicionat per les restriccions i normes en l'aplicació d'aquests per a cada país. El consum de FRs ha crescut substancialment en els darrers quatre anys, sobretot en el sector de l'electrònica, i es preveu un augment del 2,7% entre els anys 2019 i 2025. Aquests compostos són consumits principalment per la indústria del plàstic i de les resines, però també en la industria del tèxtil i del cautxú. Pel que fa al consum a escala mundial, Àsia és el continent capdavanter en consum d'aquests compostos, amb una quota del 51% per l'any

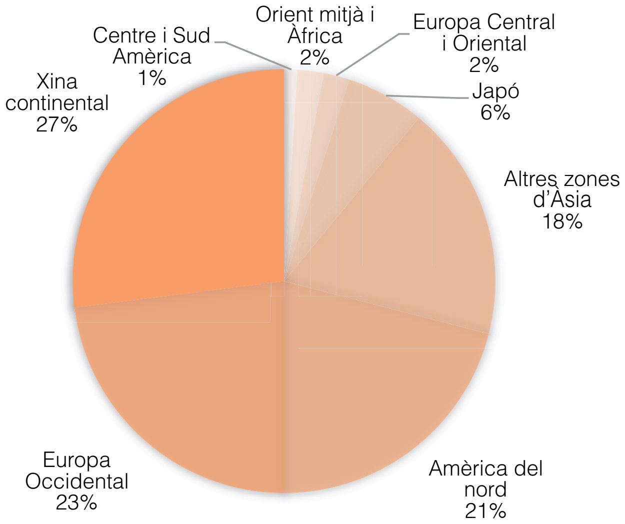


Figura 2. Consum mundial de retardants de flama per regió, 2019. Adaptació de IHS Consulting, 2020; Flameretardants-online (2019)

2019, essent la Xina la major consumidora individual amb un 27%. Pel que fa a Europa, al 2019 els països de la regió Occidental van consumir un 23% dels FRs a escala mundial, mentre que a les regions centrals i orientals només es consumí el 2% (Figura 2).

1.3 FRs i plastificants estudiats

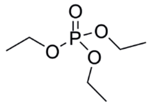
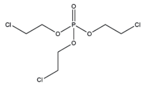
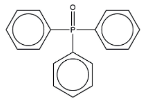
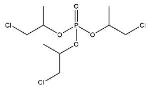
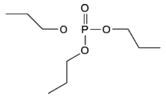
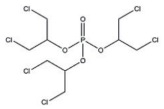
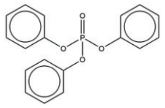
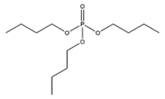
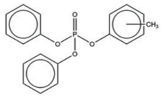
Tot i que els compostos principals d'aquesta tesi doctoral són els OPEs, en alguns dels anàlisis també s'han mirat altres FRs i plastificants, així com els BFRs legislats: PBDEs i HBCDs, els EFRs, els declorans i les SCCPs. En aquest apartat es fa una breu introducció de les característiques de tots aquests grups de compostos.

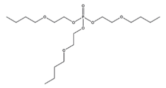
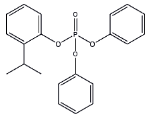
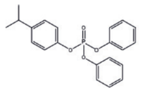
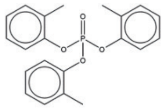
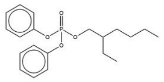
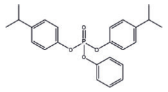
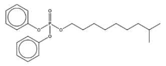
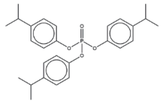
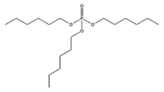
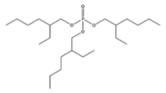
OPEs

Els OPEs són una família molt coneguda per ser la major alternativa als PBDEs. Són considerats contaminants orgànics emergents, tot i que alguns dels seus compostos poden presentar algunes característiques dels POPs. Aquests són productes sintètics que s'utilitzen per aplicacions diverses, en són exemple protegir o potenciar les propietats dels plàstics o dels teixit, com a agents antiespumants i són aplicats a multitud de materials com a plastificants i/o FRs. Degut a la seva incapacitat d'unir-se químicament als polímers, els OPEs poden alliberar-se fàcilment al medi ambient a través de la volatilització, l'abrasió i/o la dissolució (Möller et al., 2011).

Els OPEs són èsters orgànics que contenen l'àcid fosfòric com a nucli ($O{=}P(OR)_3$) i grups alquils o arils com a cadenes laterals. Poden classificar-se com a halogenats, si hi ha presència d'àtoms de clor, o no halogenats. Les propietats fisicoquímiques d'aquests compostos (Taula 1) varien segons els substituents de les cadenes laterals, donant lloc a un complex comportament ambiental. La solubilitat d'aquests compostos decreix quan la massa molecular és més gran i a la inversa. Els valors de log K_{ow} dels OPEs (Taula 1) poden variar des de 0,80 pel compost trietil fosfat (TEP, de l'anglès *triethyl phosphate*) fins a 9,49 pel compost tris (2-etilhexil) fosfat (TEHP, de l'anglès *tris(2-ethylhexyl)phosphate*), variant considerablement entre els diferents OPEs, essent menys lipofílics que els BFRs. Els OPEs alquílics i arílics amb masses moleculars grans, com el TEHP o el fosfat 2-etilhexidifenil (EHDPP, de l'anglès *2-ethylhexyldiphenyl phosphate*) són més biodisponibles degut al seu alt valor de Log K_{ow} (>5) i poden arribar a biomagnificar-se en les xarxes tròfiques aquàtiques, tot i que els OPEs són un grup que es metabolitza amb facilitat. Els OPEs clorats que presenten una alta solubilitat, generalment són difícils d'eliminar en plantes de tractament d'aigües residuals (EDAR) i per tant, s'aboquen en grans quantitats a les aigües naturals (Marklund et al., 2005).

Taula 1. Propietats fisicoquímiques i estructures dels OPEs

Acrònim	Nom químic	Estructura química	Formula molecular	Pes molecular	Solubilitat (mg L^{-1}, 25 °C)	Log (K_{OW})
TEP	Fosfat de trietil		$C_6H_{15}O_4P$	182	$5x10^5$	0,80
TCEP	Tris (2-cloroetil) fosfat		$C_6H_{12}Cl_3O_4P$	285	$7x10^3$	1,44
TPPO	Òxid de trifenil fosfà		$C_{18}H_{15}OP$	278	62,8	2,87
TCIPP	Tris (2-clorosopropil) fosfat		$C_9H_{18}Cl_3O_4P$	328	$1,6x10^3$	3,11
TPP	Fosfat de tripropil		$C_9H_{21}O_4P$	224	827	1,87
TDCIPP	Tris (1,3-dicloro-2-propil) fosfat		$C_9H_{15}Cl_6O_4P$	431	1,50	3,65
TPHP	Fosfat de trifenil		$C_{18}H_{15}O_4P$	326	1,90	4,59
TNBP	Tri-n-butyl phosphate		$C_{12}H_{27}O_4P$	266	$2,8x10^2$	4,00
DCP	Difenil cresil fosfat		$C_{19}H_{17}O_4P$	340	0,24	5,25

TBOEP	Tris(2-butoxietil)fosfat		$C_{18}H_{39}O_7P$	398	$1{,}2\times10^{3}$	3,75
2IPPDPP	2-isopropilfenil difenil fosfat		$C_{21}H_{21}O_4P$	368	-	5,30
4IPPDPP	4-isopropilfenil difenil fosfat		$C_{21}H_{21}O_4P$	368	$2{,}65\times10^{-2}$	5,70
TCP	Tricresil fosfat		$C_{21}H_{21}O_4P$	368	0,36	6,34
EHDPP	2-etilhexildifenil fosfat		$C_{20}H_{27}O_4P$	362	1.90	5,73
B4IPPPP	Bis (4-isopropilfenil) fenil fosfat		$C_{24}H_{27}O_4P$	410	-	6,03
IDPP	Isodecil difenil fosfat		$C_{22}H_{31}O_4P$	390	0,75	7,28
T2IPPP	Tris(2-isopropilfenil) fosfat		$C_{27}H_{33}O_4P$	452	-	9,10
THP	Trihexil fosfat		$C_{18}H_{39}O_4P$	350	$1{,}02\times10^{-2}$	7,45
TEHP	Tris(2-etilhexil)fosfat		$C_{24}H_{51}O_4P$	435	0,60	9,49

Retardants de flama halogenats (HFRs) legislats

Els FRs halogenats (HFRs, de l'anglès *halogenated flame retardants*) són compostos principalment bromats (tot i que també clorats), que actuen sobre l'etapa de combustió capturant els radicals lliures produïts durant la reacció, evitant la seva propagació i inclús l'aparició de les flames. A la taula següent es resumeixen les propietats fisicoquímiques dels HFRs legislats que s'expliquen a continuació.

Taula 2. Propietats fisicoquímiques dels PBDEs i HBCDs

Compost	Nom químic	Formula molecular	Pes molecular	Solubilitat (mg/L)	Log K_{ow}
PBDEs					
BDE-28	2,4,4'-tribromodifenil èter	$C_{12}H_7Br_3O$	407	$3{,}30\times10^{-1}$	5,52
BDE-47	2,2',4,4'-tetrabromodifenil èter	$C_{12}H_6Br_4O$	486	$9{,}50\times10^{-2}$	6,01
BDE-99	2,2',4,4',5-pentabromodifenil èter	$C_{12}H_5Br_5O$	565	$3{,}90\times10^{-2}$	6,71
BDE-100	2,2',4,4',6-pentabromodifenil èter	$C_{12}H_5Br_5O$	565	$5{,}40\times10^{-2}$	6,71
BDE-153	2,2',4,4',5,5'-hexabromodifenil èter	$C_{12}H_4Br_6O$	644	$1{,}70\times10^{-2}$	7,90
BDE-154	2,2',4,4',5,6'-hexabromodifenil èter	$C_{12}H_4Br_6O$	644	$8{,}7\times10^{-4}$	7,92
BDE-183	2,2',3,4,4',5',6-heptabromodifenil èter	$C_{12}H_3Br_7O$	722	$1{,}5\times10^{-3}$	7,14
BDE-209	Decabromodifenil èter	$C_{12}Br_{10}O$	959	$1{,}4\times10^{-4}$	9,97
HBCDs					
α-HBCD	alfa-hexabromociclododecà	$C_{12}H_{18}Br_6$	642	48,8	5,07
β-HBCD	beta-hexabromociclododecà	$C_{12}H_{18}Br_6$	642	14,7	5,12
γ-HBCD	gamma-hexabromociclododecà	$C_{12}H_{18}Br_6$	642	3,40	5,47

PBDEs

Els PBDEs són FRs que s'apliquen i s'utilitzen a diferents tipus de materials (aparells electrònics, mobles, vehicles, materials de construcció, teixits, plàstics, etc) per tal de prevenir-los de la ignició.

Els materials plàstics poden contenir fins a un 15% en pes de PBDEs, encara que normalment el percentatge oscil·la entre el 2 i 6%. En canvi, en altres polímers com el poliuretà el contingut de PBDEs pot arribar fins el 30%. Més concretament, la mescla comercial Penta-BDE era utilitzada principalment en materials compostos de poliuretà com matalassos, mobles, etc. Mentre que la mescla comercial Octa-BDE s'utilitzava en materials de plàstic més durs com cobertes d'ordinadors o monitors. La mescla comercial Deca-BDE és la que ha tingut un ús més estès i prolongat, representant més del 80% de la producció total de PBDEs. S'utilitzava en plàstics com el poliestirè d'alt impacte, equipament electrònic, recobriment de cables elèctrics, tèxtils i mobiliari. A més, també s'aplicava en vehicles, vaixells i en el sector de la construcció.

La seva estructura molecular està formada per dos anells de fenil units per un pont d'oxigen. Cada anell consta de cinc àtoms d'hidrogen, cadascun dels quals es pot intercanviar per un o més àtoms de brom, formant fins a 209 possibles congèneres de BDE. Com que les estructures són similars a les dels PCBs, s'utilitza la mateixa nomenclatura proposada per Ballschmiter and Zell (1980).

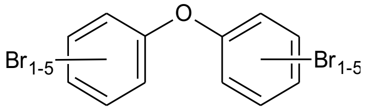


Figura 3. Estructura general dels PBDEs

Existeixen tres mescles comercials principals que presenten quantitats de brom diferents en les seves molècules, aquestes són: la Penta-BDE i la Octa-BDE que en contenen majoritàriament PBDEs amb cinc i vuit àtoms de brom respectivament i finalment, la Deca-BDE que conté el BDE-209.

HBCD

L'HBCD és un conegut grup de BFR utilitzats des de la dècada del 1960 principalment en el poliestirè expandit (EPS, de l'anglès *Expanded Polystyrene*) -popularment conegut com a porexpan- i el poliestirè extrudit (XPS, de l'anglès *Extruded Polystyrene*) (Rani et al., 2013). Amb la prohibició de les mescles comercials PentaBDE i OctaBDE al 2009, els HBCDs van passar a ocupar la tercera posició en rànquing dels FRs més utilitzats, darrera del tetrabromobisfenol-A (TBBPA) i del decabromodifenil éter (BDE-209) (Alaee et al., 2003).

L'HBCD ($C_{12}H_{18}Br_6$) és un hidrocarbur cíclic alifàtic bromat que, entre els setze possibles estereoisòmers, les mescles comercials es componen principalment de tres formes estereoisomètriques, essent el γ-HBCD el compost predominant en la mescla (75-89%), seguit de l' α-HBCD i del β-HBCD (10-13% i 1-12% respectivament). Per cadascun dels diastereoisòmers trobem una parella d'enantiòmers, el (-)- i el (+)- (Harrad et al., 2010, 2009; Janák et al., 2008; Koch et al., 2015; Li et al., 2012).

Aquests estereoisòmers són compostos hidrofòbics, amb uns valors de K_{ow} per α, β i γ de 5,07, 5,12 i 5,47 respectivament. Pel que fa a la solubilitat, l'isòmer α-HBCD és més soluble en aigua (48,8 μg/L), mentre que el γ-HBCD és el que té una solubilitat més baixa (3,40 μg/L). Aquestes diferències es traslladen també en el medi ambient, on els diferents isòmers tenen velocitats d'absorció biològica i metabòlica diferent.

Els HBCDs tenen un gran potencial de bioacumulació i biomagnificació. Són persistents en el medi ambient i tenen un potencial de transport ambiental de gran abast. Són altament tòxics per organismes aquàtics i especialment perjudicials per l'ésser humà, ja que s'ha observat toxicitat neuroendocrina i pel desenvolupament (Stockholm Convention and UNEP, 2017).

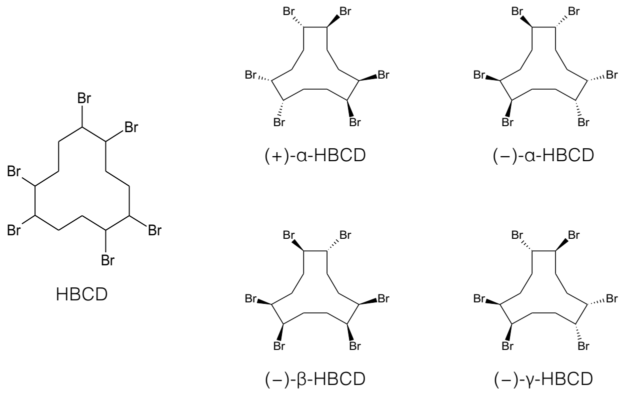


Figura 4. Estructures d'alguns isòmers de l'HBCD

FRs Emergents

Els EFRs o també coneguts com a nous FRs, són els substituts dels FRs legislats, que al entrar dins de la llista de POPs del conveni d'Estocolm van quedar regulats per la llei. Així doncs, les indústries van haver de buscar additius o reactius que satisfessin les demandes del mercat per substituir els BFRs i amb l'augment exponencial de l'ús dels EFRs es van començar a realitzar més estudis per veure i conèixer millor els efectes adversos que aquests produïen. Com ja era d'esperar, s'han acabat comportant d'una manera molt semblant als seus predecessors. Diversos estudis han demostrat que els EFRs presenten unes característiques fisicoquímiques molt semblants als PBDEs, com per exemple que són semi volàtils i lipofílics, podent-se transportar a llargues distàncies i trobant-se a diferents medis com l'aigua, els sediments, el sòl, la pols i acumulant-se a la fauna silvestre (Covaci et al., 2011; Hou et al., 2021). Alguns d'aquests compostos, com el DBDPE i l'HBB no es produeixen a Europa però són importats des de la Xina i el Japó a on es fabriquen en grans quantitats ja que són uns dels additius més utilitzats. S'estima que la producció anual d'EFRs és d'entre 100.000 i 180.000 T anuals (Zuiderveen et al., 2020).

A la taula següent es resumeixen les propietats fisicoquímiques del DBDPE, l'HBB, el PBEB i els declorans.

Taula 3. Propietats fisicoquímiques dels EFRs i declorans

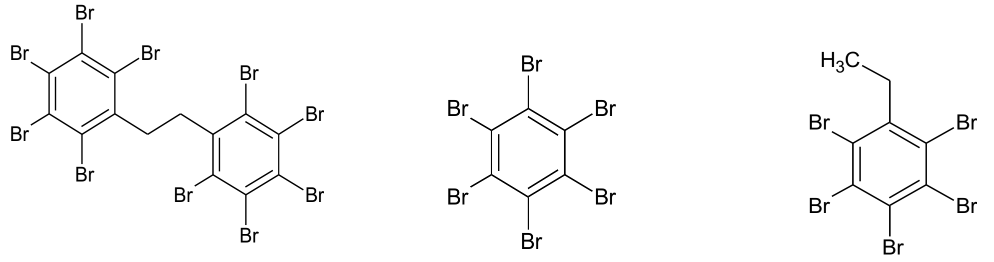
Acrònim	Nom químic	Formula molecular	Pes molecular (g/mol)	Solubilitat (µg/L)	Log K_{OW}
DBDPE	decabromodifenil età	$C_{12}Br_{10}O$	971	9,50x10^{-2}	11,2
HBB	hexabromobenzè	C_6Br_6	551	770	6,07
PBEB	pentabromoetilbenzè	$C_8H_5Br_5$	501	350	7,48
DP	decloran plus	$C_{18}H_{12}Cl_{12}$	654	4,40x10^{-2}	9,00
Dec602	decloran 602	$C_{14}H_4Cl_{12}O$	614	8,49	7,10
Dec603	decloran 603	$C_{17}H_8Cl_{12}$	638	3,00x10^{-1}	8,50
Dec604	decloran 604	$C_{13}H_4Br_4H_{16}$	692	2,21	8,50

DBDPE, HBB i PBEB

El DBDPE és un compost que degut a la seva gran mida molecular, la seva baixa solubilitat en aigua i la seva disponibilitat biològica, es creia que no tindria la capacitat d'entrar en els organismes vius i que presentava una baixa toxicitat (SUN et al., 2018), però a causa de l'augment del seu ús ha esdevingut un dels EFRs més utilitzats i en conseqüència, s'ha detectat en diferents matrius ambientals, biològiques i humanes (Aznar-Alemany et al., 2018; Chen et al., 2013; Cristale et al., 2018). El DBDPE no es produeix ni a Europa, ni als Estats Units, ni a la majoria de països desenvolupats, però s'importa principalment de la Xina, que contribueix en més d'un 50% de la producció global (Shen et al., 2019).

L'HBB també pertany als EFRs. Es produeix en menor volum que el DBDPE però s'aplica molt a la indústria del paper, del tèxtil, del plàstic i de l'electrònica. La producció principal d'aquest compost es troba localitzada a la Xina i també al Japó a on és molt utilitzat (Covaci et al., 2011). S'ha detectat a diferents mostres ambientals, des de sediments fins a peixos (Cruz et al., 2015), en els quals s'han vist efectes toxicològics, com l'estrès oxidatiu al fetge de carpes daurades (*Carassius auratus*) i fins i tot arribant a la inhibició dels canals de Na^+ i K^+ (Feng et al., 2013). També s'han trobat efectes adversos en el fetge de petits mamífers com als ratolins, a on s'ha observat la inhibició dels processos enzimàtics principals del fetge en les fases inicials d'exposició a l'HBB *in vivo* (Frydrych et al., 2005).

El PBEB és un FR de tipus additiu que s'usava en resines de polièster termostables i en resines termoplàstiques i es produïa principalment als Estats Units durant els anys 70 i 80. Tot i que actualment, segons el Sistema Europeu d'Informació sobre Substàncies Químiques, està catalogat com a producte químic de baix volum de producció fabricat per Albemarle a França (ECB-SIS, 2021). Aquest compost també és considerat persistent en el medi ambient i amb capacitat de bioacumulació (Covaci et al., 2011).



DBDPE HBB PBEB

Figura 5. Estructures dels BFRs emergents

Declorans

Dins dels EFRs, i com a part dels HFRs, hi trobem la família dels declorans o norbornens halogenats (HN), que s'utilitzen com a FRs per a cables electrònics, revestiments de cables, ordinadors i en polímers. Inclouen el decloran plus (DP) -del qual existeixen dos isòmers: el syn-DP i l'anti-DP- i els seus anàlegs, el decloran 602, 603 i 604 (Dec 602, Dec 603 i Dec 604). Aquests van sorgir com a alternativa al decloran -conegut com a Mirex- quan al 1978 el seu ús com a FR i com a pesticida es va prohibir als Estats Units a causa de la seva toxicitat, persistència al medi ambient i alt potencial de bioacumulació (Kaiser, 1978).

Una consulta recent que es va dur a terme entre abril i juny del 2020, estima que actualment s'utilitza un volum de DP d'entre 90 i 230 T/any. El sector automobilístic és el principal usuari dels DPs, amb un consum anual estimat d'entre 68 i 130 T/any el 2020 (European Chemicals Agency, 2021a). Segons el registre del reglament REACH (de l'anglès *Registration, Evaluation, Authorisation and Restriction of Chemicals*), els DPs no es fabriquen a Europa i per tant, són importants com a substàncies, mescles i en diferents articles.

Tot i no existir fonts naturals de DP, es troben en mostres ambientals, fauna i éssers humans d'arreu del món (Zaccaroni et al., 2018). S'ha observat que el DP pot provocar efectes toxicològics semblants als dels PBDEs i els HBCDs (Liang et al., 2014), essent capaç d'induir l'apoptosi i interferir en alguns processos metabòlics, així com interferir en algunes rutes d'expressió de proteïnes i en els canals de Ca^{2+} en peixos exposats a altes concentracions -entre 1,10 i 100 µg/g ww (wet weight)- de DP (Liang et al., 2014).

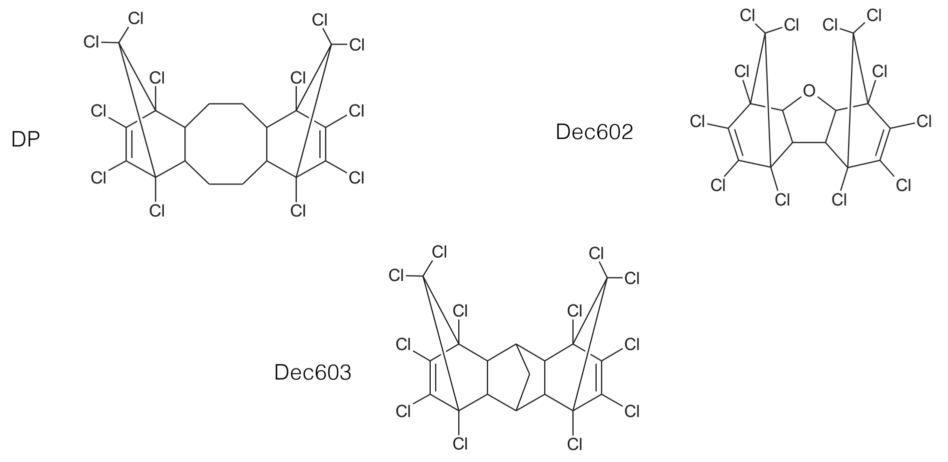


Figura 6. Estructures dels declorans

SCCPs

Les cloroparafines (CPs, de l'anglès *chlorinated paraffines*), són una família de compostos químics orgànics sintètics que consisteixen en cadenes de n-alcans amb diferents graus de cloració. Són compostos químics industrials complexes que es produeixen en grans quantitats des del 1930, amb una producció anual estimada de >1.000.000 T/any (Glüge et al., 2016), superant la quantitat total de PCBs produïda abans de la seva prohibició als anys 70-80. Es poden utilitzar com a plastificants en polímers (majoritàriament en el PVC), en el cautxú, pintures, adhesius, com a FR per a plàstics, així com en la indústria metal·lúrgica com a additius per a lubricants d'altes pressions (Stockholm Convention and UNEP, 2017). Les alliberacions d'aquests contaminants també es pot produir durant la producció, l'emmagatzematge, el transport, l'ús industrial i l'eliminació de la crema de residus.

Es produeixen per cloració de radicals lliures de diferents fraccions de n-alcans compostos entre C_{10} i C_{30} en condicions forçoses tals com la llum ultraviolada o la temperatura. El grau de cloració pot variar entre el 30 i el 72% en pes, depenent de l'aplicació prevista (Barber et al., 2005). Segons la longitud de la seva cadena de carbonis, es classifiquen en tres grups: les CPs de cadena curta ($C_{10\text{-}13}$, SCCPs), de cadena mitja ($C_{14\text{-}17}$, MCCPs) i de cadena llarga ($C_{>17}$, LCCPs).

La mirada del món científic es va centrar en les SCCPs al classificar-se com a POPs per la seva resistència a la degradació ambiental, la provocació de diferents efectes tòxics, la propensió al transport ambiental de llarg abast i la tendència a bioacumular-se en organismes vius. A més, la incineració de productes químics o residus que continguin SCCPs poden donar lloc als PCBs i policloronaftalens (PCNs). La seva estructura molecular és: $C_xH_{(2x\ y+2)}Cl_y$, on x = 10–13 i y= 1–13, podent tenir un contingut de clor entre el 50% i el 70% en pes (EC, 1999). La cloració dels n-alcans produeix mescles extremadament complexes, degut a les nombroses possibles posicions dels àtoms de clor. Les propietats fisicoquímiques dels diferents congèneres i mix dels isòmers de les SCCPs es troben resumides a la Taula 4.

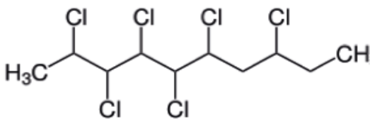


Figura 7. 2,3,4,5,6,8-hexachlorodecane, un exemple de SCCPs (61% Cl en pes) ($C_{10}H_{18}Cl_4$)

Taula 4. Propietats fisico-químiques de les SCCPs

Congèneres SCCPs	**%Cl**	**Solubilitat en aigua (µg/L)**	**Log K_{ow}**
$C_{10}H_{18}Cl_4$	50	328, 630, 2370	5,93
$C_{10}H_{17}Cl_5$	56	449–692	-
$C_{10}H_{16}Cl_6$	61	-	-
$C_{10}H_{13}Cl_9$	71	400	5,64
$^{14}C_{11}$	59	150	-
$C_{11}H_{20}Cl_4$	48	575	5,93
$C_{11}H_{19}Cl_5$	54	546-962	6,20-6,40
$C_{11}H_{18}Cl_6$	58	-	6,40
$C_{11,5}$	60	-	4,48-7,38
$^{14}C_{12}H_{21}Cl_5$	51	-	-
$C_{12}H_{20}Cl_6$	56	-	6,61
$^{14}C_{12}H_{20}Cl_6$	56	-	6,20
$C_{12}H_{19}Cl_7$	59	-	7,00
$C_{12}H_{18}Cl_8$	63	-	7,00
$C_{12}H_{16}Cl_{10}$	67	-	6,60
$C_{13}H_{23}Cl_5$	49	78	6,14
$C_{13}H_{22}Cl_6$	53	-	6,77-7,00
$C_{13}H_{21}Cl_7$	58	-	7,14
$C_{13}H_{16}Cl_{12}$	70	6.4	7,21
$C_{10}–C_{13}$	49	-	4,39-6,93
$C_{10}–C_{13}$	63	-	5,47-7,30
$C_{10}–C_{13}$	70	-	5,68-8,69
$C_{10}–C_{13}$	71	-	5,37-8,69

1.4 Context legislatiu

La classificació d'alguns d'aquests compostos com a POPs, va fer que a l'any 2004 el Penta-BDE i l'Octa-BDE es prohibissin a Europa i es retiressin del mercat dels EUA (Betts, 2008). Posteriorment, al desembre del 2006, es va aprovar el Reglament (CE) nº 1907/2006 del Parlament Europeu i del Consell de la Unió Europea, relatiu al registre, la avaluació, l'autorització i la restricció de substàncies i preparats químics (REACH). Per l'entrada en vigor d'aquest reglament, es va crear l'Agència Europea de Substàncies Químiques (ECHA), la qual és l'encarregada de la gestió d'aquest. L'objectiu d'aplicació del reglament era garantir un alt nivell de protecció de la salut humana i del medi ambient envers a les substàncies químiques i a la vegada fomentar la competitivitat i la innovació. Per tal de complir amb els objectius del REACH les empreses estan obligades a identificar i gestionar els riscos associats a les substàncies que es fabriquen i comercialitzen a la Unió Europea, demostrant com utilitzar aquestes substàncies de manera segura i portant a terme una posterior comunicació de tota la informació relativa a les mesures de gestió de riscos a les parts implicades (Parlamento Europeo y Consejo de la Unión Europea, 2007).

Amb aquesta fi, el REACH va establir que no es pot comercialitzar cap substància ni article amb un contingut de Penta-BDE i Octa-BDE major a 0,1% en massa. A l'any 2008, les SCCPs també van ser incloses dins del reglament REACH (European Chemicals Agency, 2009), dos anys més tard, al 2010 es van incloure el tris (2-cloroetil) fosfat (TCEP) i els HBCDs. Finalment, no va ser fins el 2017 que s'hi va incloure el Deca-BDE, el mateix any que també s'incloïa dins del Conveni d'Estocolm (Figura 8).

El Conveni d'Estocolm, firmat al 2001 i amb entrada en vigor al 2004, té com a objectiu protegir la salut humana i el medi ambient dels POPs i té una major importància que el reglament REACH. Quatre anys més tard de la seva aprovació, va incloure dotze compostos a la llista dels POPs. Aquests dotze, es van classificar en tres grups:

- Pesticides organoclorats: Aldrín, clordà, dieldrina, diclorodifeniltricloroetà (DDT), endrina, heptaclor, mirex i toxafè.
- Productes químics industrials: Hexaclorobenzè (HCB) i bifenils policlorats (PCB).
- Subproductes no intencionats: Dibenzodioxines policlorades o dioxines (PCDD) i dibenzofurans policlorats o furans (PCDF).

Des de la quarta reunió del conveni al 2009, es va decidir modificar els Annexes A, B i C per afegir més substàncies, les quals havien de complir els següents criteris:

- Persistència: vida mitjana de 2 mesos en aigua.
- Bioacumulació: factor de bioconcentració (BCF, de l'anglès *bioconcentration factor*) en espècies aquàtiques >5.000, o en cas de falta de dades, log K_{ow} > 5.
- Efectes adversos: evidència d'efectes adversos per a la salut humana i el medi ambient.
- Potencial de transport ambiental de llarg abast: evidència mitjançant la mesura dels nivells en punts allunyats dels focus de contaminació.

Les SCCPs es van incloure en una llista com a candidates a entrar al Conveni l'any 2008 i finalment, al 2017 ja es van incloure dins de l'Annex A. La producció i ús de la majoria de PBDEs va ser restringit i prohibit l'any 2009. Les mescles comercials més utilitzades dins d'aquest grup de compostos eren la Penta- i Octa- BDE, mentre que el Deca-BDE-209 -utilitzat com a substitut del Penta-/Octa-BDEs en diferents aplicacions i per tant, també un dels més utilitzats-, no es va prohibir fins el 2017 (Blum et al., 2019). L'entrada del Deca-BDE al Conveni d'Estocolm va marcar un abans i un després en la contaminació global per PBDEs, ja que la seva producció mundial total era deu vegades més que la resta de mescles comercials. Tot i l'aplicació de normatives que restringien l'ús dels PBDEs en productes de nova formació, aquest grup de contaminants seguia i segueix entrant al medi a través de tots aquells productes vells, com per exemple electrodomèstics, vehicles i mobles que encara estan en ús (Stockholm Convention and UNEP, 2017) o que es reciclen. Al 2013, va ser el torn dels HBCDs, que es va limitar la seva producció per a EPS i XPS en la construcció, però ja a la llista del 2017 apareixien dins l'Annex A, prohibint la seva producció i utilització.

Així doncs, al 2017 es va passar a obtenir una llista total de 28 substàncies (12 POPs antics i 16 POPs nous) (Stockholm Convention and UNEP, 2017). Aquests compostos es troben classificats en l'Annex A,B i C. En l'Annex A hi consten tots aquells contaminants que s'ha d'eliminar la seva producció i utilització, mentre que en a l'Annex B hi ha els compostos químics que s'ha de restringir la seva producció i utilització. També s'hauran d'eliminar o restringir les importacions i exportacions dels productes químics inclosos als annexos A i B, excepte per la seva eliminació ambientalment racional, finalitats o utilitzacions permeses pel Conveni, o complint determinats requisits. A l'Annex C hi ha les substàncies que se n'ha de reduir les alliberacions derivades de fonts antropogèniques no intencionals.

En conseqüència a aquestes prohibicions, es van haver de buscar compostos que els poguessin substituir, aquests són els coneguts com a EFRs, algun d'ells són l'HBB, el PBEB i el DBDPE. La producció d'aquests ha augmentat en les últimes tres dècades i per aquest motiu es troben en programes de seguiment. El DBDPE ja consta dins del reglament REACH, ja que està considerat PBT o vPvB (vPvB, de l'anglès *very persistent and very bioaccumulative*) i a més, és una substància registrada pel seu rang de tonatge total (10.000-100.000 T/any), però en canvi, els HBB i PBEB només hi estan preinscrits (Covaci et al., 2011) (Figura 8).

El DP va ser definit per la ECHA al 2018 com a substància altament preocupant (SVHC, de l'anglès *Substance of very high concern*) degut a les seves propietats persistents i bioacumulatives. Aquest compost mostra similituds estructurals amb altres POPs inclosos al reglament REACH, és per això que actualment es troba sota consulta per poder-lo adherir. Tot i així, no es fabrica dins l'Àrea Econòmica Europea però si s'importa com a substància en mescla i en articles (European Chemicals Agency, 2021b) (Figura 8).

Les SCCPs estan classificades per el REACH des del 2008 com a substàncies PBT o vPvB i també es troben dins la llista de candidats per entrar dins del reglament (de Wit et al., 2020). A més, des del 2017 que es troben dins l'Annex A del Conveni d'Estocolm. La proposta d'entrada d'aquests compostos al Conveni d'Estocolm està dirigida a les SCCPs que continguin un 48% en pes de clor (Boer et al., 2010). Per altre banda, la Organització Internacional Marítima (IMO) els ha classificat com a contaminants marins severs i per tant, es troben dins la categoria 9 de mercaderies perilloses de les Nacions Unides pel que fa al transport per carretera i ferrocarril per Europa (IMO, 1996).

A la Figura 8 trobem resumides les regulacions de les diferents famílies de contaminants estudiades excepte els OPEs,

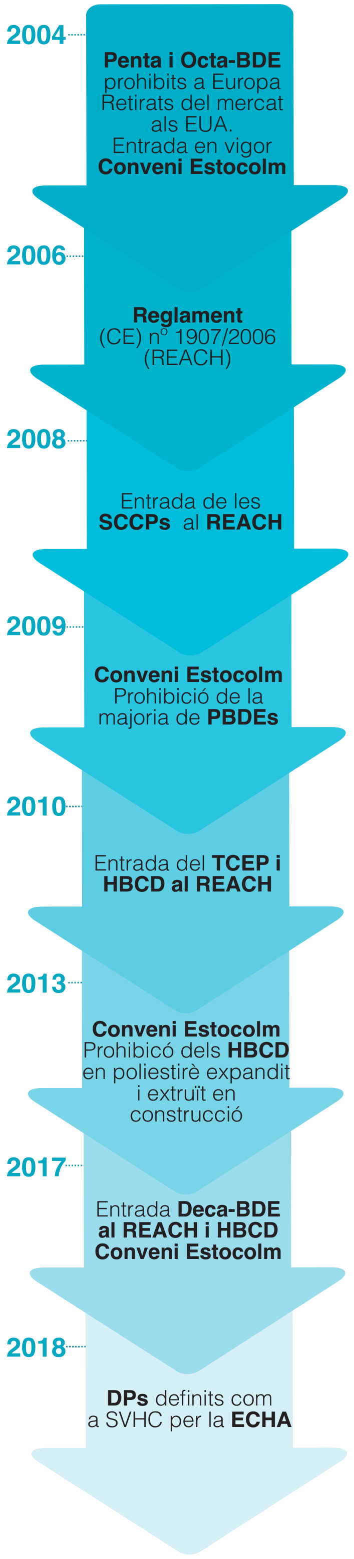


Figura 8. Resum de la legilació

que estan resumits a continuació.

Com a substituïts principals dels PBDEs es van començar a utilitzar els OPEs, que tot i no estar dins del Conveni d'Estocolm, alguns d'ells es troben registrats al REACH, com és el cas dels següents compostos (HBM4EU, 2019):

- TEP: Registrat pel REACH.
- TCEP: SVHC (Tòxic per la reproducció (Article 57c)), tots els seus usos requereixen d'una autorització sota l'Annex XIV del REACH des del 21/08/2015.
- Tris (1,3-dicloro-2-propil) fosfat (TDCIPP, de l'anglès *tris(1,3-dichloro-2-propyl) phosphate*): es troba dins la llista d'avaluació de disruptors endocrins.
- Fosfat de trifenil (TPHP, de l'anglès *triphenyl phosphate*): es troba dins la llista d'avaluació de disruptors endocrins i registrada pel seu rang de tonatge total (10.000-100.000 T/any).
- Fosfat de tri-n-butil (TNBP, de l'anglès *Tri-n-butyl phosphate*): es troba dins la llista d'avaluació de substàncies classificades com a cancerígenes, mutagèniques o tòxiques per la reproducció.
- Tris (2-butoxietil) fosfat (TBOEP, de l'anglès *tris(2-butoxyethyl) phosphate*), tricresil fosfat (TCP, de l'anglès *tricresyl phosphate*), EHDPP i TEHP : Es troben registrats al REACH pel seu rang de tonatge total (10.000-100.000 T/any).

1.5 Rellevància dels OPEs com a plastificants i FRs

Com s'ha vist en l'apartat anterior, la prohibició i restricció dels principals HFRs, degut als seus efectes perjudicials confirmats en la vida silvestre i ens els éssers humans, han aplanat el camí per a l'àmplia aplicació dels OPEs. Aquests s'han anat utilitzant com a FRs alternatius, plastificants i agents antiespumants en diferents productes industrials i de consum, portant un augment de la seva producció, ús i abocaments en el medi ambient. Tal i com s'ha explicat a l'apartat *1.2 Retardants de flama (FRs) i plastificants, definició i classificació*, els OPEs van suposar el 18% del total dels FRs consumits a nivell mundial durant l'any 2019 (IHS Consulting, 2020; Flameretardants-online, 2019).

Poden arribar a ser neurotòxics, carcinogènics i/o disruptors endocrins (Van der Veen and de Boer, 2012); alguns dels seus compostos poden arribar a bioacumular-se i/o biomagnificar-se (Zhao et al., 2018); i es poden transportar a llargues distàncies, a on s'han trobat concentracions d'OPEs en zones remotes (Casas et al., 2021; Möller et al., 2012; Xie et al., 2022).

Així doncs, és de rellevant importància analitzar aquests compostos en diferents matrius ambientals ja que encara manquen molts estudis dels OPEs sobretot en biota, i especialment en el medi marí. En aquesta tesi ens hem centrat en aquesta família de contaminants per tal de conèixer més a fons molts dels aspectes encara desconeguts sobre el seu comportament ambiental i biològic.

1.6 Entrada en el medi ambient dels OPEs

Els OPEs estudiats tenen origen antropogènic, és a dir, que no són d'origen natural, i per tant arriben al medi ambient a partir de productes, residus o fonts de contaminació. Aquests són utilitzats en diferents indústries com a additius i s'alliberen al medi a partir d'abocaments, aigües residuals, fums i gasos que generen i també a partir dels seus productes finals tant durant la seva vida útil com una vegada ja són tractats com a residus (Figura 9).

Quan aquests contaminants entren en contacte amb l'atmosfera, es poden arribar a transportar per l'aire a llargues distàncies (Castro-Jiménez et al., 2016; Möller et al., 2012). El mateix passa en el cas de l'aigua, ja que els rius poden transportar-los fins al mar i una vegada allà, les corrents oceàniques són les encarregades de transportar-los a arreu (Figura 9). Però la majoria d'aquests compostos es caracteritzen també per tenir una alta hidrofobicitat i un alt K_{ow}. Així doncs, molts d'ells quan arriben a un medi aquós busquen refugi en el teixit adipós dels organismes vius, podent bioacumular-se i/o biomaginificar-se a través de la xarxa tròfica (Figura 9) (Bekele et al., 2019), fins a poder arribar als humans a través del consum de peix i/o marisc (Bekele et al., 2021a). A més, també els podem trobar en els llots i sediments de mars (Ma et al., 2017), rius (Barón et al., 2014; Giulivo et al., 2017) i estuaris i en els sòls i les plantes dels boscos (Z. Hu et al., 2021). Els OPEs presenten una vida mitjana $t_{1/2}$ molt inferior que els PBDEs en els organismes (Hou et al., 2016) (Taula 5). En general, els OPEs amb un baix potencial de bioacumulació (log $K_{OW} < 3$) presenten una taxa d'eliminació més alta i una $t_{1/2}$ més curta (Green et al., 2007).

Taula 5. Vida mitjana ($t_{1/2}$) dels OPEs. Adaptació de Xie et al., 2022

	$t_{1/2}$ (h)		
Acrònim	**Aire**	**Aigua**	**Microbiana**
TEP	3	360	-
TCEP	12	2200	42
TPPO	-	-	-
TCIPP	7	3300	-
TPP	-	-	-
TDCIPP	12	4300	50
TPHP	24	800	98
TNBP	3	210	75
DCP	-	-	-
TBOEP	2	460	-
2IPPDPP	-	-	-
4IPPDPP	-	-	-
TCP	19	910	29, 25, 28
EHDPP	8	780	30
B4IPPPP	-	-	-
IDPP	-	-	-
T2IPPP	-	-	-
THP	-	-	-
TEHP	3	240	52

Les zones remotes, incloses les regions polars, l'alta muntanya i els oceans, tenen ecosistemes únics que tot i que sembla que es mantinguin inalterats, no es troben exemptes d'aquesta contaminació. L'entrada dels contaminants en les zones remotes ve explicada principalment pel transport a llarga distancia a través de les corrents atmosfèriques i/o oceàniques (Chokwe et al., 2020; Li et al., 2017; Na et al., 2020; Sühring et al., 2021; Xie et al., 2020).

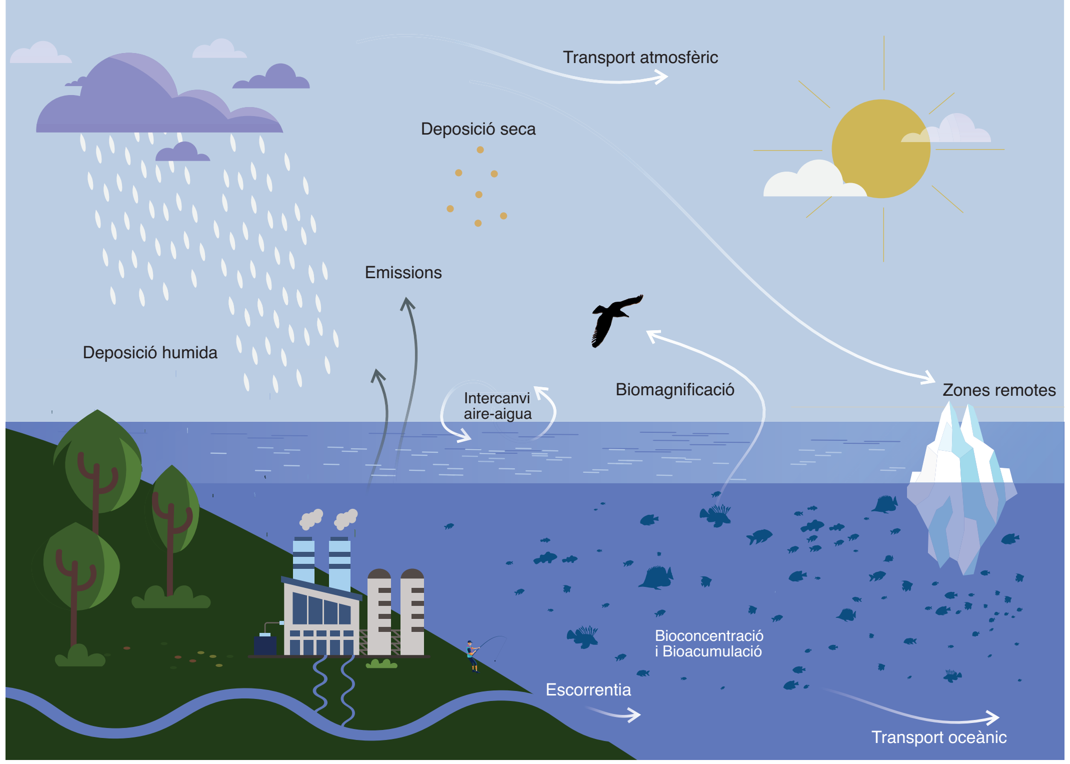


Figura 9. Esquema de les vies d'entrada dels OPEs al medi marí

Els OPEs són omnipresents en el medi marí i s'han detectat concentracions en les diferents matrius: aire, sediments, aigua i biota. Les concentracions detectades en les partícules d'aire sospeses sobre dels oceans Pacífic, Índic, Àrtic i Austral, entre els anys 2010 i 2011, van des de 120 a 2.900 pg/m^3 (Möller et al., 2012). Aquests valors es poden comparar amb els trobats a les zones tropicals i subtropicals dels oceans Atlàntic, Pacífic i Índic, a on es van trobar valors de 360-4.400 pg/m^3 (Li et al., 2018). En general, les concentracions elevades d'OPEs en aire es troben a les regions properes a la costa, especialment en zones urbanitzades i industrials, seguides pels oceans oberts de latituds mitjanes, l'Àrtic i finalment l'Oceà Austral (Xie et al., 2022).

Els OPEs, sobretot els clorats, són molt difícils d'eliminar en les EDARs i en conseqüència, es troben valors molt elevats en els efluents d'aquestes plantes (Andresen et al., 2004; Wang et al., 2020). Valors d'OPEs d'entre 10-1.000 ng/L s'han detectat en aigües superficials que acaben desembocant al mar (Fries and Püttmann, 2003; Lian et al., 2021; Rodgers et al., 2018; Schmidt et al., 2020). En les aigües de zones costeres de 7 països Europeus s'han pogut detectar valors que oscil·laven entre els 0,43 i 870 ng/L (Aznar-Alemany et al., 2018). Concentracions del mateix ordre de magnitud s'han pogut veure en zones properes a àrees urbanes del Regne Unit (280±35 ng/L), Portugal (550± 440 ng/L) i el Mediterrani nord-occidental (240±330 ng/l) (Schmidt et al., 2021). En canvi, les concentracions detectades en zones similars a la Xina són relativament superiors, amb un rang de concentració de 88–3.600 ng/L (Chen et al., 2019; Hu et al., 2014; Lian et al., 2021).

A l'igual que les aigües, els sediments marins propers a àrees amb una alta densitat de població, tendeixen a tenir altes concentracions d'OPEs. Tot i així, també s'han trobat concentracions en zones d'alta mar però d'ordres de magnitud molt més baixes. Les concentracions trobades en els sediments marins van des dels 0,35 als 71 ng/g en pes sec (dw, de l'anglès *dry weight*) (Xie et al., 2022). En un estudi recent realitzat al llarg de la costa Coreana, s'hi van detectar concentracions de fins a 350 ng/g dw. Normalment, aquests valors tant elevats venen de zones altament contaminades com són els ports marítims (Choi et al., 2020). Altres estudis han revelat que les concentracions d'OPEs en els sediments anaven disminuint a mesura que s'allunyaven de la costa (Ma et al., 2017; Y. Wang et al., 2017). A mars tancats com el Mediterrani, també s'han trobat altes concentracions d'OPEs, concretament en el Golf de Lleó on els valors oscil·laven entre 4 i 230 ng/g dw (Alkan et al., 2021).

Les concentracions d'OPEs trobades en organismes marins han revelat la presència d'aquests contaminants a través de les xarxes tròfiques oceàniques. Els estudis existents d'OPEs en biota són escassos. Els estudis publicats presenten valors molt diferents de concentracions d'OPEs, anant de les desenes als milers de ng/g en pes lipídic (lw, de l'anglès *lipid weight*). Sundkvist et al., (2010) va detectar nivells de fins a 15.000 ng/g lw a individus de peixos Eelpout (Zoarces Viviparus) de la costa de Suècia i Evenset et al., (2009) nivells de fins a 7.022 ng/g lw en bacallà Atlàntic a Kongsfjorden (Àrtic) (Figura 10). En canvi, per salmons i arengades, també de la costa de Suècia, es van trobar concentracions de 34 i 15 ng/g lw respectivament (Sundkvist et al., 2010). Dins dels organismes marins, els peixos són dels que presenten concentracions més elevades, ja que l'entrada dels contaminants pot venir tant de l'absorció dels OPEs dissolts en l'aigua a través de les brànquies i el teixit epitelial, com per la dieta (veure apartat de *1.7 Comportament en biota dels OPEs*).

En els ocells marins també se'ls hi ha trobat altes concentracions d'OPEs, ja que la seva dieta es basa principalment en petits peixos. A la Figura 10 podem veure que es van trobar concentracions mitjanes de 2.246±1.232 ng/g lw en pingüins Papua (Pygoscelis papua) de l'Antàrtida (Fu et al., 2020), unes dades molt preocupants al tractar-se d'una zona remota. Tot i la manca d'estudis d'OPEs en mamífers marins, s'han trobat concentracions considerables en foca gris (*Halichoerus grypus*) (490 ng/g lw), foca comú (*Phoca vitulina*) (320 ng/g lw) i marsopa (*Phocoena phocoena*) (280 ng/g lw) del mar Baltic (de Wit et al., 2020). Per altre banda, es van mirar concentracions d'OPEs en ossos polars de l'Àrtic i tot i que es van detectar OPEs, les concertacions reportades eren molt baixes, per sota de ppbs (de l'anglès *parts per billion*). El TEHP va ser l'únic OPE que es va poder quantificar amb valors de mitjana geomètrica de 0,163–0,308 ng/g (Letcher et al., 2018).

Observant els valors d'OPEs detectats en medis abiòtics i biòtics, veiem que generalment es troben concentracions més altes en els medis abiòtics. Cal tenir en compte que les dades disponibles en ambients biòtics és més escassa i per tant, es necessitaria seguir investigant en aquesta direcció per seguir estudiant el comportament d'aquests compostos en el medi

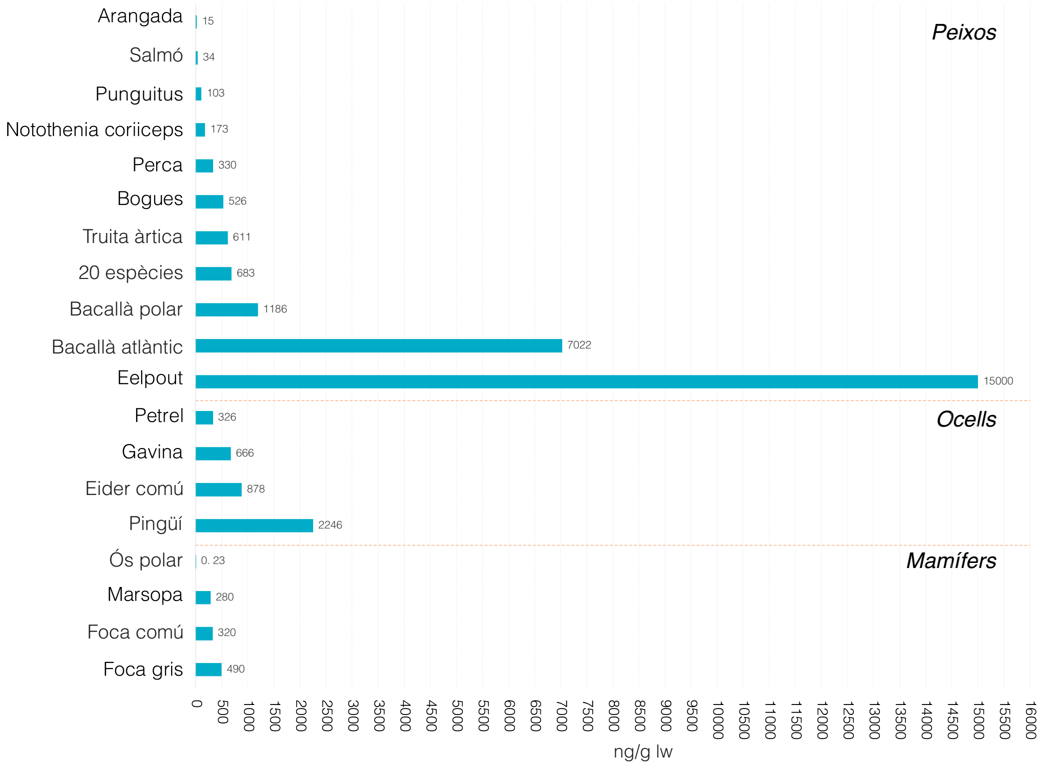


Figura 10. Resum de les concentracions d'OPEs (ng/g lw) trobades en diferents espècies de biota. (de Wit et al.,2020)

ambient. A més, els diferents estudis que mostren la presència d'OPEs en organismes vius, especialment en la biota marina, fan necessari un estudi més exhaustiu sobre el comportament i l'impacte d'aquests contaminants en el medi marí. No obstant, les concentracions baixes en individus que es troben a dalt de tot de la cadena tròfica ens fa pensar que hi pot haver una pobre assimilació de la dieta o una ràpida metabolització dels compostos (Greaves et al., 2016).

1.7 Comportament en biota dels OPEs

En l'apartat anterior hem vist les principals rutes d'entrada al medi ambient. En aquest apartat es descriuen alguns dels principals processos que componen el comportament dels OPEs en biota.

Degut a la seva naturalesa hidrofòbica, els organismes aquàtics poden arribar a acumular altes concentracions de contaminants orgànics en relació amb la concentració que es troben aquests en el medi ambient. Les vies d'exposició d'aquests organismes amb els contaminants en són varies: directament de l'aigua, a través de l'absorció de partícules en suspensió o pel consum d'organismes de nivells tròfics inferiors. Segons quina sigui aquesta via d'entrada o si en són més d'una parlarem de bioconcentració, bioacumulació o biomagnificació.

Com ja hem vist, els OPEs són omnipresents en el medi ambient i en la biota, tot i així, la informació que hi ha sobre el seu potencial de bioacumulació i biomagnificació segueix essent limitada. Només està disponible per alguns compostos i és força controvertida (Pantelaki and Voutsa, 2020).

Bioconcentració i bioacumulació

La concentració de substàncies químiques en els organismes aquàtics es poden calcular mitjançant dos factors diferents: el BCF i el factor de bioacumulació (BAF, de l'anglès *bioaccumulation factor*). Ambdós factors il·lustren la distribució de la substància química entre l'aigua i els organismes aquàtics, sovint peixos, en condicions estables. El BCF es refereix als nivells de l'organisme només degut a la absorció de l'aigua circumdant, mentre que el BAF inclou totes les fonts ambientals així com l'absorció de la dieta. Així doncs, el BCF pels animals aquàtics només es pot mesurar en estudis de laboratori en els que es pot restringir l'absorció a través de l'alimentació, mentre que el coeficient mesurat en el camp és el BAF.

Taula 6. BCF dels OPEs i PBDEs

OPEs		PBDEs	
Compost	**BCF (L/Kg)**	**Compost**	**BCF (L/Kg)**
TEP	1,40	BDE-28	$1,28 \times 10^4$
TCEP	3,46	BDE-47	$1,31 \times 10^4$
TPPO	89,4	BDE-99	$1,93 \times 10^4$
TCIPP	49,1	BDE-100	$4,44 \times 10^4$
TPP	5,32	BDE-153	$2,47 \times 10^4–1,46 \times 10^4$
TDCIPP	101	BDE-154	$3.42 \times 10^4–1,16 \times 10^4$
TPHP	73,2	BDE-183	$128–1,50 \times 10^3$
TNBP	69,6	BDE-209	$2,91 \times 10^4$
DCP	213		
TBOEP	54,2		
2IPPDPP	$6,39 \times 10^3$		
4IPPDPP	-		
TCP	$8,56 \times 10^3$		
EHDPP	269		
B4IPPPP	-		
IDPP	288		
T2IPPP	-		
THP	$2,72 \times 10^5$		
TEHP	$1,00 \times 10^6$		

En el cas dels OPEs, els BCF són força diferents entre ells (Taula 6). Hi ha estudis que han realitzat experiments controlats per avaluar el BFC dels OPEs en peixos (Arukwe et al., 2018; Hou et al., 2019; G. Wang et al., 2017b; Wang et al., 2016) i han demostrat que els OPEs tenen el potencial de acumular-se en els organismes aquàtics. N'és un exemple l'estudi realitzat per G. Wang et al. (2017) per investigar l'acumulació i depuració de set OPEs en peixos zebra (Danio rerio) durant 19 dies i 3 dies sota una prova d'exposició semiestàtica. Es va veure que en estadi estacionari, els valors de BCF dels OPEs oscil·laven entre 0,50-335 L/kg ww i que tots els compostos mostraven un alt potencial d'acumulació a baixes concentracions d'exposició.

Els OPEs arils i alquils, amb una major massa molecular, són més hidrofòbics i tenen uns valors de BFC similars, presentant així una major afinitat pels sediments i pel sòl (Van der Veen and de Boer, 2012; Wei et al., 2015). També està demostrat que els OPEs clorats són molt més solubles en aigua i es consideren una amenaça persistent pels animals aquàtics (Reemtsma et al., 2008; Van der Veen and de Boer, 2012). L'absorció per les brànquies podria ser una de les formes més comuns en que els animals aquàtics absorbeixen els OPEs dissolts en l'aigua. Alguns estudis en peixos (Kim et al., 2011; Malarvannan et al., 2015; Sundkvist et al., 2010) han vist que l'absorció d'aquests a través de les brànquies i el teixit epitelial té una gran contribució, fins i tot en alguns casos més que a través de la biomagnificació de la xarxa tròfica. Tot i així, hi ha pocs estudis que hagin investigat l'absorció dels OPEs a través de les brànquies (Hou et al., 2016).

Comparant els valors de BCF dels OPEs amb els dels seus predecessors, els PBDEs, veiem que alguns compostos dels OPEs presenten BCF comparables als dels PBDEs (Taula 6), encara que de manera general, aquests últims són més bioacumulables amb BCF que oscil·len entre 128 i 44.430 L/Kg (Mansouri et al., 2012).

Les propietats fisico-químiques d'una substància desenvolupen un paper important en el procés de bioacumulació (Figura 11). Les característiques d'aquestes substàncies que confereixen la tendència a la bioacumulació són: la lipofília, que està directament relacionada amb la magnitud de la solubilitat d'una substància química en octanol i es caracteritza per la magnitud del coeficient de partició octanol-aigua (K_{ow}); la baixa solubilitat en aigua o la hidrofobicitat, degut a la falta de grups funcionals polars; i l'estabilitat estructural que dóna lloc a la persistència en el medi ambient (anys en comptes de dies). El paper d'aquestes característiques també depèn del tipus d'organisme i de la seva estructura i metabolisme (Homem and Ratola, 2020; Schwarzenbach et al., 2003). No obstant, en el cas dels OPEs presenten una alta variabilitat en els valors de K_{ow}, oscil·lant entre 0,80 i 9,49, (Taula 1), que no necessàriament reflecteixen el seu comportament d'acumulació.

Per obtenir dades dels BAF ens hem de centrar en els estudis de camp realitzats, ja que els duts a terme en laboratoris són limitats a causa de que en la majoria el temps d'exposició és curt, hi ha dificultats per arribar a un equilibri, la via d'exposició és limitada (principalment es considera l'absorció dels OPEs en les brànquies, sense tenir en compte la ingesta per la dieta), en canvi en el medi aquàtic les limitacions són insignificants (Bekele et al., 2021b). Aquesta explicació podria ser la raó del perquè existeixen diferents BAFs entre els estudis de control en laboratoris i en els estudis de camp, per tant seria necessari prestar més atenció als mecanismes d'acumulació.

Choo et al. (2018) va informar sobre el potencial de bioacumulació dels OPEs en el carpí daurat (*Carassius auratus*) del riu Nakdong de Corea del Sud. Es van mesurar valors de BAFs de 2.440-71.000 L/Kg ww, 5.900-140.000 L/Kg ww i 1.970-59.900 L/Kg ww en el múscul, fetge i gònades respectivament, excedint els valors estàndards de bioacumulació establerts pels POPs en el Conveni d'Estocolm (BAF/BCF ≥ 5000 L/kg). Wang et al. (2019) també va reportar valors per sobre de la norma en invertebrats (2–50.119 L/Kg ww) i plàncton (24–31.623 L/Kg ww) i valors del mateix d'ordre de magnitud que el límit pels peixos bentònics (4–2.570 L/Kg ww) i peixos pelàgics (4–1.479 L/Kg ww) en la xarxa tròfica del llac Taihu, a la Xina. Valors similar s'han trobat en el zooplàncton del nord-oest del Mar Mediterrani (Schmidt et al., 2021). No obstant, altres estudis com els de Hou et al. (2017) i Liu et al. (2019) van trobar valors de BAF en peixos de rius de Pequin (Xina) i del Sud de la Xina, respectivament, per sota dels valors establerts pel conveni.

Els valors de BAF dels OPEs segueixen sent inferiors als valors de BAF dels PBDEs, que van des de log BAF 6,1 a 7,1 (He et al., 2012), demostrant una clara bioacumulació al estar per sobre del valors indicat pel conveni d'Estocolm (log BAF ≥ 3,7). El mateix passa amb el DBDPE, que també presenten valors de log BAF superiors als de la majoria d'OPEs, amb valors de log BAF d'entre 6,69 i 7,10 en peixos del riu Dongjiangb (Xina) (He et al., 2012). En canvi, per la resta d'EFRs estudiats i per les SCCPs, els rangs de log BAF són comparables amb els dels OPEs: 2,72-4,09 pel HBB, 3,31-5,54 pel PBEB -calculats en diferents espècies aquàtiques de l'estuari del riu Pearl a la Xina (Hou et al., 2021)- i 2,46-3,45 per les SCCPs en diferents espècies de peixos d'aigua dolça de la ciutat de Longtang, al sud de la Xina (Sun et al., 2017). Cal tenir en compte que els resultats de l'acumulació dels contaminants poden variar en les proves *in situ* degut a les complexes condicions ambientals, com per exemple de la quantitat de matèria orgànics dissolta.

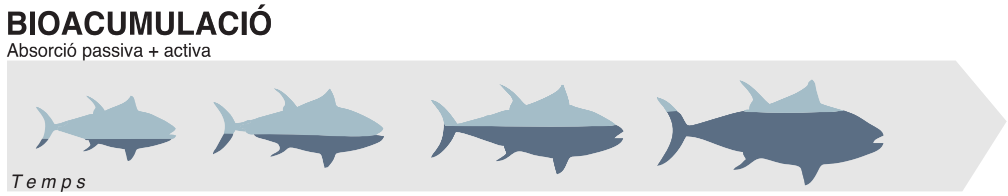


Figura 11. Representació gràfica del procés de bioacumulació d'una substància (ombra fosca) en organismes al llarg del temps

Biomagnificació

Les concentracions totals de POPs en els oceans són generalment baixes. Tot i així, els organismes aquàtics poden sovint bioconcentrar i bioacumular els baixos nivells de concentració que es troben a l'aigua en nivells relativament alts en els seus teixits. La biomagnificació (Figura 12) és el procés pel qual un compost químic augmenta la seva concentració a mesura que es troba a un nivell tròfic més alt dins una cadena tròfica. Així doncs, podem dir que la biomagnificació és un cas especial de bioacumulació en el que la concentració química en els organismes supera la de la dieta degut a l'absorció dietètica. Igual que la bioconcentració i la bioacumulació, la biomagnificació es pot expressar mitjançant el corresponent factor de biomagnificació (BMF, de l'anglès *biomagnification factor*), definit com a la concentració d'una substància química en un organisme respecte a la seva dieta. El potencial de biomagnificació d'una substància química dependrà de diferents factors, entre ells les seves propietats fisico-químiques (ja que els coeficients d'assimilació del compost variaran en funció d'aquestes), les condicions ambientals que afectaran a la biodisponibilitat del compost químic per l'organisme, les característiques dels organismes exposats i la cadena alimentària d'aquest.

Són pocs els estudis que han examinat les xarxes tròfiques aquàtiques i han informat sobre els potencials de transferència tròfica i biomagnificació dels OPEs. Un d'ells és el realitzat per Bekele et al. (2019) sobre el potencial de biomagnificació de 20 OPEs en una xarxa tròfica marina del nord de la Xina, a on es van trobar valors per sobre de 1 del TMF (de l'anglès *Trophic magnification factors*) pels Cl alquil-OPEs, els no-Cl alquil-OPEs i els aril-OPEs, demostrant una biomagnificació d'aquests (Bekele et al., 2019). Brandsma et al. (2015) també va calcular el TMF d'una cadena tròfica aquàtica trobant valors de TMFs > 1 pel TBOEP, TCIPP i el TCEP (3,5, 2,2 i 2,6 respectivament). No obstant, en el mateix estudi no es va observar biomagnificació per la resta d'OPEs analitzats. Per altre banda, es van trobar valors molt més baixos analitzats en la xarxa tròfica del llac Taihu a la Xina (Zhao et al., 2018), aquestes diferències podrien venir de les característiques que presenta cada ecosistema -com la composició de les espècies, la condició de l'hàbitat, la longitud de la cadena tròfica, el nivell de contaminació, la biotransformació dels OPEs en la biota, etc-, que determinen el comportament de la transferència tròfica dels OPEs i posteriorment el seu grau de biomagnificació (Borga et al., 2013; Sun et al., 2017, 2015; Walters et al., 2016).

Els OPEs presenten una biomagnificació menor que els PBDEs, els quals van mostrar una correlació estadística significativament positiva amb el nivell tròfic en dofins comuns (*Delphinus delphis*) i mulars (*Tursiops truncatus*) del Golf de Càdis (Barón et al., 2015a). En el cas dels EFRs, l'alt potencial de transferència tròfica ha estat recolzat per les altes concentracions reportades en animals aquàtics de nivells tròfics superiors en comparació amb els de nivell tròfic inferior de les xarxes tròfiques aquàtiques del Sud de la Xina (Wu et al., 2011; Zhang et al., 2011). Per les SCCPs, igual que en el cas dels OPEs, els coneixements que hi ha sobre la seva biomagnificació són escassos, tot i així alguns estudis han demostrat un TMF > 1, indicant una potencial magnificació tròfica de les SCCPs en les xarxes tròfiques aquàtiques (H. Hu et al., 2021; Zhou et al., 2018).

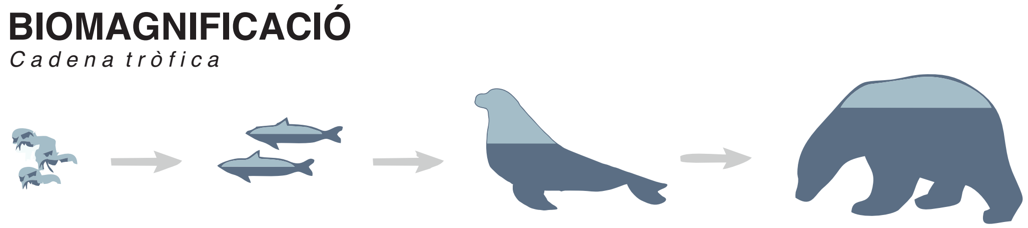


Figura 12. Representació gràfica del procés de biomagnificació d'una substància (ombra fosca) en organismes al llarg de la cadena tròfica

1.8 Metabolisme dels OPEs

El metabolisme és una part determinant sobre la bioacumulació i l'eliminació de xenobiòtics. En els estudis sobre metabolisme es fan amb enfocaments *in vivo* i *in vitro* tant en animals de laboratori com en humans. En el cas dels OPEs s'ha limitat a estudis en rates de laboratori i en models humans *in vitro*, tot i que també existeixen estudis *in situ*. S'ha demostrat que els OPEs poden passar ràpidament a través de la biotransformació de fase I i fase II en metabòlits, que són més hidrofílics i poden ser eliminats més ràpidament (Cooper et al., 2011; Van den Eede et al., 2013). Les vies metabòliques comuns pels OPEs es resumeixen a la O-desalquilació, la hidroxilació i la deshalogenació oxidativa (Hou et al., 2016) (Figura 13).

Els mecanismes metabòlics dels OPEs en organismes aquàtics com els peixos, són molt complicats. S'ha demostrat que l'activitat enzimàtica juga un paper important en l'eliminació dels OPEs. Diferents investigacions sobre el metabolisme d'aquests contaminants han pogut demostrar que els metabòlits dels OPEs es produeixen principalment en el fetge (Hou et al., 2018; G. Wang et al., 2017a). Un altre estudi realitzat en gavines va demostrar que el TNBP tenia una taxa de degradació més ràpida, seguit pel TBOEP, TCIPP, TPHP i TDCIPP (Greaves et al., 2016). No obstant, en el mateix estudi es va veure que el TEP no es degradava, resultat que també es va donar a una investigació duta a terme a mamífers, a on es mirava el metabolisme del microsoma hepàtic de foques i ossos polars (Strobel et al., 2018).

La taxa metabòlica entre els diferents OPEs varia de manera significativa, essent els tri èsters no-Cl alquil-OPEs els que es metabolitzaven més ràpidament que els tri èsters Cl alquil-OPEs (Yao et al., 2021). Així doncs, queda confirmada la dependència estructural dels OPEs en les vies metabòliques (Strobel et al., 2018; Yao et al., 2021)

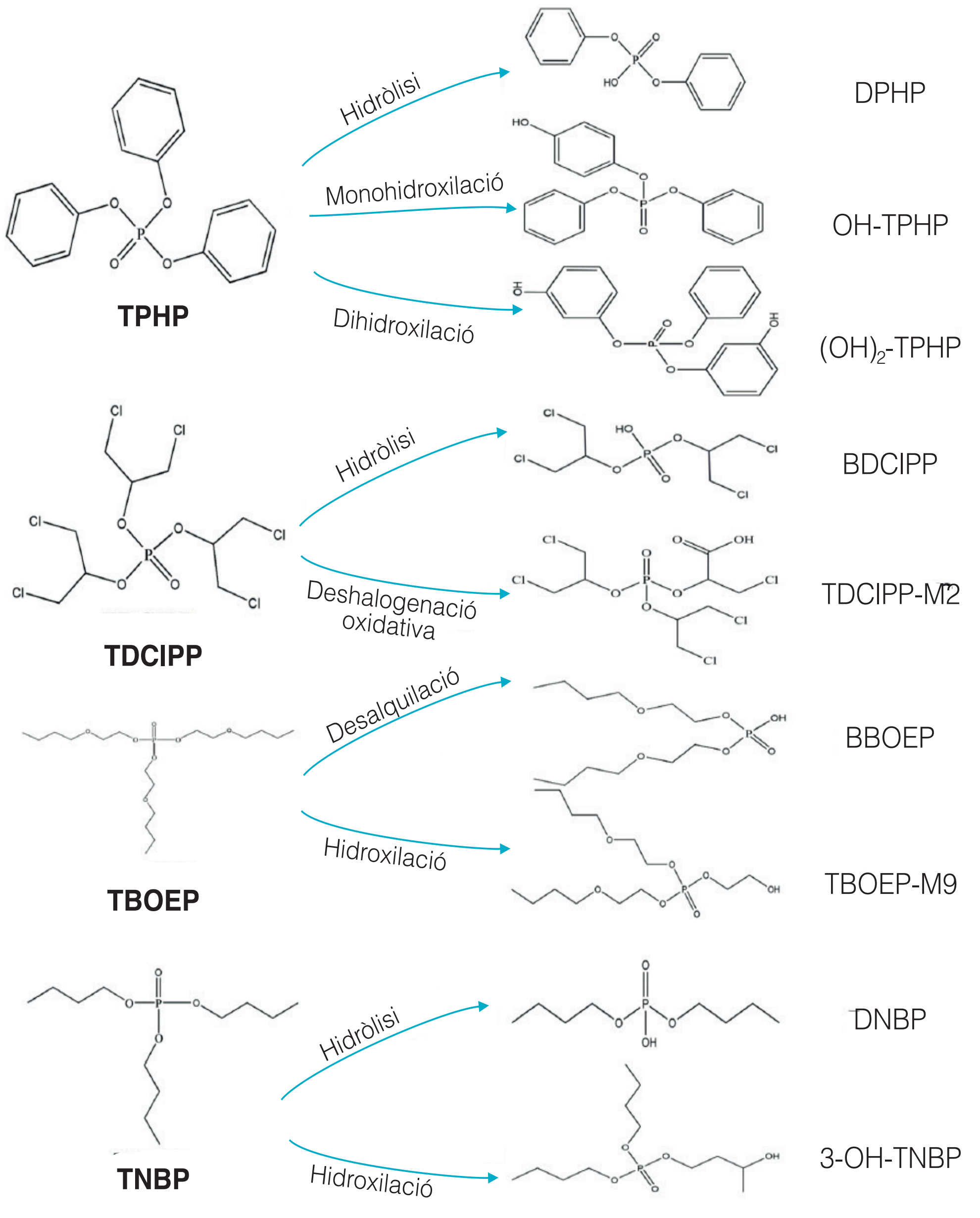


Figura 13. Els metabòlits principals dels OPEs en organismes vius. Adaptació de (Yao et al., 2021)

1.9 Avaluació del risc a l'exposició dels OPEs

Els OPEs plantegen riscos ecològics potencials pel medi ambient i la salut humana (Reemtsma et al., 2008; Van der Veen and de Boer, 2012). A més dels efectes nocius a causa de l'exposició aguda i/o de les altes dosis de FRs i plastificants, cada vegada hi ha més evidències que revelen els efectes toxicològics a llarg termini dels principals OPEs en organismes vius que actuen com a bioindicadors. Per aquest motiu, al llarg del temps s'han anant establint diferents normes per tal de regular les possibles conseqüències adverses derivades de l´ús d'aquests contaminants, amb l'objectiu de protegir el medi ambient i la salut humana.

En general els OPEs tenen un potencial d'alteració endocrina (Rosenmai et al., 2021). Yao et al. (2021) va veure que el TPHP pot provocar canvis patològics en els organismes a l'inhibir l'activitat enzimàtica i que també pot produir neurotoxicitat en el desenvolupament i alterar les hormones sexuals i el sistema immune. El mateix estudi va confirmar que per la seva banda, el TBOEP és disruptor endocrí i efecte a l'equilibri de les hormones sexuals. A més, també pot provocar danys oxidatius a les cèl·lules. El TDCIPP pot arribar a alterar l'equilibri de les hormones sexuals i el sistema endocrí de la tiroides. Altres efectes adversos que pot causar és la neurotoxicitat i pot provocar un desenvolupament anormal dels teixits i òrgans en embrions (Yao et al., 2021).

Tot i així, és molt important seguir investigant la toxicitat dels OPEs ja que per alguns dels seus compostos encara se'n desconeixen els efectes.

1.10 Metodologies d'anàlisi dels OPEs

Una vegada s'ha fet un anàlisi de la visió general i del context actual dels compostos estudiats a la tesi, en aquest apartat s'han volgut recopilar els mètodes analítics d'OPEs en biota existents fins a l'actualitat, ja que és la matriu que s'ha analitzat en aquesta tesi. El desenvolupament de metodologies per l'anàlisi d'aquests compostos en biota és complex ja que es determinen a nivell de traces.

Els mètodes que hi ha publicats inclouen un nombre diferent d'OPEs a analitzar, aquests van des de mètodes d'anàlisi de 8 OPEs (Gao et al., 2014) fins a mètodes d'anàlisi de 16 OPEs (Santín et al., 2016). Les diferents característiques i els paràmetres establerts de les metodologies d'anàlisi descrites pels OPEs, en matrius biològiques, es troben resumides a la Taula 7.

Recol·lecta i preservació de les mostres

La recol·lecta de les mostres és una de les parts més importants prèvia a l'anàlisi. Un bon planejament del mostreig i conservació de les mostres és clau per obtenir uns resultats òptims i reproduïbles ja que qualsevol error pot derivar a possibles contaminacions creuades. Així doncs, dur a terme un bon protocol és essencial en el camp de la química analítica. És fonamental escollir prèviament quina espècie es mostrejarà, així com la mida, l'edat i el sexe, o el teixit. El número d'exemplars també s'ha de tenir en compte ja que és clau per obtenir una bona reproductibilitat dels resultats. A més, la intravariabilitat dins d'una mateixa espècie i d'una mateixa zona, sol ser major en mostres de biota que en mostres ambientals.

Els estudis relatius a la biota aquàtica són els que refereixen tant a les espècies d'invertebrats com els peixos capturats en xarxes d'arrossegament i xarxes de malla de fons (Hallanger et al., 2015; McGoldrick et al., 2014; Zhao et al., 2018). Altres estudis investiguen els OPEs en altres tipus de fauna com són els ocells (gavines i els seus ous) o els mamífers tant marins com terrestres (os polar, balena, foca, etc). Les mostres que s'agafen en aquest cas van des de la sang fins als teixits i en cas que els individus es trobessin morts, també es poden analitzar alguns òrgans. Tot això es fa sota estrictes regulacions ètiques i generalment sota el permís d'agències governamentals (Guigueno et al., 2019; Hallanger et al., 2015; Letcher et al., 2018). Les mostres de sang poden ser recol·lectades de mamífers capturats vius (Hallanger et al., 2015). Pel que fa als individus morts, s'obtenen a partir de captures accidentals o avaraments, com per exemple en el cas dels dofins (Barón et al., 2015b). Així doncs, segons el mostreig que s'utilitzi s'obté una informació o una altre. En el cas de realitzar biòpsies a organismes vius -obtenció de la mostra *in situ* mitjançant una ballesta amb un dard de biòpsia-, es pot obtenir un nombre major de mostres permetent realitzar millors comparatives. Per altre banda, en aquest cas les limitacions que hi ha són la dificultat o impossibilitat d'obtenir informació sobre els individus així com el seu sexe, l'edat, mida, pes, etc. En canvi, si es treballa amb animals varats, és a dir que estan morts, aquesta informació si que es pot aconseguir i a més, en ocasions també es pot arribar a tenir mostres de diferents teixits. Tot i així, en aquest cas el nombre de mostres és limitat i es troba lligat a la casuística de cada estudi.

Per poder garantir una bona conservació de les mostres de biota, aquestes s'emboliquen en paper d'alumini per evitar la contaminació creuada amb plàstics, es col·loquen a una bossa hermètica i es congelen *in situ* per el seu posterior anàlisi. Per alguns teixits s'utilitza nitrogen líquid per tal d'evitar l'activitat enzimàtica (Letcher et al., 2018).

Degut a que aquests compostos s'extreuen utilitzant solvents orgànics, s'ha d'eliminar l'aigua de les mostres. Una de les tècniques més utilitzades en aquest cas és la liofilització que consisteix en separar l'aigua de les mostres mitjançant la congelació i posterior sublimació del gel a baixa pressió (buit). Aquest és un dels millors mètodes per assecar les mostres sense alterar la seva composició (Giulivo et al., 2016). Una vegada es té la mostra seca, es tritura i homogeneïtza per poder-se emmagatzemar a -20ºC abans de ser analitzada (Giulivo et al., 2016).

Mètodes d'extracció

Hi ha pocs mètodes desenvolupats i optimitzats per l'extracció d'OPEs en mostres de biota. El mètode més utilitzat és l'extracció accelerada amb dissolvents (ASE, de l'anglès *Accelerated Solvent Extraction*), també conegut com a extracció de líquids a pressió (PLE, de l'anglès *Pressurized Liquid Extraction*). Aquest és un sistema automatitzat que utilitza dissolvents orgànics per extreure compostos orgànics de mostres sòlides i semi-sòlides a altes pressions (500-3.000 psi) i temperatures (50-200ºC) per sobre del punt d'ebullició dels dissolvents. L'extracció assistida per ultrasons (UAE, de l'anglès *Ultrasonic-Assisted Extraction*), també és una tècnica molt utilitzada per la seva rapidesa (10-30 minuts), la seva senzillesa i la factibilitat ja que pot complir amb els estàndards de la majoria de laboratoris. L'extracció per Soxhlet no és tant usada ja és més lenta, es necessiten almenys 24 hores d'extracció, i el volum de dissolvents necessari per portar-la a terme és molt elevat (Bekele et al., 2021b). Finalment, tenim l'extracció assistida per microones (MAE, de l'anglès *Microwave-Assisted Extraction*), la qual ha demostrat ser una poderosa tècnica d'extracció degut a la seva capacitat per reduir el volum de dissolvents utilitzats i el temps d'extracció, així com la capacitat per escalfar ràpidament la mescla de mostra-dissolvent a partir de l'aplicació de microones, accelerant la transferència de massa dels compostos des de la matriu de la mostra (Ramalhosa et al., 2012).

Mètodes de purificació

Després de l'extracció de les mostres biològiques és necessari un pas de neteja per reduir la interferència de la matriu i del contingut de lípids i per tal de separar els OPEs d'altres famílies de compostos que també puguin estar presents a les mostres. Una de les metodologies més utilitzades és la SPE (de l'anglès, *solid phase extration*), que és un tipus d'extracció per adsorció que consisteix en posar les mostres en contacte amb una fase sòlida mitjançant la qual els analits s'adsorbeixen selectivament en la superfície de la fase sòlida (Bekele et al., 2021b). Una tècnica molt semblant és l'extracció en fase sòlida dispersiva (d-SPE, de l'anglès *Dispersive solid phase* *extraction*), que es basa en l'adició d'un sorbent directament en la solució analítica seguida d'una dispersió que afavoreix el contacte entre el sorbent i els analits (Chu and Letcher, 2015). Per últim, trobem la cromatografia de permeabilitat en gel (GPC, de l'anglès *Gel Permeation Chromatography*), basada en l'exclusió per la mida de les partícules, ja que les molècules orgàniques queden retingudes en una columna mentre que les molècules d'interès que són més petites, poden passar fàcilment a través dels porus de la columna. Cal tenir en compte que aquest mètode és lent ja que requereix d'injeccions individuals i seqüencials (Bekele et al., 2021b).

Giulivo et al. (2016) va optimitzar un mètode de purificació en línia a través d'unes columnes per a la purificació (TurboFlowTM), que permeten la màxima retenció i la bona elució dels analits. Aquestes són CycloneTM-P (0,5 x 50 mm) i C18-XL (0,5 × 50 mm) i s'utilitzen en combinació. La columna TurboFlowTM requereix l'activació utilitzant modificadors com ara àcid fòrmic o formiat d'amoni o acetat. El mètode optimitzat utilitza les fases mòbils d'aigua (0,1% d'àcid fòrmic) i metanol (0,1% d'àcid fòrmic) a 0,75 mL/min. Aquesta metodologia permet optimitzar i reduir el temps d'extracció dels compostos.

Anàlisi instrumental

Per un bon anàlisi de les traces ambientals es requereix d'un mètode analític fiable, selectiu, sensible i ràpid. En el cas dels OPEs, al ser molècules amb unes propietats fisicoquímiques tan variades, des de molt polars a molt hidrofòbics, fa més difícil d'establir un bon mètode d'anàlisi. Tant la cromatografia de gasos (GC, de l'anglès *gas chromatography*) com la cromatografia de líquids (LC de l'anglès *liquid chromatography*), en combinació amb espectrometria de masses (MS, de l'anglès *mass spectrometry*), es poden realitzar per a l'anàlisi d'OPEs en matrius biòtiques.

La GC s'utilitza per a determinar compostos no polars, utilitzant fases estacionaries no polars, principalment fenilmetilpolisiloxà al 5%. Els quadrupols o els triples quadrupols (QqQ) s'utilitzen habitualment com a detectors en l'espectròmetre de masses (Bekele et al., 2018; Guo et al., 2017; Liu et al., 2019a). La LC acoblada a l'espectrometria de masses també és una tècnica adequada per a la determinació dels OPEs en biota. La LC s'utilitza gairebé sempre amb el mètode de fase inversa, utilitzant fases mòbils de mescles d'aigua (acidificada amb àcid fòrmic) amb metanol o acetonitril en gradient d'elució per la separació i fases estacionàries de C18 (Giulivo et al., 2016; Hallanger et al., 2015; He et al., 2019; McGoldrick et al., 2014; Santín et al., 2016; Zheng et al., 2020). Els mètodes de LC-MS/MS que utilitzen analitzadors de masses de QqQ són els més utilitzats en determinar OPEs en matrius ambientals degut a la seva alta sensibilitat i selectivitat. L'anàlisi es porta a terme utilitzant la ionització per electroesprai (ESI, de l'anglès *electrospray ionization*) en mode de ionització positiva o negativa, depenent del compost i en el mètode d'adquisició de monitorització de reaccions múltiples seleccionades (MRM, de l'anglès *multiple selected reaction monitoring*). En general, aquests mètodes ofereixen unes bones recuperacions i uns límits de quantificació i detecció relativament baixos per la majoria dels OPEs (Taula 7).

Així doncs, els mètodes instrumentals que principalment s'utilitzen per l'anàlisi d'OPEs en mostres de biota són els GC-MS, GC-MS/MS i LC-MS/MS. L'anàlisi basat en LC-MS/MS és el més avantatjós per la seva major selectivitat i idoneïtat pels OPEs que són menys volàtils. Tot i així, l'alta proporció de lípids i la interferència de la matriu de la mostra de biota segueix sent un repte a l'hora d'analitzar OPEs, per tant és necessari un mètode robust d'extracció i neteja per la quantificació d'aquests contaminants en mostres biològiques.

Taula 7. Resum dels mètodes optimitzats fins el moment d'OPEs en biota. Adaptació de (Bekele et al., 2021b)

Mètode d'extracció	Paràmetres d'optimització	Purificació	Anàlisi instrumental	nº OPEs	Paràmetres	Recoveries i RSD (%)	Referències
ASE	Dissolvent d'extracció: aigua amb 10% d'acetonitril Temperatura: 150ºC Pressió: 1500 psi	SPME	GC-FPD (gas chromatography-flame photometric detector)	8 OPEs	mLODs 0,010–0,208ng/g	79,8% – 107,3% RSD: 2,0% –9,0%	(Gao et al., 2014)
ASE	Quantitat de mostra: 1 g Dissolvent d'extracció: DCM:Hex (1:1 v/v) Temperatura: at 100ºC Pressió: 1500 psi	SPE aminopropil silica gel columna-GPC	LC-MS/MS	14 OPEs	mLODs: 0,06 – 0,20 ng/g IDLs: 0,01–0,12 ng/mL	67 – 104% RSD< 16%	(Chen et al., 2012)
ASE	Quantitat de mostra: 3-6 g Dissolvent d'extracció: EA:cyclohexane (5:2 v/v) i cyclohexane:diethyl ether (9:1 v/v) Temperatura: 100ºC Pressió: 1500 psi	sistema GPC	GC-HRMS				(Sundkvist et al., 2010)
ASE	Quantitat de mostra: 0,5-1 g Dissolvent d'extracció: dichloromethane and n-hexane mixture (1:1, V/V) Temperatura: 100ºC Pressió: 1500 psi	SPE, columna multicapa (PSA, florisil, silicagel, alumina)	GC-MS				(Bekele et al., 2019)
ASE	Quantitat de mostra: 0,5–2,0 g Dissolvent d'extracció: DCM:Hex (1:1 v/v)	SPE Gel aminopropil silica	LC- MS/MS ESI, MRM				(McGoldrick et al., 2014)
Extracció per ultrasons	Quantitat de mostra: 1 g Dissolvent d'extracció: DCM:Hex (1:1 v/v)	d-SPE	UHPLC-MS/MS-APCI(+)	13 OPEs	mLOQs: 0,05–0,50 ng/g ww	54–113%, RSD<17%	(Chu and Letcher, 2015)
Extracció per ultrasons	Quantitat de mostra: 0,25 g Dissolvent d'extracció: Acetone:Hex (1:1 v/v)	SPE Alumina i cartutx C18	LC-MS/MS LC-QqLIT-MS	16 OPEs	mLOQs : 1,12–38,8 ng/g lw MDLs 0,34–11,6 ng/g lw,	45–115%, RSDs<25%	(Santín et al., 2016)
Extracció per Soxhlet	Quantitat de mostra: 2 g Dissolvent d'extracció: DCM	SPE en un cartutx Oasis HLB	GC-MS/MS	12 OPEs	mLODs 0,004 – 0,059 ng/g	56–108%	(Liu et al., 2018)
Extracció per Soxhlet	Dissolvent d'extracció: Acetone: Hex (1:1 v/v)	SPE columna Florisil GPC	GC-MS				(Guo et al., 2017)
MAE	Quantitat de mostra: 1 g Dissolvent d'extracció: Hex:Acetone (1:1 v/v)	SPE i GPC	GC-MS	14 OPEs	mLODs 0,006–0,021 ng/g lw	70,3–111%, excepte pel TMP (38,9–55,6%) RSDs< 14,1%	(Ma et al., 2013)

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CAPÍTOL II

OBJECTIUS I ESTRUCTURA DE LA TESI

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2.1 Objectius

La deixalla marina -definida per la ONU Medi Ambient com a "qualsevol material sòlid, persistent, fabricat o processat que es descarregui, evacuí o abandoni en el medi marí i costaner"-, està predominada pels residus plàstics, representant un greu problema pels oceans i en conseqüència, una gran amenaça per a la fauna marina. Més enllà de la ingesta de plàstics pels animals marins, hi ha una creixent preocupació per l'increment acumulació d'additius i contaminants dels plàstics i la capacitat d'alguns d'ells de bioacumular-se i/o biomagnificar-se. És necessari donar especial atenció a aquests contaminants degut a la seva difusió ambiental i els seus efectes toxicològics. És per aquest motiu, que l'objectiu general d'aquesta tesi és **estudiar l'impacte dels compostos plastificants i retardants de flama, especialment els OPEs, en la biota marina i la seva correlació amb els residus plàstics marins.** D'aquest objectiu general se'n deriven sis objectius específics, que són:

- Avaluar la distribució dels OPEs en els diferents teixits.
- Estudiar les tendències espacials mitjançant l'anàlisi de dofins de diferents mars i oceans,

i la possible correlació amb els nivells de residus plàstics.

- Estudiar les tendències temporals dels OPEs.
- Avaluar la presència d'OPEs i el seu nivell de concentració en diferents espècies marines.
- Estudiar la bioacumulació i biomagnificació dels OPEs.
- Estudiar la transferència placentària d'OPEs entre mare i fetus de mamífer marins.

2.2 Estructura de la tesi

Aquesta tesi ha estat escrita per compendi d'articles i es troba dividida en 5 Capítols principals. El primer capítol consta d'una introducció general dels FRs i plastificants en el medi ambient, a on s'ha introduït la problemàtica associada a aquests compostos, entrant més en detall en els OPEs ja que són els compostos principals d'aquest estudi. S'ha fet una breu explicació de la definició i classificació dels contaminants estudiats, passant pel context legislatiu de cadascun dels grups així com la rellevància dels OPEs com a plastificants i FRs. Centrant-nos amb aquest darrer grup, s'ha entrat més en detall en la seva entrada al medi ambient i quin és el seu comportament en la biota. També hi trobem una breu pinzellada del metabolisme dels OPEs i l'avaluació del risc a l'exposició d'aquests. Per últim, hi ha un recull de metodologies d'extracció i d'anàlisi dels OPEs en biota existents fins a data d'avui.

En el *Capítol 2*, en el que ara ens trobem, hi ha explicat l'objectiu general i els específics d'aquesta tesi així com l'estructura de la mateixa.

Els *Capítols 3 i 4* exposen la feina feta durant la tesi i els resultats obtinguts així com un resum de les metodologies utilitzades en els diferents estudis -les que no apareixen en les publicacions- i un apartat de discussió per a cada capítol. Ambdós capítols comencen amb una introducció per posar en context el treball que s'hi exposa, seguidament dels articles publicats i el desenvolupament d'una discussió global dels resultats.

En el *Capítol 3* s'analitza la tendència espai-temporal dels FRs i plastificants en diferents espècies de dofins, incloent 3 publicacions científiques d'anàlisi d'aquests contaminants en dofins a diferents zones del món. Els treballs publicats són:

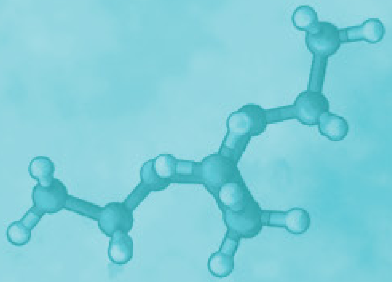
- Publicació #1: **Sala, B.,** Giménez, J., de Stephanis, R., Barceló, D., Eljarrat, E., 2019. First determination of high levels of organophosphorus flame retardants and plasticizers in dolphins from Southern European waters. Environ. Res. 172. https://doi.org/10.1016/j.envres.2019.02.027

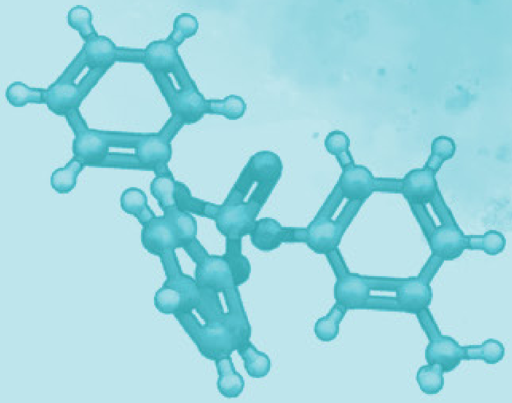
- Publicació #2: Aznar-Alemany, Ò., **Sala, B.**, Plön, S., Bouwman, H., Barceló, D., Eljarrat, E., 2019. Halogenated and organophosphorus flame retardants in cetaceans from the southwestern Indian Ocean. Chemosphere 226. https://doi.org/10.1016/j.chemosphere.2019.03.165
- Publicació #3: Aznar-Alemany, Ò., **Sala, B.**, Jobst, K.J., Reiner, E.J., Borrell, A., Aguilar, À., Eljarrat, E., 2021. Temporal trends of halogenated and organophosphate contaminants in striped dolphins from the Mediterranean Sea. Sci. Total Environ. 753, 142205. https://doi.org/10.1016/j.scitotenv.2020.142205

En el *Capítol 4* s'han realitzat estudis de la bioacumulació i la biomagnificació d'OPEs en diferents espècies de fauna marina, així com la capacitat de transferència gestacional dels contaminants. Per fer-ho s'hi ha afegit 4 publicacions científiques dutes a terme en aquesta tesi:

- Publicació #4: Garcia-Garin, O., **Sala, B.,** Aguilar, A., Vighi, M., Víkingsson, G.A., Chosson, V., Eljarrat, E., Borrell, A., 2020. Organophosphate contaminants in North Atlantic fin whales. Sci. Total Environ. 721, 137768. https://doi.org/10.1016/j.scitotenv.2020.137768
- Publicació #5: **Sala, B.**, Balasch, A., Eljarrat, E., Cardona, L., 2021. First study on the presence of plastic additives in loggerhead sea turtles (Caretta caretta) from the Mediterranean Sea. Environ. Pollut. 283, 117108. https://doi.org/10.1016/j.envpol.2021.117108
- Publicació #6: **Sala, B.,** Giménez, J., Fernández-Arribas, J., Bravo, C., Lloret-Lloret, E., Esteban, A., Bellido, J.M., Coll, M., Eljarrat, E., 2022. Organophosphate ester plasticizers in edible fish from the Mediterranean Sea: Marine pollution and human exposure. Environ. Pollut. 292. https://doi.org/10.1016/j.envpol.2021.118377
- Publicació #7: **Sala, B.**, Garcia-Garin, O., Borrell, A., Aguilar, A., Víkingsson, G.A., Eljarrat, E., 2022 Transplacental transfer of plasticizers and flame retardants in fin whales (*Balaenoptera physalus*) from the North Atlantic Ocean. Enviat a Environmental Pollution (11 d'abril 2022).

Per últim, en el *Capítol 5* s'exposen les conclusions generals d'aquesta tesi en base a la problemàtica exposada en el primer capítol, els objectius específics plantejats i els resultats obtinguts en els *Capítols 3 i 4*.





CAPÍTOL III

TENDÈNCIES ESPAI-TEMPORALS DE RETARDANTS DE FLAMA I PLASTIFICANTS ORGANOFOSFATS (OPEs) EN DOFINS

TENDÈNCIES ESPAI TEMPORALS DELS RETARDANTS DE FLAMA I PLASTIFICANTS ORGANOFOSFORATS (OPEs) EN DOFINS

3.1 Introducció

Fa dècades que els FRs i els plastificants entren al medi ambient a partir de diferents fonts de contaminació, però no va ser fins els anys 70 que el sector de la química ambiental va començar a investigar en aquesta direcció. En els darrers anys s'han fet grans avenços a mesura que s'han desenvolupat instruments i tècniques més sensibles, permetent la detecció de traces de substàncies químiques en matrius ambientals complexes, com és el cas de la biota que conté altes quantitats de lípids, implicant utilitzar una metodologia eficient d'extracció i purificació per eliminar les interferències i coel·lucions d'origen matricial. Per tal de poder analitzar i quantificar les diferents famílies estudiades s'utilitza la MS-MS i es treballa en mode de monitoratge de reaccions seleccionades (SRM) amb dues transicions per compost.

En aquesta tesi ens centrem en l'estudi dels ecosistemes marins, concretament en la fauna marina, uns dels més castigats per la contaminació per plàstics i en conseqüència de la contaminació dels compostos associats a ells. Per poder avaluar aspectes com la presència dels OPEs, la seva distribució als diferents teixits o les tendències espai-temporals, s'han escollit diferents espècies de dofins, ja que són considerats bons bioindicadors i al ser predadors de primer nivell d'una gran quantitat de peixos i calamars, poden concentrar els contaminants per bioconcentració i s'integren àmpliament en l'ecosistema en termes d'exposició dels impactes ambientals (Wells et al., 2004). La seva salut i l'estat de la població no només mostren l'estrès natural i antropogènic sobre l'espècie, sinó que serveixen d'indicadors de la salut i l'estat dels nivells tròfics inferiors dels ecosistemes marins (Wells et al., 2004). A més, al tenir una alta esperança de vida, es pot observar l'acumulació dels contaminants al llarg del temps en els seus organismes. Cal tenir en comte que totes les espècies de cetacis es troben protegides (Braulik, 2019; Braulik et al., 2021, 2017; Wells et al., 2019), per tant no es poden capturar sigui quina sigui la seva finalitat. Així doncs, les tres maneres d'aconseguir mostres d'aquests individus és a partir de biòpsies, captures accidentals o avaraments.

Per tal de poder caracteritzar correctament la problemàtica dels OPEs en mostres de biota, és necessari realitzar un estudi de distribució d'aquests contaminants en els diferents teixits. En matrius biòtiques, es poden analitzar tant els individus sencers, com seria el cas d'animals petits com els mol·luscs, o mostres concretes ja sigui plasma, ous, greix, etc. No obstant, hi ha vegades que es té l'oportunitat de poder estudiar diferents teixits d'un mateix individu, com és el cas d'aquesta tesi, a on s'han pogut analitzar mostres de greix, cervell, ronyó, fetge i múscul de dofí comú (*Delphinus delphis*). D'aquesta manera, podem avaluar a quins teixits s'acumulen els OPEs i escollir quin d'aquests és el més apropiat per dur a terme els diferents estudis. En el cas de la majoria de POPs, com per exemple els PCBs, els PBDEs i les dioxines, s'acumulen principalment en la matèria grassa i per tant, es solen analitzar teixits com l'adipós o bé amb alt contingut en greix, com en el cas del múscul (Barón et al., 2015; Dron et al., 2022; Wan et al., 2013). En canvi, compostos com els perfluorats no s'acumulen en els lípids sinó en les proteïnes (Lindh et al., 2012).

Pel que fa a la distribució espacial, s'han pogut obtenir mostres de dofins del mar Mediterrani i de l'Oceà Índic. El Mar Mediterrani, originalment "Mare Medi Terra", significa literalment mar envoltat de terra, una terra que està habitada i que per tant, exposa totalment aquestes aigües a les activitats humanes i en pateix les conseqüències directes. De manera global s'estima que el plàstic que entra al Mediterrani és aproximadament 100.000 T l'any (Jambeck et al., 2015). Liubartseva et al. (2018) va fer una estimació dels 10 inputs que més contribuïen en l'aportació de residus plàstics al Mediterrani. La llista estava encapçalada per les rutes marítimes (20%), a causa de l'efluència de vaixells mercantils, seguida del riu Nil (6,8%) i tres rius turcs (11%). De les ciutats que més hi contribueixen, Barcelona es troba en el segon lloc, amb 1.787 T/any, equivalent a l'1,8% (Liubartseva et al., 2018).

A la costa de Sudàfrica hi arriba l'Oceà Índic, el tercer oceà més gran del planeta. Les seves aigües, al contrari que el Mediterrani, es troben obertes i poden arribar a tenir una influència molt diferent a l'hora de transportar els residus que hi arriben. A Sudàfrica es generen 2.371 T/any de residus plàstics i la generació de residus per càpita és d'uns 41 Kg/hab/any, superant a la mitjana mundial de 29 Kg/hab/any (Billard et al., 2021). Del 70% dels residus plàstics generats, el 14% es recicla, el 28% s'elimina a abocadors sanitaris o instal·lacions d'incineració i el 28% restant s'elimina a abocadors oberts. En aquests abocadors també hi arriben residus exportats d'altres països més desenvolupats, molts dels quals són aparells electrònics que contenen FRs i plastificants (Kajiwara et al., 2014). La major part de plàstics que arriben al medi marí procedeix de fonts terrestres (Li et al., 2016) i s'estima que entre 14.000 i 40.000 T arriben als oceans des de Sudàfrica (Verster and Bouwman, 2020).

A més a més, cal tenir en compte que la contaminació per plàstics es desplaça pels oceans del món gràcies als vents i corrents superficials, entre d'altres processos. En les zones d'acumulació oceànica hi ha cinc grans girs, el de l'Atlàntic Nord i Sud, el del Pacífic Nord i Sud i el de l'Oceà Índic. Aquets girs són grans sistemes de corrents circulars i poden acumular elements passius com els residus marins flotants (Howell et al., 2012). Les quantitats de plàstic flotant determinades per l'hemisferi sud (Atlàntic Sud, Pacífic Sud i Oceà Índic), es troben dins del mateix rang que els de l'hemisferi nord (Atlàntic Nord, Pacífic Nord, i Mar Mediterrani) (Andrade et al., 2021). A més, les corrents atmosfèriques i oceàniques ajuden a que els additius plastificants viatgin arreu del món, fins a arribar a zones remotes (Li et al., 2017; Papachlimitzou et al., 2015; Strobel et al., 2018). Per aquest i d'altres motius, és interessant estudiar les diferents famílies de contaminants a diferents parts del món, per tal de veure com es comporten.

Com que l'ús dels additius químics varia en el temps degut bé a la seva prohibició o bé a un increment del seu ús, és important realitzar estudis per avaluar les tendències temporals i així determinar si un marc legislatiu porta a un descens dels nivells de contaminació i si un increment en l'ús porta a un augment dels nivells. Els estudis temporals s'acostumen a fer amb una diferència mínima d'una dècada per tal de poder detectar canvis significatius. En aquesta tesi es disposen de dades de dofins del Mar Mediterrani des del 1990 al 2018 i per tant, s'ha pogut dur a terme un estudi d'aquestes característiques.

3.2 Metodologia

Per analitzar els OPEs en mostres de biota, es fa una extracció sòlid-líquid amb ultrasons, una purificació de la mostra en línia i l'anàlisi es porta a terme pel sistema Thermo Scientific TurboFlowTM, que consisteix en un triple quadrupol (QqQ) MS, dues bombes LC quaternàries (Finnigan sud- Veyor L-bomba) i tres columnes de LC, dos per a la purificació de la mostra i una per la separació analítica (Giulivo et al., 2016). Aquest mètode ja havia estat desenvolupat en el laboratori per Giulivo et al. (2016), per un total de 14 compostos. En aquesta tesis hem ampliat el llistat d'OPEs fins a un total de 19 compostos.

La transició més intensa és la que anomenem de quantificació, és a dir, que es fa servir per quantificar i l'altra és la de confirmació, la qual compara la relació d'intensitats entre transicions de les mostres amb la dels patrons.

Mostreig

En aquest capítol s'han analitzat diferents espècies de dofins, que són:

- Dofí comú (*Delphinus delphis*; Linnaeus, 1758): Aquesta espècie es troba preferentment a aigües tropicals, temperades i fredes de l'Oceà Atlàntic i Pacífic. També el trobem en el Mar Mediterrani, sobretot a la part occidental (Mar d'Alboran) i al Mar Negre. Es tracta d'un dofí de mida mitja, els adults es troben entre els 1,7 i 2,7 m, i el seu pes es troba al voltant dels 200 Kg. La seva alimentació es basa en calamars i peixos petits que formen bancs, com el seitó (*Engraulis encrasicolus*) i la sardina (*Sardina pilchardus*) i no tant habitual, en alguns crustacis.

- Dofí llistat (*Stenella coeruleoalba*; Meyen, 1833): Aquesta és una espècie de dofí que habita aigües subtropicals i temperades de tot el món i és el cetaci més freqüent de la mediterrània occidental. Els adults poden arribar a mesurar 2,56 m, tot i que la seva mida mitjana és d'entre 1,8 i 2,7 m i pesen uns 150 Kg pel que fa les femelles i 160 Kg els mascles. Els animals més joves i immadurs s'alimenten d'un ventall més gran d'espècies, mentre que els individus adults mantenen una dieta menys variada formada per petits pelàgics com la sardina (*Sardina pilchardus*) o juvenils de lluç (*Merluccius merluccius*) (Gómez-Campos et al., 2011). Tot i que també s'alimenten, en menor mesura, de cefalòpodes i crustacis.

- Dofí d'estuari Indo-Pacífic (*Sousa plumbea*; Cuvier, 1829): és una espècie de dofí oceànic, viu a aigües costeres, badies i estuaris. És típic de la costa Índica de Sudàfrica, de l'est de l'Índia, la badia de Bengala i del mar Roig. Els dofins adults poden mesurar de 1,8 a 2,6 m i pesar entre 100 i 139 Kg. Són animals oportunistes, que consumeixen una àmplia varietat d'espècies a on predomina el calamar i peixos com el sabre (*Trichiurus lepturus*).

- Dofí mular Indo-Pacífic (*Tursiops aduncus*; Rice 1998): es troba a les aigües costaneres i poc profundes del voltant de l'Índia, el nord d'Austràlia, el sud de Xina, el mar Roig i la costa Oriental d'Àfrica. És un dofí petit, amb una longitud mitja de 2,6 m i un pes màxim del mascle adult de 230 Kg. S'alimenten d'una gran varietat de peixos però també mengen alguns cefalòpodes (calamars).

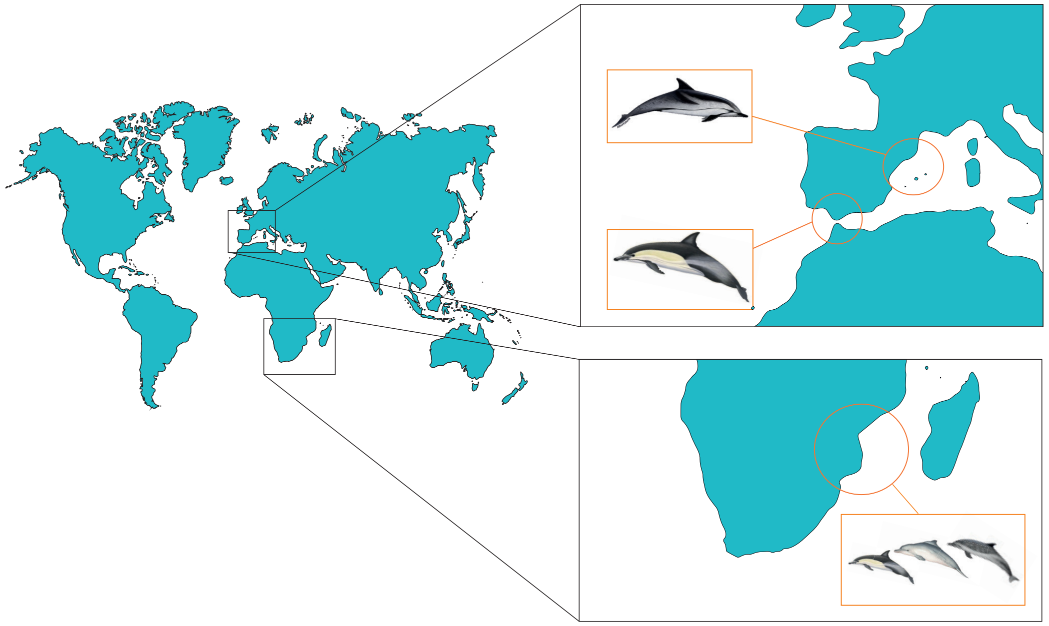


Figura 14. Localització dels mostrejos de les diferents espècies de dofins estudiades

Pel que fa a les mostres de dofins comuns procedents del Mar d'Alboran (Mediterrani Sud), es van aconseguir gràcies a la col·laboració amb investigadors de l'Estació Biològica de Doñana (EBD-CSIC). Les de dofí llistat del mar Mediterrani, era el Departament de Biologia Animal de la Universitat de Barcelona qui ens facilitava les mostres, ja que fan el seguiment dels dofins llistats i disposen de mostres de diferents períodes. Finalment, les mostres dels dofins comú, d'estuari Indo-Pacífic i mular Indo-Pacífic provinents de la costa de Sudàfrica, es van obtenir gràcies al South African Institute for Aquatic Biodiversity (SAIAB) i a la Research Unit: Environmental Sciences and Management de la North-West University de Sudàfrica.

Les mostres dels tres estudis es van aconseguir d'individus varats a les diferents costes, Mar d'Alboran, nord-oest del Mediterrani i a la costa de Sudàfrica. Una vegada els individus han estat recollits per les institucions encarregades en cada zona, es procedeix a fer la necròpsia per tal d'obtenir les mostres dels diferents teixits. A les imatges es pot veure un exemple de l'obtenció de la mostra de múscul de dofí en una necròpsia que vam poder presenciar a la facultat de veterinària de la Universitat Autònoma de Barcelona (UAB), que és la que sol proporcionar les mostres als investigadors de la Facultat de Biologia de la UB, amb els que col·laborem.

Figura 15. Necròpsia d'un dofí llistat (*Stenella coeruleoalba*)

Anàlisi químic

Tot i que els compostos principals estudiats en aquesta tesi són els OPEs, per alguns dels estudis també s'han inclòs anàlisis d'altres FRs així com els PBDEs, els EFRs i els declorans. Pels OPEs s'ha utilitzat el mètode de Giulivo et al. (2016) optimitzat prèviament al laboratori utilitzant TFC-LC-MS-MS com a instrument d'anàlisi. Per a les altres famílies de compostos, s'ha utilitzat el mateix mètode d'extracció per a tots ells, optimitzat prèviament al laboratori per a Barón et al. (2014, 2012). Les famílies dels PBDEs i els EFRs, s'analitzen mitjançant GC-MS-MS amb impacte electrònic (EI), excepte el BDE-209 i el DBDPE que s'analitzen per GC-NCI-MS ja que així s'obté una major sensibilitat. Els declorans en canvi, s'analitzen a partir de GC-NCI-MS-MS.

3.3 Resultats

Els resultats s'engloben en tres articles ja publicats, a on s'han analitzat els plastificants i FRs en diferents espècies de dofins en tres parts del món: el Mar Mediterrani pel Mar d'Alboran, El Mar Mediterrani per la costa catalana i l'Oceà Índic per la costa de Sudàfrica. Les publicacions són:

- Publicació #1: **Sala, B**., Giménez, J., de Stephanis, R., Barceló, D., Eljarrat, E., 2019. First determination of high levels of organophosphorus flame retardants and plasticizers in dolphins from Southern European waters. Environ. Res. 172. https://doi.org/10.1016/j.envres.2019.02.027

- Publicació #2: Aznar-Alemany, Ò., **Sala, B.**, Plön, S., Bouwman, H., Barceló, D., Eljarrat, E., 2019. Halogenated and organophosphorus flame retardants in cetaceans from the southwestern Indian Ocean. Chemosphere 226. https://doi.org/10.1016/j.chemosphere.2019.03.165

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PUBLICACIÓ CIENTÍFICA #1

“First determination of high levels of organophosphorus flame retardants and plasticizers in dolphins from Southern European waters”

Sala, B., Giménez, J., de Stephanis, R., Barceló, D., Eljarrat, E.

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First determination of high levels of organophosphorus flame retardants and plasticizers in dolphins from Southern European waters

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A R T I C L E I N F O

Keywords:
Brain
Plastic debris
Tissue distribution
Tri-n-butyl phosphate
Tris(chloroethyl) phosphate

A B S T R A C T

This study evaluates for the first time organophosphorus flame retardant (OPFR) occurrence in the Alboran Sea delphinids (Spain). OPFRs were detected in all the individuals with concentration levels up to 24.7 µg/g lw. Twelve out of sixteen tested analytes were detected, being TBOEP which presented the highest detection frequency, and IDPP which presented the highest levels of concentration. OPFR distribution in different tissues (blubber, brain, kidney, muscle and liver) was evaluated. The pattern distribution showed the highest contribution for blubber (mean value of 68%) and the lowest contribution for liver (mean value of 2%). Seven OPFRs were detected in brain samples showing their capacity to surpass the blood-brain barrier and reach the brain. Moreover, high affinity for the brain tissue was observed. This is extremely important due to the neurotoxic effects of several compounds such as TCEP and TNBP. OPFR levels were compared with previously published PBDE concentrations, and no significant differences were observed. Taking into account the lower use and lower bioaccumulation and biomagnification capacities of OPFRs, this could indicate an additional OPFR source of pollution in addition to their use as FRs.

1. Introduction

Organophosphorus flame retardants (OPFRs) have been used since the 1960s. OPFRs are a large class of flame retardants (FRs), which are also used as plasticizers, antifoaming agents and as performance additives in consumer products. Halogenated OPFRs are frequently used as additive FRs applied to polyurethane and other polymers for use in furniture, construction, textile industry and electronic equipment. In addition, the non-halogenated OPFRs are primarily employed as plasticizers, lubricants, antifoaming agents, and present as additives in lacquers, hydraulic fluids and floor polishing products (Andresen et al., 2007). The worldwide total FR use in 2013 was reported to be greater than 2 million tonnes, of which halogenated flame retardants (HFRs) made up ~31%, while OPFRs corresponded to ~16% of the total volume (IHS consulting, 2014). Approximately, 85% of FR use is in the production of plastics, while rubber and textile products account for most of the rest (IHS consulting, 2014). The production of OPFRs as alternatives to polybrominated diphenyl ethers (PBDEs, the previous most used HFRs banned by the Stockholm Convention in 2009 (Stockholm-Convention, 2010)) has increased from 186,000 t in 2001–680,000 t in 2015 (Pantelaki and Voutsa, 2019). Moreover, the global OPFR market is forecasted to grow at an annual rate of 5.2% from 2016 to 2021.

One of the most prominent toxic effects of OPFRs on human and experimental animals is developmental neurotoxicity. OPFRs can cause neurodevelopmental effects similar to organophosphate pesticides. Neurotoxic effects have been observed for some OPFRs such as tris (chloroethyl) phosphate (TCEP) (*practical abbreviations for OPFRs proposed by* Bergman et al., 2012 *were adopted*), tri-n-butyl phosphate (TNBP) and tris(phenyl) phosphate (TPHP) (Meeker and Stapleton, 2010). Besides neurotoxicity, some OPFRs are also known for being endocrine disruptors, affecting thyroid glands and some reproductive functions, and may be involved in the development of diabetes (Liu et al., 2012). Some chlorinated OPFRs such as TCEP and tris(1,3-dichloro-2-propyl) phosphate (TDClPP) are suspected carcinogens affecting the liver, kidney and testes tissue (EPA, 2015).

The occurrence of OPFRs in natural environments was first reported in the late 1970s (Saeger et al., 1979; Sheldon and Hites, 1978). Since then OPFRs have been detected in numerous environmental samples such as in air, wastewater effluent, household dust, sediment, and biota

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(Brandsma et al., 2015; Giulivo et al., 2017; Kademoglou et al., 2017; Kim et al., 2017; Aznar-Alemany et al., 2018; Herrero et al., 2018). However, their occurrence in the marine environment is not widely reported.

In recent years, the scientific community has become aware of the problem of marine litter, and specifically of plastics. The physical damages caused by the presence of plastics have been reported for different marine organisms (de Stephanis et al., 2013). However, chemical damage due to the presence of additives in plastics has not been studied as much. For instance, mussels exposed to microplastics contaminated with polyaromatic hydrocarbons, showed bioaccumulation of these chemicals in both digestive gland and gills (Avio et al., 2015). The purpose of this study was to determine the bioaccumulation of OPFRs in common dolphin tissues from the Alboran Sea. This species was chosen as a case study due to its high trophic level in the food web and then acting as sentinels of the marine environment.

2. Materials and methods

2.1. Standards and reagents

Sixteen OPFRs were included in our analytical work. Analytical standards were obtained from different companies: tris(2-butoxyethyl) phosphate (TBOEP), TCEP, tris(2-chloroisopropyl) phosphate (TClPP), trihexyl phosphate (THP) and tris(2-ethylhexyl)phosphate (TEHP) from Santa Cruz Biotechnology (Santa Cruz, CA, USA); tetrakis(2-chlorethyl) dichloroisopentyl-diphosphate (V6), 2-ethylhexyldiphenyl phosphate (EHDPP), isodecyldiphenyl phosphate (IDPP) and tris(tribromoneopentyl) phosphate (TBNPP) from AccuStandard (New Haven, CT, USA); diphenyl cresyl phosphate (DCP), TNBP, TPHP, triphenylphosphine oxide (TPPO) and TDClPP from Sigma-Aldrich (St. Louis, MO, USA); tricresyl phosphate (TMCP) from Dr. Ehrenstorfer (Augsburg, Germany); and, tris(isopropyl-phenyl) phosphate (IPPP) from Chiron (Trondheim, Norway). Labeled standards used for quantification were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada) (d_{15}-TDClPP, d_{27}-TNBP, d_{12}-TCEP and $^{13}C_2$-TBOEP) and from Cambridge Isotope Laboratories Inc. (Andover, MA, USA) (d_{15}-TPHP).

C18 cartridges were obtained from Biotage (Uppsala, Sweden) and basic alumina cartridges were purchased from Interchim (Montluçon, France). Acetonitrile and hexane solvents for organic trace analysis were purchased from J.T. Baker (Center Valley, PA, USA). Methanol and water solvent for trace analysis as well as ammonium acetate and formic acid were obtained from Merck (Darmstadt, Germany).

2.2. Sample collection

Eleven common dolphins (*Delphinus delphis*) were found stranded in the coast of the Alboran Sea in the autonomous community of Andalusia (Spain) from 2004 to 2010. The Alboran Sea connects the Mediterranean Sea with the Atlantic Ocean and provides an important corridor for migratory species. In fact, it presents one of the highest densities of cetacean populations in the Mediterranean Sea (Cañadas et al., 2005, 2014). Different kinds of tissue were removed in situ, preserved in aluminum foil and transported to the laboratory where they were frozen. The available samples were 43: 9 blubbers, 10 muscles, 9 livers, 10 kidneys and 5 brains (Table 1).

2.3. Sample preparation

Frozen samples were lyophilized, homogenized and stored at − 20 °C until analysis. 0.25 g of dry weight (dw) were spiked with labeled standards of d_{12}-TCEP, d_{15}- TDClPP, d_{27}-TNBP, d_{15}-TPhP and $^{13}C_2$-TBOEP as internal standards. Samples were kept overnight to equilibrate prior to the extraction with 15 mL of acetone:hexane (1:1) using an ultrasound system. The extraction was carried out twice, and both extracts were combined in a vial. The 30 mL extract was dried under a purified nitrogen stream, and then it was reconstituted with 60 mL of acetonitrile. Extracts were then passed through a tandem of SPE cartridges of 5 g of basic alumina and 2 g of C18, previously conditioned with 20 mL of acetonitrile. OPFRs were eluted with additional 60 mL of acetonitrile. The collected extract was evaporated under a purified nitrogen stream. Finally the sample was reconstituted to 200 µL with methanol.

Table 1
Summary of collected samples.

Individual	Tissues				
	Blubber	Brain	Kidney	Liver	Muscle
A	X	X	X	X	X
B	X	X	X	X	X
C	X		X	X	X
D			X		X
E	X		X	X	X
F	X	X	X	X	X
G	X	X	X	X	X
H	X		X	X	X
I	X		X	X	X
J			X	X	X
K	X	X			
Total	9	5	10	9	10

Lipid weight (lw) was determined as follows: one gram of sample was extracted using the same methodology described above. The solvent was evaporated using a nitrogen stream and after that dried in an oven at 100 °C. The lipid weight was then determined gravimetrically.

2.4. Instrumental analysis

Instrumental analysis was performed by LC, using a Symbiosis™ Pico (SP104.002, Spark, Holland), connected in series with a 4000 QTRAP Hybrid Triple Quadrupole - Linear Ion Trap-MS equipped with a Turbo Ion Spray source (Applied Biosystems-Sciex, Foster City, CA, USA). Target compounds were separated on a Purospher Star RP-18 endcapped column (125 mm × 2.0 mm, particle size 5 µm) with a C18 guard column (4 × 2.0 mm), both supplied by Merck (Darmstadt, Germany). The optimized separation conditions were as follows: solvent (A) water (0,1% formic acid) and (B) methanol (10 mM ammonium acetate) at a flow rate of 0.25 mL/min. The gradient elution was: 50% (B) for initial and hold for 1 min; 80% (B) at 3 min and hold for 1 min; 90% (B) at 9 min and hold for 8 min; 100% (B) at 22 min and hold for 9 min 50% (B) at 32 min and hold for 5 min to return to initial mode. The total chromatographic time was 37 min. The sample injection was 10 µL (Santín et al., 2016).

2.5. Quality assurance

Throughout all sampling and analysis processes, plastic material was avoided due to potential contamination, as some of our analytes are used as FRs but also as plasticizers. However, OPFR contamination can come from different places that cannot be controlled, like indoor or nitrogen from the evaporator. A realistic goal is to minimize as much as possible the blank signal, i.e., heating all the non-volumetric material at 340 °C and rinsing with an appropriate solvent just before use. In any case, for each batch of samples, a blank was included. Blank levels were subtracted from corresponding samples, only if blank signals do not exceeded 10% of sample signals. If blank values are greater than 10%, then the sample is discarded and re-analysed in another batch of samples.

Instrumental parameters such as recoveries, limits of detection (LODs) and limits of quantification (LOQ) are summarised in Supporting information (Table S1). Recoveries ranged between 48%

Table 2
OPFR median levels obtained in different common dolphin tissues (expressed in ng/g dw and lw) collected from the Alboran Sea.

	Muscle		Liver		Kidney		Blubber		Brain	
	dw	lw	dw	lw	dw	lw	dw	lw	dw	lw
TCEP	0.35	32.1	5.85	61.8	nq	nd	10.4	38.1	0.76	21.6
TClPP	nd	nd	60.2	529	nd	nd	61.5	119	41.2	847
TPPO	5.68	226	nq	nd	3.25	19.5	nd	nd	nd	nd
TBOEP	1.78	66.9	1.91	29.8	2.51	24.5	1.41	3.90	0.71	19.9
TNBP	16.8	1309	24.5	98.4	7.51	73.1	54.4	110	18.3	501
DCP	10.1	293	nd	nd	13.5	132	nd	nd	nd	nd
TPHP	nd	nd	nd	nd	40.1	306	nd	nd	nd	nd
TMCP	6.13	308	5.66	78.5	5.60	46.4	5.58	92.4	4.69	122
EHDPP	9.60	439	nq	nd	0.60	5.44	9.66	29.9	2.77	66.1
IDPP	1.93	86.0	2.28	20.0	21.3	308	13.2	26.5	606	9149
IPPP	34.6	1390	nd	nd	nd	nd	nd	nd	121	3649
THP	0.10	3.74	nd	nd	nq	nd	nd	nd	nd	nd
ΣOPFRs	20.1	645	5.51	66.0	12.8	127	127	267	74.4	1527
Range	1.92–64.7	69.5–2939	1.04–81.1	9.7–712	nd – 57.6	nd-789	12.6–1222	27.2–2450	nd-820	nd-24729
Frequency	100%		100%		90%		100%		60%	

and 102%, always being within the range of acceptability (40–120%) for analytical methods based on quantification by isotopic dilution, with relative standard deviation always below 10%. LODs and LOQs ranged between 0.34 and 11.6 ng/g lw and 1.12–38.8 ng/g lw, respectively, with the exception of TBNPP (37.4 and 125 ng/g lw, respectively) and IPPP (51.6 and 172 ng/g lw, respectively) which had higher limits.

3. Results and discussion

3.1. OPFR levels

OPFRs were detected in all the individuals analysed with total OPFR concentrations up to 24.7 µg/g lw. Twelve out of sixteen tested analytes were detected. Only V6, TDClPP, TBNPP and TEHP were not detected. Table 2 summarises the results obtained in the different individuals as well as in the different tissues, indicating the detection frequency and concentration ranges as well as median values (for individual sample results see Supporting information, Table S2).

Compounds with higher detection frequencies were TBOEP with 77% of positive samples, followed by TNBP, IDPP, EHDPP, TClPP, TCEP and TMCP with 40%, 37%, 30%, 28%, 23% and 21%, respectively. Moreover, TBOEP, TNBP, IDPP and TMCP were detected in all the different tissues. The remaining analytes were detected in less than 10% of the samples. As regards concentration levels, the highest values were obtained for IDPP (mean value of all samples analysed = 516 ng/g lw), followed by TNBP (174 ng/g lw), IPPP (142 ng/g lw) and TClPP (127 ng/g lw). The most contaminated sample was a brain tissue with total OPFR concentration of 24.7 µg/g lw, in which IDPP reached a value of 18.3 µg/g lw.

There is not an easy explanation for the observed OPFR pattern. There are many factors that can affect their presence in dolphin tissues. First of all, the presence in the marine environment that will be directly related with the different uses and applications in the studied area. Then, bioconcentration and biomagnification capacities of the different molecules, as well as metabolic processes, must be taken into account. It is important to note that studied compounds include molecules with a wide range of molecular mass (from 266 g/mol for TNBP to 453 g/mol for IPPP) and log K_{ow} (from 1.44 for TCEP to 9.49 for TEHP) (Wei et al., 2015). Neither molecular mass nor K_{ow}, cannot explain results observed for compounds with highest frequency of detection or with highest levels of contamination. Bioaccumulation factors (BCF) also showed a wide range, between 1.37 for TCEP to 10^6 for TEHP (van der Veen and de Boer, 2012). The BCF generally increases with increasing molecular mass, except for chlorine containing compounds for which no relation can be found between the BCF, the molecular mass or the amount of chlorine in the molecule.

Published works on OPFR levels in biota are scarce, and even more for marine mammals. Papachlimitzou et al. (2015) determined twenty OPFRs in blubber and liver tissue of harbour porpoises stranded or by caught in the UK during 2012. Six OPFRs were detected at maximum concentrations, between 6.7 and 246 ng/g wet weight (ww): triethyl phosphate (TEP), tributyl phosphine oxide (TrBuPO), TPPO, TPHP, TBOEP and EHDPP. Comparison is unfeasible as they provide their data in a wet basis. However, we have made an approximate calculation of our values expressed in ww basis, assuming water content of 80%. Thus, our blubber samples would reach a mean value of 60 ng/g ww with a maximum concentration of 244 ng/g ww, while liver samples, a mean value of 3.1 ng/g ww and a maximum concentration of 16.2 ng/g ww, being concentration levels similar to those reported in UK.

Hallanger et al. (2015) investigate the occurrence of OPFRs in different species (blubber of ringed seals, and plasma of harbour seals and polar bears) within the Svalbard Archipelago (Norway) between 2008 and 2010. Eight of the 14 OPFRs examined were detected: TNBP, TCEP, TCIPP, TDClPP, TEHP, TPHP, EHDPP and TMCP. However, the highest number of compounds was detected in harbour seal plasma, whereas in blubber of ringed seal, only TEHP (up to 1.96 ng/g lw) and EHDPP (up to 9.60 ng/g lw) were detected, with a detection frequency of 10% and 20%, respectively. In our study, TEHP was not detected in any sample, but detection frequency as well as concentration levels for EHDPP were higher (30% and up to 349 ng/g lw). Another study with blubber samples of polar bears and ringed seals from East Greenland (Strobel et al., 2018) showed low concentrations, ranging from nq to 0.57 ng/g ww for TEP, nd to 54 ng/g ww for TDClPP, nd to 0.65 ng/g ww for TEHP, nd to 7.2 ng/g ww for TPHP, nd to 3.10 ng/g ww for TNBP and nd to 2.5 ng/g ww for TBOEP. If we compare with our results, TEP was not included in our analytical work, TDClPP, TEHP and TPHP were not detected in our blubber matrices, but recalculating our TNBP and TBOEP values expressed in ww (between 2.03 and 59.4 and nd-2.52 ng/g ww, respectively), higher values were found in our study. In any case, it is expected that the Mediterranean Sea presented higher levels of contamination than polar areas, as seen for other persistent organic pollutants (POPs) such as PBDEs and dechloranes (Aznar-Alemany et al., 2019).

3.2. Tissue distribution

Concentration levels found in each tissue were transformed from ng/g lw to ng/g dw (Table 2) to make comparisons. Otherwise tissues with very low fat content could be misinterpreted as more contaminated than others. As we can see, the most contaminated tissue regarding total OPFR concentration is blubber, with a median

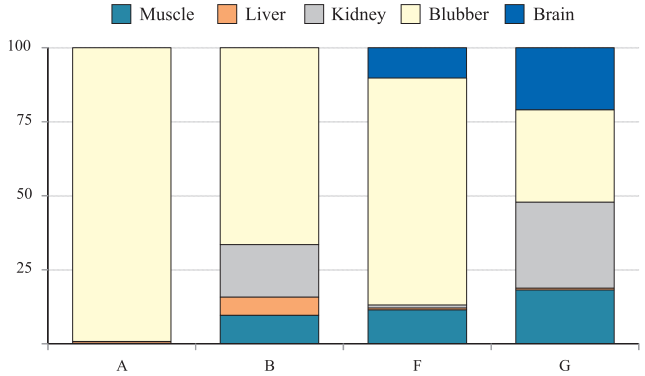

Fig. 1. Percentage contribution of OPFRs (expressed in ng/g dw) in each tissues, for individuals A, B, F and G.

concentration of 127 ng/g dw. This means that these compounds apparently have the same bioaccumulation behaviour as other FRs such as PBDEs. The next most contaminated tissue was brain, followed by muscle, kidney and finally liver. In order to have a more realistic comparison, we selected only the four individuals of which we have available samples of the five different studied tissues. Fig. 1 showed the comparison of the OPFR contribution in each tissue. As we can see, the pattern is dominated by a high contribution in blubber, ranging between 31% and 99%, with a mean value of 68%. The rest of tissues presented mean contributions lower than 12%, up to a minimum of 2% found for the liver. This tissue distribution, where highest levels were found in blubber and lowest concentrations in liver, could indicate the rapid metabolism of these compounds, with OPFRs found in storage fat tissues, but not in high metabolic activity tissues like liver. Similar findings were observed by Greaves and Letcher (2014) when they studied tissue distribution of OPFRs in herring gulls.

It is important to note that 7 different OPFRs (TCEP, TClPP, TBOEP, TNBP, TMCP, EHDPP and IDPP) were found in brain tissue. The existence of the blubber-brain barrier (BBB) should prevent the organic contaminants to enter the brain thanks to an active transport mechanism mediated by the P-glycoprotein (Pardridge, 2005). Several factors, such as molecular weight, lipid solubility, geometry, halogenation degree, or polarity, are key factors to determine the BBB permeation capacity of a compound (Grumetto et al., 2014). However, our data demonstrated that some OPFRs are able to surpass this BBB and reach the brain. This is highly relevant for compounds with potential neurological toxicity such as TCEP and TNBP (Meeker and Stapleton, 2010). Moreover, it seems that these OPFRs have a high potential to cross the BBB, because their levels (normalised in lw) were higher in brain than in blubber. Fig. 2 showed the comparison between OPFR levels found in brain and blubber samples corresponding to the same individual. We have only taken into account those individuals in which we have detected OPFRs in both tissues. As we can see, for all the seven OPFRs, concentrations in brain were always higher than in blubber, showing more affinity for the brain tissue. The same behaviour was found for BDE-153 and hexabromobenzene (Barón et al., 2015). In contrast, levels of other halogenated contaminants were higher in blubber than in brain samples (Corsolini et al., 2014). In fact, more than 90% of the total POP burden in cetaceans is concentrated in blubber due to its high lipid content (Yordy et al., 2010).

3.3. OPFRs vs PBDEs

Samples of blubber and brain included in our study were previously also analysed for determining their content on PBDEs and emerging HFRs (Barón et al., 2015). PBDEs levels in blubber ranged from 93.3 to 2045 ng/g lw, with a mean value of 1001 ng/g lw, whereas brain levels ranged from 6.87 to 791 ng/g lw, with a mean value of 205 ng/g lw. These values increased slightly if we also take into account the contamination by emerging HFRs, with mean values of 1092 and 316 ng/g lw for blubber and brain, respectively. Comparing with our OPFR data, mean level in blubber was slightly lower (633 ng/g lw), whereas mean value in brain was higher (1093 ng/g lw) (Fig. 3); however differences were not significant ($t = 1.262$, $df = 17$, $p > 0.1$, and $t = 1.047$, $df = 13$, $p > 0.1$ for blubber and brain, respectively).

If we take into account that OPFRs represent around 15% by volume of the FR total global production, whereas HFRs represent around 30% by volume, the environmental occurrence of HFRs should be something higher. Moreover, higher bioaccumulation potential of HFRs versus OPFRs has been previously described (Giulivo et al., 2017), as well as limited OPFR biomagnification through food web (Hallanger et al., 2015) probably due to biotransformation processes (Strobel et al., 2018). All these data suggested that HFR levels in dolphins, with a high trophic level, should be higher than those of OPFRs. Thus, the similarity on the range of concentrations for both groups of pollutants could indicate an additional OPFR source of pollution in addition to their use as FRs.

OPFRs are also used as plasticizers, antifoaming agents and performance additives in consumer products. Precisely, its use as plasticizers as well as the large amount of marine plastic debris could contribute to the OPFR levels found in dolphin tissues. The Mediterranean Sea, a semi-enclosed sea with an intensive use of plastic, was modelled to be a potentially important accumulation zone of plastic debris (Lebreton et al., 2012). Cózar et al. (2015) found that the total load of floating plastic debris in the Mediterranean is comparable to that in the accumulation zone of the five subtropical gyres, and it can be considered as an additional great accumulation zone of floating plastic debris (average plastic concentration of 423 g/Km^2 or 243,853 items/Km^2). If we focus on the Alboran sea, it should be noted that greenhouse cultivation has spread rapidly over the last years, where the mild winter temperatures allow the production of low-cost vegetables all year round. In western Almeria approximately 25,902 ha of crops were ground under plastics in the 2005 season (Sanjuan, 2007). Greenhouses use many plastic materials with different utilities. And it has been observed that many of these plastics end up floating on the marine coast. Precisely, two out of the four compounds with higher concentration levels in our dolphin samples, corresponded to compounds used only as plasticizers (TNBP and IPPP), while the others are used as FR and plasticizers (IDPP and TClPP).

Obviously, some key questions remain to be determined such as to what extent do plastics transfer additives to organisms upon ingestion.

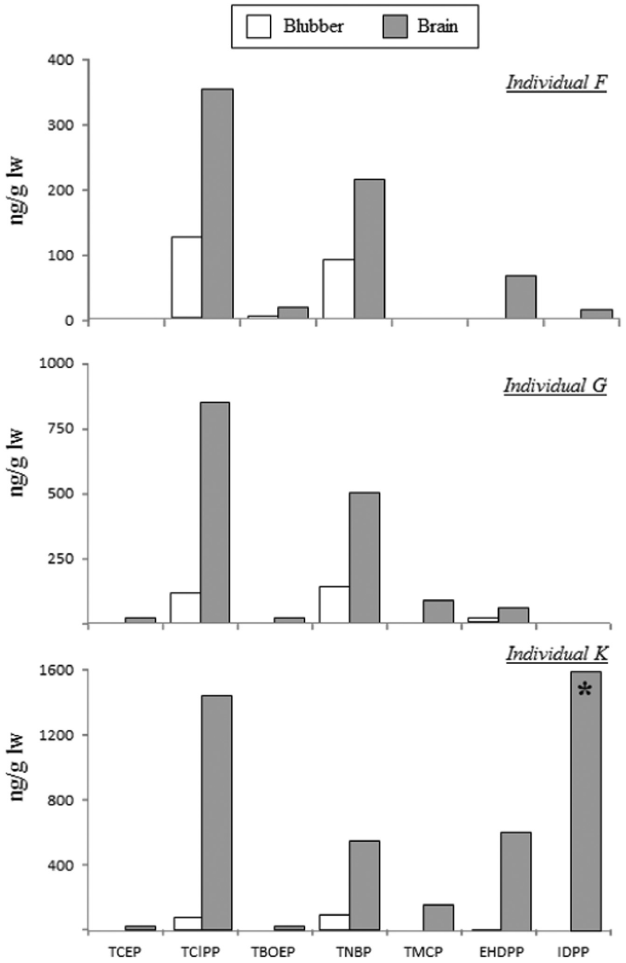

Fig. 2. Concentration levels of OPFRs in blubber and brain of individuals F, G and K. (* the real value is 18,285 ng/g lw; however it is not represented in scale to be able to observe the rest of compounds with very lower levels).

Some studies have examined the potential link between the chemical effects of plastic ingestion and the risk of bioaccumulation across the trophic web. Bakir et al. (2014), simulating physiological conditions in the gut, suggested that chemicals in plastics might be released to organisms after ingestion. Moreover, Tanaka et al. (2013) detected higher-brominated PBDE congeners (BDE-183 and BDE-209) in oceanic seabirds, which were not present in their natural prey (pelagic fish). The same compounds were present in plastic found in their stomachs, suggesting the transfer of plastic-derived chemicals from ingested plastics to the tissues of marine-based organisms. Similarly, phthalate concentrations in birds have been correlated with numbers of pieces of plastic ingested by birds (Hardesty et al., 2015). In another study, a significant correlation has been demonstrated among different phthalate esters present in samples taken in the same area of microplastics, plankton and bubbler samples of different cetacean species (Baini et al., 2017).

In addition to clarify the extent to which plastics transfer additives to organisms after ingestion, it is also necessary to clarify the origin of these additives accumulated in tissues of marine organisms. We must take into account also the contributions of water pollution and/or food chain, and to assess which is the main source for each of the different additives present in plastics. Future investigation is strongly recommended in this sense.

4. Conclusions

This study shows for the first time the OPFR accumulation in marine mammals, with a 100% detection frequency and total OPFR concentrations up to 24.7 μg/g lw. Moreover, new data regarding the OPFR

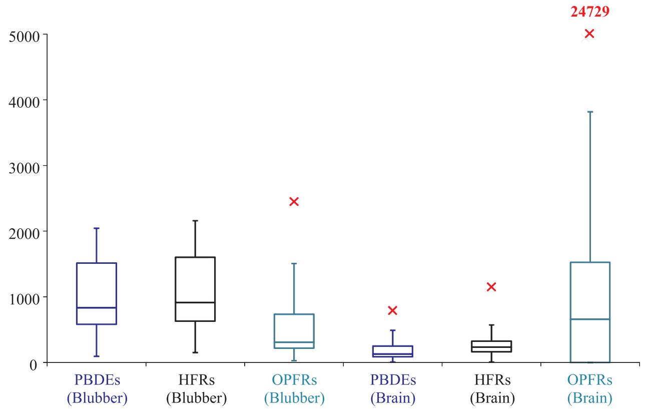

Fig. 3. Box plot of concentration levels (expressed in ng/g lw) by families of FRs and tissues (24,729 ng/g lw, the outlayer value in OPFRs in brain, it's not in scale).

distribution between different tissues were presented. Tissue distribution showed the highest levels in blubber and lowest concentrations in liver, indicating OPFR storage in fat tissues, but not in high metabolic activity tissues like liver. Moreover, seven OPFRs show the capacity to cross the BBB, deserving a special interest those compounds with potential neurological toxicity such as TCEP and TNBP. In addition, OPFRs presented a high potential to cross the BBB, because their levels were higher in brain than in blubber. These data express the need for further study of the neurotoxic properties of these products, and the permeation mechanisms that allow these compounds to surpass the BBB.

OPFR levels were not significant different from HFR concentrations found in the same individuals. Considering that production volume of OPFRs destined for FR purposes is approximately half that of HFRs, and that bioaccumulation and biomagnification capacities are lower for OPFRs, the similarity in dolphin levels would indicate an additional source of contamination of OPFRs in addition to their use as FR. In this sense and taking into account that OPFRs are also used as plasticizers, it is necessary to carry out studies investigating and evaluating the impact of marine plastic debris on different marine organisms, such other cetacean species.

Finally, more studies are needed to assess whether the presence of OPFRs in marine organisms can be proposed as an indicator of plastic exposure. Thus, we would be able to propose a new methodological approach for the assessment of plastic litter in the seas.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envres.2019.02.027.

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“Halogenated and organophosphorus flame retardants in cetaceans from the southwestern Indian Ocean”

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Halogenated and organophosphorus flame retardants in cetaceans from the southwestern Indian Ocean

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HIGHLIGHTS

- PBDE levels in South African dolphins were as high as in more industrialised areas.
- HBCD, DBDPE, PBEB and HBB were rarely detected or at very low concentrations.
- Dec 602 was the only quantifiable dechlorane at 232 ± 549 ng g^{-1} lw.
- OPFR levels were one or two orders of magnitude higher than PBDE levels.
- Natural MeO-PBDEs, analogous to man-made PBDEs, showed outstanding levels.

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ABSTRACT

PBDEs, HBCD, DBDPE, PBEB and HBB, dechloranes and OPFRs, as well as natural MeO-PBDEs were monitored in muscle tissue of three dolphin species from the southwestern Indian Ocean (*Delphinus delphis*, *Sousa plumbea* and *Tursiops aduncus*) collected between 2012 and 2015. The mean PBDE concentration was 416 ± 333 ng g^{-1} lw. BDE-47 was found in all samples and was almost half the total PBDE contamination. BDE-209, BDE-100 and BDE-99 were present in ≥85% of the samples. HBCD was detected in just two samples at 20 and 330 ng g^{-1} lw. PBEB and HBB were not detected, while DBDPE was in all samples but always below its limit of quantification. Dec 602 was the only quantifiable dechlorane at 232 ± 549 ng g^{-1} lw. Mean OPFR concentration was 10452 ± 11301 ng g^{-1} lw. TBOEP was found in all samples making up most of the total OPFR contamination. MeO-PBDEs were detected in all samples at 114 ± 137 ng g^{-1} lw. Data on flame retardants in biota and environmental samples from the southwestern Indian Ocean are scarce and, as a result, comparisons are difficult. However, data from other marine predators in the region, such as penguins, suggest that further studies are needed to determine if these concentrations are the consequence of a high local contamination or widespread thoughout the Indian Ocean.

1. Introduction

Flame retardants (FRs) are compounds applied to all kind of materials, such as household appliances, office electronics, textiles and furniture, to prevent them from ignition. Polybrominated diphenyl ethers (PBDEs) are the most used FRs in a great variety of indoor and outdoor products (Alaee et al., 2003). Penta-BDE, Octa-BDE and Deca-BDE are PBDEs commercial mixtures produced at different levels of bromination. PBDEs leach out of materials since they are not covalently bonded with the polymers, but simply blended with them (Alaee et al., 2003). Hence, PBDEs have been found in all kinds of environmental matrices (Harner et al., 2006; Guerra et al., 2010; Sánchez-Avila et al., 2011; Gorga et al., 2013) and biological matrices (Norén and Meironyté, 2000; Lacorte et al., 2010; Guerra et al., 2012). PBDEs persist in the environment,

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bioaccumulate through the food chain and show toxicity on hormonal regulation, neuronal, thyroid and liver activity (Branchi et al., 2003; Mikula and Svobodova, 2006; Costa and Giordano, 2011).

The effects of FRs on human health and the environment have been a growing concern through time. In 2009, Penta-BDE and Octa-BDE were added to the Stockholm Convention on Persistent Organic Pollutants (POPs) (SC, 2008). There were a few exceptions for specific uses, and North America and Europe decided to reduce their presence in formulations and committed to stop producing them by 2013 (Schecter et al., 2010; EPA, 2014). Appart from their persistence, bioaccumulation and adverse effects, POPs also have potential for long-range transport. POPs are often halogenated and have high lipid solubility, leading to their bioaccumulation in fatty tissues. They are also semi-volatile, which promotes air transportation before deposition far from their sources. The use of Penta-BDE, Octa-BDE and Deca-BDE has also been restricted or banned by several European directives and regulations (REACH, 2006; HSEEE, 2011; PSWP, 2013). After ten years included in the REACH regulation, Deca-BDE was also added to the Stockholm Convention in 2017.

Previously, in 2013 hexabromocyclododecane (HBCD), a FR already included in the REACH regulation, had also been added to the Stockholm Convention (REACH, 2006; SC, 2008). Like PBDEs, HBCD is likely to leach out of the materials and can be transported long distances (Alaee et al., 2003; de Wit et al., 2006). HBCD is very toxic to aquatic organisms and neuroendocrine and developmental toxicity has been observed in humans (SC, 2008). It has been detected in environmental and biotic samples (Eljarrat et al., 2004, 2009).

Novel FRs act as substitutes for the banned compounds due to their health and environmental concerns (Betts, 2008). Some of them are decabromodiphenyl ethane (DBDPE), pentabromoethylbenzene (PBEB) and hexabromobenzene (HBB). DBDPE is the marketed alternative to Deca-BDE as their structures are similar; therefore their properties are also expected to be (Hardy et al., 2002). With a production peak in the 1970s, PBEB persists in environmental matrices and bioaccumulates (Covaci et al., 2011). Japan and China are the main producers of HBB (Cruz et al., 2015). Additionally, Dechlorane Plus (DP) and dechloranes 602, 603 and 604 (Dec 602, Dec 603, Dec 604) are chlorinated alternatives to mirex, which was banned in the United States of America due to its toxicity. Research groups around the world are currently studying novel FRs to assess their behaviour and occurrence in the environment. Brominated novel FRs have been found in environmental matrices and biota (Zhu et al., 2014; Barón et al., 2015b; Aznar-Alemany et al., 2017; Giulivo et al., 2017) and so have dechloranes (Hong et al., 2010; Torre et al., 2010; Guerra et al., 2011; Houde et al., 2014).

A growing alternative to halogenated flame retardants (HFRs), are organophosphorus flame retardants (OPFRs). They comprised 20% of the amount of FRs used in 2006 in Europe —doubling the amount of brominated FRs used that year— and the ban on PBDEs increased their popularity (Van der Veen and de Boer, 2012). OPFRs are also released from materials and access environmental matrices through washout, infiltration, deposition, etc. (Andresen et al., 2004; Schreder and La Guardia, 2014). Moreover, OPFRs are used as plasticisers that can leak from the tones of plastic that reach seas and oceans. Their presence has been reported in sediments, water and fish (Chung and Ding, 2009; Gao et al., 2014; Giulivo et al., 2016). OPFRs show toxic effects on the reproductive and endocrine systems, as well as systemic and carcinogenic effects (Van der Veen and de Boer, 2012; Hou et al., 2016).

Finally, methoxylated PBDEs (MeO-PBDEs) are natural analogs to PBDEs that are synthesized by some marine sponges, algae and their associated cyanobacteria (Malmvarn et al., 2008). They have been found in cetaceans and seafood (Losada et al., 2009; Alonso et al., 2014; Aznar-Alemany et al., 2017). MeO-PBDEs can be detected in marine mammals at similar levels to manufactured halogenated organic compounds (Vetter et al., 2002; Vetter, 2006).

The present article assesses the occurrence of the aforementioned compounds in three species of dolphin from the Indian Ocean; the long-beaked common dolphin (*Delphinus delphis*), Indian Ocean humpback dolphin (*Sousa plumbea*) and the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*). This study also provides data about FRs in marine mammals from a region with little data published about these contaminants in environmental samples and little to none in biota.

2. Materials and methods

2.1. Sampling

Thirteen muscle samples of three species of dolphin were collected from individuals accidently caught in shark nets of KwaZulu-Natal (east-coast South Africa), in the Indian Ocean (Fig. 1), between 2012 and 2015. The samples included two individuals of long-beaked common dolphin, five individuals of Indian Ocean humpback dolphin and six individuals of Indo-Pacific bottlenose dolphin. For brevity purposes in this article, the species will be referred to as simply common dolphin, humpback dolphin and bottlenose dolphin. The samples contained individuals of different age groups. Age groups were assigned according to the size of the dolphins (Cockcroft and Ross, 1990; Best, 2007; Plön et al., 2015). Samples were freeze-dried prior to shipping to the analytical laboratory. Lipid content referenced to dry weight (dw) was between 0.65 and 11.7%. See Table 1 for details.

2.2. Standards and reagents

Native and ^{13}C-labelled standard mixtures of PBDEs (BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183 and BDE-209); *syn*-DP, *anti*-DP and ^{13}C-*syn*-DP; α-, β- and γ-HBCD and their deuterated congeners were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Dec 602 (95%), Dec 603 (98%) and Dec 604 (98%) were purchased from Toronto Research Chemical Inc. (Toronto, ON, Canada). HBB, DBDPE, PBEB and the standard mixture of MeO-PBDEs (5-MeO-BDE-47, 6-MeO-BDE-47, 4′-MeO-BDE-49, 2′-MeO-BDE-68, 5′-MeO-BDE-99, 5′-MeO-BDE-100, 4′-MeO-BDE-101 and 4′-MeO-BDE-103) were bought from Wellington Laboratories Inc. (Guelph, ON, Canada). Tris(2-butoxyethyl) phosphate (TBOEP), tris(chloroethyl) phosphate (TCEP), tris(chloroisopropyl) phosphate (TCIPP), trihexyl phosphate (THP) and tris(2-ethylhexyl) phosphate (TEHP) were purchased from Santa Cruz Biotechnology (SantaCruz, CA, USA). Isodecyldiphenyl phosphate (IDPP) and 2-ethylhexyldiphenyl phosphate (EHDPP) were purchased from AccuStandard (New Haven, CT, USA). Diphenylcresyl phosphate (DCP), tri-*n*-butyl phosphate (TNBP), triphenyl phosphate (TPHP), triphenylphosphine oxide (TPPO) and tris(1,3-dichloro-2-propyl) phosphate (TDCPP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tricresyl phosphate (TMCP) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Isopropyl phenyl phosphate (IPPP) was purchased from Chiron (Trondheim, Norway). Labelled d_{15}-TDCPP, d_{27}-TNBP, d_{12}-TCEP and $^{13}C_2$-TBOEP were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). Labelled d_{15}-TPHP was obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Dichloromethane (DCM), methanol and sulphuric acid were purchased from Merck (Darmstadt, Germany). Acetone and hexane for organic trace analysis were purchased from J.T. Baker (Center Valley, PA, USA).

Fig. 1. Sample location for the present study and other studies discussed.

Table 1
Sampling data.

species	gender	size (cm)	age group	year	fat (%) from dry weight
Long-beaked common dolphin (*Delphinus delphis*)	female	222	adult	2012	1.04
	male	239	adult	2012	2.19
Indian Ocean humpback dolphin (*Sousa plumbea*)	female	125	new-born	2015	4.53
	male	183	juvenile	2014	1.60
		214	juvenile	2014	1.68
		245	adult	2013	0.74
		249	adult	2015	11.7
Indo-Pacific bottlenose dolphin (*Tursiops aduncus*)	female	146	calf	2013	8.97
		154	juvenile	2014	8.50
		205	juvenile	2014	1.48
	male	180	juvenile	2014	0.65
		224	juvenile	2014	2.26
		248	adult	2014	1.74

2.3. Sample preparation

The extraction of OPFRs was carried out by ultrasound assisted extraction according to an existing method (Giulivo et al., 2016). Freeze-dried muscle tissue (0.5 g dw) was extracted twice by sonication with hexane:acetone (1:1 v/v). The combined extract was reconstituted in 5 ml of hexane:methanol (1:3 v/v). The solution was centrifuged and an aliquot of 200 μl was used for the instrumental analysis. Labelled OPFR standards were added prior to analysis by turbulent flow chromatography-(TFC)-LC-MS/MS.

For all the other FRs, sample extraction was carried out with a different existing method (de la Cal et al., 2003; Labandeira et al., 2007). Freeze-dried sample (1.5 g dw) was spiked with the ^{13}C-labelled standards. Pressurized liquid extraction (PLE) was used with a mixture of hexane:DCM (1:1 v/v). Afterwards, the lipid content was determined gravimetrically and the extract was redissolved in hexane and treated with concentrated sulphuric acid to remove the fat. Then, the organic phase was cleaned by solid phase extraction (SPE) using Al–N cartridges (5 g) and eluted with hexane:DCM (1:2 v/v). The extract was reconstituted with 40 μl of toluene prior to the instrumental analysis.

2.4. Instrumental analysis

For OPFRs, online sample purification and analysis was performed with a Thermo Scientific TurboFlow™ system (Giulivo et al., 2016). CycloneTM-P (0.5 × 50 mm) and C18-XL (0.5 × 50 mm) columns were used in combination for purification. Chromatographic separation was achieved with an analytical column Purosphere Star RP-18 (125 mm × 0.2 mm). Mobile phase was a gradient of water (0.1% formic acid) and methanol (ammonium acetate) at 0.75 ml min^{-1}. Spectrometric analysis was performed with a triple quadrupole with a heated-electrospray ionization source. LC flow rate was 5 μl min^{-1}, ion transfer tube temperature was 320 °C and vaporizer temperature was 50 °C. For all compounds, selective reaction monitoring (SRM) mode was used with two transitions monitored for each one. Recoveries for individual compounds ranged 47–98% and RSDs were 2.4–16%. LOQs and LODs were, respectively, 0.97–24.8 ng g^{-1} lipid weight (lw) and 0.19–19.3 ng g^{-1} lw.

PBDEs, MeO-PBDEs, HBB, DBDPE and PBEB were analysed with an Agilent 7890A gas chromatograph coupled to an Agilent 7000B triple quadrupole mass spectrometer (Eljarrat et al., 2002, 2007). Chromatographic separation was carried out with a DB-5ms column (15 m × 0.25 mm × 0.1 μm of film thickness). For the spectrometric determination (Barón et al., 2014), electronic ionization at 300 °C was used, with helium as carrier gas. BDE-209 and DBDPE were analysed with the same chromatographic conditions and the same column as PBDEs using an Agilent 5975A mass spectrometer because of better sensibility. For the spectrometric determination (Eljarrat et al., 2004), negative chemical ionization at 250 °C was used. For the analysis of dechloranes (Barón et al., 2012), the same column and negative chemical ionization at 175 °C was used, with methane as ionization gas and helium as carrier gas. SRM mode was used for all compounds, except for BDE-209 and DBDPE, analysed using selected ion monitoring (SIM). Recoveries for individual compounds ranged from 51 to 99% and RSDs were 1.1–22%. LOQs and LODs were, respectively, 7.7 pg g^{-1} lw to 35.4 ng g^{-1} lw and 2.3 pg g^{-1} lw to 10.6 ng g^{-1} lw.

After analysis of the previous HFRs, extracts were redissolved in methanol and 1 ng of d_{18}-HBCD was added. HBCD was analysed using an Agilent HP 1100 binary pump LC system coupled to a hybrid triple quadrupole/linear ion trap 4000QTRAP (Guerra et al., 2008). Recoveries for α-, β- and γ-HBCD ranged 85–105% and RSDs were 3.1–8.3%. LOQs and LODs were, respectively, 0.4–4.4 ng g^{-1} lw and 0.2–2.0 ng g^{-1} lw.

2.5. Data analysis

A t-test was used for the statistical analysis taking $p < 0.05$ as the criterion for statistical difference. In box plots figures, outliers (×) were calculated as values above Q3 + 1.5 IQR and below Q1 − 1.5 IQR (Q3 = third quartile, IQR = interquartile range, Q1 = first quartile). For all means and standard deviations (SD), concentrations below LOQ were given the LOD value and concentrations below LOD were considered to be 10% of the LOD.

To compare the proportional compositions or *fingerprints* of the different species, sexes, and age classes, we used multivariate ordinations (non-metric multidimensional scaling (NMS), with Sørensen as distance measure) using PC-Ord version 6.20 (MjM Software Design, www.pcord.com). Concentration data were relativized per sample to compensate for the large differences in concentrations. Of the 18 compounds analysed, we eliminated those compounds with less than three quantifiable values; 12 remained. A maximum of six axes were allowed, random starting conditions, 200 maximum number of iterations, 50 runs with real data followed by 50 runs with randomised data, the latter to stress-test the final ordination. Convex hulls were drawn on the same ordination for species, sex and age class. Final stress of less than five gives an excellent representation of the relationships between samples (McCune and Grace, 2002).

3. Results and discussion

FRs concentrations in muscle of Indian Ocean dolphins are summarized in Table 2 (for individual compound results see *Supplementary information*). Results are expressed in ng g^{-1} lw. FRs were detected in all samples. PBDEs and OPFRs were detected in all samples ranging from 33.3 to 1309 ng g^{-1} lw and from 1630 to 31861 ng g^{-1} lw, respectively. HBCD was detected in just two samples at 20 and 330 ng g^{-1} lw. DBDPE was detected in all samples, but always below its LOQ (0.26 ng g^{-1} lw). The novel FRs PBEB and HBB were not detected in any sample (<0.2 ng g^{-1} lw). Dechloranes were present in 84.6% of the samples. However, Dec 602 was the only quantifiable congener at concentrations from 58.0 to 2034 ng g^{-1} lw in 69.2% of the samples. One sample contained Dec 603 and *anti*-DP, and two other samples contained *anti*-DP too, both compounds always below their LOQs (0.02 and 0.01 ng g^{-1} lw). MeO-PBDEs were quantifiable in all the samples with a mean concentration of 114 ± 137 ng g^{-1} lw.

3.1. Legacy HFRs

Mean PBDE concentration was 416 ± 333 ng g^{-1} lw (Table 2). BDE-47 was found in all samples and was almost half the total PBDE contamination (42 ± 16%). BDE-209, BDE-100 and BDE-99 were present in 100, 92 and 85% of the samples, respectively, representing an average 20, 9 and 19% of the PBDE contamination. BDE-183 was not detected.

Published data about FRs in dolphins from the Indian Ocean is very scarce. Comparisons should be made with caution since different factors such as species analysed, sampling period or tissue studied, can affect the levels of contamination. Total PBDEs in blubber of Indo-Pacific humpback dolphin (*Sousa chinensis*, 1992) and Irrawaddy dolphin (*Orcaella brevirostris*, 2000–2001) from India were always below 20 ng g^{-1} lw (Kannan et al., 2005; Kajiwara et al., 2006). One would expect PBDE levels on the Asian coast to be higher than on the African coast, but counterintuitively, these levels were much lower than the ones we obtained in the present study. Similarities could be found, though, as BDE-47, BDE-99 and BDE-100 were detected in all the samples and accounted for 66, 16 and 9% of the total PBDE contamination. BDE-209 was not analysed by Kannan et al. (2005) and Kajiwara et al. (2006). However, our results were lower than those obtained in blubber of Indo-Pacific humpback dolphin (*Sousa chinensis*, 1997–2001) from Hong Kong, which ranged from 280 to 6000 ng g^{-1} lw (Kajiwara et al., 2006). Some studies from the last two decades on different dolphin species showed mean concentrations values between 420 and 880 ng g^{-1} lw in Europe, with the highest values at 2340 ng g^{-1} lw (Barón et al., 2015a, 2015b) and of 166 ng g^{-1} lw in Brasil, ranging from 6 to 1800 ng g^{-1} lw (Alonso et al., 2012). These results seem to be similar to the ones of the present study.

Table 2
Concentration of contaminants (ng g^{-1} lw).

species	gender	age group	∑PBDEs[a]	HBCD	DBDPE[b]	Dec 602[c]	∑MeO-PBDEs[d]	∑OPFRs[e]
Delphinus delphis	female	adult	97.7	< LOD	< LOQ	150	52.4	1961
	male	adult	165	< LOD	< LOQ	< LOQ	112	2030
Sousa plumbea	female	new-born	494	< LOD	< LOQ	58.0	37.6	1630
	male	juvenile	244	< LOD	< LOQ	127	64.0	3965
		juvenile	573	20.7	< LOQ	321	220	25239
		adult	667	< LOD	< LOQ	< LOQ	132	14212
		adult	33.3	< LOD	< LOQ	49.1	23.1	3449
Tursiops aduncus	female	calf	563	< LOD	< LOQ	81.8	76.8	3772
		juvenile	382	330	< LOQ	132	529	3083
		juvenile	264	< LOD	< LOQ	< LOD	65.3	31861
	male	juvenile	1309	< LOD	< LOQ	2034	1.50	30595
		juvenile	189	< LOD	< LOQ	< LOD	51.3	7951
		adult	424	< LOD	< LOQ	59.5	123	6131
		LOD	0.04	0.20	0.08	0.02	0.43	0.19
		LOQ	0.12	0.40	0.26	0.07	1.42	1.03
frequency of detection (%)			100	15.4	100	69.2	100	100

[a] BDE-183 was not detected (BDE-183 < 6.24 ng g^{-1} lw).
[b] The other novel brominated FRs were not detected (PBEB, HBB < 0.2 ng g^{-1} lw).
[c] The other dechloranes were mostly not detected (<0.002–0.007 ng g^{-1} lw).
[d] 5-MBDE-47, 4-MBDE-99, 4-MBDE-100, 5-MBDE-99 and 4-MBDE-101 were not detected (<0.43–3.75 ng g^{-1} lw).
[e] THP, TCEP, TCIPP, DCP and IDPP were not detected (<0.88–2.96 ng g^{-1} lw).

Looking at the admittedly limited sample size from South Africa, sewage sludge and effluent samples of a wastewater treatment plant in Cape Town (west South Africa) showed levels of the same sum of congeners of 400 ± 490 ng g^{-1} dw and 5100 ± 6900 ng l^{-1}, respectively, with BDE-47 and BDE-209 as the main contributors (Daso et al., 2012). Sludge from wastewater treatment plants from 26 cities in China were reported to have a mean of 94 ng g^{-1} dw (5.1–1115 ng g^{-1} dw), including five PBDE congeners more (Wang et al., 2007). On the other hand, sediments and leachate from Gauteng (Fig. 1), closer to KwaZulu-Natal, showed maximum PBDE concentrations of 114 ng g^{-1} dw and 3.7 ng l^{-1} (Olukunle et al., 2015). Eggs of African penguin (*Spheniscus demersus*) from Robben Island (Cape Town, n = 10) collected during the same period as the present samples had a mean PBDE concentration (including BDE-209) of 15 ng g^{-1} lw (between 1.8 and 120 ng g^{-1} lw), and eggs from Bird Island (Port Elizabeth, n = 10) had a mean (including BDE-209) of 3.8 ng g^{-1} lw (ranging between 3.5 and 7.2 ng g^{-1} lw) (Bouwman et al., 2015). Both dolphins and penguins are predators consuming similar prey, such as sardine and squid. Penguins are, however, more restricted in their feeding ranges than dolphins. The ‘fingerprints’ of the two breeding populations of penguin (Robben Island and Bird Island) based on POPs showed distinct differences. The overlap of the dolphin convex hulls (section 3.5 of this article) probably indicates much greater feeding ranges.

In short, a lack of published information on PBDEs in South Africa makes it hard to comment on the high levels in the present study. However, some of the available data on environmental samples show high levels, suggesting high environmental contamination in the area, which would explain the results from the present study.

Possible explanations for high levels of HFRs would be atmospheric transportation, air-water exchange, deposition and exposure (direct or indirect) to plastics (Bouwman et al., 2015). Asia has been reported as a source of marine pollution for HFRs with a stress on BDE-209 and the shift to alternative FRs (Möller et al., 2011). Another reason could be the city of Durban as a local source. PBDE levels in dust collected in Durban in 2012 and 2013 reached concentrations of 27.5 ng g^{-1} in e-waste recycling sites samples and of 11.8 ng g^{-1} in automobile samples (Abafe and Martincigh, 2015, 2016).

HBCD was detected in just two juvenile dolphins; α-HBCD in a female humpback dolphin (20.7 ng g^{-1} lw) and β- and γ-HBCD in a male bottlenose dolphin (173 and 158 ng g^{-1} lw). HBCD was quantifiable in only one African penguin egg from Robben Island (0.15 ng g^{-1} lw) and in seven eggs from Bird Island (mean 0.12 ng g^{-1} lw, ranging between 0.10 and 0.13 ng g^{-1} lw) (Bouwman et al., 2015). The difference of three orders of magnitude in concentrations between dolphins and penguins suggests trophic differences, differences in prey size with larger prey presumably having larger amounts of accumulated pollutants or that dolphins (mammal) and penguins (bird) accumulate and metabolize POPs differently.

3.2. Emerging HFRs

PBEB and HBB were not detected, while DBDPE was in all samples but always below its LOQ, 0.26 ng g^{-1} lw. As DBDPE is the marketed alternative to Deca-BDE (which consists in 97–98% of BDE-209), it seems logical to detect the presence of DBDPE in modern samples where BDE-209 accounts for 20% of the total PBDEs. This percentage of BDE-209 is quite high for biota samples, suggesting a previous abundant usage of Deca-BDE, which would have been replaced by the usage of DBDPE, hence its presence in these samples.

Dec 602 was the only quantifiable dechlorane at 232 ± 549 ng g^{-1} lw. Dec 602 has a higher bioaccumulation potential than other dechloranes (Shen et al., 2011). Dec 603 was detected in one adult common dolphin and anti-DP was found in both adult common dolphins and an adult humpback dolphin, both compounds below their LOQ. It must be noted, however, that other species of dolphins from the Mediterranean Sea had total dechloranes levels in blubber below 60 ng g^{-1} lw (Barón et al., 2015a).

3.3. Organophosphorus flame retardants

The mean OPFR concentration was 10452 ± 11301 ng g^{-1} lw. TBOEP was found in all samples making 82 ± 28% of the total OPFR contamination. TPPO and TDCPP were detected in 53.8% of the samples. TDCPP was always below 1.03 ng g^{-1} lw, while TPPO mean concentration was 27.6 ± 45.9 ng g^{-1} lw. IPPP, TEHP, TNBP, EHDPP and TMCP were detected in one to three samples at concentrations up to 1749 ng g^{-1} lw; except for IPPP and TEHP in a juvenile male bottlenose dolphin with 17591 and 5581 ng g^{-1} lw, respectively. Published works on OPFR levels in biota are scarce, and even more for marine mammals. A study from 2012 on blubber and liver of harbour porpoises from the United Kingdom also found TBOEP to be present at higher concentrations than the other OPFRs included in our study (Papachlimitzou et al., 2015). Their highest concentration was 205 ng g^{-1} ww in blubber. Assuming an approximate 80% of water content and a 5% of lipid from dw (based on data from 42 Mediterranean dolphins from a different study), that would translate to 20500 ng g^{-1} lw, in line with our highest values 23489 and 31841 ng g^{-1} lw. A study including seals (blubber and plasma), Artic foxes (liver) and polar bears (plasma) from Norway showed concentrations below 10 ng g^{-1} ww for most OPFRs (Hallanger et al., 2015). Conversely, TCIPP reached 372 ng g^{-1} ww in seal plasma and TBOEP reached 590 ng g^{-1} ww in seal blubber and 2198 ng g^{-1} ww in fox liver. In our study, TCIPP was never detected and the conversion of the maximum TBOEP values in seal blubber and fox liver would clearly exceed our results. On the other hand, a different study on polar bear fat and ringed seal blubber from East Greenland found concentrations of individual OPFRs mostly below limits of quantification (Strobel et al., 2018). For both species TPHP showed the highest levels (up to 7.2 ng g^{-1} ww), while TPHP was only detected in two of our samples. Our results are in the same order of magnitude as other studies in mammals, except for the study from East Greenland, where the impact of human activity might be weaker.

A recent work (Sala et al., 2019) carried out by our group reported OPFRs in common dolphin (*Delphinus delphis*) samples from the Alboran Sea, western Mediterranean Sea (2004–2010). OPFR concentrations in muscle tissue ranged between 69.5 and 2939 ng g^{-1} lw, with a mean value of 994 ng g^{-1} lw. These values were one order of magnitude lower than those obtained in the present study (mean value of 10452 ng g^{-1} lw).

It is important to note that OPFRs were at concentrations clearly higher than dechloranes (t-test $t = 3.07$, df = 22, $p < 0.05$) and even PBDEs ($t = 2.97$, df = 22, $p < 0.05$). Higher bioaccumulation potential of HFRs versus OPFRs has been described (Giulivo et al., 2017), as well as limited OPFR biomagnification through the food web (Hallanger et al., 2015) probably due to biotransformation processes (Strobel et al., 2018). This would suggest that OPFR levels in dolphins, belonging to a high trophic level, should be lower than those of HFRs. Therefore, the present results might indicate a greater input of OPFRs to the environment, arguably as a result of OPFRs not being used only as FRs but also as plasticisers. This draws the attention to further monitoring of these compounds.

The present study was conducted with individuals that were accidently caught in shark nets. Thus, the number of samples, the species, the sex and the maturity stage of the dolphins were impossible to control. POPs levels should show a characteristic trend during the lifetime of the dolphins, with males showing increasing levels with age, as POPs are bioaccumulated. Females should show a decrease after giving birth due to the mother-to-calf transfer.

With the present samples, no differences among age groups could be assessed statistically for any family of compounds, except for OPFRs (Fig. 2). Female adult dolphins were excluded in case they had reproduced and transferred their contamination to their calves.

As seen in Fig. 2, OPFRs showed an increase between calves and juveniles ($t = 2.56$, df = 6, $p < 0.05$), but there was no increase from juveniles to adults ($t = 1.73$, df = 8, $p > 0.1$). Some congeners (e.g. TBOEP, TCIPP, TDCPP, TPHP) can be metabolised and they would not follow the POP increasing pattern with age, but their levels could stabilise or decrease in male individuals (Van den Eede et al., 2013; Greaves et al., 2016). TBOEP accounted for 82 ± 28% of the total OPFR contamination, greatly influencing this pattern. The increase in OPFR concentrations from calves to juveniles that did not occur from juveniles to adults hints at the metabolic transformation of this congener after reaching maturity.

3.4. Natural compounds

MeO-PBDEs showed in all the samples with a mean concentration of 114 ± 137 ng g^{-1} lw. These levels are similar to those found in other dolphin species from Tanzania (65 ± 43 ng g^{-1} lw), in the same region of the Indian Ocean (Rayne et al., 2004; Mwevura et al., 2010). There are reports of MeO-PBDEs in delphinids from more distant regions, like the Mediterranean Sea (Barón et al., 2015a, 2015b) or Brazil (Alonso et al., 2012). In agreement with the most occurring congeners in those studies, 6-MBDE-47 and 2-MBDE-68 were detected in 92.3% of our samples. Additionally, 5-MBDE-100 was found in 69.2% of them. Levels reported in blubber of the Mediterranean delphinids were up to 2506 ng g^{-1} lw and in liver of Franciscana dolphin from Brazil, mostly up to 3939 ng g^{-1} lw. Contrary to what happened with the relative contribution of the congeners, the levels of contamination in those more distant regions are quite different. MeO-PBDEs were present in the dolphins from this study in the same order of magnitude as the FRs, including the analogous PBDEs. Sharing levels with a POP of similar structure and properties is a good reason to consider these natural compounds in routine monitoring. While compounds of natural origin cannot be controlled by regulation, it is important to take into account that MeO-PBDEs' effects on marine biota could add to PBDEs' effects. High levels of the natural compounds could make a case for a tougher legislation on the analogous anthropogenic contaminants in order to keep the balance.

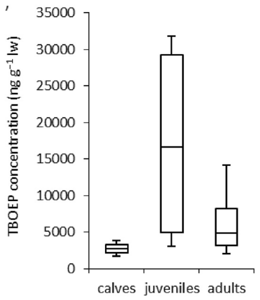

Fig. 2. Box plots of TBOEP concentrations for all maturity stages. Adults include only males.

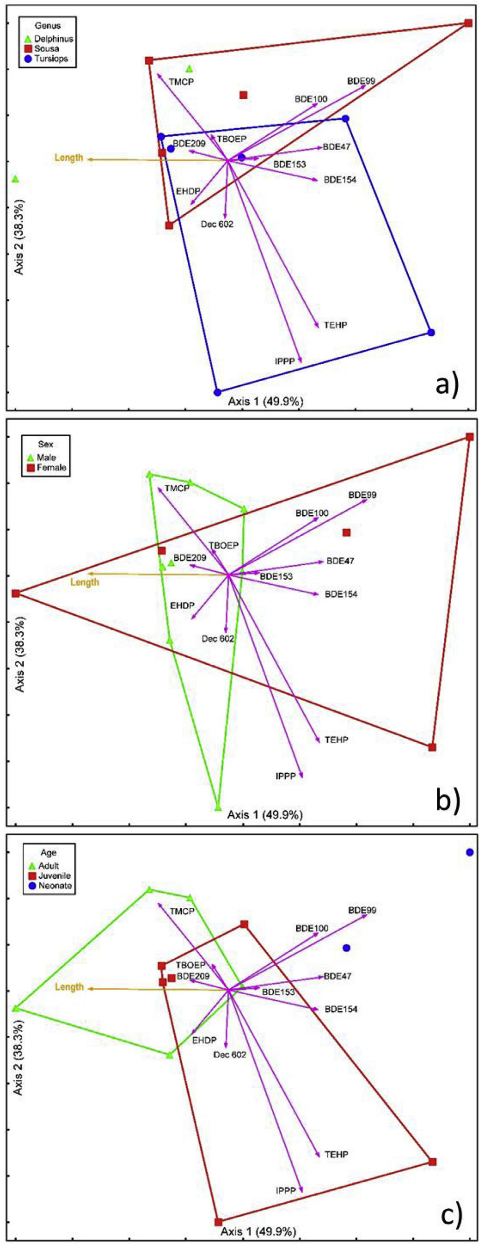

Fig. 3. NMS ordination for species, sex and age group.

3.5. Ordination of proportional composition

To compare the proportional compositions or *fingerprints* of the different species, sexes, and age classes, we used multivariate ordinations (see 2.5. Data analysis). Although the number of individuals was limited, this analysis serves as a preliminary assessment of similarities or differences among the mentioned groups.

The NMS ordination required three axes, with axis 1 explaining 59.9% of the variation, axis 2 explained 49.9% and axis 3, 38.3%, for a cumulative explanation of 88.2%. Axes 1 and 2 are represented in Fig. 3. The closer each sample point is to another, the more commonality in relative composition. The final instability was 0.647, indicating an excellent representation. The final stress was 0.00000, reached after 77 iterations, with no improvement in stress versus iteration after 48 iterations. The convex hulls of the species overlapped (common dolphin had only two samples, so no convex hull drawn) indicating no differences in proportional composition of the compounds in the samples, relative to species (Fig. 3a). Similarly, there was no differences between sexes (Fig. 3b), nor for the age classes, except for the two neonate samples (Fig. 3c).

The low stress value indicates that the ordination is an excellent representation of the relative positions or *fingerprints* of each sample (Fig. 3). Neither species (Fig. 3a) nor sex (Fig. 3b) showed any differences, as the convex hulls overlapped. This may indicate similar exposures to the compounds, possibly through shared or similar prey. The two neonates (the blue dots in Fig. 3c) did show some separation from the older age classes, strongly associated with higher relative proportions of BDE-99 and BDE-100.

IPPP, TEHP and Dec 602 ordinated perpendicularly to PBDEs and opposite to TBOEP. This suggests three different sources of the compounds from the prey that is consumed.

4. Synthesis and conclusions

PBDEs and dechloranes (only Dec 602) were found in dolphins from the eastern coast of South Africa at levels as high as in more industrialised areas, such as Europe. BDE-47, as well as BDE-209, BDE-100 and BDE-99, dominated the PBDE profiles.

DBDPE was the only brominated alternative FR detected in the samples, but always below its LOQ. HBCD was detected in just two samples. Mean OPFR concentration was 10452 ± 11301 ng g^{-1} lw, with TBOEP as the main contributor. These concentrations were one or two orders of magnitude higher than PBDE concentrations. This highlights the need for further monitoring of OPFRs. Additionally, adult dolphins might be metabolising TBOEP successfully as the bioaccumulation trend from calves to juveniles seemed to stop in the adult age.

Finally, the natural MeO-PBDEs showed outstanding levels for a compound that is analogue to a family of POPs.

Reports about FRs in biota and environmental samples from South Africa and the Indian Ocean are scarce. This region should be further studied to see if these levels are the consequence of a high local contamination, if they are an isolated case influenced by the small number of samples or the variations between individuals, if bioaccumulation occurs, and if these compounds contribute towards population declines.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2019.03.165.

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PUBLICACIÓ CIENTÍFICA #2

Supplementary information

“Halogenated and organophosphorus flame retardants in cetaceans from the southwestern Indian Ocean”

Aznar-Alemany, Ò., **Sala, B.**, Plön, S., Bouwman, H., Barceló, D.,
Eljarrat, E.

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Table S1. Concentrations of legacy FRs (ng g^{-1} lw)

species	sex	age	length (cm)	% fat dw	BDE-28	BDE-47	BDE-100	BDE-99	BDE-154	BDE-153	BDE-183	BDE-209	α-HBCD	β-HBCD	γ-HBCD
Delphinus capensis	female	adult	222	1.0	nd	nq	7.9	17.0	nd	nd	nd	72.1	nd	nd	nd
Delphinus capensis	male	adult	239	2.2	nd	67.1	nq	23.4	26.5	12.8	nd	34.1	nd	nd	nd
Sousa plumbea	female	newborn	125	4.5	nd	151	56.2	269	nd	nd	nd	17.4	nd	nd	nd
Sousa plumbea	male	juvenile	183	1.6	nd	147	42.8	nd	nq	nd	nd	52.8	nd	nd	nd
Sousa plumbea	male	juvenile	214	1.7	7.7	268	72.8	167	nd	nd	nd	58.0	20.7	nd	nd
Sousa plumbea	male	adult	245	0.7	nd	253	76.0	57.1	64.6	128	nd	87.1	nd	nd	nd
Sousa plumbea	male	adult	249	12	nd	14.1	5.0	nq	nq	nd	nd	12.7	nd	nd	nd
Tursiops aduncus	female	calf	146	9.0	6.2	299	47.6	85.8	97.7	0.6	nd	25.4	nd	nd	nd
Tursiops aduncus	female	juvenile	154	8.5	nd	239	nd	nd	85.2	28.9	nd	27.9	nd	173	158
Tursiops aduncus	female	juvenile	205	1.5	nd	114	44.1	57.7	nd	nd	nd	48.1	nd	nd	nd
Tursiops aduncus	male	juvenile	180	0.7	nd	457	178	540	nd	nd	nd	133	nd	nd	nd
Tursiops aduncus	male	juvenile	224	2.3	nq	97.6	nq	54.1	nq	nq	nd	35.3	nd	nd	nd
Tursiops aduncus	male	adult	248	1.7	nq	203	nq	72.6	82.5	nq	nd	64.2	nd	nd	nd
				LOD	0.04	0.05	0.20	0.29	0.43	0.64	6.24	0.16	0.2	2.0	0.2
				LOQ	0.12	0.18	0.67	0.97	1.42	2.13	20.8	0.52	0.4	4.4	0.4

nd = < LOD, nq = < LOQ

Table S2. Concentrations of emerging HFRs (ng g^{-1} lw)

species	sex	age	length (cm)	% fat dw	PBEB	HBB	DBDPE	Dec 602	Dec 603	Dec 604	*syn*-DP	*anti*-DP
Delphinus capensis	female	adult	222	1.0	nd	nd	nq	150	nq	nd	nd	nq
Delphinus capensis	male	adult	239	2.2	nd	nd	nq	nd	nd	nd	nd	nq
Sousa plumbea	female	newborn	125	4.5	nd	nd	nq	58.0	nd	nd	nd	nd
Sousa plumbea	male	juvenile	183	1.6	nd	nd	nq	127	nd	nd	nd	nd
Sousa plumbea	male	juvenile	214	1.7	nd	nd	nq	321	nd	nd	nd	nd
Sousa plumbea	male	adult	245	0.7	nd	nd	nq	nd	nd	nd	nd	nq
Sousa plumbea	male	adult	249	12	nd	nd	nq	49.1	nd	nd	nd	nd
Tursiops aduncus	female	calf	146	9.0	nd	nd	nq	81.8	nd	nd	nd	nd
Tursiops aduncus	female	juvenile	154	8.5	nd	nd	nq	132	nd	nd	nd	nd
Tursiops aduncus	female	juvenile	205	1.5	nd	nd	nq	nd	nd	nd	nd	nd
Tursiops aduncus	male	juvenile	180	0.7	nd	nd	nq	2034	nd	nd	nd	nd
Tursiops aduncus	male	juvenile	224	2.3	nd	nd	nq	nd	nd	nd	nd	nd
Tursiops aduncus	male	adult	248	1.7	nd	nd	nq	59.5	nd	nd	nd	nd
				LOD	0.18	0.20	0.08	0.02	0.007	0.007	0.006	0.002
				LOQ	0.61	0.67	0.26	0.07	0.024	0.024	0.018	0.008

nd = < LOD, nq = < LOQ

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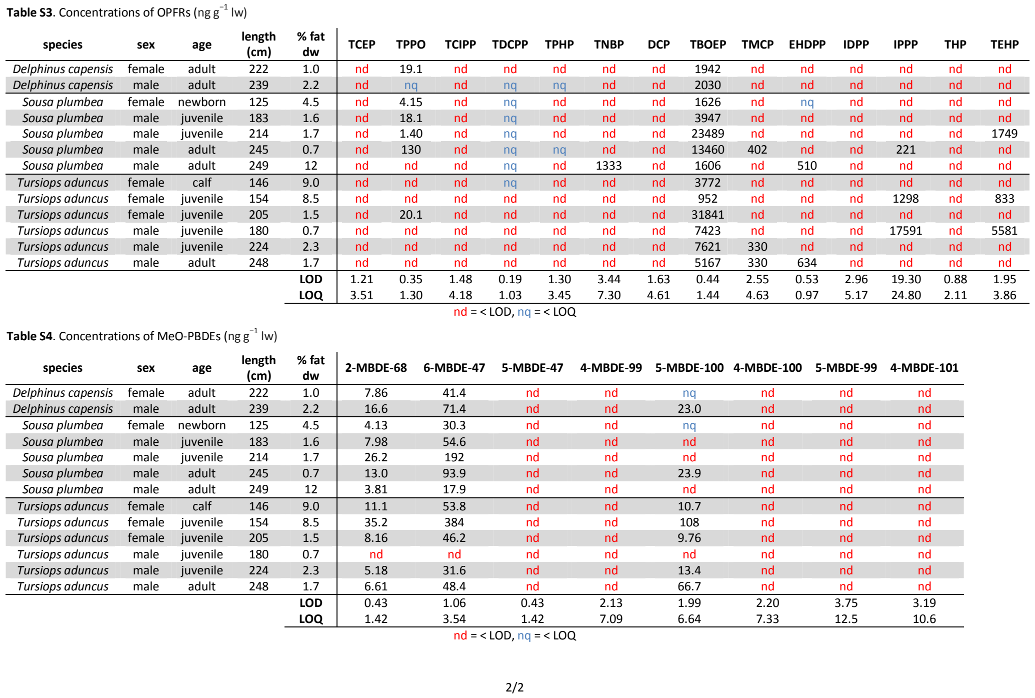

Table S3. Concentrations of OPFRs (ng g^{-1} lw)

species	sex	age	length (cm)	% fat dw	TCEP	TPPO	TCIPP	TDCPP	TPHP	TNBP	DCP	TBOEP	TMCP	EHDPP	IDPP	IPPP	THP	TEHP
Delphinus capensis	female	adult	222	1.0	nd	19.1	nd	nd	nd	nd	nd	1942	nd	nd	nd	nd	nd	nd
Delphinus capensis	male	adult	239	2.2	nd	nq	nd	nq	nq	nd	nd	2030	nd	nd	nd	nd	nd	nd
Sousa plumbea	female	newborn	125	4.5	nd	4.15	nd	nq	nd	nd	nd	1626	nd	nq	nd	nd	nd	nd
Sousa plumbea	male	juvenile	183	1.6	nd	18.1	nd	nq	nd	nd	nd	3947	nd	nd	nd	nd	nd	nd
Sousa plumbea	male	juvenile	214	1.7	nd	1.40	nd	nq	nd	nd	nd	23489	nd	nd	nd	nd	nd	1749
Sousa plumbea	male	adult	245	0.7	nd	130	nd	nq	nq	nd	nd	13460	402	nd	nd	221	nd	nd
Sousa plumbea	male	adult	249	12	nd	nd	nd	nq	nd	1333	nd	1606	nd	510	nd	nd	nd	nd
Tursiops aduncus	female	calf	146	9.0	nd	nd	nd	nq	nd	nd	nd	3772	nd	nd	nd	nd	nd	nd
Tursiops aduncus	female	juvenile	154	8.5	nd	nd	nd	nd	nd	nd	nd	952	nd	nd	nd	1298	nd	833
Tursiops aduncus	female	juvenile	205	1.5	nd	20.1	nd	nd	nd	nd	nd	31841	nd	nd	nd	nd	nd	nd
Tursiops aduncus	male	juvenile	180	0.7	nd	nd	nd	nd	nd	nd	nd	7423	nd	nd	nd	17591	nd	5581
Tursiops aduncus	male	juvenile	224	2.3	nd	nd	nd	nd	nd	nd	nd	7621	330	nd	nd	nd	nd	nd
Tursiops aduncus	male	adult	248	1.7	nd	nd	nd	nd	nd	nd	nd	5167	330	634	nd	nd	nd	nd
				LOD	1.21	0.35	1.48	0.19	1.30	3.44	1.63	0.44	2.55	0.53	2.96	19.30	0.88	1.95
				LOQ	3.51	1.30	4.18	1.03	3.45	7.30	4.61	1.44	4.63	0.97	5.17	24.80	2.11	3.86

nd = < LOD, nq = < LOQ

Table S4. Concentrations of MeO-PBDEs (ng g^{-1} lw)

species	sex	age	length (cm)	% fat dw	2-MBDE-68	6-MBDE-47	5-MBDE-47	4-MBDE-99	5-MBDE-100	4-MBDE-100	5-MBDE-99	4-MBDE-101
Delphinus capensis	female	adult	222	1.0	7.86	41.4	nd	nd	nq	nd	nd	nd
Delphinus capensis	male	adult	239	2.2	16.6	71.4	nd	nd	23.0	nd	nd	nd
Sousa plumbea	female	newborn	125	4.5	4.13	30.3	nd	nd	nq	nd	nd	nd
Sousa plumbea	male	juvenile	183	1.6	7.98	54.6	nd	nd	nd	nd	nd	nd
Sousa plumbea	male	juvenile	214	1.7	26.2	192	nd	nd	nd	nd	nd	nd
Sousa plumbea	male	adult	245	0.7	13.0	93.9	nd	nd	23.9	nd	nd	nd
Sousa plumbea	male	adult	249	12	3.81	17.9	nd	nd	nd	nd	nd	nd
Tursiops aduncus	female	calf	146	9.0	11.1	53.8	nd	nd	10.7	nd	nd	nd
Tursiops aduncus	female	juvenile	154	8.5	35.2	384	nd	nd	108	nd	nd	nd
Tursiops aduncus	female	juvenile	205	1.5	8.16	46.2	nd	nd	9.76	nd	nd	nd
Tursiops aduncus	male	juvenile	180	0.7	nd	nd	nd	nd	nd	nd	nd	nd
Tursiops aduncus	male	juvenile	224	2.3	5.18	31.6	nd	nd	13.4	nd	nd	nd
Tursiops aduncus	male	adult	248	1.7	6.61	48.4	nd	nd	66.7	nd	nd	nd
				LOD	0.43	1.06	0.43	2.13	1.99	2.20	3.75	3.19
				LOQ	1.42	3.54	1.42	7.09	6.64	7.33	12.5	10.6

nd = < LOD, nq = < LOQ

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PUBLICACIÓ CIENTÍFICA #3

“Temporal trends of halogenated and organophosphate contaminants in striped dolphins from the Mediterranean Sea”

Aznar-Alemany, Ò., **Sala, B.**, Jobst, K.J., Reiner, E.J., Borrell, A.,
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Temporal trends of halogenated and organophosphate contaminants in striped dolphins from the Mediterranean Sea

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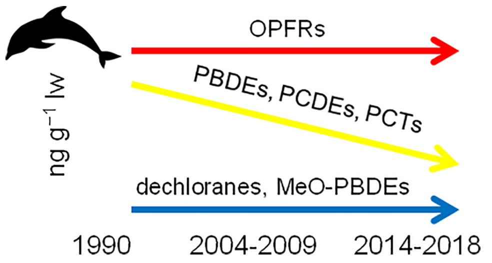
HIGHLIGHTS

- Target and non-target analysis to monitor halogenated/organophosphate pollutants
- Muscles from 42 striped dolphins from three different periods were analysed.
- PCDEs and PCTs were determined for the first time in dolphin samples.
- PBDE, PCDE and PCT declined from 1990 to 2018, and OPFRs had a steady concentration.
- Despite level decrease, these compounds remain a risk to Mediterranean dolphins.

GRAPHICAL ABSTRACT



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ABSTRACT

PBDEs, HBCD, novel DBDPE, PBEB and HBB, dechloranes, OPFRs and natural MeO-PBDEs were monitored in muscle of striped dolphins (*Stenella coeruleoalba*) from the Mediterranean Sea collected in three time periods (1990, 2004–2009 and 2014–2018). PBDEs levels decreased about 60% in under three decades, from 5067 ± 2210 to 2068 ± 2642 ng g^{-1} lw, evidencing the success of their ban. Most PBDEs were found in all the samples, with BDE-47, -99, -154, -100 and -153 as the main contributors. Found in 71.4% of the samples, α-HBCD was stable through time and usually <LOQ. DBDPE concentrations decreased by 89% from 1990 to 2004–2009 and have remained stable since. HBB occurred rarely and decreased by 94% to a current few ng g^{-1} lw. Dec 602 was the main dechlorane with stable concentrations around 1200 ng g^{-1} lw, but a declining trend might have started in the last years. OPFRs concentrations were stable and showed the highest concentrations of all FRs in 2014–2018: 6253 ± 11,293 ng g^{-1} lw. TBOEP and TNBP contributed to most of the OPFR concentration, the former with decreasing levels by 96%. MeO-PBDEs showed mean concentrations between 600 and 700 ng g^{-1} lw in all periods. Non-targeted analysis allowed the identification and semi-quantification of additional chlorinated pollutants, such as polychlorinated terphenyls (PCTs) (levels decreasing by 81% to a current 770 ng g^{-1} lw mean) and polychlorinated diphenyl ethers (PCDEs) (decreasing by 83% to a current 3200 ng g^{-1} lw) in Mediterranean marine mammals for the first time.

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1. Introduction

Nowadays there is an obvious concern about the human impact on the environment, including marine pollution. Regulation such as the Stockholm Convention on Persistent Organic Pollutants (POPs) and European directives have restricted or banned the use of some contaminants of concern. The impact of these regulations should translate into decreasing time trends for the regulated contaminants and alternative compounds should appear. Cetaceans are long-lived, highly mobile top predators that have been used as bioindicators of the environmental pollutant loads and temporal trends because their tissues integrate the environmental characteristics of the water masses in which they live (Borrell and Aguilar, 2007).

During the last decade, polybrominated diphenyl ethers (PBDEs) have been added to the Stockholm Convention and their use has been restricted or banned by several European directives and regulations (REACH, 2006; HSEEE, 2011; PSWP, 2013). PBDEs are flame retardants (FRs), which are applied to all kind of materials to prevent them from ignition. In the past, PBDEs have been the most used FRs (Alaee et al., 2003). They are available as mixtures at different levels of bromination. PBDEs leach out of materials since they are not bonded but simply blended with the polymers (Alaee et al., 2003). Hence, PBDEs have been found in environmental (Guerra et al., 2010; Sánchez-Avila et al., 2011) and biological matrices (Lacorte et al., 2010). PBDEs persist in the environment, bioaccumulate through the food web and show toxicity (Mikula and Svobodova, 2006).

Hexabromocyclododecane (HBCD) is another FR than can leach out of the materials and is toxic to aquatic organisms, albeit less than PBDEs (Alaee et al., 2003; SC, 2008; Lu et al., 2018). It has been detected in environmental and biotic samples (Eljarrat et al., 2004a; Eljarrat et al., 2009) and included in the REACH regulation and the Stockholm Convention.

Substitutes for the banned FRs include decabromodiphenyl ethane (DBDPE), pentabromoethylbenzene (PBEB) and hexabromobenzene (HBB) (Hardy et al., 2002; Covaci et al., 2011; Cruz et al., 2015). Additionally, Dechlorane Plus (DP) and dechloranes 602, 603 and 604 (Dec 602, Dec 603, Dec 604) are chlorinated alternatives to Mirex. These alternative compounds have been also found in environmental matrices and biota (Hong et al., 2010; Guerra et al., 2011; Aznar-Alemany et al., 2017a, 2017b; Giulivo et al., 2017).

A growing alternative to halogenated flame retardants (HFRs) are organophosphorus flame retardants (OPFRs). They represented 20% of all FRs used in Europe in 2006; twice the quantity of brominated FRs (Van der Veen and de Boer, 2012). OPFRs are also released from materials and access environmental matrices through washout, infiltration, deposition, etc. (Schreder and La Guardia, 2014). Moreover, OPFRs are used as plasticisers, so they leak from the tons of plastic that reach the seas and oceans. Their presence has been reported in sediments, water, fish and marine mammals (Giulivo et al., 2016; Sala et al., 2019; Garcia-Garin et al., 2020) and their toxicity has been reported (Van der Veen and de Boer, 2012).

Methoxylated PBDEs (MeO-PBDEs) are natural analogues to PBDEs that are synthesized by some marine organisms (Malmvarn et al., 2008). They have been found in cetaceans and seafood (Alonso et al., 2014; Aznar-Alemany et al., 2017a, 2017b). Being natural compounds, their presence cannot be controlled. However, their structural similitude to PBDEs might confer them similar negative effects that would add to those of the anthropogenic FRs. As they can be analysed using the same method, it makes sense to collect the data.

This study assesses the occurrence of the aforementioned FRs in striped dolphins (*Stenella coeruleoalba*) from the Mediterranean Sea through three time periods. A non-targeted analysis for halogenated contaminants was also performed in search for compounds that occurred frequently and at apparent great concentrations and that might be currently overlooked. Some suspects were confirmed and semi-quantified.

2. Materials and methods

2.1. Sampling

Samples of dorsal muscle were collected from 42 striped dolphins that were found stranded on the Catalan coast, in the North Western Mediterranean Sea. The samples were collected in three different periods, including 15 samples from 1990, 15 samples from 2004 to 2009 and 12 samples from 2014 to 2018. All individuals were adult males ranging from 164 to 224 cm of body length (Calzada et al., 1997). Samples were kept frozen at −20 °C and were freeze-dried prior to analysis. Lipid content referenced to wet weight (ww) was mostly between 0.25 and 1.21%, with three samples between 3.98 and 6.51% (see Table 1 for details). The nature of these samples makes it impossible to design a sample plan choosing all the characteristics of the samples. The co-authors from the Universitat de Barcelona collect the dolphin samples as part of a monitoring programme. Having adult male and three different time periods as key requirements, samples were selected when there were at least 5 g available.

2.2. Standards and reagents

Native and ^{13}C-labelled standard mixtures of PBDEs (both including BDE-28, -47, -99, -100, -153, -154, -183 and -209); *syn*-DP, *anti*-DP and ^{13}C-*syn*-DP; α-, β- and γ-HBCD and their deuterated congeners were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Dec 602 (95%), Dec 603 (98%) and Dec 604 (98%) were purchased from Toronto Research Chemical Inc. (Toronto, ON, Canada). HBB, DBDPE, PBEB and the standard mixture of MeO-PBDEs (5-MeO-BDE-47, 6-MeO-BDE-47, 4′-MeO-BDE-49, 2′-MeO-BDE-68, 5′-MeO-BDE-99, 5′-MeO-BDE-100, 4′-MeO-BDE-101 and 4′-MeO-BDE-103) were bought from Wellington Laboratories Inc. (Guelph, ON, Canada). Tris (2-butoxyethyl) phosphate (TBOEP), tris(chloroethyl) phosphate (TCEP), tris(chloroisopropyl) phosphate (TCIPP), trihexyl phosphate (THP) and tris(2-ethylhexyl) phosphate (TEHP) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Isodecyldiphenyl phosphate (IDPP) and 2-ethylhexyldiphenyl phosphate (EHDPP) were purchased from AccuStandard (New Haven, CT, USA). Diphenylcresyl phosphate (DCP), tri-*n*-butyl phosphate (TNBP), triphenyl phosphate (TPHP), triphenylphosphine oxide (TPPO) and tris(1,3-dichloro-2-propyl) phosphate (TDCPP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tricresyl phosphate (TMCP) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Isopropyl phenyl phosphate (IPPP) was purchased from Chiron (Trondheim, Norway). Labelled d$_{15}$-TDCPP, d$_{27}$-TNBP, d$_{12}$-TCEP and ^{13}C$_2$-TBOEP were purchased from Wellington Lab-oratories Inc. (Guelph, ON, Canada). Labelled d$_{15}$-TPHP was obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). ^{13}C-Labelled standards for the non-targeted analysis (PCB-101, PCB-118, PCB-153, BDE-47, BDE-99 and BTBPE) were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). To confirm the suspected compounds, 3,3′,4,4′-tetrachlorodiphenyl ether (CDE-77), 2,2′,4,4′,5-pentachlorodiphenyl ether (CDE-99), 2,2′,4,4′5,5′-hexachlorodiphenyl ether (CDE-153) and polychlorinated terphenyl mixtures Aroclor 5432 and Aroclor 5460 were obtained from AccuStandard. Dichloromethane (DCM), methanol and sulphuric acid were purchased from Merck (Darmstadt, Germany). Acetone and hexane for organic trace analysis were purchased from J.T. Baker (Center Valley, PA, USA).

Table 1
Sampling information.

Year	*n*	Size (cm)	Water content (%)	Lipids (referred to ww) (%)
1990	15	181–216	65.1–73.8	0.25–1.18
2004–2009	15	166–224	66.3–72.1	0.26–1.21, *3.98*, *6.51*
2014–2018	12	164–210	71.0–73.6	0.19–1.07, *5.88*

2.3. Sample preparation

For HFRs and MeO-PBDEs, sample extraction was carried out according to previous works (de la Cal et al., 2003; Labandeira et al., 2007). Freeze-dried sample (1.5 g dry weight (dw)) was spiked with the labelled standards. Pressurized liquid extraction (PLE) was used with hexane:dichloromethane (1:1). Lipid content was determined gravimetrically weighting the dried extract every 30 min until steady weight. The extract underwent an acid attack to remove the fat. The organic phase was cleaned by solid phase extraction (SPE) using Al–N cartridges (5 g) and eluted with hexane:dichloromethane (1:2). The extract was reconstituted with 40 μL of toluene.

The extraction of OPFRs was carried out by ultrasound assisted extraction according to an existing method (Giulivo et al., 2016). Freeze-dried sample (0.5 g dw) was extracted by sonication with hexane:acetone (1:1). The extract was reconstituted in 5 mL of hexane: methanol (1:3) and centrifuged and an aliquot of 200 μL was used for the instrumental analysis. Purification was performed on-line at the beginning of the instrumental analysis. Labelled OPFRs standards were added prior to analysis by turbulent flow chromatography (TFC) coupled to LC-MS-MS.

For the non-target analysis, a softer version of the method for HFRs was used, avoiding the acid attack. Freeze-dried sample (1 g dw) was spiked with the labelled standards. The extraction was carried out with a Buchi SpeedExtractor E-914/E-916 using hexane:dichloromethane (1:1) in two cycles of 20 min at 100 °C. The extracts were re-dissolved in hexane before undergoing a SPE using acidic alumina cartridges and eluting with hexane:dichloromethane (1:2). Extracts were evaporated to incipient dryness and reconstituted with toluene to a final volume of 600 μL prior to the instrumental analysis.

2.4. Instrumental analysis

HFRs and MeO-PBDEs (except for HBCD) were analysed by GC–MS-MS using an Agilent 7890A gas chromatograph coupled to an Agilent 7000B triple quadrupole mass spectrometer and a DB-5 ms column. Brominated compounds were analysed using electronic ionization (EI). The instrumental conditions and the spectrometric determination are described in previous publications (Eljarrat et al., 2007; Barón et al., 2014). Due to their low sensitivity with GC-EI-MS-MS, BDE-209 and DBDPE were analysed by GC–MS with the same chromatographic conditions in an Agilent 5975A mass spectrometer using negative ion chemical ionization (NICI) (Eljarrat et al., 2004b). The analysis of dechloranes was performed by GC-NICI-MS-MS as described in a previous article (Barón et al., 2012).

After analysis of the previous HFRs, extracts were re-dissolved in methanol. HBCD was analysed using an Agilent HP 1100 binary pump LC system coupled to a hybrid triple quadrupole/linear ion trap 4000QTRAP (Guerra et al., 2008).

For OPFRs, online sample purification and analysis was performed with a Thermo Scientific TurboFlow™ system (Giulivo et al., 2016). CycloneTM-P and C18-XL columns were used in combination for purification. Chromatographic separation was achieved with an analytical column Purosphere Star RP-18, starting at a flow rate of 0.25 mL/min and performed under gradient elution conditions using 0.1% formic acid in water (A) and ammonium acetate in methanol (B) as mobile phases. Initially, solutions A and B were used at 50%, then the gradient increased up to 80% of B, and finally up to 100% of B. Spectrometric analysis was performed with a triple quadrupole with a heated-electrospray ionization source.

2.5. Quality assurance

Throughout all sampling and analysis processes, plastic material was avoided due to potential contamination, as some of our analytes such as OPFRs are used as FRs but also as plasticizers. However, OPFR contamination can come from different places that cannot be controlled, like indoor or nitrogen from the evaporator. A realistic goal is to minimize as much as possible the blank signal, i.e., heating all the non-volumetric material at 340 °C and rinsing with an appropriate solvent just before use. In any case, for each batch of samples, a blank was included. Blank levels were subtracted from corresponding samples, only if blank signals do not exceeded 10% of sample signals. If blank values are greater than 10%, then the sample is discarded and re-analysed in another batch of samples.

Quality parameters such as recoveries, limits of detection (LODs) and limits of quantification (LOQ) are summarized in *Supporting information* (Table S1).

2.6. Non-targeted analysis

GC × GC-HRQTOFMS analysis was performed with an Agilent 7890B gas chromatograph (Agilent Technologies, CA, USA) fitted with a Zoex ZX2 GC × GC thermal modulator (Zoex, TX, USA) and interfaced to a Waters Xevo G2-XS quadrupole time-of-flight mass spectrometer (Waters Corporation, Wilmslow, UK). The first-dimension column was an Rtx-5 (60 m × 0.25 mm × 0.25 μm film) followed by a Restek Siltek deactivated guard column (1 m × 0.15 mm) in the modulator loop. The second-dimension column was an Rtx-17 SIL (1 m × 0.15 mm × 0.15 μm film) and was placed in a secondary oven. The secondary column was then connected to a Custom MXT tubing (sulfinert treated, 0.8 m × 0.18 mm), which was inserted into the transfer line. Helium was used as the carrier gas and the flow was 1.5 mL min^{-1}. The injector temperature was 280 °C. The initial oven temperature was held at 40 °C for 1 min, and then ramped at 3 °C min^{-1} to 310 °C and held for 10 min. The secondary oven was at 10 °C offset, relative to the primary oven. The modulator was at a 15 °C offset relative to the primary oven and the modulation period was of 4 s. The transfer line was at 340 °C. The cone gas flow rate was 100 L hr^{-1} and the auxiliary gas flow was 150 L hr^{-1}. The ion source temperature was 150 °C. The acquisition range was 50–1200 amu and the acquisition rate was 30 Hz. The mass spectrometer was operated at a resolving power of >20,000 (FWHM). Data processing was conducted using GC Image HRMS R2.5 (Zoex). Confirmation of compounds identified by non-targeted analysis was performed in the single dimension GC mode using a 15 m db-5ht (0.25 mm × 0.1 um) column and the GC conditions described by Di Lorenzo et al. (2019).

A composite mass spectrum was generated by combining all mass spectra recorded during the chromatographic separation. Mass measurements, recorded using the IUPAC mass scale were converted to the H/Cl mass scale, which is defined by the substitution of a hydrogen atom by a chlorine atom being equal to 34.000 Da (Taguchi et al., 2010). H/Cl mass is calculated by applying a 34/33.96102 factor to IUPAC mass. The H/Cl mass defect plot enables the efficient and comprehensive characterisation of halogenated compounds (Jobst et al., 2013) and helped guide the extraction of mass chromatograms from the GC × GC contour plot (not shown).

The ions with the exact masses of the mass defect plot were extracted from the corresponding TIC of the specific samples. The mass spectra were interpreted and elemental compositions were assigned using the following criteria: C 0–100; N 0–5, O 0–5, S 0–5, Cl 0–12, Br 0–12. The measured and theoretical masses and isotope ratios were within 5 ppm and ± 15% respectively.

2.7. Statistical analysis

As mentioned in the sampling section, the nature of the samples resulted in a small sample size and high standard deviations. While simple data observation would be appropriate, some statistical analysis was performed as a contrast. Since the results of both methods agreed, it was decided to add the statistical information in the results, albeit with this reminder of the limitations of the data set.

Table 2
Contaminant concentrations ($ng\,g^{-1}$ lw) and frequencies of detection (FD) by periods.

Period		∑PBDEs	DBDPE	HBB	PBEB	α-HBCD	∑Dechloranes	∑OPFRs	∑MeO-PBDEs	∑PCDEs	∑PCTs
1990	Median[a]	4551	<LOQ	56.3	<LOD	<LOD	1050	2810	615	14,500	3600
	Mean[a]	5067	85.6	24.1	<LOD	16.3	1227	7288	626	19,000	4000
	SD[a]	2210	99.1	41.4	–	63.0	754	10,021	198	10,000	2300
	FD (%)	100	100	26.7	0.00	60.0	100	100	100	100	100
2004–2009	Median	2142	<LOQ	1.25	<LOD	<LOD	1050	1227	406	4200	1100
	Mean	2722	9.10	4.30	0.37	358	1244	3980	682	7500	1400
	SD	2435	20.3	9.74	0.87	1135	940	8737	894	7800	1300
	FD (%)	100	86.7	20.0	20.0	73.3	100	100	100	100	100
2014–2018	Median	1041	<LOQ	<LOQ	<LOD	<LOD	318	2513	362	670	340
	Mean	2068	27.7	1.46	<LOD	82.5	1245	6253	687	3200	770
	SD	2642	74.0	4.28	–	153	2246	11,293	774	4400	980
	FD (%)	100	75.0	16.7	0.00	83.3	100	100	100	91.7	100

[a] Means, median and SDs are calculated including all samples, <LOQ values as equal to the LOD and <LOD values as 10% of the LOD.

The data were divided into three groups according to the sampling period. The results for each pollutant family and the sampling period were checked first to detect possible outliers. Extreme outliers, which differ in at least 3 IQR units beyond Q1 and Q3, were excluded from the statistical analysis. The normality within the groups and the homoscedasticity between the groups were evaluated using the Kolmogorov-Smirnov and Levene tests, respectively. All the compounds showed a normal distribution in each group of samples and only three of them showed homoscedasticity between the groups.

For each family of compounds separately, differences between sampling periods were investigated using one-way analysis of variance (ANOVA), followed by multiple comparisons based on the Tukey HSD post hoc test for compounds with homogeneity of variances (i.e. PBDEs and HBB and dechloranes) and the Games-Howell post hoc test for compounds with variance heterogeneity (i.e. OPFRs, PCDEs, PCTs, MeO-PBDEs). For those families of compounds that showed a normal distribution for the total number of samples (i.e. PBDEs, HBB, dechloranes, PCDEs, PCTs and MeO-PBDEs), the temporal trends of their concentrations were also examined by linear regression analysis with the year as independent variable.

For means, standard deviations (SD) and box plots, concentrations below LOQ were given the LOD value and concentrations below LOD were considered to be 10% of the LOD. All statistical calculations were carried out using the statistical package IBM SPSS 20.

3. Results and discussion

Concentrations of FRs in muscle of striped dolphins are summarized in Table 2 (see Supporting Information for results of compound groups and individual compounds in each sample (Tables: S2, groups, S3, legacy FRs; S4, emerging HFRs; S5, OPFRs, and S6, MeO-PBDEs)). Due to the intrinsic variability of results from individuals that could differ in even a couple of decades of age, the standard deviation within a period is comparable to the contamination level. This variability of the data might obscure mild time trends. However, observing a difference between periods despite this variability evidences that a change has clearly occurred.

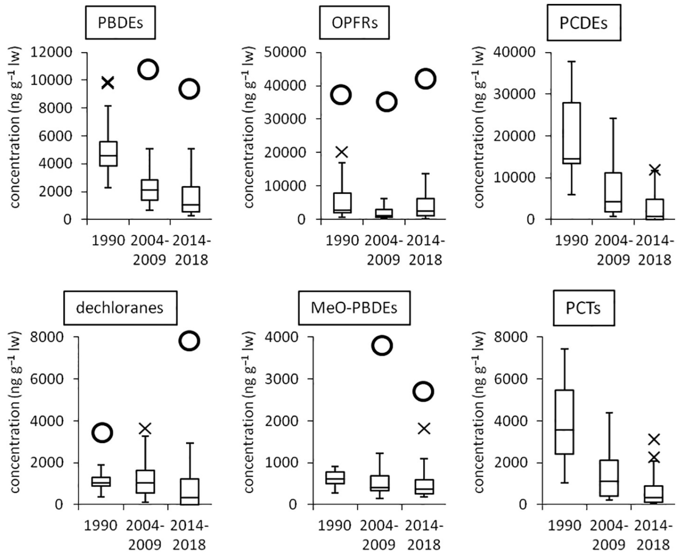

Fig. 1. Temporal trends of contaminants in dolphin muscle showed in the three periods. Two types of outliers are distinguished: mild outliers (X) and extreme outliers (O); the latter were excluded from the statistical analyses.

3.1. Legacy BFRs

PBDE levels decreased from 5067 ± 2210 $ng\,g^{-1}$ lw in 1990 to 2068 ± 2642 $ng\,g^{-1}$ lw in 2004–2009 (Tukey test, $p < 0.001$) and no significant decrease has been observed from 2004–2009 to 2014–2018 (Table 2, Fig. 1). However, it must be taken into account that 1990 and 2004 are separated by 14 years, while 2009 and 2014 are only separated by 5 years. Therefore, assuming a regular decrease in contamination levels as reported in Canada (ECCC, 2016), a statistically significant change in concentrations would be easier to detect between 1990 and 2004–2009 as a bigger time gap would allow for greater differences in concentrations. Being the 2004–2009 and 2014–2018 periods as long as the separation between them, it made sense to analyse the trend along the whole three-decade period through a regression analyses. The result showed a highly decreasing of trend of PBDE concentrations of about 60% from 1990 to 2018. ($R = 708$, $p > 0.001$) (Fig. 1) and it is reasonable to think that the trend continues. The bans in Europe and North America in the early 2000s and the inclusion of PBDEs in the Stockholm Convention in 2011 may account for a decrease in the emission of these pollutants and, therefore, the amount detected in biota. These findings could validate the success of the prohibition.

But what do these levels represent in terms of risk to marine mammals? PBDEs have been related to disruption of thyroid functions, of neurobiological development and to foetal toxicity/teratogenicity in mammals (Alonso et al., 2014). Hall et al. (2003) established the threshold level for PBDEs in blubber (1500 $ng\,g^{-1}$ lw) at which thyroid endocrine disruption in juvenile grey seals was observed. If we take this threshold as valid also for dolphins, we observe that 100% of analysed individuals from 1990 presented levels higher than 1500 $ng\,g^{-1}$ lw, indicating high risk of suffering hyperthyroidism and associated thyrotoxicosis. The percentage drops to 73% of individuals from 2004 to 09. However, and despite the observed PBDE level decrease, and after 15 years of the ban of these pollutants in Europe, currently even 33% of dolphins are at risk of hyperthyroidism.

PBDE time trends keep a similarity with those of polychlorinated biphenyls (PCBs) in the same species and area. PCBs peaked between 1960 and the late 1970s before being prohibited and PCB levels in blubber of Mediterranean striped dolphin showed a marked decline from 1988, but then stabilized from 2001 to 2009, but with concentrations ranging from 7000 to 153,000 $ng\,g^{-1}$ lw (Castrillon et al., 2010).

Regarding PBDE congeners, BDE-28, -47, -99, -100 and -154 were found in all the samples. BDE-153 and BDE-209 had high frequencies of detection (98 and 95%, respectively) and BDE-183 was found in 45% of the samples. BDE-47 was the main contributor to the PBDEs profile (≈40%) followed by BDE-154 (≈20%) and BDE-99 and -100 (≈14% each). BDE-154 is not a typical major contributor to the PBDE profiles. While no conclusive explanation could be found, it has been observed that several higher-brominated PBDEs (including half of the congeners in the Octa-BDE commercial mixture) debrominate preferably into BDE-154, while further debrominations do not clearly favour the other congeners (Zeng et al., 2010). On the other hand, since the concentrations of PBDE congeners did not follow a normal distribution (Shapiro Wilks $p < 0.05$), Spearman's bivariate correlation was calculated. All PBDEs were highly correlated with each other ($p < 0.01$) except the BDE-209, which was not correlated with BDE-28, -100, -154 and -183, but it was with the rest of congeners BDE-99, -153 (both $p < 0.05$) and − 47 ($p < 0.01$). It makes sense that BDE-209 be the less correlated congener as, differently from the other PBDEs, this congener is more commonly associated with sediments than biota.

In 28.6% samples, α-HBCD was quantifiable ranging from 1.99 to 4425 $ng\,g^{-1}$ lw (550 ± 1233 $ng\,g^{-1}$ lw). The number of samples with quantifiable concentrations in each period was 1, 7 and 4, in chronological order. Frequencies of detection in each period were 60.0, 73.3 and 83.3. Several tests were performed including either all samples or all samples except outliers or samples above LOD or samples above LOQ and no variation with time could be seen in any case ($p > 0.1$).

Dolphin blubber from the same and other species sampled from the south of Spain between 2004 and 2012 showed levels of HBCD in the same range as the dolphins from those years here studied and levels of PBDEs in dolphin blubber were in the lower half of our results (Barón et al., 2015a; Barón et al., 2015b). Those two studies included common dolphins (*Delphinus delphis*), bottlenose dolphins (*Tursiops truncatus*), pilot whales (*Globicephala melas*) and one of them also included Risso's dolphins (*Grampus griseus*) and striped dolphins.

3.2. Emerging HFRs

Just as α-HBCD and PBDEs showed different time trends, brominated emerging FRs can also be divided between unchanged and decreased levels. PBEB was detected in only 20% of the samples from 2004 to 2009 within the range of 0.86–3.20 $ng\,g^{-1}$ lw, not far from its LOQ, 0.61 $ng\,g^{-1}$ lw. With PBEB not being detected in 92.9% of the dolphins in the study, the only time trend observed was a constant absence of it. HBB was detected in a reduced and decreasing number of dolphins with time and in decreasing concentrations, 26.7% through 16.7% and 24.1 ± 41.4 $ng\,g^{-1}$ lw through 1.46 ± 4.28 $ng\,g^{-1}$ lw (Table 2). Taking into account only the values above the LOQ, results from the ANOVA showed that the concentrations of HBB decreased from 1990 to 2004–2009 ($p < 0.001$) and then remained steady until 2014–2018 ($p = 0.5$). However, when analysing the trend for the whole period (with year as the independent variable), a significant decrease was observed ($R = 0.942$, $p < 0.001$).

DBDPE showed the same pattern as HBB, albeit with higher frequencies of detection and concentrations: 100% through 75.0% and 85.6 ± 99.1 $ng\,g^{-1}$ lw through 27.7 ± 74.0 $ng\,g^{-1}$ lw (Table 2). With these higher values, the concentrations of DBDPE decreased from 1990 to 2004–2009 ($p < 0.01$) and then remained steady until 2014–2018 ($p = 0.15$).

Concentrations of dechloranes in Mediterranean dolphins also seem to have been steady for three decades ($p > 0.1$ for all Tukey post hoc comparisons between groups) at 1238 ± 1372 $ng\,g^{-1}$ lw (Table 2, Fig. 1). The same regression analyses applied before to PBDEs was applied to dechloranes to provide further insight, showing no trends in the whole period ($R = 146$, $p = 0.37$) (Fig. 1). However, dechlorane levels appear to be lower in 2017–2018 than in the rest of years (Fig. 1). Whether this is the beginning of a decreasing trend or a one-time variation remains to be seen by samples to be collected during the next decade.

Dec 602 was detected in all the samples and accounted for 90.4 to 100% of the total dechlorane contamination (median = 100%). The range of concentrations of Dec 602 for all dolphins in this study is 55.2–7880 $ng\,g^{-1}$ lw, while the range for the other dechloranes goes from not detected to 51.6 $ng\,g^{-1}$ lw. Dec 602 was found in all the samples, Dec 603 and *anti*-DP were detected in 19.5% of the samples and Dec 604 and *syn*-DP in just 4.9% and 7.3% of them, respectively. Therefore, the time trend of dechloranes is namely a time trend for Dec 602. The median of the concentrations for each congener in the few quantifiable samples seem to somewhat decrease. Here they are expressed in $ng\,g^{-1}$ lw and in chronological order. Dec 603: 30.1 to 2.1 to <LOD. Dec 604: 8.2 to <LOD to 0.6. *syn*-DP: 11.6 to 5.0 to <LOD. *anti*-DP: 26.0 to 8.19 to 4.2. On the other hand, it is important to note that these compounds were detected in very few samples and at very low levels compared to Dec 602 in all periods.

Levels of dechloranes in dolphin blubber sampled from the south of Spain between 2004 and 2012 were an order of magnitude below those found in the present study (Barón et al., 2015a; Barón et al., 2015b). Levels of PCBs in biopsy samples have previously been reported to be lower than in stranded dolphins (Jepson et al., 2016). This could explain the difference between our results and the levels found in biopsy samples. The smaller difference between North and South Mediterranean stranded striped dolphins might be related to the geographical habitat. As previously described, northern Spain is more industrialized than southern Spain and thus striped dolphins from the northwestern Mediterranean showed PCB concentrations that were double of those in their conspecific from the Alboran Sea (Marsili et al., 2018).

3.3. OPFRs

OPFRs appeared to have a steady concentration over time of 5811 ± 9831 ng g^{-1} lw (Table 2, Fig. 1). While in 1990 both OPFRs and PBDEs were the major contributors to the FR contamination in these dolphins, the decreasing levels of PBDEs have left OPFRs alone in that position. This makes sense as in 2006 OPFRs already doubled the amount of PBDEs used in Europe and in 2009 the Stockholm Convention started including PBDEs (Van der Veen and de Boer, 2012).

TBOEP was found in all samples from 1990 and 2004–2009 and in 66.7% from 2014 to 2018. TBOEP concentrations decreased from 1990 (2825 ± 3600 ng g^{-1} lw) to 2004–2009 (504 ± 416 ng g^{-1} lw) and 2014–2018 (132 ± 178 ng g^{-1} lw). TNBP and TCEP show steady levels at the orders of magnitude of 10^3 and 10^2 ng g^{-1} lw, respectively. EHDPP and IDPP were found in concentrations over 1000 ng g^{-1} lw in five and three samples, respectively. TDCPP and IPPP in less than 17% of the samples at concentrations below 20 ng g^{-1} lw, mostly below their LOQs. THP was found in one sample from 1990 at 112 ng g^{-1} lw.

Data about OPFRs in dolphins are scarce. There are currently two reports studying common dolphins that were found stranded in south of Spain between 2004 and 2010 (Sala et al., 2019) and three dolphin species from the southwestern Indian Ocean (*Delphinus capensis*, *Sousa plumbea* and *Tursiops aduncus*) accidentally caught in shark nets between 2012 and 2015 (Aznar-Alemany et al., 2019). Like in the present study, the samples were muscle tissue of dead individuals. Levels in the common dolphins ranged from 69.5 to 2939 ng g^{-1} lw, with a mean value of 994 ng g^{-1} lw. Levels in the dolphins from the Indian Ocean ranged from 1630 to 31,861 ng g^{-1} lw, with a mean value of 10,452 ng g^{-1} lw. The levels reported in this article for striped dolphin were slightly higher than the levels in common dolphin, sampled 800 km south from our location. The difference in species and location might account for this. The range of levels in the Indian Ocean reached a higher maximum concentration. Levels of PBDEs in those dolphins were also higher than one would expect and further research is required to assess the reason for that difference. Additionally, and coinciding with the present work, TBOEP presented the highest frequencies of detection in both studies, 77 and 82%, respectively.

3.4. MeO-PBDEs

Finally, one could expect MeO-PBDEs to show stable levels through time as they are natural compounds independent from anthropogenic pressure and legislation. In fact, the ANOVA did not show variation in the three periods ($p > 0.1$, for all Games-Howell post hoc comparisons between groups). Agreeing with previous studies with delphinids from the Mediterranean Sea (Barón et al., 2015a; Barón et al., 2015b), Brazil (Alonso et al., 2012) and South Africa (Aznar-Alemany et al., 2019), the most frequently occurring congeners were 6-MBDE-47 (124–2839 ng g^{-1} lw, in 100% of the samples) and 2-MBDE-68 (23.4–894 ng g^{-1} lw, 95.2%). Additionally, 5-MBDE-100 and 4-MBDE-100 were found in 83.3 and 59.5% of the samples at concentration one or two orders of magnitude lower. Total concentrations of MeO-PBDEs reported in blubber of the Mediterranean delphinids sampled between 2004 and 2012 were in the same range.

3.5. Non-targeted compounds: PCTs and PCDEs

A high-resolution mass spectrum may be visualized by constructing a Kendrick mass defect plot. Visual interrogation of such a plot enables efficient discovery and annotation of (unknown) chemical compounds in complex environmental samples. Kendrick realised that by replacing the International Union of Pure and Applied Chemistry (IUPAC) mass scale (C = 12.000 Da) with the Kendrick scale (CH_2 = 14.000 Da), organic ions belonging to homologous series could readily be identified on the basis of their mass defect (Kendrick, 1963). The conversion is achieved applying a 14/14.01565 factor to IUPAC mass and the utility of this approach is demonstrated by the fact that Kendrick mass defect of methyl, ethyl and propyl naphthalene is 0.9195 Da. The same approach also works well for Cl, Br congeners (Taguchi et al., 2010; Jobst et al., 2013) and CF_2 homologues (Myers et al., 2014) using other non-standard mass scales. The Kendrick mass defect plot represents the Kendrick mass defect of each mass spectral peak (y axis) plotted against the nominal mass (x axis) (Hughey et al., 2001). In this study, the H/Cl mass scale proposed by Taguchi et al. (2010) was employed. Consequently, Br/Cl congener series appear as a band of peaks parallel to the x axis in a specific region of the mass defect plot. Additionally, isotope patterns, like those of bromine and chlorine, are easily recognizable.

Due to the complexity of the GC × GC-HRQTOFMS analysis, five samples of each period were selected for the analysis of non-targeted compounds. Two groups of peaks showing chlorine isotope patterns and separated 34 mass units from each other were present in the mass defect plots of all of the samples (Fig. 2, Table S7).

With $C_{18}H_{14\text{-}x}Cl_x$ (x = 5–7) as a formula, compound group A was thought to correspond to polychlorinated terphenyls (PCTs). PCTs are similar to PCBs, in terms of chemical properties and toxicity (Jensen and Jørgensen, 1983). Long-term toxic effects of PCTs in animals have shown to be of most importance. PCTs may cause liver damage, including tumours, and disturb the hormonal, immunological and reproductive systems. Few publications address the occurrence of PCTs in the Mediterranean Sea. They were detected in shellfish collected between 1989 and 1991 from the Catalan coast in levels lower than PCBs' (Galcerán et al., 1993a; Galcerán et al., 1993b). PCTs were also found in clams, mussels, tuna and salmon purchased in markets in Madrid (Spain) (Fernández et al., 1998).

Compound group B was identified as having the formula $C_{12}H_{10\text{-}x}OCl_x$ (x = 4–8), which likely corresponded to polychlorinated diphenyl ethers (PCDEs). Due to their higher log K_{ow} values, PCDEs bioaccumulate in organisms at a much higher level than hydroxyl PCBs (Koistinen, 1998).

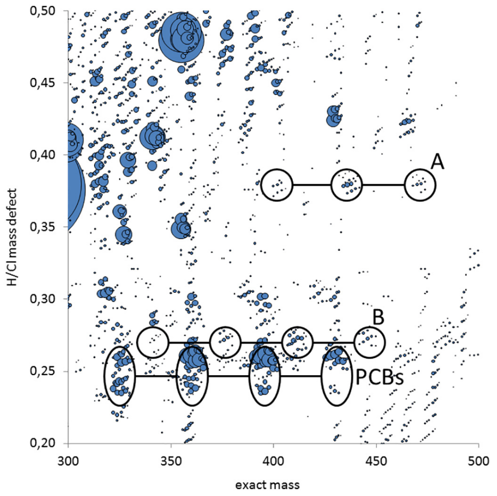

Fig. 2. H/Cl mass defect plot of a sample: A and B are unknown compounds.

Despite their connection with PCBs and PBDEs, the occurrence of PCDEs is not as well documented as that of the other compounds. PCDEs might also be environmentally significant due to their conversion into polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in photolytic reactions and industrial processes (Becker et al., 1991; Domingo, 2006). They do not seem to have been documented in the Mediterranean Sea yet.

Two PCT mixtures (di- to penta-PCTs Aroclor 5432, hepta- to undeca-PCTs Aroclor 5460) and PCDEs with different levels of chlorination (tetra-CDE-77, penta-CDE-99, hexaCDE-153) were purchased to confirm the identity of the unknown compounds. Full scan at low and high energy were registered for all standards and samples. Runs at low and high energy provided the molecular ion of the analytes and a confirmation fragment (generally by loss of two chlorine atoms), respectively. The suspected compounds matched the retention times and the mass spectra of the standards (Fig. S1). Lacking the corresponding standards, hexa-CTs, hepta-CDEs and octa-CDEs were identified by their molecular mass, matching their mass spectra with modelled mass spectra and at times of retention consistent with the regular separation observed between the available congeners.

Instrumental blanks showed no compounds. Hepta- and octa-CDE and tri-, tetra-, penta-, hexa- and deca-CT showed in one, two or three procedure blanks at 0.59% ± 0.96 of the samples' response.

After the confirmation of PCTs and PCDEs, semi-quantitative determination was performed (Table 2). An external calibration line using the labelled internal standard with the closest retention time was used. As PCDE standards of different chlorination degree were purchased separately, it was assumed that all PCDEs of the same chlorination degree would have the same analytical response and equal to the purchased standard. Lacking hepta- and octa-CDE standards, having observed that sensitivity seemed to increase with the degree of chlorination and bearing in mind that this was a semi-quantification with the aim to set a tentative order of magnitude and detect temporal trends, hepta- and octa-CDE were semi-quantified using the hexa-CDE calibration. Regarding PCTs, which came in two Aroclor mixtures, it was assumed that all degrees of chlorination in a mixture had the same analytical response; therefore, the proportion of each group's area relative to the sum of areas equalled the proportion in the composition of the mixture. It was observed that the semi-quantification of hexa-CT, which was not included in any of the mixtures, was similar using penta- and hepta-CT (average difference of 2.7%). Therefore, it was semi-quantified as an average of both results bearing in mind the tentative order of magnitude and the focus on temporal trends. Values were rounded as they are an indication of magnitude rather than an exact quantity. The determined concentrations are probably somewhat lower than the true values due to incomplete recovery of the compounds in the extraction. See Supporting Information for results of individual compounds in each sample (Tables: S8, PCTs, and S9, PCDEs).

Tri-CTs were found in 23.8% of the samples, deca- and nona-CTs were in 66.7 and 71.4% of them, tetra- to octa-CTs were in over 95% of the samples. Hexa-CTs were semi-quantified as the mean of the values obtained using the calibration lines of penta- and hepta-CTs (most values differed by less than 30%).

Tetra-CDEs were not detected, penta-CDEs were found in three samples and hexa-CDEs were in all but the sample from 2018. Hepta-CDEs were present in 38.1% of the samples and octa-CDEs were in all but the sample from 2018. The total peak group of penta- and hexa-CDEs was semi-quantified with their respective standard assuming a similar response for the congeners with the same degree of chlorination. Hepta- and octa-CDEs were semi-quantified using the calibration line of hexa-CDEs.

Both groups of compounds (PCTs and PCDEs) showed clearly declining levels between 1990 and 2014–2018 ($p < 0.01$, Games-Howell post hoc comparison, Fig. 1). Moreover, both groups showed a significant decrease through all periods (linear regression applied to concentrations versus year: $R = 56$, $p < 0.001$).

In 1990, PCT levels were comparable to PBDE levels. In 2014–2018, they are comparable to dechloranes and MeO-PBDEs. In 1990, PCDE levels were roughly three or four times the levels of OPFRs and PBDEs. In 2014–2018, they seemed to be somewhere between the two families of FRs.

Studies on PCTs in marine biota are very scarce. They have been reported in Spanish seafood samples ranging between 9.84 and 17.6 ng g^{-1} wet weight (ww) in mussels, 1.24–24.2 ng g^{-1} ww in clams, 21.1–64.2 ng g^{-1} ww in salmon and 63.4–259 ng g^{-1} ww in tuna. Expressed in ww, the present semi-quantitative results in Mediterranean striped dolphins sampled in 1990 ranged from 6 to 36 ng g^{-1} ww. These levels were in the same order of magnitude as those of the other Mediterranean organisms. PCTs were reported in 1981 in grey seals from the Baltic Sea at 500–1000 ng g^{-1} ww (Renberg et al., 1978). Levels in fat of gulls from the Mediterranean Sea (1978) and the Baltic Sea (1980) were 610–10,510 ng g^{-1} ww (range) and 1800 ng g^{-1} ww (mean), respectively (Vannucchi et al., 1978; Falandysz, 1980). If the similar concentrations in gulls were an indication that the order of magnitude of the environmental contamination in both seas was comparable, similar concentration may be found in marine mammals. Assuming a broad comparison of grey seals with striped dolphins as marine mammals and taking into account the decline in levels observed in the present study, PCT semi-quantitative concentrations in the dolphins from 1990 are consistent with the concentrations in the grey seals from 1987.

Studies on PCDEs in marine biota are just as scarce. Total concentrations of PCDEs in seals from Lake Saimaa (Finland, 1991–1992) and Baltic seals (1993) were 220–460 ng g^{-1} lw and 30–380 ng g^{-1} lw (Koistinen et al., 1995; Koistinen et al., 1997). Those levels were much lower than the levels obtained for the Mediterranean dolphins from 1990, 6000–38,000 ng g^{-1} lw. Other compounds have previously been found at high levels in the Mediterranean Sea. A study on the impact of PCBs on cetaceans in European waters reported that striped dolphins and other species around the Iberian Peninsula had their mean and median PCB concentrations among the highest published for cetaceans globally (Jepson et al., 2016). PBDEs in seafood showed higher levels in the Mediterranean Sea than in other European waters and high levels of pyrethroid pesticides have been reported in striped dolphins from the south of the Iberian Peninsula (Aznar-Alemany et al., 2017a; Aznar-Alemany et al., 2017b).

A PCB toxicity threshold for the onset of physiological impacts in dolphins is 10 mg kg^{-1} lw (Hall et al., 2006), and a higher threshold was reported for reproductive impairment in ringed seals (*Phoca hispida*) from the Baltic Sea at 41 mg kg^{-1} lw (Helle et al., 1976). If PCTs are similar to PCBs in terms of toxicity (Jensen and Jørgensen, 1983), their levels were always below the lower threshold. However, assuming some similarity with PBDEs, approximately 82% of dolphins exceeded the PBDE threshold (1500 ng g^{-1} lw) in 1990 compared with 17% in 2014–2018. Regarding PCDEs, over 90% of the individuals exceeded the lower PCB and the PBDE thresholds in 1990, whereas in 2014–2018 the percentage decreased to 17% and 42%, respectively.

4. Conclusions

In this study, target and non-target analysis were carried out, obtaining contamination levels from a wide variety of halogenated and organophosphate contaminants in dolphin samples from the Mediterranean Sea. For some compounds such as PBDEs, previous studies have been already published. However, for other families of emerging compounds such as OPFRs, or less studied pollutants such as PCTs and PCDEs, our results are the first ones reported in this type of samples. Furthermore, we have been able to characterize time trends of the different compounds.

PBDEs and OPFRs showed the highest concentrations in the orders of magnitude of 10^3–10^5 ng g^{-1} lw. However, while PBDE levels have decreased about 60% from 1990 to 2018, probably due do the relevant legislation, OPFRs showed steady concentrations over time becoming the predominant family of these FRs to take into account for environmental management. Dechloranes contamination was mainly attributed to Dec 602. Dec 602 reached maximum concentrations in the low $10^3\,\mathrm{ng\,g^{-1}}$ lw values. Although Dec 602 levels seemed stable, lower levels in 2017 and 2018 hinted at a possible decrease that should be assessed in future studies. PBDEs, OPFRs and Dec 602 were found in all samples, as well as natural MeO-PBDEs. Being naturally occurring, hence independent from human activity, MeO-PBDEs showed steady levels below $1000\,\mathrm{ng\,g^{-1}}$ lw. Legacy α-HBCD and emerging BFRs were detected in fewer samples and at lower levels. No time trend was observed for α-HBCD or PBEB, the latter mostly undetected. On the other hand, concentrations of DBDPE and HBB decreased between 1990 and 2018. Finally, PCTs and PCDEs showed clearly declining levels between time period evaluated.

Although there is a trend of decreasing concentration levels of certain pollutants over time, they still remain a health risk for dolphins. For instance, and currently, 33% of dolphins are at risk of hyperthyroidism due to PBDE levels. Similarly, from 17 to 42% of dolphins still have PCDE risk levels. The present study suggests that we will have to wait at least another decade for PBDEs and PCDEs do not pose a threat to Mediterranean striped dolphins.

CRediT authorship contribution statement

Òscar Aznar-Alemany: Conceptualization, Formal analysis, Investigation, Writing – Original Draft, Writing – Review & Editing, Visualization. **Berta Sala:** Methodology, Formal analysis (OPFRs). **Karl J. Jobst:** Methodology, Formal analysis (Non-targeted analysis), Investigation, Writing – Original Draft. **Eric J. Reiner:** Methodology, Validation, Investigation, Resources, Supervision, Project administration, Funding acquisition. **Asunción Borrell:** Conceptualization, Resources, Writing – Original Draft, Writing – Review & Editing, Supervision, Project administration, Funding acquisition. **Alex Aguilar:** Resources, Writing – Original Draft, Writing – Review & Editing, Supervision, Project administration, Funding acquisition. **Ethel Eljarrat:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing – Original Draft, Writing – Review & Editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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PUBLICACIÓ CIENTÍFICA #3

Supplementary information

“Temporal trends of halogenated and organophosphate contaminants in striped dolphins from the Mediterranean Sea”

3

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Table S1. Recoveries, limits of detection (LODs) and quantification (LOQs) of selected pollutants in dolphin samples.

		Recoveries (%)	**ng/g lw**	
			LOD	**LOQ**
PBDEs	BDE-28	72	0.04	0.12
	BDE-47	77	0.05	0.18
	BDE-100	67	0.20	0.67
	BDE-99	63	0.29	0.97
	BDE-154	59	0.43	1.42
	BDE-153	73	0.64	2.13
	BDE-183	57	5.12	18.0
	BDE-209	61	0.16	0.52
MeO-PBDEs	2-MBDE-68	97	0.43	1.42
	6-MBDE-47	85	1.06	3.54
	5-MBDE-47	88	0.43	1.42
	4-MBDE-99	80	2.13	7.09
	5-MBDE-100	82	1.59	6.64
	4-MBDE-100	83	2.20	7.33
	5-MBDE-99	102	3.75	12.5
	4-MBDE-101	109	3.19	10.6
HBCDs	α-HBCD	79	0.10	0.20
	β-HBCD	109	1.00	3.30
	γ-HBCD	81	0.10	0.30
Emerging BFRs	HBB	80	0.20	0.67
	PBEB	70	0.18	0.61
	DBDPE	58	0.08	0.26
HNs	Dec 602	97	0.02	0.07
	Dec 603	88	0.007	0.024
	Dec 604	99	0.007	0.024
	syn-DP	86	0.006	0.018
	anti-DP	82	0.002	0.008
OPFRs	DCP	70	1.63	4.61
	EHDP	60	0.53	0.97
	IDPP	86	2.96	5.17
	TBP	70	3.44	7.30
	THP	80	0.88	2.11
	TPHP	53	1.30	3.45
	TPPO	49	0.35	1.30
	TBOEP	63	0.44	1.44
	TCEP	67	1.21	3.51
	TCIPP	63	1.48	4.18
	TDCPP	55	0.19	1.03
	TEHP	97	1.95	3.86
	IPPP	81	19.3	24.8
	TMCP	77	2.55	4.63

Table S2. Total concentrations of all compound groups (ng g^{-1} lw). nd = < LOD, nq = < LOQ

Year	Size (cm)	Water (%)	Fat[a] (%)	PBDEs	PBEB	HBB	DBDPE	HBCDs	Dechloranes	OPFRs	MeO-PBDEs	PCDEs	PCTs
1990	181	69.1	1.43	2898	nd	86.8	181	nd	726	6245	400	6035	1400
1990	187	69.4	2.69	5354	nd	nd	38.6	nd	1279	2498	331	14843	2805
1990	191	70.4	1.08	3595	nd	nd	nq	nd	908	37390*	751	10834	2942
1990	193	70.0	1.63	9862	nd	nd	49.8	nq	1103	1707	906	30715	4630
1990	193	71.5	1.74	4280	nd	93.4	137	nq	1294	2515	615	14489	3578
1990	194	66.1	1.72	9798	nd	nd	121	nq	1000	2955	673	26524	2055
1990	197	69.0	1.15	2988	nd	nd	nq	nd	1101	835	894	13983	7451
1990	200	69.6	1.45	4139	nd	nd	nq	244	377	670	587	14385	4395
1990	206	73.8	1.54	4551	nd	nd	56.3	nq	924	20180	480	-	-
1990	208	69.9	1.40	4598	nd	82.5	94.4	nq	1668	2810	552	-	-
1990	210	65.1	0.71	5800	nd	nd	nq	nd		2282	534	37848	7320
1990	210	68.5	1.79	5314	nd	nd	117	nq	1757	9673	832	29440	6302
1990	211	66.8	3.55	2305	nd	nd	nq	nd	650	857	281	12767	1044
1990	212	68.1	1.62	6116	nd	98.0	118	nq	3506*	14322	746	-	-
1990	216	71.9	1.45	4393	nd	nd	371	nq	879	4361	802	-	-
2004	194	71.4	2.38	2142	1.24	10.0	11.1	21.3	608	3094	404	4237	561
2007	170	71.4	1.03	2729	3.20	31.0	79.0	nq	1660	1227	839	1553	1354
2007	185	68.4	1.25	1548	nd	nd	nq	nd	737	722	220	3140	1257
2007	194	68.3	3.42	1038	0.86	nd	3.84	16.9	520	948	233	2022	300
2007	198	70.7	1.63	10706*	nd	nd	nq	4425	1266	35057*	3797*	16448	2845
2007	210	71.4	0.91	3522	nd	nd	nq	nd	2598	1828	991	24134	3035
2008	166	68.7	20.8	675	nd	nd	3.06	251	98.8	523	448	1631	214
2008	185	67.2	1.65	1111	nd	nd	nd	nq	460	533	343	709	323
2008	185	72.1	1.98	2988	nd	nd	nd	nq	3655	3710	337	1692	1114
2008	190	69.5	13.1	1843	nd	nd	1.25	544	518	211	668	5936	350
2008	194	71.7	1.11	2493	nd	nd	nq	nd	1050	1738	322	5876	3300
2008	204	69.0	1.47	4447	nd	nd	nq	nd	1924	5811	699	20273	4375
2008	205	70.0	2.41	2658	nd	nd	11.2	54.7	1623	2686	411	4223	787
2009	205	66.3	3.58	1697	nd	nd	21.7	54.5	1205	1191	339	16664	1129
2009	224	67.9	2.64	1210	nd	23.4	4.93	nq	739	375	147	3787	474
2014	204	71.0	20.3	394	nd	nd	nq	228	55.2	7639	2678*	114	35
2015	204	71.5	2.34	1347	nd	nd	nq	nd	599	160	187	1835	809
2015	205	73.6	1.01	9461*	nd	nd	nq	384	2639	6703	1828	7154	1137
2015	210	73.4	0.71	3070	nd	nd	nq	nd	7880*	41190*	836	11326	3115
2016	198	72.8	2.35	4416	nd	nd	nq	375	1842	2148	509	11909	2264
2017	164	72.7	2.48	630	nd	nd	nd	nq	86.2	1906	254	506	203
2017	183	72.2	3.83	435	nd	nd	nd	1.99	65.8	502	200	398	120
2017	196	73.5	2.63	985	nd	14.9	3.04	nq	143	6081	376	823	168
2017	198	71.9	2.51	1096	nd	nd	2.73	nq	493	2876	343	342	480
2017	-	71.5	3.17	586	nd	2.45	253	nq	73.4	641	301	327	74
2017	-	72.8	3.20	2131	nd	nd	72.9	nq	999	1408	472	4149	816
2018	-	72.5	3.14	268	nd	nd	nd	nq	62.1	3739	234	nd	49

[a] Lipid content referenced to dry weight; * Excluded from data analysis

Table S3. Concentrations of legacy FRs (ng g^{-1} lw). nd = < LOD, nq = < LOQ

Year	Size (cm)	Water (%)	Fat[a] (%)	BDE -28	BDE -47	BDE -100	BDE -99	BDE -154	BDE -153	BDE -183	BDE -209	α-HBCD	β-HBCD	γ-HBCD
1990	181	69.1	1.43	33.0	1072	396	574	504	275	nq	43.7	nd	nd	nd
1990	187	69.4	2.69	24.7	1815	654	955	1109	607	184	5.51	nd	nd	nd
1990	191	70.4	1.08	36.3	1996	nq	474	477	332	nd	280	nd	nd	nd
1990	193	70.0	1.63	68.6	3837	1154	2076	1560	974	192	nq	nq	nd	nd
1990	193	71.5	1.74	35.2	1663	540	894	718	383	46.9	nq	nq	nd	nd
1990	194	66.1	1.72	55.2	2272	713	1059	844	473	68.4	4313	nq	nd	nd
1990	197	69.0	1.15	34.6	2003	nq	nq	542	279	36.4	93.0	nd	nd	nd
1990	200	69.6	1.45	27.3	2451	481	560	574	nd	nd	45.4	244	nd	nd
1990	206	73.8	1.54	48.9	1870	559	878	684	371	32.6	108	nq	nd	nd
1990	208	69.9	1.40	43.3	1758	611	832	839	468	45.3	nq	nq	nd	nd
1990	210	65.1	0.71	29.9	2728	514	917	927	578	nd	105	nd	nd	nd
1990	210	68.5	1.79	40.1	2121	712	1147	821	428	36.7	8.32	nq	nd	nd
1990	211	66.8	3.55	19.2	1397	nq	270	264	303	nd	51.4	nd	nd	nd
1990	212	68.1	1.62	40.7	2178	851	1169	1214	576	85.0	3.20	nq	nd	nd
1990	216	71.9	1.45	52.1	1970	600	802	656	291	nd	21.9	nq	nd	nd
2004	194	71.4	2.38	19.2	604	310	265	741	198	nd	3.91	21.3	nd	nd
2007	170	71.4	1.03	38.4	782	419	293	812	350	nd	33.5	nq	nd	nd
2007	185	68.4	1.25	7.39	424	166	367	366	157	nd	60.5	nd	nd	nd
2007	194	68.3	3.42	11.9	361	204	94.6	256	109	nd	1.11	16.9	nd	nd
2007	198	70.7	1.63	34.6	3178	1110	2589	2732	1015	nq	47.0	4425	nd	nd
2007	210	71.4	0.91	27.9	1471	413	620	628	278	18.7	65.1	nd	nd	nd
2008	166	68.7	20.8	13.5	288	119	74.5	135	41.5	nd	3.14	250	nd	1.17
2008	185	67.2	1.65	24.6	417	200	162	218	89.0	nd	nd	nq	nd	nd
2008	185	72.1	1.98	17.1	840	526	268	922	414	nq	nd	nq	nd	nd
2008	190	69.5	13.1	17.6	661	330	284	393	158	nq	nq	544	nd	nd
2008	194	71.7	1.11	6.56	481	240	635	649	325	35.3	121	nd	nd	nd
2008	204	69.0	1.47	7.10	1340	449	1031	1075	498	nq	46.6	nd	nd	nd
2008	205	70.0	2.41	16.4	738	461	263	751	304	nd	124	54.7	nd	nd
2009	205	66.3	3.58	11.1	641	360	124	384	167	nd	10.7	54.5	nd	nd
2009	224	67.9	2.64	12.4	393	249	91.8	297	158	nd	8.68	nq	nd	nd
2014	204	71.0	20.3	6.06	187	37.9	68.5	71.3	19.7	nd	3.40	228	nd	nd
2015	204	71.5	2.34	3.08	433	180	290	296	114	nd	30.5	nd	nd	nd
2015	205	73.6	1.01	22.3	2486	892	1472	1531	668	41.1	2348	384	nd	nd
2015	210	73.4	0.71	8.94	603	313	833	849	344	18.0	102	nd	nd	nd
2016	198	72.8	2.35	5.84	731	449	1314	1380	464	28.0	45.1	375	nd	nd
2017	164	72.7	2.48	19.9	224	133	54.1	146	52.6	nd	nq	nq	nd	nd
2017	183	72.2	3.83	20.6	158	89	30.6	97.4	36.0	nd	2.79	1.99	nd	nd
2017	196	73.5	2.63	11.3	321	201	86.7	264	101	nd	nq	nq	nd	nd
2017	198	71.9	2.51	10.6	261	183	81.7	420	140	nd	nq	nq	nd	nd
2017	-	71.5	3.17	9.91	232	115	59.9	110	41.2	nd	17.3	nq	nd	nd
2017	-	72.8	3.20	10.8	601	377	184	688	270	nd	nq	nq	nd	nd
2018	-	72.5	3.14	9.34	104	50.9	34.9	46.2	23.4	nd	nq	nq	nd	nd

[a] Lipid content referenced to dry weight

Table S4. Concentrations of emerging HFRs (ng g^{-1} lw). nd = < LOD, nq = < LOQ

Year	Size (cm)	Water (%)	Fat[a] (%)	PBEB	HBB	DBDPE	Dec 602	Dec 603	Dec 604	syn-DP	*anti*-DP
1990	181	69.1	1.43	nd	86.8	181	715	nd	nd	10.9	nd
1990	187	69.4	2.69	nd	nd	38.6	1279	nd	nd	nd	nd
1990	191	70.4	1.08	nd	nd	nq	830	51.6	nd	nd	26.0
1990	193	70.0	1.63	nd	nd	49.8	1093	10.4	nd	nd	nd
1990	193	71.5	1.74	nd	93.4	137	1294	nd	nd	nd	nd
1990	194	66.1	1.72	nd	nd	121	993	nd	nd	nd	7.38
1990	197	69.0	1.15	nd	nd	nq	1101	nd	nd	nd	nd
1990	200	69.6	1.45	nd	nd	nq	341	nd	8.17	nd	28.1
1990	206	73.8	1.54	nd	nd	56.3	917	7.72	nd	nd	nd
1990	208	69.9	1.40	nd	82.5	94.4	1668	nd	nd	nd	nd
1990	210	65.1	0.71	nd	nd	nq	-	-	-	-	-
1990	210	68.5	1.79	nd	nd	117	1757	nd	nd	nd	nd
1990	211	66.8	3.55	nd	nd	nq	600	49.9	nd	nd	nd
1990	212	68.1	1.62	nd	98.0	118	3506	nd	nd	nd	nd
1990	216	71.9	1.45	nd	nd	371	867	nd	nd	12.4	nd
2004	194	71.4	2.38	1.24	10.0	11.1	608	nd	nd	nd	nd
2007	170	71.4	1.03	3.20	31.0	79.0	1660	nd	nd	nd	nd
2007	185	68.4	1.25	nd	nd	nq	737	nd	nd	nd	nd
2007	194	68.3	3.42	0.86	nd	3.84	520	nd	nd	nd	nd
2007	198	70.7	1.63	nd	nd	nq	1266	nd	nd	nd	nd
2007	210	71.4	0.91	nd	nd	nq	2598	nd	nd	nd	nd
2008	166	68.7	20.8	nd	nd	3.06	99	nd	nd	nd	nd
2008	185	67.2	1.65	nd	nd	nd	460	nd	nd	nd	nd
2008	185	72.1	1.98	nd	nd	nd	3651	3.64	nd	nd	nd
2008	190	69.5	13.1	nd	nd	1.25	516	2.4	nd	nd	nd
2008	194	71.7	1.11	nd	nd	nq	1033	nd	nd	nd	17.1
2008	204	69.0	1.47	nd	nd	nq	1924	nd	nd	nd	nd
2008	205	70.0	2.41	nd	nd	11.2	1613	1.88	nd	nd	8.19
2009	205	66.3	3.58	nd	nd	21.7	1195	nd	nd	5.03	4.77
2009	224	67.9	2.64	nd	23.4	4.93	739	0.89	nd	nd	nd
2014	204	71.0	20.3	nd	nd	nq	55	nd	nd	nd	nd
2015	204	71.5	2.34	nd	nd	nq	599	nd	nd	nd	nd
2015	205	73.6	1.01	nd	nd	nq	2639	nd	nd	nd	nd
2015	210	73.4	0.71	nd	nd	nq	7880	nd	nd	nd	nd
2016	198	72.8	2.35	nd	nd	nq	1842	nd	nd	nd	nd
2017	164	72.7	2.48	nd	nd	nd	81	nd	0.58	nd	5.00
2017	183	72.2	3.83	nd	nd	nd	62	nd	nd	nd	3.32
2017	196	73.5	2.63	nd	14.9	3.04	143	nd	nd	nd	nd
2017	198	71.9	2.51	nd	nd	2.73	493	nd	nd	nd	nd
2017	-	71.5	3.17	nd	2.45	253	73	nd	nd	nd	nd
2017	-	72.8	3.20	nd	nd	72.9	999	nd	nd	nd	nd
2018	-	72.5	3.14	nd	nd	nd	62	nd	nd	nd	nd

[a] Lipid content referenced to dry weight

Table S5. Concentrations of OPFRs (ng g^{-1} lw). nd = < LOD, nq = < LOQ

Year	Size (cm)	Fat[a] (%)	TCEP	TPPO	TCIPP	TDCPP	TPHP	TNBP	DCP	TBOEP	TMCP	EHDPP	IDPP	IPPP	THP	TEHP
1990	181	1.43	620	nd	nd	nd	nd	2467	nd	3157	nd	nd	nd	nd	nd	nd
1990	187	2.69	376	nd	nd	nd	nd	1133	nd	989	nd	nd	nd	nd	nd	nd
1990	191	1.08	nd	19.1	10293	nq	nq	nd	627	6962	669	18819	nd	nq	nd	nd
1990	193	1.63	nd	nd	nd	nd	nd	1310	nd	397	nd	nd	nd	nd	nd	nd
1990	193	1.74	nd	nd	nd	nd	nd	1542	nd	862	nd	nd	nd	nd	112	nd
1990	194	1.72	nd	nd	nd	nd	nd	1983	nd	972	nd	nd	nd	nd	nd	nd
1990	197	1.15	nd	nd	nd	nd	nd	nd	nd	835	nd	nq	nd	nd	nd	nd
1990	200	1.45	nd	15.3	nd	nq	206	nd	nd	449	nd	nq	nd	nd	nd	nd
1990	206	1.54	nd	nd	nd	nd	nd	1947	nd	5707	nd	12526	nd	nd	nd	nd
1990	208	1.40	nd	nd	nd	nd	nd	nd	nd	2810	nd	nd	nd	nd	nd	nd
1990	210	0.71	nd	nd	nd	nd	nq	nd	nd	2282	nd	nd	nd	nd	nd	nd
1990	210	1.79	309	nd	nd	nd	nd	nd	nd	750	nd	nd	8614	nd	nd	nd
1990	211	3.55	nd	nd	nd	nd	nd	nd	nd	857	nd	nd	nd	nd	nd	nd
1990	212	1.62	299	nd	nd	nd	nd	255	nd	13768	nd	nd	nd	nd	nd	nd
1990	216	1.45	773	nd	nd	nd	nd	2010	nd	1579	nd	nd	nd	nd	nd	nd
2004	194	2.38	421	nd	nd	nd	nd	2432	nd	241	nd	nd	nd	nd	nd	nd
2007	170	1.03	nd	nd	nd	nd	nd	70.4	nd	1157	nd	nd	nd	nd	nd	nd
2007	185	1.25	nd	nd	nd	nd	nd	nd	442	279	nd	nd	nd	nd	nd	nd
2007	194	3.42	nd	nd	nd	nd	nd	795	nd	153	nd	nd	nd	nd	nd	nd
2007	198	1.63	nd	78.9	nq	nq	1769	23228	348	1533	nd	8100.5	nd	nq	nd	nd
2007	210	0.91	nd	32.7	nq	nd	nd	850	569	376	nd	nq	nd	nq	nd	nd
2008	166	20.8	nd	64.1	148	2.06	nd	nd	nd	309	nd	nd	nd	nd	nd	nd
2008	185	1.65	260	nd	nd	nd	nd	nd	nd	273	nd	nd	nd	nd	nd	nd
2008	185	1.98	378	nd	nd	nd	nd	2328	nd	1004	nd	nd	nd	nd	nd	nd
2008	190	13.1	nd	158	nd	3.07	nd	nd	nd	49.9	nd	nd	nd	nd	nd	nd
2008	194	1.11	nd	nd	nd	nd	nd	nd	557	485	695.4	nq	nd	nd	nd	nd
2008	204	1.47	nd	14.1	nq	nd	nd	5250	nd	547	nd	nd	nd	nd	nd	nd
2008	205	2.41	368	nd	nd	nd	nd	1591	130	596	nd	nd	nd	nd	nd	nd
2009	205	3.58	223	nd	nd	nd	nd	585	nd	384	nd	nd	nd	nd	nd	nd
2009	224	2.64	173	23.5	nd	nd	nd	nd	nd	178	nd	nd	nd	nd	nd	nd
2014	204	20.3	nd	nq	nq	nd	nd	nd	nd	nd	nd	7639	nd	nd	nd	nd
2015	204	2.34	nd	16.6	nq	nq	nd	nd	nd	143	nd	nq	nd	nd	nd	nd
2015	205	1.01	nd	nq	nd	nd	nd	nd	nd	nd	nd	6703	nd	nq	nd	nd
2015	210	0.71	nd	nq	nq	nq	nd	40734	nd	455	nd	nd	nd	nq	nd	nd
2016	198	2.35	nq	nd	nd	nd	nd	1904	nd	244	nd	nd	nd	nd	nd	nd
2017	164	2.48	nd	nd	nd	nd	nd	1414	nd	492	nd	nd	nd	nd	nd	nd
2017	183	3.83	nd	nd	nd	nd	nd	502	nd	nd	nd	nd	nd	nd	nd	nd
2017	196	2.63	469	nd	nd	nd	nd	2452	nd	nd	nd	nd	3161	nd	nd	nd
2017	198	2.51	402	nd	nd	nd	nd	2356	nd	118	nd	nd	nd	nd	nd	nd
2017	-	3.17	nd	nd	nd	nd	nd	638	nd	2.98	nd	nd	nd	nd	nd	nd
2017	-	3.20	229	nd	nd	nd	nd	1060	nd	120	nd	nd	nd	nd	nd	nd
2018	-	3.14	nd	nd	nd	nd	nd	2679	nd	8.41	nd	nd	1052	nd	nd	nd

[a] Lipid content referenced to dry weight

Table S6. Concentrations of MeO-PBDEs (ng g^{-1} lw). nd = < LOD, nq = < LOQ

Year	Size (cm)	Water (%)	Fat[a] (%)	2-MBDE -68	6-MBDE -47	5-MBDE -47	4-MBDE -99	5-MBDE -100	4-MBDE -100	5-MBDE -99	4-MBDE -101
1990	181	69.1	1.43	87.5	313	nd	nd	nq	nq	nd	nd
1990	187	69.4	2.69	47.9	256	nd	nd	10.1	17.6	nd	nd
1990	191	70.4	1.08	180	570	nd	nd	nd	nd	nd	nd
1990	193	70.0	1.63	194	692	nd	nd	10.5	9.71	nd	nd
1990	193	71.5	1.74	102	494	nd	nd	8.3	10.3	nd	nd
1990	194	66.1	1.72	112	541	nd	nd	7.6	12.0	nd	nd
1990	197	69.0	1.15	84.0	784	nd	nd	26.9	nd	nd	nd
1990	200	69.6	1.45	140	447	nd	nd	nd	nd	nd	nd
1990	206	73.8	1.54	108	355	nd	nd	7.63	8.77	nd	nd
1990	208	69.9	1.40	84.6	443	nd	nd	11.4	13.0	nd	nd
1990	210	65.1	0.71	118	392	nd	nd	23.8	nd	nd	nd
1990	210	68.5	1.79	134	676	nd	nd	7.29	15.1	nd	nd
1990	211	66.8	3.55	64.8	206	nd	nd	9.71	nd	nd	nd
1990	212	68.1	1.62	117	605	nd	nd	13.3	10.8	nd	nd
1990	216	71.9	1.45	117	665	nd	nd	11.3	8.60	nd	nd
2004	194	71.4	2.38	96.4	299	nd	nd	nq	8.75	nd	nd
2007	170	71.4	1.03	178	638	nd	nd	7.68	14.7	nd	nd
2007	185	68.4	1.25	nd	220	nd	nd	nd	nd	nd	nd
2007	194	68.3	3.42	35.1	198	nd	nd	nq	nq	nd	nd
2007	198	70.7	1.63	894	2839	nd	nd	64.0	nd	nd	nd
2007	210	71.4	0.91	181	786	nd	nd	24.1	nd	nd	nd
2008	166	68.7	20.8	109	340	nd	nd	nq	nq	nd	nd
2008	185	67.2	1.65	71.3	272	nd	nd	nq	nq	nd	nd
2008	185	72.1	1.98	nd	326	nd	nd	nq	11.1	nd	nd
2008	190	69.5	13.1	159	509	nd	nd	nq	nq	nd	nd
2008	194	71.7	1.11	64.6	242	nd	nd	15.4	nd	nd	nd
2008	204	69.0	1.47	81.9	595	nd	nd	22.3	nd	nd	nd
2008	205	70.0	2.41	52.7	348	nd	nd	nq	9.52	nd	nd
2009	205	66.3	3.58	43.7	296	nd	nd	nq	nq	nd	nd
2009	224	67.9	2.64	23.4	124	nd	nd	nq	nq	nd	nd
2014	204	71.0	20.3	634	2020	nd	nd	24.7	nd	nd	nd
2015	204	71.5	2.34	33.3	154	nd	nd	nd	nd	nd	nd
2015	205	73.6	1.01	450	1378	nd	nd	nd	nd	nd	nd
2015	210	73.4	0.71	nd	836	nd	nd	nd	nd	nd	nd
2016	198	72.8	2.35	77.4	431	nd	nd	nd	nd	nd	nd
2017	164	72.7	2.48	51.6	202	nd	nd	nq	nq	nd	nd
2017	183	72.2	3.83	38.3	161	nd	nd	nq	nd	nd	nd
2017	196	73.5	2.63	80.0	296	nd	nd	nq	nq	nd	nd
2017	198	71.9	2.51	70.4	273	nd	nd	nq	nd	nd	nd
2017	-	71.5	3.17	76.5	224	nd	nd	nq	nq	nd	nd
2017	-	72.8	3.20	77.1	385	nd	nd	nq	10.0	nq	nd
2018	-	72.5	3.14	72.9	161	nd	nd	nq	nq	nd	nd

[a] Lipid content referenced to dry weight

Table S7. Data of the untargeted analysis

Compound	Mass defect	Masses	Suggested formula	Suggested compound
A	0.38	400 435 470	$C_{18}H_{14-x}Cl_x$ (x = 5-7)	Polychlorinated terphenyls (PCTs)
B	0.27	341 376 411 446	$C_{12}H_{10-x}OCl_x$ (x = 4-8)	Polychlorinated diphenyl ethers (PCDEs)

Table S8. Concentrations of PCTs (ng g^{-1} lw). nd = < LOD, nq = < LOQ

Year	Size (cm)	Fat[a] (%)	Di-CTS	Tri-CTS	Tetra-CTS	Penta-CTS	Hexa-CTS	Hepta-CTS	Octa-CTS	Nona-CTS	Deca-CTS	Undeca-CTS
1990	181	1.43	nd	nd	170	304	556	285	79	5.9	nd	nd
1990	187	2.69	nd	nd	264	519	1368	554	100	nd	nd	nd
1990	191	1.08	nd	261	327	561	1253	409	116	10	3.4	nd
1990	193	1.63	nd	nd	574	948	1920	943	227	17	nd	nd
1990	193	1.74	nd	nd	467	794	1446	691	168	13	nd	nd
1990	194	1.72	nd	nd	401	570	701	312	66	5.5	nd	nd
1990	197	1.15	nd	474	823	1464	3232	1140	276	35	6.8	nd
1990	200	1.45	nd	669	574	784	1513	548	261	38	7.3	nd
1990	206	1.54	-	-	-	-	-	-	-	-	-	-
1990	208	1.40	-	-	-	-	-	-	-	-	-	-
1990	210	0.71	nd	nd	1108	1529	3191	1113	340	30	9.6	nd
1990	210	1.79	nd	nd	719	1383	2971	1043	185	nd	1.8	nd
1990	211	3.55	nd	nd	nd	217	498	247	81	nd	1.5	nd
1990	212	1.62	-	-	-	-	-	-	-	-	-	-
1990	216	1.45	-	-	-	-	-	-	-	-	-	-
2004	194	2.38	nd	nd	69	117	223	107	42	3.0	0.4	nd
2007	170	1.03	nd	nd	88	278	586	269	123	8.0	1.3	nd
2007	185	1.25	nd	nd	139	283	532	213	81	5.9	3.0	nd
2007	194	3.42	nd	nd	39	58	116	63	22	1.6	nd	nd
2007	198	1.63	nd	nd	503	711	1178	365	78	7.2	2.4	nd
2007	210	0.91	nd	364	409	410	1021	569	223	32	6.8	nd
2008	166	20.8	nd	31	48	38	55	28	11	1.7	nd	nd
2008	185	1.65	nd	nd	26	58	117	75	42	3.4	0.8	nd
2008	185	1.98	nd	nd	75	260	449	229	101	nd	nd	nd
2008	190	13.1	nd	nd	79	88	119	46	17	nd	0.3	nd
2008	194	1.11	nd	261	301	640	1411	517	148	18	4.1	nd
2008	204	1.47	nd	nd	540	1143	2038	555	97	nd	2.1	nd
2008	205	2.41	nd	nd	106	190	328	123	40	nd	nd	nd
2009	205	3.58	nd	nd	195	270	433	193	38	nd	nd	nd
2009	224	2.64	nd	nd	62	106	165	106	35	nd	nd	nd
2014	204	20.3	nd	nd	5.0	5.0	11	7.5	6.0	0.8	0.2	nd
2015	204	2.34	nd	77	76	109	249	201	85	12	nd	nd
2015	205	1.01	nd	193	210	315	34	241	124	15	3.7	nd
2015	210	0.71	nd	218	365	636	1119	558	193	23	3.8	nd
2016	198	2.35	nd	nd	349	546	880	389	86	11	3.0	nd
2017	164	2.48	nd	nd	11	13	45	70	55	8.2	1.4	nd
2017	183	3.83	nd	nd	13	12	31	35	27	2.6	nd	nd
2017	196	2.63	nd	nd	11	25	57	43	29	2.1	0.5	nd
2017	198	2.51	nd	nd	24	77	212	114	52	nd	1.0	nd
2017		3.17	nd	nd	4.3	6.0	17	25	22	nd	0.6	nd
2017		3.20	nd	nd	124	205	323	134	29	nd	nd	nd
2018		3.14	nd	6.7	nd	nd	7.4	14	19	1.0	0.3	nd

[a] Lipid content referenced to dry weight

Table S9. Concentrations of PCDEs (ng g^{-1} lw). nd = < LOD, nq = < LOQ

Year	Size (cm)	Water (%)	Fat[a] (%)	Tetra-CDEs	Penta-CDEs	Hexa-CDEs	Hepta-CDEs	Octa-CDEs
1990	181	69.1	1.43	nd	nd	4394	120	1520
1990	187	69.4	2.69	nd	nd	9596	639	4608
1990	191	70.4	1.08	nd	2742	6313	nd	1779
1990	193	70.0	1.63	nd	nd	22121	nd	8594
1990	193	71.5	1.74	nd	nd	10880	413	3196
1990	194	66.1	1.72	nd	nd	19793	1178	5553
1990	197	69.0	1.15	nd	nd	9935	515	3532
1990	200	69.6	1.45	nd	nd	11060	15	3310
1990	206	73.8	1.54	-	-	-	-	-
1990	208	69.9	1.40	-	-	-	-	-
1990	210	65.1	0.71	nd	nd	25567	1341	10941
1990	210	68.5	1.79	nd	nd	23266	nd	6175
1990	211	66.8	3.55	nd	nd	8067	nd	4700
1990	212	68.1	1.62	-	-	-	-	-
1990	216	71.9	1.45	-	-	-	-	-
2004	194	71.4	2.38	nd	nd	2987	74	1176
2007	170	71.4	1.03	nd	nd	692	nd	861
2007	185	68.4	1.25	nd	nd	2220	nd	920
2007	194	68.3	3.42	nd	nd	1509	nd	513
2007	198	70.7	1.63	nd	nd	11734	732	3983
2007	210	71.4	0.91	nd	nd	17393	nd	6741
2008	166	68.7	20.8	nd	505	902	nd	224
2008	185	67.2	1.65	nd	nd	551	nd	158
2008	185	72.1	1.98	nd	nd	1124	nd	567
2008	190	69.5	13.1	nd	925	4145	nd	867
2008	194	71.7	1.11	nd	nd	4190	nd	1686
2008	204	69.0	1.47	nd	nd	14987	nd	5286
2008	205	70.0	2.41	nd	nd	3076	63	1084
2009	205	66.3	3.58	nd	nd	11439	865	4360
2009	224	67.9	2.64	nd	nd	2568	135	1084
2014	204	71.0	20.3	nd	nd	92	nd	22
2015	204	71.5	2.34	nd	nd	1268	nd	567
2015	205	73.6	1.01	nd	nd	5099	112	1943
2015	210	73.4	0.71	nd	nd	6503	407	4415
2016	198	72.8	2.35	nd	nd	7130	564	4215
2017	164	72.7	2.48	nd	nd	371	nd	135
2017	183	72.2	3.83	nd	nd	326	nd	72
2017	196	73.5	2.63	nd	nd	658	nd	165
2017	198	71.9	2.51	nd	nd	121	nd	221
2017	-	71.5	3.17	nd	nd	301	nd	26
2017	-	72.8	3.20	nd	nd	2960	157	1033
2018	-	72.5	3.14	nd	nd	nd	nd	nd

[a] Lipid content referenced to dry weight

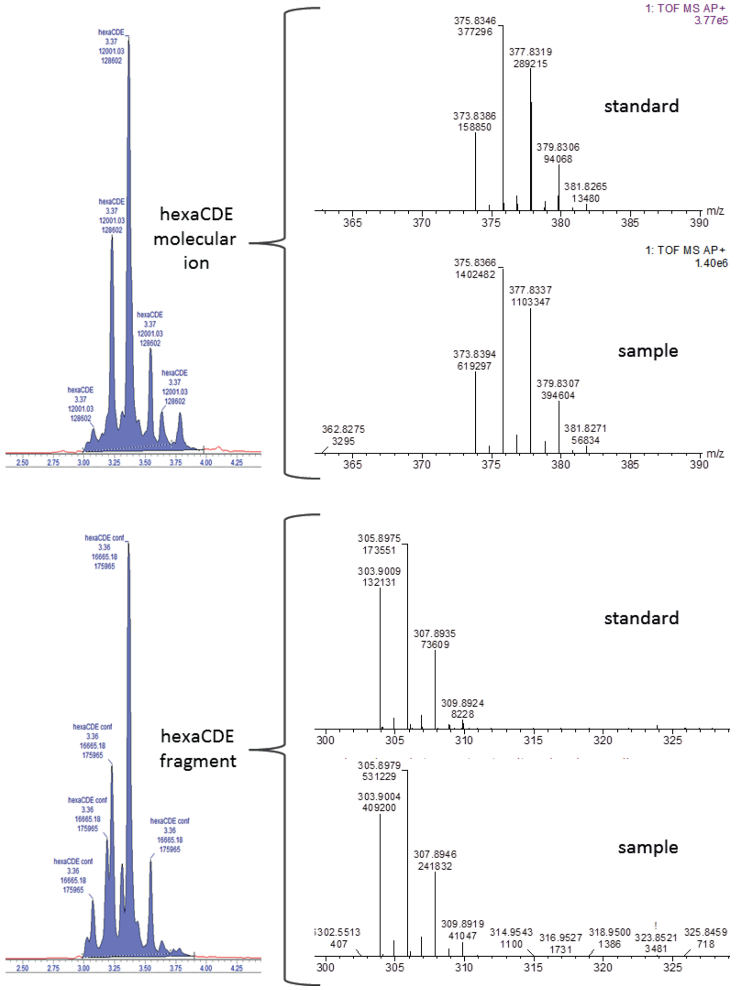

Figure S1. Standard's chromatograms of hexa-CDEs molecular ion and confirmation fragment and matching mass spectra for the standard and a dolphin sample.

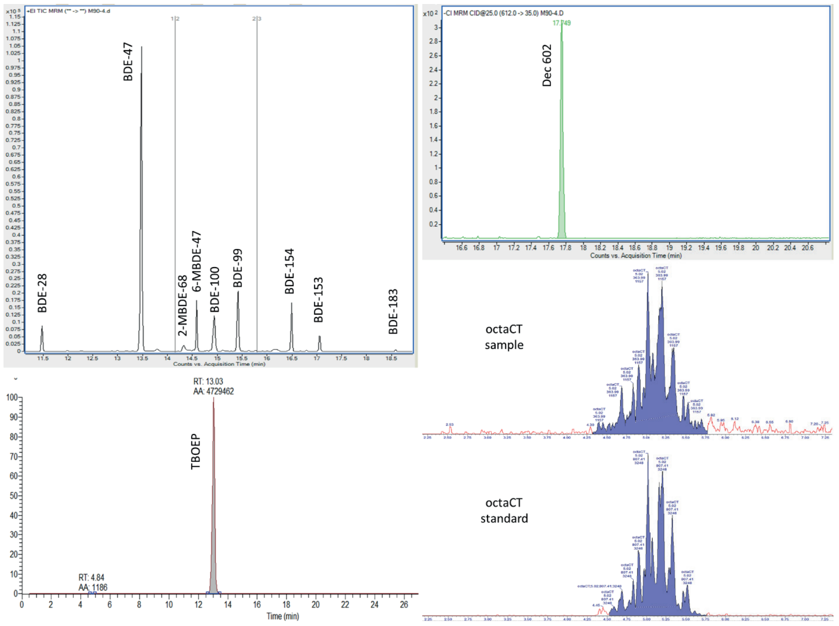

Figure S2. Chromatograms for several analytes in a 1990 sample and comparison of a non-targeted Octa-CT sample chromatogram with the analyte chromatogram.

3.4 Discussió

En aquesta tesi s'hi inclouen les primeres publicacions –i les úniques fins el moment– de concentracions d'OPEs en diferents espècies de dofins tant de l'hemisferi nord (Mar Mediterrani) com del sud (Oceà Índic). Els treballs del Mar Mediterrani (Costa Catalana) i Oceà Índic estaven inclosos a la tesi doctoral "*Contaminants orgànics persistents i pseudopersistents en medi marí. Estudi en biota i en consum humà*" de Aznar Alemany. (2017), en la qual es feia referència a la problemàtica dels BFRs. Però tota la part corresponent als OPEs és part del treball que s'ha dut a terme en aquesta tesi doctoral.

Distribució dels OPEs en els diferents teixits

En el primer estudi que es va realitzar, es van analitzar OPEs en diferents teixits (greix, cervell, ronyó, múscul i fetge) de dofí comú (*Delphinus delphis*) del Mar d'Alboran. Amb aquest estudi es pretenia escollir quin era el teixit idoni per analitzar i estudiar la concentració d'OPEs en animals marins. Els resultats van demostrar que els nivells més alts es trobaven a la grassa (valor mitjà del 68%) i els més baixos al fetge (valor mitjà del 2%), indicant que els OPEs s'emmagatzemen més en teixits grassos que en teixits que presenten una alta activitat metabòlica, com és el cas del fetge (Figura 16). Una vegada que les dades s'han normalitzat segons el contingut lipídic, veiem que el múscul és també un bon teixit per avaluar la concentració d'OPEs en mostres biològiques, amb l'avantatge de que es tracta d'una matriu molt més neta que el greix i en la qual també s'ha obtingut el 100% de freqüència de detecció en les mostres de dofí. Per aquest motiu, en els següents estudis que vam portar a terme la matriu analitzada va ser múscul de dofí.

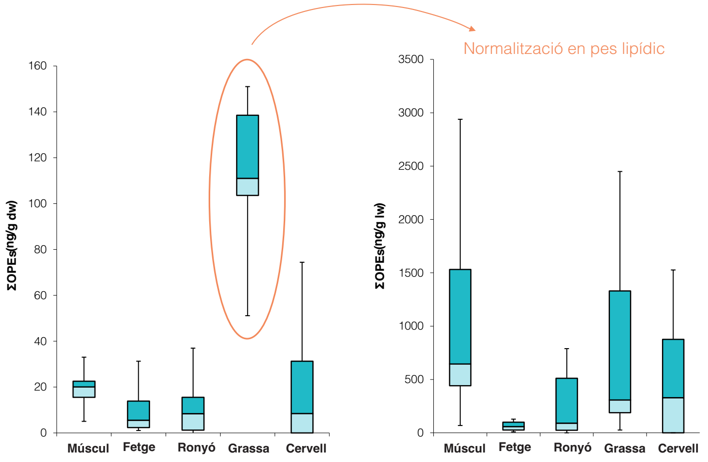

Figura 16. Comparació de les concentracions trobades en els diferents teixits

Els OPEs tendeixen a acumular-se més en el múscul i el greix que en la sang (plasma), ronyons i fetge (Bekele et al., 2021; Greaves and Letcher, 2014). Aquests darrers teixits, en comparació amb el múscul i el greix, com a teixits de mobilització a curt termini, tenen una capacitat de metabolització dels OPEs més ràpida (Fu et al., 2021). L'activitat enzimàtica dels teixits juga un paper clau en l'eliminació d'aquests contaminants (Hou et al., 2018; Yao et al., 2021).

Tendències espacials dels OPEs

Poder disposar de mostres de dofins de diferents mars i oceans ens ha permès realitzar un estudi de tendència espacials dels OPEs. No obstant, s'ha de tenir en compte que s'han comparat espècies diferents de dofí, ja que no es disposava de mostres d'una mateixa espècie per les diferents zones, tal i com seria favorable. Així doncs, l'estudi de tendències espacials s'ha dut a terme a partir de mostres de dofins de diferents espècies, suposant que totes elles corresponien a un nivell tròfic similar per tal de poder tractar les dades conjuntament independentment de l'espècie que es tractés.

Per altre banda, la disponibilitat de les mostres recol·lectades durant diferents anys compresos entre el 1990 i el 2018 ens permeten realitzar un estudi de tendències temporals. Així doncs, en aquest capítol es fa un anàlisi espai-temporal dels OPEs en una mateixa matriu, múscul de dofí. En aquests estudis també disposem dels resultats d'un grup de BFRs clàssic, els PBDEs, dades que utilitzarem per comparar el seu comportament amb el del nostre grup de contaminants, els OPEs, ja que es tracta dels seus substituts. Per mirar les diferències entre contaminants, s'ha utilitzat el test no-paramètric Kruskal-Wallis, ja que les nostres dades són no-normals. S'han descartat les dades atípiques per fer els estudis estadístics.

Per tal d'avaluar els nivells de concentració en diferents zones i veure si hi ha o no diferències, disposem de dades del Mar Mediterrani per la costa catalana, el Mar mediterrani pel Mar d'Alboran i l'Oceà Índic per la costa de Sudàfrica. En el cas de la costa catalana, tot i tenir dades des del 1990 fins el 2018, s'han utilitzat només les més actuals (2014-2018) per tal de poder-les comparar amb les dels altres indrets.

A la Figura 17 trobem representades les concentracions de PBDEs i OPEs pel Mar Mediterrani (Costa Catalana) i l'Oceà Índic (Costa de Sudàfrica). S'han agafat només aquests dos punts ja que són els únics dels quals disposem de dades d'una franja temporal més semblant per poder-les comparar: 2012-2015 per Sudàfrica i 2014-2018 per la costa catalana. Si observem la Figura 17, veiem que per ambdues zones la família que presenta una major concentració són els OPEs amb una mitjana de 10.452±11.301 ng/g lw per l'Oceà Índic i 6.249±11.287 ng/g lw pel Mediterrani. Tot i la gran diferència de concentracions d'OPEs entre una zona i l'altre, no s'observen diferències significatives entre si (Kruskal-Wallis, p-valor=0,460).

Pel que fan els PBDEs en canvi, predominen en el mar Mediterrani amb una mitjana de 2.068±2.642 ng/g lw i un rang de concentració de 268-9.461 ng/g lw. En canvi, a la costa de Sudàfrica es va detectar una mitjana de 416±333 ng/g lw en un rang de 33,3-1.309 ng/g lw de PBDEs. En aquest cas si que hi veiem diferències significatives entre les concentracions de cada zona (Kruskal-Wallis, p-valor=0,006).

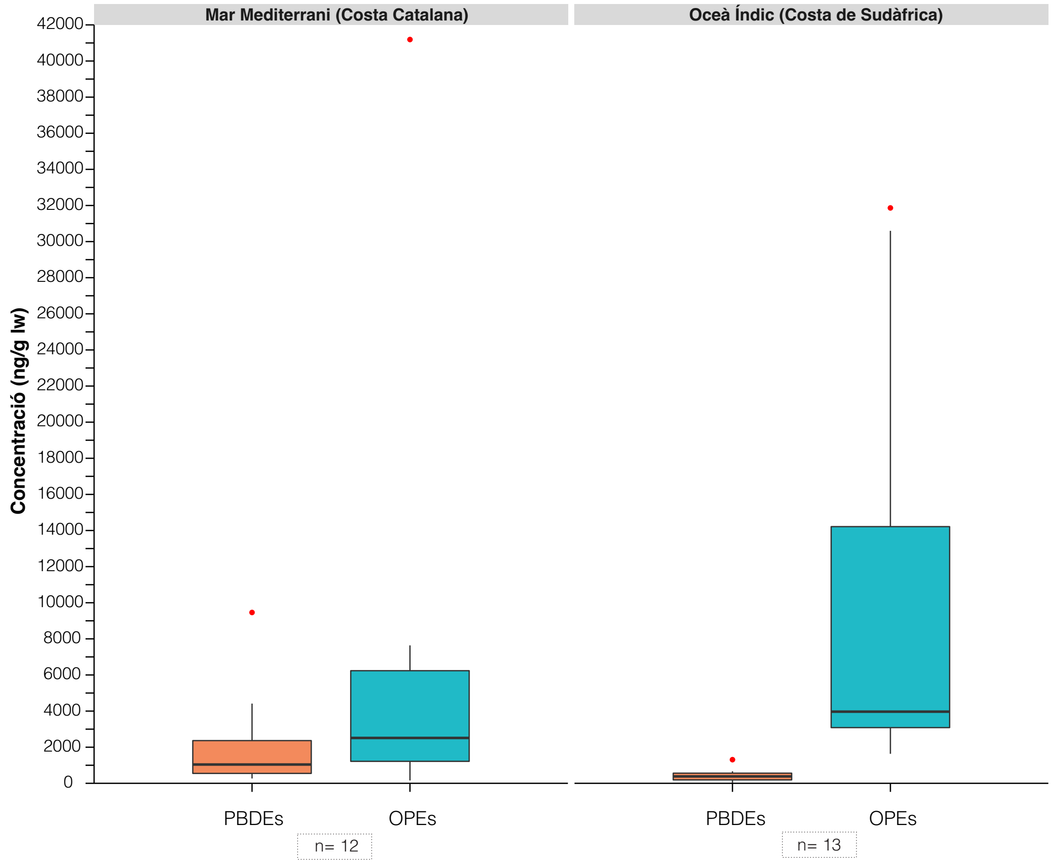

Figura 17. Diagrama de caixes de les concentracions de PBDEs i OPEs en el Mar Mediterrani (Costa Catalana) (n=12) i a l'Oceà Índic (Costa de Sudàfrica) pels períodes (n=13) 2014-2018 i 2012-1015 respectivament. Els punts vermells indiquen les dades atípiques.

Tant els PBDEs com els OPEs s'utilitzen com a FRs (Quintana et al., 2007; Rauert et al., 2018). Els PBDEs han estat els BFRs més utilitzats durant dècades (Ohoro et al., 2021) i els seus nivells ens poden donar una idea del grau d'impacte urbà i industrial d'una zona (Yogui and Sericano, 2009). Així doncs, els nivells superiors del Mediterrani podrien estar indicant una major activitat urbana i industrial en aquesta zona comparat amb la de la costa de Sudàfrica. Tot i així, els OPEs s'utilitzen como FRs però també com a plastificants (Andresen et al., 2004) i per tant, la seva presència també pot estar lligada a l'alt nivell de residus plàstics en els mars i oceans (Andrade et al., 2021).

Hem calculat la relació entre els nivells d'OPEs i de PBDEs ($R_{OPEs/PBDEs}$), dividint la concentració d'OPEs entre la de PBDEs en el mateix individu. A la Taula 8 s'hi troben resumides les $R_{OPEs/PBDEs}$ per a cada lloc. Podem observar que els valors de la costa de Sudàfrica, amb una mitjana de 33,6±37,1, són més elevats que els de la costa catalana (5,23±6,61) i que la concentració a Sudàfrica d'OPEs és significativament superior a la de PBDEs (Kruskal-Wallis, p-valor<0,0001).

Taula 8. Any, espècie i $R_{OPEs/PBDEs}$ per les mostres de dofins de la Costa Catalana i de costa de Sudàfrica

Mar Mediterrani (Costa Catalana)			Oceà Índic (Costa de Sudàfrica)		
Any	Espècie	$R_{OPEs/PBDEs}$	Any	Espècie	$R_{OPEs/PBDEs}$
2014	*Stenella coureloalba*	19,4	2012	*Delphinus delphis*	20,1
2015	*Stenella coureloalba*	0,12	2012	*Delphinus delphis*	12,4
2015	*Stenella coureloalba*	0,71	2014	*Sousa plumbea*	16,3
2015	*Stenella coureloalba*	13,4	2014	*Sousa plumbea*	44,0
2016	*Stenella coureloalba*	0,49	2013	*Sousa plumbea*	21,3
2017	*Stenella coureloalba*	3,02	2015	*Sousa plumbea*	104
2017	*Stenella coureloalba*	1,16	2013	*Tursiops aduncus*	6,71
2017	*Stenella coureloalba*	6,17	2014	*Tursiops aduncus*	8,07
2017	*Stenella coureloalba*	2,62	2014	*Tursiops aduncus*	120
2017	*Stenella coureloalba*	1,09	2014	*Tursiops aduncus*	23,4
2017	*Stenella coureloalba*	0,66	2014	*Tursiops aduncus*	42,1
2018	*Stenella coureloalba*	13,9	2014	*Tursiops aduncus*	14,5
	Mitjana	5,23	2015	*Sousa plumbea*	3,31
	Mediana	1,89		Mitjana	33,6
	SD	6,61		Mediana	20,1
				SD	37,1

Aquests valors ens estarien indicant que la costa del sud-oest de l'Índic pateix un major impacte degut a la contaminació per plàstics. Si ens fixem en dades referents a aquesta contaminació veiem que a Sudàfrica, les estimacions de fuga de plàstic a les vies fluvials i als oceans són de 79.000 T, contribuint al 35% del total de les fugues de plàstic dins la regió d'Àfrica oriental i meridional (Pucino et al., 2020). Al voltant del 58% dels residus plàstics es gestionen malament i la crema de residus és una pràctica que encara es porta a terme, provocant grans quantitats de contaminació (Billard et al., 2021). Tal i com veiem representat en el mapa (Figura 18), les fuites de plàstic més importants de Sudàfrica tenen lloc a la província de KwaZulu-Natal, la costa de la qual es van obtenir les mostres de dofins analitzats. La zona urbana de KwaZulu-Natal, que també és la més propera a la costa, és la que més quantitat de residus genera: 6.677.966 T per l'any 2020. De les quals se'n gestiona un 71% i un 4% acaba arribant als mars i oceans. Pel que fa les zones rurals, els números no són iguals, tot i generar quasi la meitat de tones de residus (4.305.262 per l'any 2020), només un 4% és gestionat de manera adequada i hi ha una fuita de plàstics de fins el 8% (Billard et al., 2021). Si ajuntem les fuites de plàstic del 2020 d'ambdues zones, veiem que només de la província de KwaZulu-Natal van arribar 19.670,64 T, el 5,5% del total de residus generats, a l'Oceà Índic. A més, al ser un poble de costa, un dels motors econòmics principals és la pesca, i només d'aquesta activitat ja arriben 115 T al mar cada any (Figura 18). Les concentracions trobades al Mediterrani són molt inferiors que les de Sudàfrica, encara que tampoc són òptimes i també es creu que poden estar provocades per l'afectació de la contaminació marina, ja que és un dels mars més contaminats (García-Rivera et al., 2018a). Les zones més afectades per la contaminació són: el Mar d'Alboran, el Golf d'Alacant i les costes a prop de Barcelona, segurament relacionades amb les activitats pesqueres i industrials i les altes densitats poblacionals (Martín-Lara et al., 2021). Els residus plàstics generats per càpita anuals són 39 Kg/hab/any i arriben al mar 570 T/any dels quals 405 T/any provenen de les zones costeres, 34 vegades menys que a la costa de KwaZulu-Natal. Així doncs, sembla que els valors de $R_{OPEs/PBDEs}$ superiors a la costa de l'Índic, podrien estar relacionats a una major exposició als OPEs degut també a una major presència de residus plàstics marins en aquesta zona.

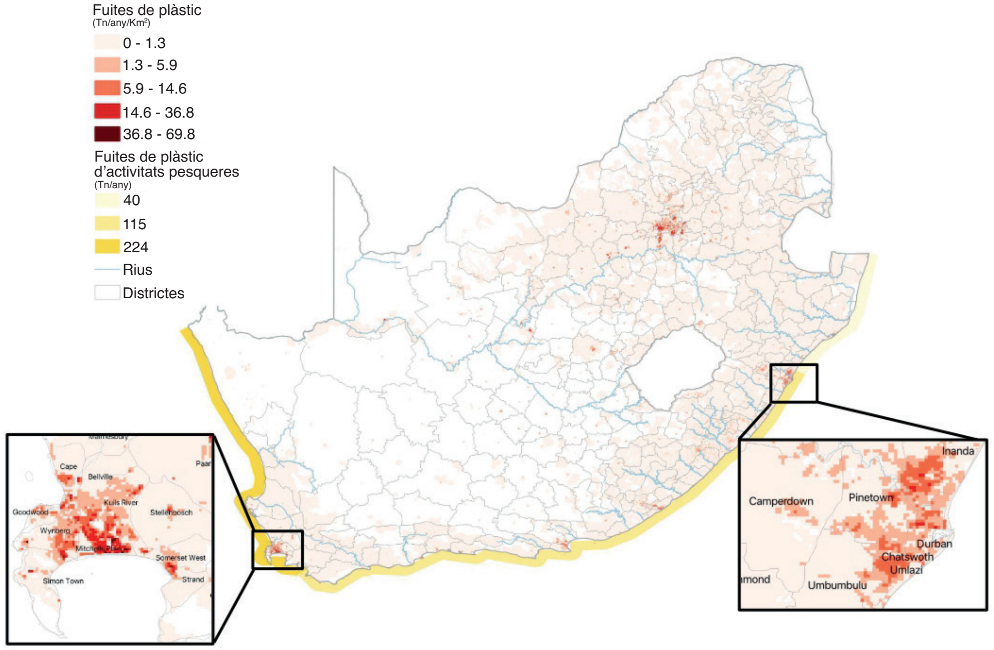

Figura 18. Zones costeres contaminades per plàstics a Sudàfrica. Adaptació de: (Billard et al., 2021)

Per altre banda, també hem volgut valorar els patrons d'OPEs observats en cada zona d'estudi. S'ha calculat el percentatge de contribució d'aquests en les mostres de les diferents localitzacions estudiades, partint dels valors mitjans de concentració obtinguts. Pels compostos que no s'han detectat (nd) o no s'han pogut quantificar (nq), hem utilitzat el valor de ½ LOD i de LOD respectivament. Pel que fa al Mar Mediterrani de la costa catalana, s'han agafat només les dades del període 2004-2018, ja que era l'interval de temps que més s'assemblava al mostrejat al Mar d'Alboran (2004-2010) i a la costa de Sudàfrica (2012-2015).

Fixant-nos en la Figura 19, veiem que les dues zones del Mar Mediterrani presenten un perfil molt més similar entre elles que no pas amb la de l'Índic. En el Mediterrani occidental hi predomina el TNBP, tant a la zona nord com a la sud, amb concentracions mitjanes de 3.366±8.687 ng/g lw per la costa catalana i 443±901 ng/g lw pel Mar d'Alboran, essent els valors de la costa catalana significativament superiors als del Mar d'Alboran (Kruskal-Wallis, p-valor = 0,0034). En aquestes zones també hi trobem presència de TBOEP, EHDPP i IDPP, tot i que en concentracions molt més baixes.

En canvi, a la costa de Sudàfrica s'hi han detectat altes concentracions de TBOEP, amb una mitjana de 8.067±9.506 ng/g lw. El segon compost més abundant en aquesta regió és e T2IPPP que també hi és present al Mar d'Alboran, amb concentracions mitjanes de 1.477±4.854 ng/g lw i 162±431 ng/g lw respectivament.

En definitiva, si analitzem el gràfic comparant els dos mars: Mediterrani i Oceà Índic, veiem que els perfils són diferents entre ells i una possible explicació podria ser la diferència de materials que es generen com a residu en cadascuna d'elles i els additius que se'ls hi aplica. En el cas del Mediterrani predomina el TNBP el qual s'utilitza principalment com a plastificant tot i que també com a agent antiespumant (Chokwe et al., 2020). Tant el EHDPP i el TBOEP, tot i que en menor mesura, són els següents OPEs que més trobem al Mediterrani occidental. En el cas de l'EHDPP s'utilitza només com a plastificant però el TBOEP també pot ser usat com a FR (Chokwe et al., 2020). Aquest últim, és el contaminant que mostra un impacte més rellevant a la costa de Sudàfrica, seguit pel T2IPPP utilitzat també com a plastificant i pel TEHP que es fa servir per ambdues coses. Així doncs, podríem pensar que el Mar Mediterrani tant per la costa catalana com pel mar d'Alboran està més contaminat per additius plastificants i en canvi, a la costa de Sudàfrica tant per additius plastificants com per FRs.

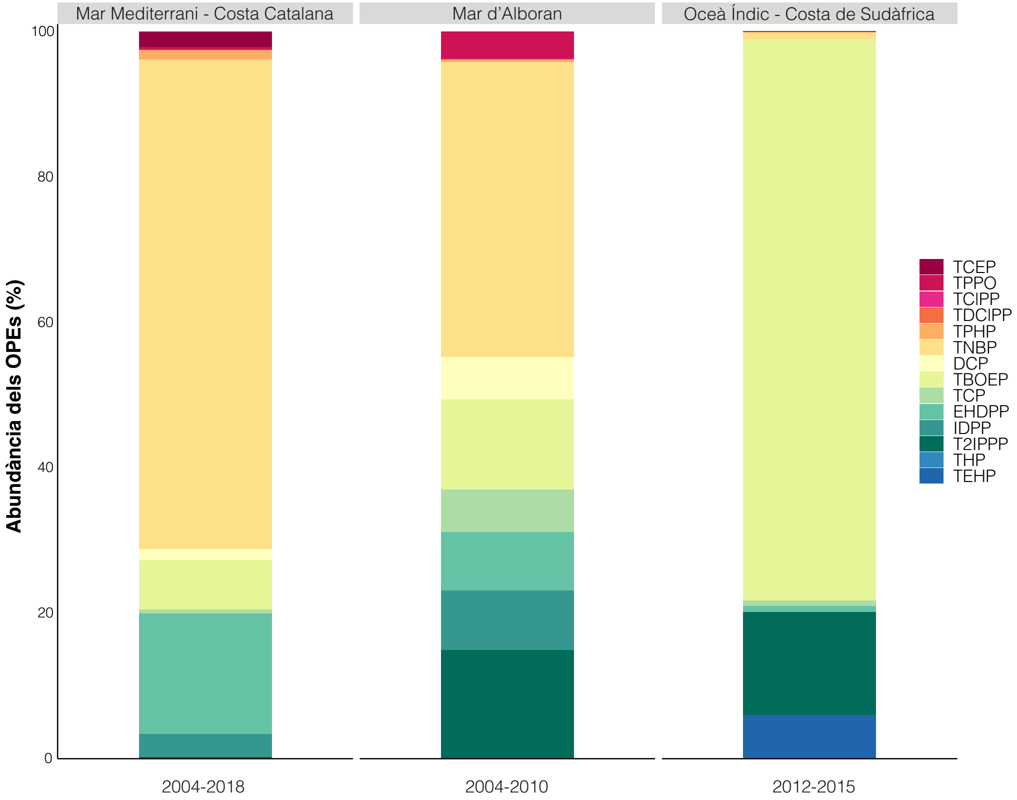

Figura 19. Abundància dels OPEs en % de cada zona estudia. nd = ½ LOD i nq = LOD

Tendències temporals dels OPEs

Al disposar de mostres recol·lectades durant diferents períodes pel Mar Mediterrani de la costa catalana, s'ha pogut dur a terme un anàlisi temporal dels OPEs trobats en aquesta zona. S'ha fet un anàlisi de les concentracions totals trobades per cada any des del 1990 fins el 2018 en els dofins i s'ha comparat amb els nivells de plàstics trobats en un estudi realitzat per García-Rivera et al. (2018b) a on es van recollir escombraries del fons marí del Mediterrani espanyol durant 11 anys (2007 al 2017). La quantitat total de residus marins trobada en aquella zona va ser de 2.197,8 kg i estava compost per plàstic (29,3%), clinker –roca sintètica– (28,4%), fusta (10,2%), metalls (9,7%) i vidre (6,2%). En el nostre cas només hem utilitzat les dades del plàstic i s'han representat les quantitats trobades en Kg en la Figura 20, a on també hi trobem representades les mitjanes de les concentracions d'OPEs (ng/g lw) trobades en les mostres de dofí llistat (*Stenella coeruleoalba*) des del 1990 fins el 2018. Cal tenir en compte que el número de mostres per cada any és molt variable ja que es tracta d'individus varats a les costes i és una dada que no podem controlar. Així que obtenir un nombre de mostres representatiu per cadascun dels anys és realment complicat.

A l'estudi dels dofins del Mar Mediterrani (*Publicació #3*), es va concloure que els PBDEs, utilitzats com a FRs, disminuïen fins un 60% des del 1990 al 2018, en canvi tal i com s'observa a la Figura 20, les concentracions d'OPEs es mantenen constants. La mateixa tendència segueixen els residus plàstics recollits per García-Rivera et al. (2018b) a la mateixa zona, a on les quantitats trobades (exceptuant el pic del 2007) s'han mantingut estables al llarg dels anys. Aquest fet ens reafirma la nostre hipòtesi de que l'aportació d'OPEs al medi marí va més enllà del seu ús com a FRs i que aquells que són utilitzats com a plastificants hi juguen un paper important. A més, dos dels OPEs predominants en el nostre estudi (TNBP, TBOEP) s'utilitzen principalment com a plastificants (Chokwe et al., 2020).

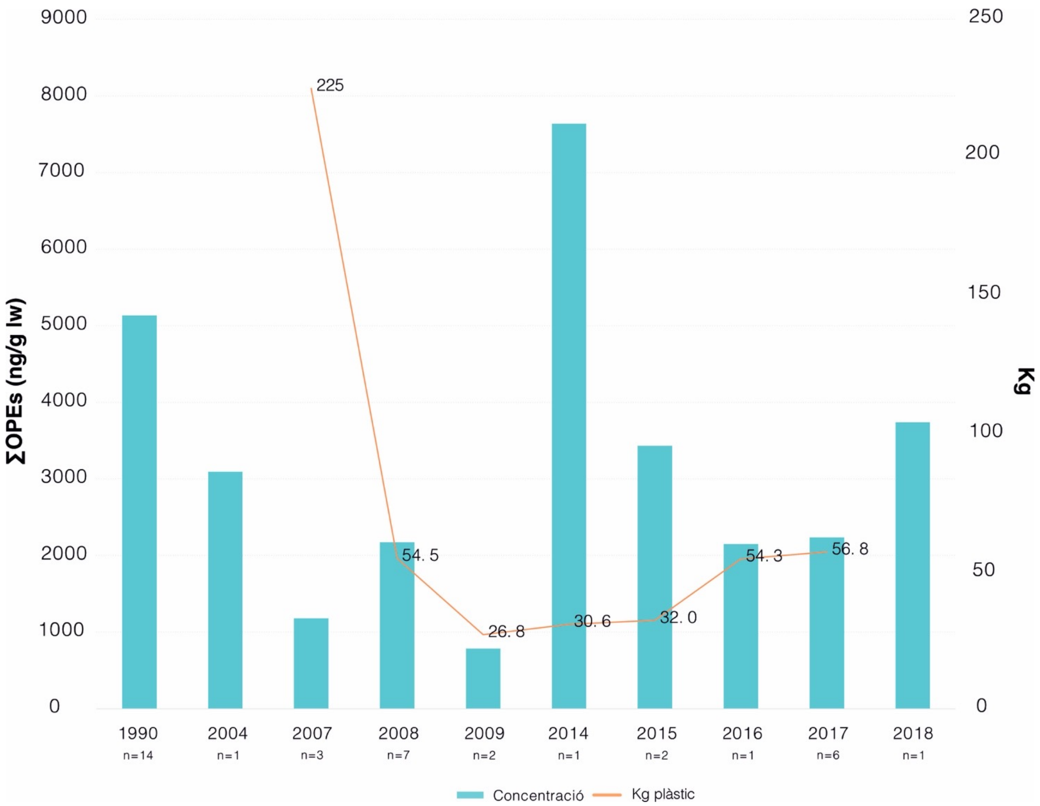

Figura 20. Concentracions mitjanes d'OPEs (eliminant dades atípiques) en dofins llistats (*Stenella coeruleoalba*) del Mar Mediterrani, expressades en ng/g lw, i Kg de plàstic trobats al fons marí del Mediterrani, en el període de temps des del 1990 fins el 2018. Dades dels Plàstics extretes de: García-Rivera et al. (2018b)

Un altre estudi que avalua la tendència temporal de contaminació per plàstics en el medi marí, és el presentat per Matsuguma et al. (2017), a on es va mirar la presència de microplàstics (<5 mm) en cores de sediments a Japó, Tailàndia, Malàisia i Sudàfrica, datats des del 1950 per les capes més profundes fins a la dècada dels 2000 per les capes superficials. En els cores de sediments més antics (fins els anys 80) es va veure que l'abundància de microplàstics augmentava. En canvi, pels cores de les capes superiors (més recents) la concentració per microplàstics es mantenia constant (Figura 21). Així doncs, tenint en compte que les dades d'aquest estudi coincideixen en temps amb les del nostre estudi temporal en OPEs, l'estabilització de la concentració de microplàstics podria ser una possible explicació del perquè tampoc es veu una tendència temporal creixent en l'estudi temporal dels OPEs en dofins (Figura 20).

A més de la presència en sediments, també són importants els nivells d'aquests contaminants en la columna d'aigua i en les aigües superficials. Tot i així, hi ha estudis que no veuen relació entre la concentració d'additius plàstics en les aigües superficials i l'abundància de partícules microplàstiques (Schmidt et al., 2021). Seguir investigant en aquesta línia és clau per poder esbrinar les vies reals d'entrada d'aquests contaminants en el medi marí, ja que les concentracions trobades en els dofins de les diferents zones no són gens menyspreables.

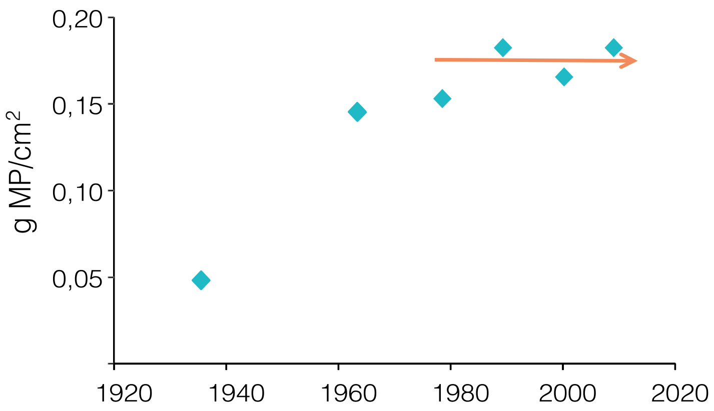

Figura 21. Anàlisi temporal de les concentracions de microplàstics (MP) en els core dels sediments analitzats per Matsuguma et al. (2017)

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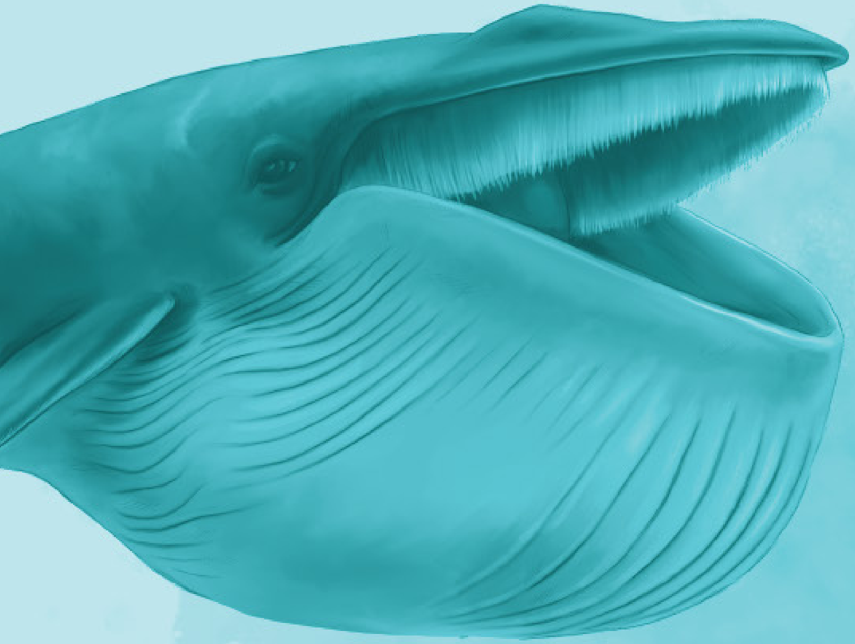

CAPÍTOL IV

BIOACUMULACIÓ, BIOMAGNIFICACIÓ I TRANSFERÈNCIA MATERNA D’OPEs EN BIOTA MARINA

BIOACUMULACIÓ, BIOMAGNIFICACIÓ I TRANSFERÈNCIA MATERNA D'OPES EN BIOTA MARINA

4

4.1 Introducció

L'ús dels OPEs a gran escala ha portat a la seva prevalença en el medi ambient, comportant impactes més amplis encara desconeguts (Xie et al., 2022). L'efecte que tenen aquests contaminants sobre la fauna marina encara s'està estudiant i hi ha arguments contradictoris sobre la seva bioacumulació i biomagnificació. Així doncs, en aquest *Capítol 4* de resultats s'ha volgut estudiar la capacitat de bioacumulació i de biomagnificació dels diferents OPEs en els organismes marins analitzats. També s'ha volgut avaluar altres possibles vies d'entrada dels OPEs, talment com a través de la ingesta de plàstic. A més, gràcies a disposar de mostres de balenes i els seus fetus s'ha pogut avaluar si es produeix transferència d'aquests contaminants de mares a fetus.

Les fonts d'entrada dels additius plàstics al medi marí en són vàries i una d'elles és la de contaminació per plàstics als mars i oceans, on la descomposició biofísica dels plàstics és prolongada (Derraik, 2002; Thompson et al., 2004), els efectes sobre la fauna són greus (Wilcox et al., 2015) i les opcions d'eliminació són molt limitades (Jambeck et al., 2015). Els efectes de l'enredament i la ingesta de restes de plàstics està molt més documentada que els efectes tòxics tot i afectar a centenars d'espècies (Worm et al., 2017). Avui en dia, encara hi ha algunes llacunes sobre el coneixement al voltant de les vies que segueixen els residus plàstics i els seus contaminants associats, que poden anar des de les aigües superficials fins als hàbitats d'aigua més profunda i els sediments, i també al voltant de la forma en que aquests contaminants es mouen a través de les xarxes tròfiques. Així doncs, s'ha volgut estudiar l'origen dels additius químics associats als plàstics acumulats en els teixits de la fauna marina, avaluant les diferents vies d'exposició, des de la contaminació de l'aigua i de la xarxa tròfica, fins a la transferència d'additius als organismes marins a través de la ingesta de residus plàstics marins.

En aquest capítol es volia avaluar la capacitat dels OPEs per acumular-se en diferents nivells tròfics tant en les preses com en els seus predadors. Tot i que la informació sobre la bioacumulació i biomagnificació ja està ben documentada pels PBDEs, no hi ha gairebé informació sobre els OPEs. Per aquest motiu tots els estudis d'aquest apartat s'ha analitzat els OPEs en diferents espècies amb dietes diferent per així poder veure quin és el seu impacte. De la mateixa manera, i encara que per PBDEs ja està estudiat, s'ha volgut realitzar un estudi per valorar la transferència mare-fetus dels OPEs. Degut a que l'obtenció d'aquest tipus de mostres és realment complicat, hem aprofitat la disponibilitat per portar a terme l'avaluació de la transferència, no només dels OPEs, sinó que també d'altres FRs i plastificants com són els EFRs i les SCCPs.

4.2 Metodologia

Mostreig

En aquesta capítol s'han analitzat diferents espècies de fauna marina, entre les quals hi trobem una espècie de balena, una de tortuga marina i tres espècies de peixos. A més, també s'ha analitzat la dieta dels següents animals:

- Balena, i la seva dieta principal, krill, a la zona d'Islàndia.
- Tortugues, i la seva dieta principal, calamars, meduses i sardines, a la zona de les Illes Balears.

- Lluç, i la seva dieta principal, sardines i anxoves, a la zona del Mediterrani occidental.

Les espècies estudiades viuen en diferents hàbitats i presenten característiques diverses, a continuació es fa un breu resum de cadascuna d'elles:

- Rorqual comú *(Balaenoptera physalus*; Linnaeus, 1758): Aquesta espècie de mamífer marí és un animal pelàgic que viu majoritàriament en aigües oceàniques tot i que també el podem trobar més a prop de la costa, a plataformes continentals. Habiten principalment en oceans de zones temperades i polars. La seva longitud pot arribar als 18 m de llargada tot i que les femelles poden arribar als 19 m i poden pesar fins a 90 T. La seva alimentació es basa en petits invertebrats com el krill, peixos petits i calamars.

- Krill (*Meganyctiphanes norvegica*; Sars, 1856): El krill són petits crustacis que es troben a les zones costeres dels mars i oceans d'arreu del planeta. Es distribueixen a tots els oceans del món i desenvolupen un paper essencial dins de la cadena tròfica de l'ecosistema oceànic. L'espècie *Meganycti phanes norvegica* és coneguda com el krill Atlàntic o krill del nord i ocupa les aigües de la plataforma i del talús de les costes occidentals i orientals de l'Atlàntic nord. És l'espècie més gran de les 86 espècies descrites de Euphausiacea, podent arribar a una mida total d'entre 40 i 50 mm per un individu adult. S'alimenta principalment per filtració de fitoplàncton i zooplàncton.

- Tortuga babaua (*Caretta caretta*; Linnaeus, 1758): És una espècie de tortuga que, depenent del seu cicle de vida, la podem trobar a gairebé tots els mars i oceans càlids del planeta: a l'Oceà Atlàntic, al Pacífic, a l'Índic i al Mar Mediterrani. Es distribueix tant en zones de mar obert, com zones més properes a la costa, així com estuaris i badies. Es tracta de la tortuga marina més petita i abundant del Mediterrani, tot i que pot arribar a mesurar 120 cm i a pesar 120 Kg. La seva dieta és molt variada ja que són omnívores, mengen des d'algues, coralls i esponges fins a peixos petits, meduses, crustacis, gasteròpodes, etc.

- Calamar (*Loligo vulgaris*; Lamarck, 1798): El calamar és un cefalòpode que es troba a prop de la costa i viu entre els 100 m (o més) de fondària durant el dia i a prop de la superfície durant la nit. Es distribueix per tot l'Atlàntic oriental, des de les Illes Britàniques fins a Angola, així com per tot el Mediterrani. Es tracta d'un animal nocturn que s'alimenta de peixos, crustacis i fins i tot d'altres cefalòpodes. Viuen en grup o també poden arribar a formar petits bancs. La seva longitud és d'entre 15 i 30 cm tot i que poden arribar als 60 cm.

- Medusa luminiscent *(Pelagia noctiluca*; Forsskal, 1775): La medusa luminescent s'anomena així ja que és fluorescent en la foscor. Es tracta d'una espècie de cnidari que es troba tant a la costa de l'oceà Atlàntic com del Mar Mediterrani, ocupant preferentment hàbitats pelàgics tot i que poden formar masses de meduses que poden arribar a la línia de costa. La mida de la seva ombrel·la pot arribar als 10 cm i els tentacles poden arribar a fer 1 m. S'alimenta principalment d'altres petites meduses i també d'ascidis.

- Lluç Europeu (*Merluccius merluccius*; Linnaeus, 1758): Aquesta és una espècie que presenta una àmplia distribució geogràfica i que viu entre 30 i 1000 m de profunditat. Habita principalment les costes de l'Oceà Atlàntic Nord-oriental, des d'Islàndia fins a Mauritània, i al Mar Mediterrani. Poden créixer fins a 180 cm i la seva dieta varia segons la mida de l'individu, dominant els crustacis a la dieta dels individus juvenils petits i els clupèids, com les sardines i les anxoves, en els individus juvenils i adults de mida més gran.

- Anxova Europea (*Engraulis encrasicolus*; Linnaeus, 1758): L'anxova europea és un peix de la família dels clupèids, que es distribueix per tot el Mediterrani i la costa atlàntica oriental, des de Noruega fins a Angola. La trobem més aviat a les zones costeres i pot habitar àrees de condicions ambientals molt diverses. Es tracta d'una espècie pelàgica que forma grans bancs, principalment per reproduir-se i alimentar-se. Pot arribar a mesurar un màxim d'entre 18 i 20 cm. Durant el dia, s'alimenten del zooplàncton que es troba a 40-60 m de profunditat, majoritàriament de copèpodes (crustacis). Tot i així, s'ha vist que esporàdicament durant la nit es poden arribar a alimentar d'espècies de crustaci més grans, com les larves de decàpodes i misidacis.

- Sardina Europea (*Sardina pilchardus*; Walbaum, 1792): Les sardines també són peixos clupèids i es troben distribuïdes per sobre la plataforma continental de l'oceà Atlàntic oriental, des del Mar del Nord fins a Senegal i en el Mar Mediterrani. És una espècie pelàgica formadora de bancs. La seva longitud pot ser de fins a 25 cm. És filtradora i s'alimenta de plàncton i zooplàncton, principalment de copèpodes tot i que en les zones de més al sud consumeixen preses més grans com eufausiacis. Les sardines es troben als nivells inferiors de la xarxa tròfica marina.

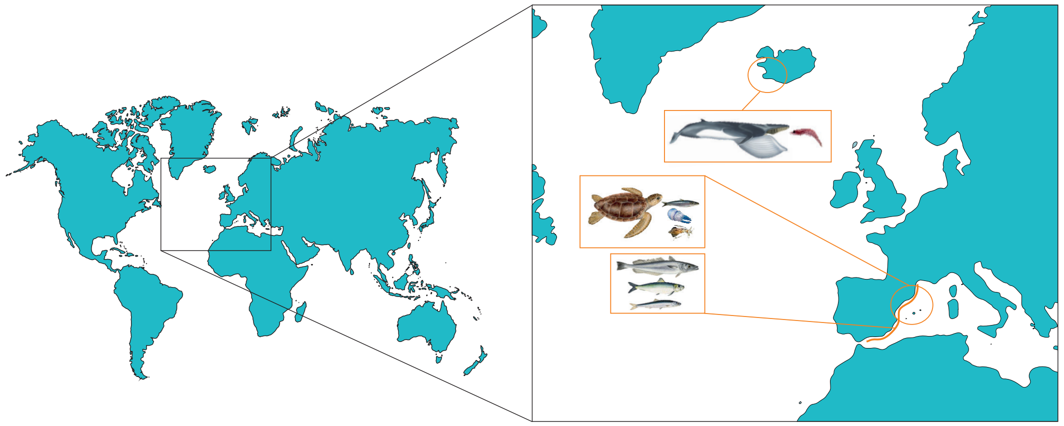

Figura 22. Mapa dels punts de mostreig de les diferents espècies estudiades

Les mostres de rorqual comú i les de krill, es van aconseguir a través d'una companyia Balenera (Hvalur H/F) que col·labora amb un centre d'investigació d'Islàndia i proporciona les mostres a la Universitat de Barcelona amb la qual hem col·laborat per dur a terme aquest estudi. De la mateixa manera també es van aconseguir les mostres de les balenes embarassades, on es va mostrejar el greix de les mares i la pell dels fetus.

Pel que fa a les mostres de tortuga babaua, van ser avaraments de la costa catalana i les Illes Balears que va dur a terme l'Institut de Recerca de la Biodiversitat (IRBio) de la UB, així com les mostres de dieta de les tortugues de les Illes Balears (calamar, medusa i sardina). En aquest mateix estudi, també es van analitzar mostres de residus plàstics recollides en la mateixa zona de mostreig i pel mateix Institut.

Les mostres de sardina, anxova i lluç de la costa del Mediterrani occidental, es van aconseguir a partir dels projectes MEDITS 2019 Survey (MEDiterranean International bottom Trawl Survey) i PELCAT (Declivi dels petits pelàgics al mar català: rol potencial del canvi en el plàncton i la contaminació) financiat per la Generalitat de Catalunya, en el qual hi ha participat l'Institut de Ciències del Mar (ICM) amb els que hem col·laborat per dur a terme l'anàlisi en aquestes espècies. Les espècies analitzades en aquest estudi són de consum humà i per tant es va poder dur a terme també un estudi d'afectació dels OPEs en la dieta en humans, a partir del càlcul de consum de peix diari.

Anàlisi químic

Mètode en biota pels OPEs

Pels OPEs s'ha utilitzat el mètode de Giulivo et al. (2016) també optimitzat prèviament al laboratori. En aquest darrer mètode, es van optimitzar un total de 14 OPEs, mentre que en aquesta tesi se n'han analitzat 19. Així doncs, s'ha ampliat el mètode amb cinc compostos nous, aquests són: el TEP, el TPP, el 2IPPDPP, el 4IPPDPP i el B4IPPDPP. A més a més, s'han inclòs també dos nous patrons, el d_{15}-TEP i el d_{21}-TPP. A la Taula 9 hi consten els paràmetres instrumentals optimitzats per a cada compost nou.

Taula 9. Paràmetres instrumentals TFC-LC-MS-MS: Temps de retenció (Rt), transicions (SMR1 i SMR2), potencial de *declustering* (DP) i energia de col·lisió (CE1 i CE2) per cada analit d'OPE optimitzat

Analit	Rt (min)	SRM_1/SRM_2	DP (V)	CE_1/CE_2 (eV)
TEP	4,53	183→99	36	35
		183→81		19
d_{15}-TEP	4,50	198→102	44	29
		198→83		40
TPP	9,51	225→99	38	37
		225→81		26
d_{21}-TPP	9,60	246→102	44	42
		246→83		29
2IPPDPP	14,0	369→327	77	39
		369→152		19
4IPPDPP	14,5	369→327	86	39
		369→152		20
B4IPPDPP	17,5	411→327	87	44
		411→152		24

Es van calcular els LODs i LOQs instrumentals (iLOD i iLOQ), amb valors que van des de 0,09 a 0,49 pg injectats pels iLOD i de 0,17 a 1,64 pg injectats pels iLOQ (Taula 10). Aquesta valors de sensibilitat són similars als obtinguts pels 14 OPEs del mètode original que van des de 0,01 a 1,56 pg injectats els iLOQ i de 0,02 a 3,43 pg injectats els iLOQ.

Una vegada es va optimitzar el mètode instrumental, es va verificar el bon funcionament de les etapes d'extracció i purificació online, mitjançant un test de recuperacions en els que es van afegir els OPEs en estudi a nivells de concentració de 200 ng/g dw. A continuació, es mostren els resultats obtinguts de recuperació, LODs i LOQs del mètode (mLODs i mLOQs) (Taula 10).

Taula 10. Paràmetres de qualitat del mètode instrumental i del mètode d'anàlisi en mostres de biota, mitjançant LC-MS-MS pels OPEs seleccionats.

Analit	Instrumental (pg injectats)		Mètode			
			(%)		(ng/g lw)	
	iLOD	iLOQ	Recuperació	RSD	mLOD	mLOQ
TEP	0,15	0,50	59	15	5,52	18,4
TPP	0,09	0,31	40	16	0,60	2,00
2IPPDPP	0,48	1,58	42	2,9	0,36	1,19
4IPPDPP	0,49	1,64	41	6,7	0,63	2,09
B4IPPPP	0,26	0,17	61	5,1	2,16	7,20

Els valors dels paràmetres analítics dels nous 5 OPEs optimitzats, es troben en el mateix ordre de magnitud que els calculats per Giulivo et al. (2016). La recuperació de tots es troba alguns valors per sota dels optimitzats en el mètode dels 14 compostos. Tant el TPP, com el 2IPPDPP i el 4IPPDPP presenten valors al límit d'acceptabilitat. No obstant, gràcies a que hem inclòs també dos nous patrons interns, el d15-TEP i el d21-TPP, les possibles pèrdues de recuperació queden automàticament corregides. Pel que fa el TEP, tot i tenir valors acceptables de recuperació, presenta valors més alts que els altres de LOD i LOQ.

Per tots els estudis s'ha valorat la presència d'OPEs. A més, per l'estudi de transferència mare-fetus també es van incloure els EFRs i les SCCPs. Pel que fa a l'anàlisi de EFRs, ja ha quedat descrit en el capítol anterior (*Capítol 3*). No obstant, a continuació es procedeix a explicar el treball desenvolupat en el que es refereix a la metodologia analítica emprada per les SCCPs.

Mètode en biota de les SCCPs

El mètode de SCCPs en biota que s'ha utilitzat es basa en l'adaptació de la Norma UNE-EN ISO 12010, norma espanyola per a la "*Determinación de alcanos policlorados de cadena corta (SCCP) en agua. Método por cromatografía de gases-espectrometría de masas (GC-MS) con ionización química negativa (NCI)*", del desembre del 2014 (UNE-EN ISO 12010, 2020), que es basa en una quantificació mitjançant una regressió lineal múltiple.

La Taula 11 recull la informació relativa a les solucions patró necessàries per poder dur a terme la determinació de les SCCPs. El Mix 1 conté un contingut en clor del 49%, el Mix 3 del 66,7% i el Mix 4 del 56%. D'aquesta manera, amb aquestes 3 solucions patró abastem un ampli rang de contingut en clor. Al laboratori també disposàvem d'altres solucions patró que ens servíran per tal de verificar l'aplicabilitat del mètode desenvolupat. El contingut en clor d'aquestes solucions es mou entre el 51,5 i el 63%.

Taula 11. Informació sobre les solucions patró utilitzades (natius i patró intern)

Solucions patró		Concentració	Dissolvent
Cloroparafina C10-C13 Mix 1	Hordalub 17	10 ng/μL	Ciclohexà
Cloroparafina C10-C13 Mix 3	Cereclor 70	10 ng/μL	Ciclohexà
Cloroparafina C10-C13 Mix 4	Hordalub 80	10 ng/μL	Ciclohexà
C10-C13 51.5% Cl	SCCP 51.5%	10 ng/μL	Ciclohexà
C10-C13 55.5% Cl	SCCP 55.5%	10 ng/μL	Ciclohexà
C10-C13 63% Cl	SCCP 63%	10 ng/μL	Ciclohexà
1,1,1,3,10,11-Hexachloroundecane	IS	100 ng/μL	Acetona

En primer lloc, es va desenvolupar el mètode d'anàlisi instrumental, així com el mètode de quantificació:

Condicions instrumentals

Les SCCPs s'analitzen per GC-NCI-MS. L'instrument que s'ha utilitzat és un Agilent 6890 GC connectat a un Agilent 5973 Network MS.

Condicions del GC

Es treballa amb una columna capil·lar DB5ms de 15 m, 0,25 mm de diàmetre interior i 0,1 μm d'espessor de la fase. Altres condicions del GC són les següents:

- Temperatura d'injecció: 100ºC
- Injecció: Polsat sense divisió de flux (*Pulse splitless*); temps de *splitless* de 0,5 min
- Volum d'injecció: 2 μL
- Programa de temperatura: 100ºC, 2min; a 70ºC/min fins 280ºC, 2,5min; a 70ºC/min fins a 320ºC, 7min. Temps total de 15 min

Condicions del MS

El mètode de ionització emprat per l'anàlisi de les SCCPs és la NCI, utilitzant metà com a gas de ionització, a un flux de 5 mL/min. Altres condicions del MS són les següents:

- Temperatura de la línia de transferència: 280ºC
- Temperatura de la font de ionització: 150ºC
- Temperatura del quadrupol: 150ºC
- Baixa resolució, 0,9 unitats de massa

Es treballa en mode SIM, monitoritzant les masses *m/z* 327, 375, 409 i 423 per les SCCPs, i la massa *m/z* 364 pel patró intern, l'1,1,1,3,10,11-hexaclorundecà. A la Figura 23 es mostren els cromatogrames obtinguts per les diferents solucions patró a una concentració de 0,1 ng/μL mitjançant aquestes condicions experimentals.

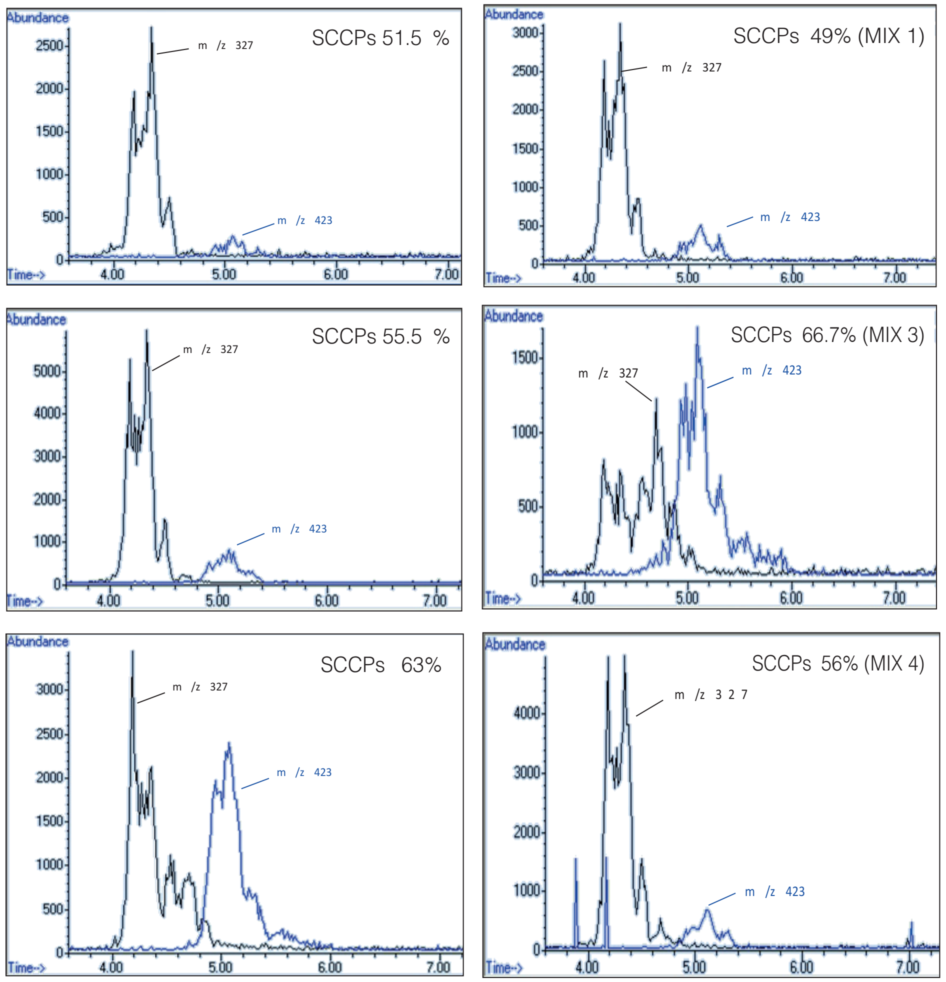

Figura 23. Cromatogrames obtinguts per les 6 diferents solucions patró de SCCPs a una concentració de 0.1 ng/μL.

Mètode quantitatiu mitjançant una regressió lineal múltiple

Es va utilitzar un mètode quantitatiu basat en la regressió lineal múltiple, ja que s'ha vist que aquest mètode comporta un menor error en la quantificació d'aquestes mescles complexes. Aquest model es va utilitzar seguint l'equació següent (Geiß et al., 2010):

$CSCCP_{sum} = b_0 + b_1A_{m1} + b_2A_{m2} + \ldots$

$CSCCP_{sum}$: concentració de la suma de SCCPs

b_0 : intercepció

b_1 , b_2 : coeficients de regressió en µg/mL

A_{m1} , A_{m2} : àrees dels pics de les masses m_1 i m_2

Tal i com indica la Norma UNE-EN ISO 12010, el calibratge s'ha de realitzar a partir de, com a mínim, 3 mescles patrons a 3 nivells de concentració diferents. En el nostre cas, hem utilitzat el MIX1, MIX3 i MIX4, i hem preparat 5 nivells de concentració de cadascuna. La Taula 12 mostra les solucions de calibratge que s'han preparat i s'han utilitzats per dur a terme el calibratge mitjançant una regressió lineal múltiple.

Taula 12. Solucions de calibratge utilitzades per la regressió múltiple lineal

Mescla	Hordalub 17 (MIX1)	Cereclor 80 (MIX3)	Hordalub 80 (MIX4)	Patró intern (1,1,1,3,10,11-Hexacloroundecà)
Suma de SCCPs ng/µL	ng/µL	ng/µL	ng/µL	ng/µL
0,15*	0,15			0,1
0,15*		0,15		0,1
0,15*			0,15	0,1
0,3	0,3			0,1
0,3		0,3		0,1
0,3			0,3	0,1
0,6*	0,6			0,1
0,6*		0,6		0,1
0,6*			0,6	0,1
1*	1			0,1
1*		1		0,1
1*			1	0,1
5	5			0,1
5		5		0,1
5			5	0,1

* Nivells de concentració proposats a la Norma UNE-EN ISO 12010

Un cop preparades i injectades aquestes solucions de calibratge, es realitzen diferents regressions múltiples lineals, utilitzant les diferents masses monitoritzades. Val a dir que segons la Norma UNE-EN ISO 12010, la quantificació s'ha de realitzar utilitzant les masses *m/z* 327 i 423, ja que aquesta combinació és la que produeix resultats més precisos. La Taula 13 mostra els resultats obtinguts per cadascuna de les combinacions, utilitzant els 3 nivells de concentració proposats a la Norma UNE-EN ISO 12010 (9 punts) més els 2 nivells de concentració preparats per nosaltres amb un total de 15 punts. Com es pot apreciar, en tots els casos s'obtenen coeficients de correlació superiors a 0,98.

Taula 13. Paràmetres de les regressions múltiples lineals

Recta amb 15 punts			
	***m/z* 327 i 423**	***m/z* 375 i 423**	***m/z* 327 i 409**
Coeficient de correlació	0,989	0,981	0,984
Variable X1	0,445	0,662	0,439
Variable X2	0,340	0,195	0,201
Error típic	0,289	0,389	0,358

També s'ha avaluat si es compleixen tots els criteris d'identificació que estableix la Norma UNE-EN ISO 12010, que són els següents:

- El perfil cromatogràfic es troba dins de la finestra específica de temps de retenció establerta.

Com es pot observar, es compleix el requisit establert segons el qual el perfil cromatogràfic, és a dir, la major part les SCCPs pel fragment mesurat es troba dins de la finestra específica de temps de retenció següents:

Taula 14. Temps de retenció per a cada *m/z*

Valors *m/z*	Rang aproximat de tR (min)	Rang aproximat de tR màxim* (min)
327	4,0 a 5,0	4,2 a 4,5
375	4,5 a 5,5	4,6 a 5,0
409	4,7 a 5,4	4,8 a 5,2
423	4,5 a 6,0	4,9 a 5,2

*Rang corresponent a la major part les SCCPs pel fragment mig. Està representat per una mescla complexa de pics no resolts.

- El resultat calculat basat en les masses *m/z* 327 i 409 no ha de diferir en més d'un ±50% del resultat basat en les masses *m/z* 327 i 423.

- El resultat calculat basat en les masses *m/z* 375 i 423 no ha de diferir en més d'un ±60% del resultat basat en les masses *m/z* 327 i 423.

Aquests dos criteris si que es compleixen, tal i com queda reflectit en els resultats obtinguts mostrats a la Taula 15.

Taula 15. Càlcul de les desviacions obtingudes en el càlcul de les concentracions (expressades en ng/µL) de les solucins patrons, mitjançant les regressions múltiples lineals establertes utilitzant les masses *m/z* 327 i 423, 327 i 409, i 375 i 423

Recta amb 15 punts					
	Concentració *m/z* 327 i 423	Concentració *m/z* 327 i 409	% desviació	Concentració *m/z* 375 i 423	% desviació
R1	8,383	7,696	8	7,129	14
R2	9,177	8,191	11	6,616	28
R3	9,432	7,903	16	6,290	33
R4	7,757	6,942	8	6,728	11

Avaluació de les recuperacions

Una vegada avaluat l'anàlisi instrumental, així com el mètode quantitatiu, procedim a l'avaluació de les recuperacions dels SCCPs amb el mètode d'extracció i purificació utilitzat, que és el mateix que l'utilitzat per determinar els PBDEs (Barón et al., 2014). Altres estudis com el de Sun et al. (2016) també han fet petites adaptacions per extreure les SCCPs en biota de mètodes propis desenvolupats per compostos com els PBDEs, entre d'altres contaminants (R. X. Sun et al., 2015a, 2015b).

Es van afegir 600 ng totals (200 ng de cada MIX) de solucions de patrons natius (SCCP 51,5%, SCCP 55,5% i SCCP 63%) en 1g de mostra liofilitzada de múscul de rorqual comú (*Balaenoptera physalus*). Es va dur a terme una extracció per ASE amb hexà:DCM (1:1), seguida d'un tractament àcid per eliminar la matèria orgànica amb H_2SO_4 i posteriorment una purificació amb SPE utilitzant cartutxos d'alumina neutra (Al-N) i realitzant l'elució amb hexà:DCM (1:2). Finalment, s'evapora l'extracte sota una corrent de nitrogen fins a sequedat i es reconstitueix amb toluè, afegint 20 ng de patró intern marcat (1,1,1,3,10,11-hexaclorundecà), per poder dur a terme l'anàlisi instrumental amb un GC-MS-MS (EI) (Barón et al., 2014).

Es duen a terme 4 rèpliques diferents a on se'ls hi afegeix el patró natiu i una cinquena en la que no s'hi afegeix natiu ja que és la que s'utilitzarà com a blanc per saber les concentracions reals de SCCPs de la matriu.

Els resultats de recuperacions obtinguts, calculats amb la recta de regressió lineal múltiple de 15 punts, són de 69, 73 , 75 i 59%, amb una mitjana de 74% i un RSD del 9,62%, considerats uns resultats de recuperació i reproductibilitat acceptables.

Avaluació de la sensibilitat

Una vegada comprovades les recuperacions satisfactòries, s'han calculat els LOD i LOQ del mètode. I s'han calculat a partir dels ions *m/z* 327 i 423 ja que aquest són els ions que s'han d'utilitzar per fer la quantificació, segons la Norma UNE-EN ISO 12010. Pel que fa al ió *m/z* 327, obtenim valors de LOD i LOQ de 0,77 ng/g lw i 2.58 ng/g lw, respectivament. I pel que fa al ió m/z 423, els valors de LOD és de 0,46 ng/g lw i els de LOQ, de 1,55 ng/g lw.

4.3 Resultats

Els resultats es troben recollits en quatre articles, tres dels quals estan publicats i un d'enviat:

- Publicació #4: Garcia-Garin, O., **Sala, B.**, Aguilar, A., Vighi, M., Víkingsson, G.A., Chosson, V., Eljarrat, E., Borrell, A., 2020. Organophosphate contaminants in North Atlantic fin whales. Sci. Total Environ. 721, 137768. https://doi.org/10.1016/j.scitotenv.2020.137768
- Publicació #5: **Sala, B.**, Balasch, A., Eljarrat, E., Cardona, L., 2021. First study on the presence of plastic additives in loggerhead sea turtles (Caretta caretta) from the Mediterranean Sea. Environ. Pollut. 283, 117108. https://doi.org/10.1016/j.envpol.2021.117108
- Publicació #6: **Sala, B.**, Giménez, J., Fernández-Arribas, J., Bravo, C., Lloret-Lloret, E., Esteban, A., Bellido, J.M., Coll, M., Eljarrat, E., 2022. Organophosphate ester plasticizers in edible fish from the Mediterranean Sea: Marine pollution and human exposure. Environ. Pollut. 292. https://doi.org/10.1016/j.envpol.2021.118377
- Publicació #7: **Sala, B**., Garcia-Garin, O., Borrell, A., Aguilar, A., Víkingsson, G.A., Eljarrat, E., 2022 Transplacental transfer of plasticizers and flame retardants in fin whales (Balaenoptera physalus) from the North Atlantic Ocean. Enviada a Environmental Pollution (11 d'abril 2022).

PUBLICACIÓ CIENTÍFICA #4

“Organophosphate contaminants in North Atlantic fin whales”

Garcia-Garin, O., **Sala, B.**, Aguilar, A., Vighi, M., Víkingsson, G.A., Chosson, V., Eljarrat, E., Borrell, A.

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Organophosphate contaminants in North Atlantic fin whales

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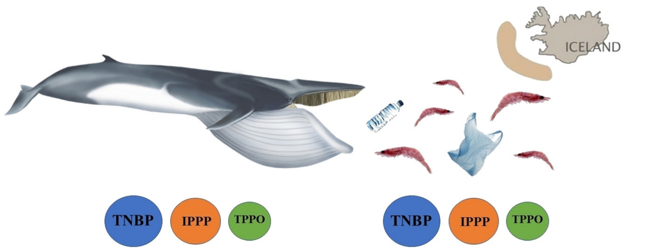
HIGHLIGHTS

- We provide the first evidence of OPFR presence in the tissues of fin whales.
- OPFRs were detected in the fin whale main prey, the krill *Meganyctiphanes norvegica*.
- TNBP, IPPP and TPPO are the most abundant OPFRs in both fin whales and krill.
- OPFRs do not seem to bioaccumulate or biomagnify.

GRAPHICAL ABSTRACT



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ABSTRACT

Pollution of the marine environment by litter composed of plastics is a growing concern. Chemical additives such as organophosphate flame retardants (OPFRs), which are added to plastics to improve their qualities, are in focus because they allegedly cause adverse effects on marine fauna. Here we analyse OPFR levels in the muscle of fin whales because, as a mysticete, this cetacean obtains its food by filter-feeding and is thus highly vulnerable to marine litter. Moreover, the fin whale performs long-range migrations from low-latitude areas in winter to high-latitude areas in summer, a trait that makes it a potentially good large-scale biomonitor of pollution. We also analyse OPFR levels in its main prey, the krill *Meganyctiphanes norvegica*, to assess transfer through diet. The samples analysed consisted of muscle tissue from 20 fin whales and whole-body homogenates of 10 krill samples, all collected off West Iceland. From the 19 OPFRs analysed, we detected 7 in the fin whale and 5 in the krill samples. Tri-n-butyl phosphate (TNBP), Isopropylated triphenyl phosphate (IPPP) and Triphenylphosphine oxide (TPPO) were the most abundant compounds found in both species. Mean $\sum$OPFR concentration, expressed on a lipid weight basis, was 985 (SD = 2239) ng g^{-1} in fin whale muscle, and 949 (SD = 1090) ng g^{-1} in krill homogenates. These results constitute the first evidence of the presence of OPFRs in the tissues of fin whales. Furthermore, they seem to support the non-significance of bioaccumulation of OPFRs through lifespan and of biomagnification trough the food web.

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1. Introduction

Marine litter, and particularly that composed of plastics, is increasing in oceans worldwide (*e.g.*, Lebreton et al., 2018). The physical adverse effects of this type of pollution on marine fauna are well known and include entanglement, suffocation, and obstruction of the digestive system (*e.g.*, De Stephanis et al., 2013; Di-Méglio and Campana, 2017). However, chemical effects related with the ingestion of plastics have been mostly associated with the toxicity of plasticizers and are still under assessment (Hermabessiere et al., 2017). Among the constituents of plastics, polybrominated diphenyl ethers (PBDEs), phthalates and organophosphate flame retardants (OPFRs) have been claimed to induce organic damage to marine fauna (Aznar-Alemany et al., 2019; Fossi et al., 2016). PBDEs were banned by the Stockholm Convention in 2009 (Stockholm-Convention, 2010) and, since then, the production and use of OPFRs as alternative flame retardants and plasticizers has dramatically increased (Pantelaki and Voutsa, 2019).

The effects of exposure to OPFRs are poorly understood, although some of these compounds, such as tri-n-butyl phosphate (TNBP) and tris(phenyl) phosphate (TPHP), have been observed to cause developmental neurotoxicity and to have endocrine, carcinogenic and adverse reproductive effects in experimental animals (Bruchajzer et al., 2015; van der Veen and de Boer, 2012). Toxicological studies on diphenylcresyl phosphate (DCP), another OPFR which is also used as flame retardant and plasticizer (van der Veen and de Boer, 2012), have elicited concern for the aquatic toxicity of this compound (Washington State, 2006). Sigma-Aldrich (2019) also pointed its potentially toxic impact on aquatic organisms because of alleged effects on reproduction and development (Washington State, 2006). Moreover, Chlorinated OPFRs are suspected carcinogens. In rats and mice they have been found to induce tumour growth in kidneys, liver and thyroid apparently associated to exposition to Tris(chloroethyl) phosphate (TCEP) and Tris(chloroisopropyl) phosphate (TCIPP), and to have similar effects in brain and testes, this time associated to exposition to Tris (1,3-dichloro-2-propyl) phosphate (TDCPP) (van der Veen and de Boer, 2012).

The occurrence of OPFRs has been investigated in detail in humans, air, sediment, freshwater and some terrestrial biota (Hou et al., 2016; Pantelaki and Voutsa, 2019), but there are few reports on the occurrence of OPFRs in the marine environment and, particularly, in marine organisms. Some studies have focused on OPFR occurrence in cetaceans (*e.g.*, Aznar-Alemany et al., 2019; Sala et al., 2019) although the scope of species analysed has been restricted to dolphins, which are top predators and often have a limited distribution range. OPFR occurrence in the long-ranging oceanic mysticetes, or baleen whales, has not so far been reported despite they obtain their food by filtering out small organisms from the water, a feature that makes them highly vulnerable to the ingestion of marine plastics.

The fin whale (*Balaenoptera physalus*; Linnaeus, 1758) is a cosmopolitan mysticete that performs annual migrations from low-latitude breeding areas in winter to high-latitude feeding areas in summer (Aguilar and García-Vernet, 2018). During these long-range migrations, fin whales cross water masses that may be contaminated by different types of pollutants, including the ever-increasing plasticizers (*e. g.* Franeker and Law, 2015; Strobel et al., 2018). Consequently, they have the capability of integrating the incidence of these chemicals over large geographical scales averaging the heterogeneity of environmental local signals to which other organisms of more restricted distribution would be subject. From this perspective, fin whales may be considered potentially good indicator species to assess the presence of toxic compounds related to plastic pollution, such as OPFRs, in large oceanic bodies of water, like they have been used to monitor the chemo-physical characteristics of water masses (Borrell et al., 2018). In the North Atlantic Ocean, the fin whale has a broad distribution that extends from about 80° N (Svalbard archipelago) to almost the Equator (south of the islands of Cape Verde), including the Mediterranean and Baltic seas (Aguilar and García-Vernet, 2018). With a population estimated at about 70,000 individuals and structured in at least 7 discrete stocks (International Whaling Commission, 2009), it is one of the most abundant mysticetes in this Ocean.

The aim of this study was to investigate the presence and concentration of OPFRs in the fin whale and in its main prey, the krill *Meganyctiphanes norvegica*, off West Iceland, to assess the potential incidence of bioaccumulation and biomagnification processes.

2. Materials and methods

2.1. Sampling

Muscle samples were collected from fin whales caught by the Hvalur H/F whaling company off western Iceland (Denmark Strait) during the summer of 2015. Samples from 20 whales (13 males and 7 females) were analysed. The body length of these whales ranged from 16.8 to 20.4 m and their reproductive status was varied (Table 1). The reproductive status of females was established by examination of the ovaries and mammary glands and that of males through the histological examination of the testicle, as described by Lockyer (1984). Samples of the whale prey, consisting of different sets of samples of whole body homogenates of euphausiids, were collected from the forestomach (first chamber) of ten fin whales caught during the 2009–2013 summer seasons. The body size of the euphausiids and their aspect suggested that they belonged to the species *Meganyctiphanes norvegica*, which is the most abundant prey consumed by fin whales off western Iceland (Víkingsson, 1997). All samples were kept frozen until analysis.

2.2. Standards and reagents

A total of nineteen OPFRs were analysed in the present study. Analytical standards were purchased from different companies: Tris(2-butoxyethyl) phosphate (TBOEP), Tris(chloroethyl) phosphate (TCEP), Tris(chloroisopropyl) phosphate (TCIPP), Trihexyl phosphate (THP) and Tris(2-ethylhexyl) phosphate (TEHP) were obtained from Santa Cruz Biotechnology (SantaCruz, CA, USA). Isodecyldiphenyl phosphate (IDPP) and 2-ethylhexyldiphenyl phosphate (EHDPP) were purchased from AccuStandard (New Haven, CT, USA). Diphenylcresyl phosphate (DCP), Tri-*n*-butyl phosphate (TNBP), Triphenyl phosphate (TPHP), Triphenylphosphine oxide (TPPO), Tris(1,3-dichloro-2-propyl) phosphate (TDCPP), Triethyl phosphate (TEP) and Tri-n-propyl phosphate (TnPP) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Tricresyl phosphate (TMCP) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). 2-isopropylphenyl diphenyl phosphate (2IPPDPP), 4-isopropylphenyl diphenyl phosphate (4IPPDPP) and Bis (4-isopropylphenyl) phenyl phosphate (B4IPPPP) were purchased from Wellington Lab-oratories Inc. (Guelph, ON, Canada). Isopropyl phenyl phosphate (IPPP) was purchased from Chiron (Trondheim, Norway). Labelled d$_{15}$-TDCPP, d$_{27}$-TNBP, d$_{12}$-TCEP and ^{13}C$_2$-TBOEP were obtained from Wellington Lab-oratories Inc. (Guelph, ON, Canada). Labelled d$_{15}$-TPHP was purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA).

Table 1
Biological variables and ∑OPFR concentrations (expressed in ng g^{-1} lipid weight (lw)) for each fin whale studied.

ID	Sex	Reproductive status	∑OPFRs
F15007	Male	Mature	270
F15017	Male	Mature	36.4
F15031	Male	Mature	824
F15049	Male	Mature	508
F15071	Male	Mature	1329
F15088	Male	Mature	162
F15144	Male	Mature	161
F15026	Male	Immature	10,232
F15034	Male	Immature	470
F15063	Male	Immature	31.9
F15085	Male	Immature	313
F15116	Male	Immature	216
F15151	Male	Immature	248
F15013	Female	Mature	507
F15025	Female	Mature	363
F15028	Female	Mature	87.0
F15015	Female	Immature	117
F15059	Female	Immature	1875
F15096	Female	Immature	1619
F15146	Female	Immature	339

2.3. Sample preparation

Samples of approximately 10 g of frozen muscle and whole-krill homogenates were subject to lyophilisation for 48 h. Sample preparation was done according to Giulivo et al. (2016): 0.5 dry weight (dw) samples were extracted by sonication using 15 mL hexane:acetone (1:1) during 15 min. The extraction was carried out twice, and both extracts were combined. The resulting extract was reduced under a gentle nitrogen stream in order to change the solvent, and it was reconstituted in 5 mL hexane:methanol (1:3). The solution was centrifuged during 10 min at 4000 rpm and an aliquot of 200 μL was used for instrumental analysis. Lipid weight (lw) was determined gravimetrically from the remaining 4.8 mL by evaporating the solvent using a nitrogen stream and drying it in an oven at 90 °C until a constant weight was reached. Labelled OPFR standards were added prior to analysis by turbulent flow chromatography (TFC) coupled to LC-MS/MS.

2.4. Instrumental analysis

Purification was performed on-line at the beginning of the instrumental analysis with a Thermo Scientific TurboFlow™ system (Giulivo et al., 2016). Columns used were CycloneTM-P (0.5 × 50 mm) and C18-XL (0.5 × 50 mm) in combination for purification. An analytical column (Purosphere Star RP-18, 125 mm × 0.2 mm) was used for chromatographic separation. The mobile phase was a gradient of water (0.1% formic acid) and methanol (ammonium acetate) at 0.75 mL min^{-1} (Santín et al., 2016). Mass spectrometric analysis was performed with a triple quadrupole with a heated-electrospray ionization source. For all compounds, selective reaction monitoring (SRM) mode was used with two transitions.

2.5. Quality assurance

A blank was included every 10 samples. If the blank signal did not exceed 10% of sample signals, OPFR levels of the blank were subtracted from the corresponding batch of samples. If the blank signal was higher, samples were re-analysed. All the non-volumetric material was heated at 340 °C and rinsed with the appropriate solvent before use, and no plastic material was used to avoid contamination. Recoveries were 48–102%, with RSDs between 0.29 and 24.7%. Limits of detection (LODs) and limits of quantification (LOQs) were 0.19–19.3 ng g^{-1} lw and 0.97–24.8 ng g^{-1} lw, respectively.

2.6. Expression of concentrations

OPFRs are lipophilic compounds and, as such, their concentrations are usually recommended to be expressed on a lw basis to control for varying lipid content between species, individuals and tissues (Krahn et al., 2003). However, here we detail the concentrations of OPFRs expressed on three bases: extractable lipid basis (lw), fresh weight basis (fw), and dry weight basis (dw) to allow comparison with previous studies. Irrespective of this, when the evaluation of concentrations involves different tissues, the data used are always those expressed on a lw basis; if they are not given in the original source, conversion from other bases is made using relevant data on tissue lipid content available in the literature.

2.7. Statistical analysis

For statistical analyses, the whales were divided into three growth stages: sexually immature whales, sexually mature females and sexually mature males. This grouping was done because the three groups usually present different burdens of lipophilic pollutants. In young whales the pollutant concentrations in specimens from the two sexes are usually indistinguishable. In females, pollutants tend to decrease due to the transfer to offspring which occurs during pregnancy and lactation. Obviously, the transfer begins at the onset of reproductive activity and, because of this, mature females tend to have lower levels than males and juveniles (Aguilar and Borrell, 1988). The normality and heteroscedasticity of the distribution of ΣOPFR (the sum of all OPFR compounds) concentrations in fin whales muscle and in krill were preliminary tested using the Shapiro Wilk and Levene's tests, respectively. Whenever the tests showed that data distribution departed from normality, comparisons were made using the non-parametric Kruskal-Wallis rank sum test. The significance level was set at $p < .05$. Calculations were carried out with the programming environment R (R Core Team, 2018).

3. Results

ΣOPFR concentration per individual whale is shown in Table 1. Mean concentrations of the single OPFR compounds in fin whales and in krill are summarized in Table 2. OPFRs were detected in all samples, and at concentrations ranging between 31.9 and 10,232 ng g^{-1} lw in fin whales (Table 1), and between 74.8 and 3764 ng g^{-1} lw in krill. Out of 19 OPFR compounds, 7 were detected in the fin whale samples and 5 in the krill samples. IPPP, TNBP and TPPO were the most abundant compounds in both species (Fig. 1) and they were detected, respectively, in 10, 65 and 55% of fin whale samples and in 50, 70 and 50% of krill samples. 4IPPDPP was the most frequent OPFR in fin whales, being detected in 90% of samples, while 4IPPDPP and 2IPPDPP were the most frequent OPFRs in krill, being detected in 90% and 100% of samples, respectively. TPP and DCP were only detected in fin whales and showed a frequency of detection of 35 and 5%, respectively.

ΣOPFR concentrations had homogeneous variances in fin whales and krill ($p = .63$, Levene test), and followed a normal distribution ($p = .15$, Shapiro Wilk test) in mature fin whales, while they did not follow a normal distribution in immature fin whales and krill ($p < .05$, Shapiro Wilk test). Taking this into account, ΣOPFR concentrations between fin whale sexes and reproductive status, and between fin whales and krill, were compared using the non-parametric Kruskal-Wallis rank sum test. No differences were detected in ∑OPFR concentrations in fin whales between immatures, mature females and mature males (1546 ± 3117; 319 ± 214 and 470 ± 462 ng g^{-1} lw, respectively; $p = .90$, Kruskall-Wallis rank sum test; Fig. 2). Similarly, no differences were detected in ∑OPFR concentrations between fin whale and krill (985 ± 2238 and 949 ± 1090 ng g^{-1} lw, respectively; $p = .29$, Kruskall-Wallis rank sum test; Table 2).

4. Discussion

In this study, the presence and concentrations of plasticizers and flame retardants of the OPFR family were investigated in North Atlantic fin whales and their main prey, the krill *Meganyctiphanes norvegica*. Three main results were obtained: 1) OPFRs are present both in North Atlantic fin whales and in krill, 2) OPFRs did neither bioaccumulate nor biomagnify in fin whales and their prey, and 3) considering both the concentration and the frequency of detection, TNBP was the main OPFR detected in both species.

Table 2
Individual OPFR-compound concentrations expressed in ng g^{-1} ww, dw and lw (mean ± SD) in fin whale muscle and krill samples collected off Iceland.

	Fin whale			Krill		
	ww (ng g^{-1})	dw (ng g^{-1})	lw (ng g^{-1})	ww (ng g^{-1})	dw (ng g^{-1})	lw (ng g^{-1})
TPPO	1.84 ± 1.72	7.67 ± 7.15	128 ± 189	1.35 ± 1.48	7.52 ± 7.93	114 ± 155
TPP	0.74 ± 1.13	2.82 ± 4.17	59.2 ± 94.9	nd[a]	nd[a]	nd[a]
TNBP	3.21 ± 2.59	13.1 ± 10.2	214 ± 281	2.71 ± 2.60	15.7 ± 12.6	165 ± 158
DCP	0.29 ± 1.30	1.17 ± 5.25	8.79 ± 39.3	nd[a]	nd[a]	nd[a]
2IPPDPP	0.41 ± 0.69	1.77 ± 2.95	39.6 ± 75.0	1.75 ± 1.17	9.92 ± 7.35	116 ± 108
4IPPDPP	0.83 ± 0.48	3.40 ± 1.85	69.4 ± 61.8	1.42 ± 0.82	7.80 ± 4.62	97.6 ± 107
IPPP	5.89 ± 26.3	24.0 ± 107	465 ± 2076	5.39 ± 8.12	31.1 ± 43.8	457 ± 792
ΣOPFRs	13.2 ± 27.8	54.0 ± 113	985 ± 2238	12.6 ± 9.46	72.1 ± 50.6	949 ± 1090
Range	0.51–129	2.24–528	31.9–10,232	2.66–36.2	13.0–186	74.8–3764

[a] nd: non detected (below detection limits).

4.1. OPFR concentrations

In the present study we report the first evidence of OPFR presence in fin whales and in their main prey. Data about OPFRs in marine mammals is scarce: to our knowledge, only 6 studies have been published; two of them on polar bears and phocids, and four on odontocetes, but none on a mysticete as the fin whale is.

The specific OPFR compounds detected in the plasma of polar bears (*Ursus maritimus*) and phocids from Norway by Hallanger et al. (2015) were different from those found in the current study, although in both studies were analysed TCEP, TCIPP, TBOEP, TEHP, TPHP, EHDPP and TNBP. In another study done in polar bears and phocids from Greenland (Strobel et al., 2018), TNBP was found to be the highest and most frequent compound, a result consistent with the present study, in which TNBP ranked as second compound with highest concentrations. However, a direct comparison of these results with those from the present study cannot be made because concentrations are expressed on a ww basis, so we recalculated the data using a value of 89.3% ($n = 92$) for Greenland polar bears (Dietz et al., 2007) and 93% ($n = 100$) for Greenland ringed seals (Cleemann et al., 2000). Resulting concentrations on a lw basis were 0.87 ng g^{-1} and 1.4 ng g^{-1}, respectively, far lower than those found in the current study, suggesting that Greenland would be less affected by plastic pollution than Iceland.

The first report on OPFRs in odontocetes was that by Papachlimitzou et al. (2015), who analysed flame retardants and plasticizers in the liver and blubber of harbour porpoises (*Phocoena phocoena*) from the UK. These authors detected 6 OPFR compounds out of the 20 analysed and found TPPO in 11 out of 19 blubber samples. Again, we had to transform the original values, expressed on a ww basis, into lw basis assuming a mean lipid content in blubber of 85%, as reported from individuals from the same species and population (Law et al., 2010). the converted results indicate that TPPO concentrations were more than three times lower in the blubber of porpoises (38 ng g^{1} lw) than in the muscle of fin whales (128 ng g^{1} lw). Putting aside potential differences in the analytical procedures between laboratories, the difference is large enough and it may be taken as an indication that, in the North Atlantic, fin whales are more contaminated by OPFRs than porpoises. However, we should consider this conclusion with caution because the comparison involves different tissues and our present understanding on the dynamics and compartmentation of OPFRs in the body of mammals is poor.

Two recent studies on OPFRs in cetaceans have focused on Mediterranean dolphins. Sala et al. (2019) detected 12 out of the 16 OPFR compounds analysed in the muscle, liver, kidney, blubber and brain of common dolphins (*Delphinus delphis*) from the Alboran Sea. They found, among other compounds, TNBP, TPPO, DCP and IPPP, consistently with what we found in fin whales. Mean ∑OPFRs in dolphin muscle was similar to the values we found in the fin whale muscle (994 and 985 ng g^{-1} lw, respectively). Also, Aznar-Alemany et al. (2019) analysed OPFR compounds in three dolphin species (*Delphinus capensis*, *Sousa plumbea* and *Tursiops aduncus*) from the Indian Ocean, and, consistently with our results, they detected TNBP, TPPO, and IPPP. In this case, ∑OPFR concentrations in the muscle of the various dolphin species ranged between 1630 and 31,861 ng g^{-1} lw, with a mean value of 10,452 ng g^{-1} lw. These concentrations are one order of magnitude higher than those observed in the present study and in the Mediterranean dolphins, this time more clearly supporting that pollution by plastic derivatives is higher in the Indian Ocean than in the Atlantic Ocean.

If information on OPFR concentrations in marine mammals is scarce, data about OPFRs in plankton is even scarcer: Zhao et al. (2018) analysed OPFR levels in plankton and crustaceans from the Taihu lake, in China, detecting 13 out of the 14 OPFRs analysed. In consistency with our results, authors also reported TNBP to be one of the major contributors to the ∑OPFR, although mean TNBP concentration in North Atlantic krill (2.71 ng g^{-1} ww) was 5 times higher than the concentrations reported by Zhao et al. (2018) (0.54 ng g^{-1} ww).

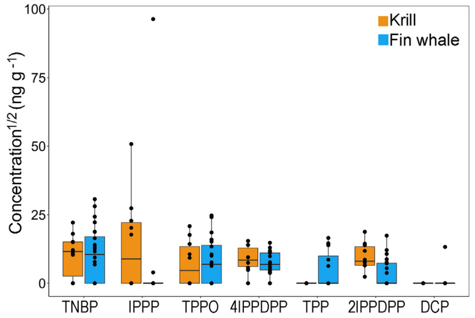

Fig. 1. Concentrations of OPFR compounds (lipid weight basis) detected in fin whales and krill off Iceland. OPFR concentrations were normalized using the square root.

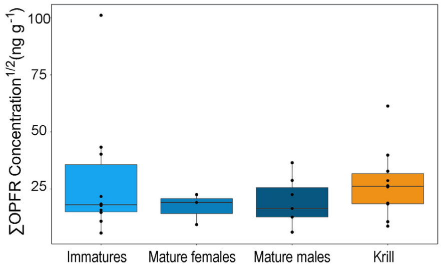

Fig. 2. Box-plots illustrating the ∑OPFR concentrations in fin whales grouped by immatures, mature females and mature males, and ∑OPFR concentrations in krill. ΣOPFR concentrations were normalized using the square root.

The information on potential health effects caused by TPPO, TPP, IPPP, 2IPPDPP and 4IPPDPP, which are commonly used as flame retardants and plasticizers, is insufficient. Conversely, toxic effects of DCP and TNBP are better studied. In rats, mice, rabbits and guinea pigs, the DCP median lethal dose (LD_{50}) has been reported to be higher than 1000 ng g^{-1} (US-EPA, 2007; van der Veen and de Boer, 2012), which is three orders of magnitude higher than the concentrations found in the tissues of fin whales. TNBP toxicity in biota is also well established (van der Veen and de Boer, 2012), with LD_{50} ranging from 1400 to 3200 ng g^{-1} in different animals (Bruchajzer et al., 2015). In rats, TNBP may produce organ damages (kidneys, liver and urinary bladder epithelium), neurotoxic effects, cancer of urinary bladder and impairment of fertility (Arnold et al., 1997; Auletta et al., 1998a; Auletta et al., 1998b; Bergman et al., 2012; Bruchajzer et al., 2015; Tyl et al., 1997). TNBP concentrations found in the muscle of fin whales ranged from nd to 6.56 ng g^{-1} ww: values three orders of magnitude lower than those referred to as LD_{50} in laboratory animals.

From this, we can conclude that the potential toxic impact of OPFRs on fin whales is limited. However, OPFR concentrations are likely to increase in the marine environment and in marine organisms as pollution from plastic marine litter increases in seas and oceans worldwide. As seen, the fin whale, similarly to other filter-feeding and long-range migrating mysticetes, stand as a collective of species potentially susceptible to be severely affected by these emerging contaminants. Moreover, their long life span (~80–90 years, Aguilar and García-Vernet, 2018), would extend fin whales exposure to these pollutants over very long periods of time. Thus, these species should be monitored with particular attention, and the effects of OPFRs must be assessed on a medium – large temporal scale, as they may lead to chronic toxicity.

OPFR concentrations are likely to increase in the marine environment and in marine organisms as pollution from plastic marine litter increases in seas and oceans worldwide. As seen, the fin whale, similarly to other mysticetes that are also filter-feeding and engage in long-range annual migrations across potentially highly polluted areas, stand as a collective of species potentially susceptible to be severely affected by these emerging contaminants and, as such, should be monitored with particular attention.

4.2. Bioaccumulation and biomagnification of OPFRs

Although a few studies in biota have suggested that OPFRs such as TNBP or DCP may bioaccumulate, this is, increase with lifespan (Giulivo et al., 2017), others show that they can be readily metabolised and that, as a result, their concentrations do not build-up with age (Greaves et al., 2016; Regnery and Püttmann, 2010; Strobel et al., 2018; Van den Eede et al., 2013). In this latter line of findings, Aznar-Alemany et al. (2019) did not observe differences in ∑OPFR concentrations between juveniles and adult male dolphins. Consistently, our results on fin whales did not show significant variation in ∑OPFR concentrations between immature individuals and reproductive mature males or females, and thus did not suggest the occurrence of bioaccumulation processes through lifespan, consistently with what happens in other mammals (Aznar-Alemany et al., 2019).

Mean ∑OPFR concentrations were similar in mature females and mature males, although they were slightly higher in males (470 ± 462 and 319 ± 214 ng g^{-1} lw, respectively). In several mammals, including cetaceans, organochlorine and other lipophilic pollutants, such as PCB or DDT, are transferred from mothers to their offspring during gestation and lactation (Aguilar and Borrell, 1994; Borrell and Aguilar, 2005; Borrell et al., 1995). Therefore, the concentration of these pollutants in mature females is usually lower than that in mature males. Along with the age-related decline in pollutant body loads in females, the transfer rates during reproduction tend to decrease with the mother's age, all which result in the first calf delivered being the one receiving the largest pollutant loads (Aguilar and Borrell, 1994; Borrell et al., 1995). The current results, with an absence of significant differences in OPFR tissue concentrations between mature males and mature females, appears to indicate that any reproductive transfer is occurring, or that if it does, is very limited. However, because mother-calf transfer of OPFRs has not been assessed in any mammal species and, given the potential parallelism in the dynamics of OPFRs with that of other lipophilic pollutants, its assessment and potential toxicological implications should be a priority for future research.

Evidences for biomagnification, this is, the building-up of ∑OPFR concentrations through the food web, are limited and conform an unclear picture (Du et al., 2019). Hallanger et al. (2015) found weak biomagnification of OPFRs from fish to their predators, and Strobel et al. (2018) also found limited OPFR biomagnification from ringed seals to polar bears. However, Brandsma et al. (2015) and Zhao et al. (2018) analysed the trophic transfer through entire marine food webs and found that the majority of OPFRs exhibit trophic dilution and thus do not biomagnify or even follow negative relationships between concentration and aquatic species trophic level. One likely explanation for these latter results may be the rapid metabolism of OPFRs into other compounds (Hou et al., 2016; Strobel et al., 2018; Zhao et al., 2018). Consistently, the fact that in our study the mean ∑OPFR concentrations did not significantly differ between the fin whale muscle and krill, seems to indicate that biomagnification through the food web is limited for these compounds.

Of the 7 OPFR compounds detected in fin whale, 5 were also detected in krill samples, suggesting that the OPFR intake in fin whales is mainly derived from the diet. However, DCP and TPP were detected in fin whale muscle but not in krill samples, indicating a different source of uptake for these compounds. A possible explanation is the direct intake of the chemical additives (*i.e.*, TPP and DCP) from the ingested micro- or macro-plastic in the fin whale stomach, and subsequent transfer to the whale tissues. Although no studies have been specifically conducted in fin whales from Icelandic waters, others carried out in other areas (*i.e.*, Besseling et al., 2015; Fossi et al., 2014; Baulch and Perry, 2014) have reported the presence of plastics in the gastrointestinal tract of baleen whales, reinforcing this argument. Similarly, other pollutants, such as higher-brominated diphenyl ethers (Tanaka et al., 2013) and phthalates (Hardesty et al., 2015) have been detected in seabirds but not in their prey, suggesting a direct intake of these compounds from ingested plastics.

2IPPDPP and IPPP were found in both the fin whales and the krill samples, but they were detected in a smaller proportion in the former (40% and 10%, respectively) than in the latter (100% and 50%, respectively). These results may be related to a more efficient metabolism and excretion of these compounds in fin whales than in krill. In addition, IPPP was only found in immature fin whales, suggesting that the capacity of metabolising this compound is acquired during lifespan. An alternative explanation may be the partial contribution of ingested plastics to the overall pollutant load built by krill ingestion. Focused research is needed to clarify this hypothesis, to better understand the processes involved in the metabolism of OPFRs in fin whales and the mechanisms through which these compounds are transferred to the whale tissues and at what magnitude.

5. Conclusions

Results of this paper provide the first evidence of OPFR presence in the muscle of North Atlantic fin whales and in their main prey, the krill *Meganyctiphanes norvegica*, with TNBP being the most concentrated OPFR in both organisms. OPFR bioaccumulation in fin whales and biomagnification from krill to whales was not observed. Our results remark the potential of fin whales to be used as biomonitors of OPFR pollution at large-scale, due to their wide distribution, high potential susceptibility to plastic-related pollutants as filter-feeding animals, and high mobility. Further research is needed to assess the potential toxic effects of TNBP to fin whales and krill, and to investigate the processes of OPFR intake, metabolism, reproductive transfer and overall dynamics in long-lived, filter-feeding cetaceans performing long-range migrations, such as fin whales.

CRediT authorship contribution statement

Odei Garcia-Garin: Conceptualization, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Berta Sala:** Methodology, Validation, Supervision. **Alex Aguilar:** Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Morgana Vighi:** Writing - original draft, Writing - review & editing, Supervision. **Gísli A. Víkingsson:** Resources. **Valerie Chosson:** Resources, Writing - original draft. **Ethel Eljarrat:** Conceptualization, Methodology, Validation, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Asunción Borrell:** Conceptualization, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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PUBLICACIÓ CIENTÍFICA #5

“First study on the presence of plastic additives in loggerhead sea turtles (*Caretta caretta*) from the Mediterranean Sea”

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First study on the presence of plastic additives in loggerhead sea turtles (*Caretta caretta*) from the Mediterranean Sea[☆]

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ABSTRACT

Loggerhead turtles (*Caretta caretta*) voluntarily ingest floating plastic debris and hence are chronically exposed to plastic additives, but very little is known about the levels of these compounds in their tissues. This work studied the presence of organophosphate esters (OPEs) on sea turtles collected from two different areas in the western Mediterranean, some of their prey and some floating plastic debris. OPEs were detected in all the samples analysed and $\sum$OPEs ranged from 12.5 to 384 ng/g wet weight (ww) in the turtles from the Catalan coasts, with a mean value of 21.6 ng/g ww, and from 6.08 to 100 ng/g ww in the turtles the Balearic Islands, with a mean value of 37.9 ng/g ww. Differences in $\sum$OPEs were statistically significant, but turtles from the two regions did not differ in their OPE profiles. As per turtle's prey, $\sum$OPEs ranged from 4.55 to 90.5 ng/g ww. Finally, marine plastic litter showed $\sum$OPEs concentrations between 10.9 and 868 ng/g. Although most compounds were present in both potential sources of contamination, prey and plastic debris, the OPE profiles in loggerhead turtles and these sources were different. Some OPEs, such as tris(2-isopropylphenyl) phosphate (T2IPPP), tripropyl phosphate (TPP) and tris(2-butoxyethyl) phosphate (TBOEP), were detected in plastic debris and turtle muscle but not in their prey, thus suggesting that ingestion of plastic debris was their main source. Contrarily, the levels of triethyl phosphate (TEP), diphenyl cresyl phosphate (DCP), 2-isopropylphenyl diphenyl phosphate (2IPPDPP) and 4-isopropylphenyl diphenyl phosphate (4IPPDPP) in turtle muscle were much higher than in jellyfish, their main prey, thus indicating a biomagnification potential. Regular ingestion of plastic debris and contamination from their prey may explain why $\sum$OPEs in loggerhead turtles is much higher than the values reported previously for teleost fishes and marine mammals from the western Mediterranean.

1. Introduction

Marine litter, dominated by plastic debris, is a global threat for marine wildlife, coastal communities and maritime activities (Veiga et al., 2016). Plastics are made of polymers derived from the crude oil and also incorporate other chemical ingredients known as additives, many considered hazardous for live beings (Halden, 2010). Nevertheless, plastic production has expanded continuously to reach global production levels of more than 350 million tons in 2018 (Europe Plastics, 2019).

Organophosphate esters (OPEs) are a major group of additives used primarily as plasticizers, flame retardants, lubricants and antifoaming agents, and are present as additives in lacquers, hydraulic fluids and floor polishing products (Andresen et al., 2004). They are applied to polyurethane and other polymers for use in furniture, construction, textile industry and electronic equipment. These compounds have been used since the 1960s, but their use increased dramatically when polybrominated diphenyl ethers (PBDEs) were banned by the Stockholm Convention in 2009 (UNEP, 2009).

The OPEs encompass a wide variety of chemical compounds, also including a broad range of physico-chemical properties (Table S1). Molecular masses range between 182.5 g/mol for TEP and 452 g/mol for T2IPPP. The solubility in water decreases with

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increasing molecular mass and ranges from 1.02 × 10^{-2} mg/L for trihexyl phosphate (THP) to 5.00 × 10^5 mg/L for TEP, at 25 °C (Wei et al., 2015). They are less lipophilic than brominated flame retardants (BFRs) (Blum et al., 2019) and their log octanol-water partition coefficient (log K_{ow}) values range between 0.80 for TEP and 9.49 for tris(2-ethylhexyl) phosphate (TEHP) (Reemtsma et al., 2008).

Developmental toxicity is one of the most prominent toxic effect of OPEs on humans and experimental animals, similar to that of organophosphate pesticides. Besides neurotoxicity, some OPEs are endocrine disruptors impacting the thyroid gland and causing diabetes and some reproductive malfunctions (Liu et al., 2012). Moreover, a recent study (Liu et al., 2021) showed that 2-ethylhexyldiphenyl phosphate (EHDPP) was associated with breast cancer, while exposure to tri-n-butyl phosphate (TNBP), triphenyl phosphate (TPHP) and EHDPP was associated with cervical cancer. Moreover, tris(2-chloroethyl)-phosphate (TCEP), tris(1,3-dichloro-2-propyl) phosphate (TDClPP) and tris(chloroisopropyl)-phosphate (TClPP) have been restricted in the European Union due to their potential toxicity on humans (Programme, 2004).

OPEs are released to the environment via volatilization, abrasion or leaching. Consequently, different OPEs have been reported in several environmental matrices such as air and dust (Abafe and Martincigh, 2019; Wang et al., 2020), water (Na et al., 2020), sediment (Giulivo et al., 2017; Liao et al., 2020) and even in the human body (Liu et al., 2021; Luo et al., 2020). Moreover, some studies also reported the occurrence of OPEs in marine vertebrates such as teleost fish (Garcia-Garin et al., 2020b), dolphins (Sala et al., 2019; Aznar-Alemany et al., 2019), harbour porpoises (Papachlimitzou et al., 2015) and fin whales (Garcia-Garin et al., 2020a). OPEs were detected in almost all the samples analysed in those studies, with levels up to 32 μg/g lipid weight (lw).

Marine turtles occur in all the warm temperate and tropical oceans (Wallace et al., 2010) and actively ingest macroplastics while feeding, perhaps mistaken them for food (Fukuoka et al., 2016). As a result, plastic debris occurs regularly in the gut contents of six out of seven species of extant sea turtles, although there is a broad variability in prevalence across species and oceanic basins (Nelms et al., 2016; Schuyler et al., 2016; Lynch, 2018). Furthermore, there is a strong selectivity according to debris type and the risk of ingestion decreases with turtle size (Schuyler et al., 2012; Fukuoka et al., 2016; Duncan et al., 2019). Beside the potential physical damage (Nelms et al., 2016), the ingestion of plastic debris has been suggested to increase the exposure of marine wildlife to a diversity of plastic-born organic pollutants (Teuten et al., 2009; Engler, 2012). Nevertheless, information about organic pollutants in marine turtles is scarce (D'ilio et al., 2011; Wyneken et al., 2013; Clukey et al., 2017; Novillo et al., 2017), evidence linking the levels of organic pollutants and the amount of plastic debris ingested by marine turtles is weak (Clukey et al., 2017) and we are not aware of any study on the occurrence of OPEs in marine turtles.

The loggerhead turtle (*Caretta caretta*) is the most abundant marine turtle in the Mediterranean Sea (Casale et al., 2018) and the frequency of occurrence of plastic debris in gut contents is high, although the total volume of plastic debris ingested is usually small (Tomas et al., 2002; Revelles et al., 2007a; Lazar and Gračan, 2011; Campani et al., 2013; Domènech et al., 2019). The purpose of this study is to determine the occurrence of OPEs in the muscle of loggerhead turtles from two distinct areas in the western Mediterranean Sea, their prey and some types of marine plastic debris collected in the same sampling areas to better identify the sources of contamination affecting these marine animals.

2. Materials and methods

2.1. Standards and reagents

Nineteen OPEs were included in this analytical study. Different analytical standards were used. TCEP, TBOEP, THP, TEHP and TClPP were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). T2IPPP was purchased from Chiron (Trondheim, Norway). EHDPP and isodecyldiphenyl phosphate (IDPP) were purchased from AccuStandard (New Haven, CT, USA). Tricresyl phosphate (TCP) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). DCP, TPHP, TDClPP, TNBP, triphenylphosphine oxide (TPPO), TEP and TPP were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2IPPDPP, 4IPPDPP and bis(4-isopropylphenyl) phenyl phosphate (B4IPPPP) were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada).

Labelled internal standards (IS) were also used. d_{15}-TPHP was obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). d_{27}-TNBP, d_{15}-TDClPP, $^{13}C_2$-TBOEP and d_{12}-TCEP were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada).

Acetone and hexane solvents for organic trace analysis were purchased from J.T. Baker (Center Valley, PA, USA). Methanol and water solvent were obtained from Merck (Darmstadt, Germany).

2.2. Sample collection

Loggerhead turtles occur through the western Mediterranean Sea, but habitat use patterns and diet differ across sub-basins (Casale et al., 2018). Turtles occurring off mainland Spain remain mostly over the continental shelf and rely more heavily on fish and squids than on jelly plankton (Tomas et al., 2002; Cardona et al., 2009). Conversely, loggerhead turtles occurring off the Balearic Islands are primarily oceanic foragers inhabiting the Algerian Basin and rely largely on jelly plankton, although they also consume pelagic fish and squids (Revelles et al., 2007a; 2007b).

Muscle samples were collected from 44 loggerhead turtles dead stranded from 2014 to 2017 along the Catalan coast (Northeastern mainland Spain) and the Balearic Islands (Table S2). Most of the turtles ranged 40–60 cm in curved carapace length, which corresponds to 7–20 years old individuals, depending of their natal origins (Piovano et al., 2011). However, age was unknown, as can be assessed only through skeletochronology (Piovano et al., 2011). Plastic debris, including bottle caps, ear sticks, plastic bags and broken fragment of unknown origin, were also collected at sea off Menorca Island (Balearic Islands). Finally, five specimens of the jellyfish *Pelagia noctiluca*, the European sardine *Sardina pilchardus* and the European squid *Loligo vulgaris* were collected from the Balearic Islands to characterize OPE levels in major dietary components. Turtle muscle samples and prey samples were wrapped in aluminium foil and stored frozen (−20 °C) until analysis. Debris samples were stored at room temperature.

2.3. Sample preparation

In order to prevent potential OPE contamination of samples throughout all the processes of sampling and analysis, plastic material was not used. A blank (including the whole analytical process, that is, both sample preparation and instrumental analysis) was included in each batch of samples.

Turtle and diet samples were transferred to the laboratory wrapped in aluminium foil at a temperature of 4 °C. Then, samples were frozen at −20 °C before being freeze dried. The lyophilized samples were grounded and homogenized and stored in sealed containers at −20 °C until analysis.

An ultrasound extraction with 0.5 g dry weight (dw) of sample, using 15 mL of hexane:acetone (1:1) as solvent, for 15 min was performed. After this, a centrifugation step for 20 min at 4000 rpm and 22 °C was carried out. This extraction procedure was performed twice for each sample, and both obtained extracts were combined and evaporated to dryness using a purified nitrogen stream in TurvoVap LV. Then, samples were reconstituted with 5 mL of hexane:methanol (1:3), and centrifuged for 10 min at 4000 rpm and 18 °C. A 200 μL aliquot was transferred to a vial, and 10 μL of a mixture of 1 ng/μL of each IS were added (Giulivo et al., 2016).

Samples of marine plastic debris were cut into small fragments with scissors (around 0.5 cm of diameter). Twenty ng of IS mixture were added and left conditioning overnight. The extraction was carried out with 5 mL of ethyl acetate using an ultrasonic bath for 15 min (Llompart et al., 2013). Afterward, samples were centrifugated for 20 min at 4000 rpm and 20 °C. Then, the process was repeated, and the final 10 mL of obtained extracts were evaporated to dryness using a purified nitrogen stream in TurvoVap LV. Finally, extracts were reconstituted with 500 μL of methanol. It should be pointed out that, considering that some plastic debris, such as bottle caps, are very dense and have low solvent permeability, it is very difficult to completely extract polymer additives from analysed fragments.

2.4. Instrumental analysis

Instrumental analysis of OPEs was carried out using a turbulent flow chromatography - high pressure liquid chromatography - tandem mass spectrometry (TFC-HPLC-MS-MS), using the system Thermo Scientific TurboFlowTM, which allows perform an online sample purification and analysis. This system consisted of two C quaternary pumps and three LC columns, the first two for purification and the last one for separation. The purification columns used were CyclonTM-P (0.5 × 50 mm) and C18-XL (0.5 × 50 mm), and the chromatographic separation was done using the column Purosphere Star RP-18 (125 mm × 0.2 mm), which has a particle size of 5 μm. The tandem MS of this system was a triple quadrupole (QqQ) that uses heated-electrospray ionisation source (H-ESI). Chromatographic separation and mass spectrometry detection parameters can be found in a previous work (Giulivo et al., 2016).

2.5. Quality assurance

Instrumental parameters such as recoveries, limits of detection (LODs) and limits of quantification (LOQ) are summarized in Supporting information (Table S3). For recovery tests, 5 ng of each analyte were added in each sampling replica (n = 5) before sample extraction, and 2.5 ng of each labelled standard were added before the instrumental analysis. Recoveries ranged between 40% and 98%, always being within the range of acceptability (40–120%) for analytical methods based on quantification by isotopic dilution, with relative standard deviation always below 16%. LODs and LOQs ranged between 0.02 and 0.95 ng/g wet weight (ww) and 0.04–2.62 ng/g ww respectively.

2.6. Statistical analysis

Student test was used to compare the mean ΣOPE levels in the loggerhead turtles from the Catalan coast and the Balearic Islands and ANOSIM was used to assess the similarity in the OPE profiles within and between the loggerhead turtles from the two areas. Principal component analysis (PCA) was used to assess which potential sources of contaminants (three prey species and five types of plastic debris) laid closer to the loggerhead turtles after identifying the directions of higher variance and projecting the data on a new subspace with fewer dimensions. ANOSIM was run in R using the package "vegan" and PCA was run using the package "R stats".

3. Results and discussion

3.1. OPE levels in turtle muscles

OPEs were detected in all the individuals analysed with total OPE concentrations ranging between 6.08 and 100 ng/g ww. Fifteen out of nineteen tested analytes were detected. Only B4IPPPP, IDPP, THP and TEHP were not detected. Table 1 summarises the results obtained (for individual sample results see Supporting information, Table S4). Sample CC3 was considered an outlier because ΣOPEs was more than 1.5 interquartile ranges (IQRs) above the 3rd quartile and hence was removed from further analysis.

Individual variability in ΣOPEs was not different between the turtles from the two areas (F-test; $^{F}\text{tab}^{=0.46>F}\text{cal}^{=0.10),}$ but mean ΣOPEs (Fig. 1) was significantly higher in the turtles from the

Table 1
OPE levels (expressed in ng/g ww) in muscles of turtles from the Catalan coast and the Balearic Islands (one *outlier (sample CC3) was not considered in these results*).

	Catalan coastline					Balearic Islands				
	Min.	Max.	Mean	Median	DF* (%)	Min.	Max.	Mean	Median	DF (%)
TEP	nd	10.5	6.21	6.06	90	nd	42.7	10.6	7.16	95
TCEP	nd	nq	nq	nq	85	nd	nq	nq	nq	86
TPPO	nd	nq	nq	nq	85	nd	nq	nq	nq	95
TClPP	nd	8.54	0.65	0.02	10	nd	22.9	2.91	0.03	36
TPP	nd	2.83	0.70	0.23	40	nd	3.56	0.91	0.16	45
TDClPP	nd	2.37	0.28	0.02	30	nd	12.2	1.32	0.72	68
TPHP	nd	1.08	0.15	0.03	20	nd	1.34	0.31	0.03	45
TNBP	nd	0.91	0.23	0.07	50	nd	0.69	0.15	0.01	45
DCP	nd	8.44	6.53	6.98	95	1.68	24.1	7.40	7.18	100
TBOEP	nq	0.23	0.03	0.01	15	nd	0.40	0.07	0.01	45
2IPPDPP	nq	17.9	1.83	0.08	80	nd	40.7	6.07	2.98	73
4IPPDPP	nd	9.11	3.29	3.64	85	nd	18.0	3.56	1.54	68
TCP	nd	nq	nq	nq	40	nd	23.8	1.66	0.03	41
EHDPP	nd	2.79	0.31	0.01	15	nd	2.52	0.24	0.01	41
T2IPPP	nd	12.0	2.04	0.47	15	nd	27.7	3.25	0.47	23
ΣOPEs	12.5	39.5	21.6	20.5	100	6.18	100	37.9	31.9	100

*DF – Detection frequency
nd: not detected, below LOD; nq: not quantifiable, below LOQ.
**Mean and median calculated with nd = ½ LOD; nq = LOD.

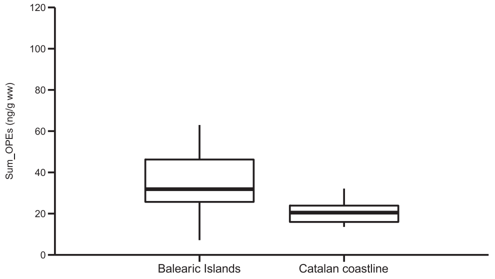

Fig. 1. Box plot of ∑OPE concentrations in Catalan and Balearic turtles.

Balearic Islands than in the turtles from the Catalan coast (Balearic Islands: 37.9 ± 22.5 ng/g ww; Catalan coast: 21.6 ± 7.04 ng/g ww; Student t-test; t_{tab}(2 tail) = 2.02 < t_{cal} = 3.04, 0.05 significance level). However, no consistent differences were observed in the OPE profiles of loggerhead turtles from the Balearic Islands and the Catalan coast, as the similarity among samples from the two regions was not lower than the similarity among samples from the same region (ANOSIN, R = −0.028, p = 0.927). Exchange of turtles between the Catalan coast and the Balearic Islands is limited (Revelles et al., 2007b; Cardona et al., 2009), but these similarities suggest that they are exposed to the same type of OPEs in both areas, which is hardly surprising considering that the same manufactured products are sold in both regions. However, the higher levels of OPEs in the loggerhead turtles from the Balearic Islands are in accordance with a higher concentration of floating plastic debris in the western Algerian Basin than along the Catalan coast (Cózar et al., 2015) and very high levels of floating plastic debris in the coastal strip off the Balearic Islands (Ruiz-Orejón et al., 2018). It should be noted that the lower frequency of occurrence of plastic debris in the gut contents of loggerhead turtles from the Balearic Islands compared to those from mainland Spain is likely a sampling artefact. This is because Revelles et al. (2007a) analysed only the stomach contents of turtles from the Balearic Islands, whereas Domènech et al. (2019) analysed the entire digestive tract of turtles from mainland Spain, thus resulting in a much higher detection rate in the latter.

Information on the presence of organic pollutants accumulated in turtle tissues is very scarce, and this is the first study reporting OPE concentration levels. There are only some previous works reporting polychlorinated biphenyls (PCBs) and DDTs data. D'ilio et al. (2011) analysed PCBs and DDTs in muscle from loggerheads turtles collected from the Mediterranean Sea, the Atlantic and the Pacific Ocean and reported values ranging between 0.6 and 23.5 ng/g ww for PCBs and 0.3 and 4.9 ng/g ww for DDTs. Those levels were slightly lower than those found in our study for OPEs (from 6.18 to 384 ng/g ww). Gardner et al. (2003) also analysed PCBs, DDTs and chlordane (cis-chlordane, trans-chlordane, cis-nonachlor, trans-nonachlor, oxychlordane, heptachlor and heptachlor epoxide) in the tissues of three species of sea turtle (seven green turtles *Chelonia mydas*, one loggerhead turtle, and one olive ridley turtle *Lepidochelys olivacea*) from the Baja California peninsula (Mexico). They reported levels in the muscle of loggerhead turtles of 49.2 ng/g ww for PCBs and did not detect DDTs and chlordane. These levels are in the same order of magnitude than the OPE levels reported in this study.

Regarding OPE levels in the muscle of other species of marine wildlife, the loggerhead turtles studied here have the highest levels reported to date. Garcia-Garin et al. (2020b) detected a mean concentration of 35.7 ng/g dry weight (dw) a in a teleost fish from the North Western Mediterranean Sea. Sala et al. (2019) reported a mean value of 20.1 ng/g dw in short-beak common dolphins *Delphinus delphis* from the Alboran sea and Garcia-Garin et al. (2020a) found a mean value of 54.0 ng/g dw in fin whales *Balaenoptera physalus* from the North Atlantic. These figures were much lower than the levels reported for loggerhead turtles in the present study, equivalent to 86.2 and 158 ng/g dw for the loggerhead turtles from the Catalan coast and the Balearic Islands, respectively. This is probably because macroplastics occur regularly in the gut of loggerhead turtles from the two areas (Tomas et al., 2002; Revelles et al., 2007a; Domènech et al., 2019), likely as a result of voluntary ingestion (Fukuoka et al., 2016), whereas only microplastics, probably ingested involuntarily with prey, occur regularly in the gut of fish and dolphins from the western Mediterranean (Garcia-Garin et al., 2020b; Novillo et al., 2020).

As regards the OPE distribution patterns, compounds with the higher detection frequencies in samples were similar in both groups of turtles: DCP was the most frequently detected (95% in the Catalan coast and 100% in the Balearic Islands), followed by TPPO (85% and 95%) and TCEP (85% and 86%) and TEP (74% and 82%). Likewise, the highest levels were obtained from the same OPE compounds in both study areas: DCP (mean value of Catalan coast and Balearic Islands = 6.53 ± 2.08 and 7.40 ± 4.54 ng/g ww, respectively), TEP (5.58 ± 3.02 and 10.1 ± 10.6 ng/g ww), 2IPPDPP and 4IPPDPP (1.83 ± 4.37 and 3.29 ± 2.81, 6.07 ± 9.76 and 3.56 ± 4.71 ng/g ww), and T2IPPP (2.04 ± 3.76 and 3.25 ± 6.41 ng/g ww).

Currently, there is not enough information to assess whether the OPE concentration levels detected in turtles may represent a risk for them. There is insufficient knowledge related to OPEs toxicity, and there are no data regarding potential effects on turtles. Among the OPEs studied to date, the DCP median lethal dose (LD50) has been reported to be higher than 1000 ng/g (Environmental and Agency, 2007), and LD50 for TNBP is also established, ranging from 1400 to 3200 ng/g in different animals (Bruchajzer et al., 2015). OPEs found in the muscle of turtles ranged from 12.5 to 384 ng/g ww, which are values several orders of magnitude lower than LD50 in laboratory animals. However, it should be pointed out that potential effects may happen at a medium-large temporal scale as they may lead to chronic toxicity, particularly in long lived species such loggerhead turtles, that they reach sexual maturity at 20 years old in the Mediterranean Sea (Piovano et al., 2011).

3.2. Potential sources of OPE contamination

Total OPE concentrations ranged from 4.55 to 90.5 ng/g ww for prey samples, and from 10.9 to 868 ng/g for plastic debris samples (Table 2). In prey samples, 15 compounds were detected, 3 of them below LOQs and nine of them had a 100% of detection frequency (TEP, TCEP, TDClPP, TPHP, TNBP, DCP, 2IPPDPP, 4IPPDPP and B4IPPPP).The highest values corresponded to TDClPP (mean value of 8.90 ng/g ww), followed by 2IPPDPP (4.67 ng/g ww), TEP (4.51 ng/g ww) and DCP (4.45 ng/g ww) (Table 2).

The present study showed for the first time the presence of 16 OPEs in marine plastic debris. TBOEP and T2IPPP had the highest detection frequency (100%), followed by B4IPPPP, TPHP, TNBP and DCP (80%). The highest concentrations corresponded to T2IPPP with 247 ± 409 ng/g. However, our results were one order of magnitude lower than levels reported previously. Zhang et al. (2018) presented results of four OPEs (TCEP, TClPP, tri-isobutyl phosphate (TiBP) and TNBP) from beached microplastics collected from the Bohai and Yellow Sea in north China, with maximum levels of 84596 ng/g. Similarly, Cao et al. (2020) on five OPEs in stranded foams collected from an island in the South China Sea reported TCEP, TCIPP and TPHP the main chemicals with ∑OPEs in plastic surface of 175, 4540 and 855 μg/g, respectively. As mentioned before, it is very difficult to completely extract polymer additives from analysed fragments. Therefore, our reported low OPE concentrations in marine plastic debris may be the consequence of this incomplete extraction.

Table 2
OPE levels in prey and plastic debris samples collected from the Balearic Islands.

	Prey (*ng/g ww*)				Plastic Debris (*ng/g*)					
	Sardine	Squid	Jellyfish	Mean*	Bottle caps	Fishing net	Ear sticks	Bags	Pieces of plastic	Mean*
TEP	4.67	8.21	nq	4.51	nd	nq	11.1	nd	54.4	13.3
TCEP	nq	1.40	0.10	0.51	nd	nd	13.5	nd	9.26	4.57
TPPO	nq	nq	nd	nq	nd	nd	nd	nd	nd	nd
TClPP	nq	8.01	nd	2.71	nd	8.75	nd	nd	nd	1.78
TPP	nd	nd	nd	nd	nd	nd	nd	nq	nd	nq
TDClPP	9.78	15.4	1.52	8.90	nd	nd	21.1	nd	0.98	4.43
TPHP	1.15	2.17	0.44	1.25	nd	0.17	2.02	nq	1.62	0.77
TNBP	0.87	1.45	0.17	0.83	0.99	4.19	nd	0.73	2.88	1.76
DCP	1.92	11.3	0.11	4.44	3.39	0.40	nd	0.35	nd	0.84
TBOEP	nd	nd	nd	nd	4.77	3.89	8.70	3.43	9.35	6.03
2IPPDPP	1.48	11.9	0.61	4.66	nd	nd	6.96	nd	nd	1.42
4IPPDPP	0.87	7.09	0.27	2.74	nd	nd	6.42	nd	nd	1.32
TCP	nd	13.3	0.49	4.61	nd	31.4	nd	nd	nd	6.31
EHDPP	nd	nq	0.84	0.29	nd	nd	nd	1.55	nd	0.32
B4IPPPP	0.91	10.3	nq	3.83	nd	2.39	5.86	3.65	3.02	3.31
IDPP	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
T2IPPP	nq	nq	nq	nq	nq	12.5	100	857	18.3	198
THP	nd	nq	nd	nq	nd	nd	nd	nd	nd	nd
TEHP	nd	nd	nd	nq	1.70	nd	nd	1.15	nd	0.59
ΣOPEs	21.7	90.5	4.55	6.69	10.9	63.7	176	868	99.8	64.3

nd: not detected, below LOD; nq: not quantifiable, below LOQ.
*Mean values were calculated assuming that nd = ½ LOD and nq = LOD.

The OPE profiles of turtle muscle, prey samples and plastic debris exhibited some remarkable differences (Tables 1 and 2, Fig. 2). Ten compounds (TEP, TClPP, TDClPP, TPHP, TNBP, DCP, 2IPPDPP, 4IPPDPP, TCP and EHDPP) were present in plastic debris, turtle muscle and prey and for that reason turtles might acquire them from plastic debris, diet or both. TEP, TClPP, TDClPP and TNBP have log K_{ow} values lower than 4.5 as well as bioconcentration factors (BCFs) below 2000 (Table S1), and hence a lack of bioaccumulative properties (EU REACH) and are more likely to be derived from plastic debris. TEP is the best example, as it is the most abundant OPE in turtle muscle and pieces of plastic, the most common type of plastic debris in the stomach contents of turtles from the Balearic Islands (Revelles et al., 2007a). Conversely, TEP levels in squid and sardine were lower than those of other OPEs. More importantly, TEP was not detected in jellyfish, the main prey of turtles in the Balearic Islands (Revelles et al., 2007a) and hence the available evidence strongly supports the hypothesis that pieces of plastic were the main source of TEP for turtles. A mixed origin is more likely for moderate bio-accumulative OPEs, such as TPHP, DCP and EHDPP (log K_{ow} values between 4.5 and 6 and BCFs < 2000). DCP is of particular interest, because is the second most abundant OPE in turtle muscle and occurs at moderate levels in all the prey species, but has been detected only in plastic bags and fishing nets. Diet is the most likely source, but contamination from plastic bags cannot be ruled out. Finally, diet is the most likely origin for the most bio-accumulative compounds found in turtle muscle, TCP and isopropylated triarylphosphates 2IPPDPP and 4IPPDPP (log K_{ow}

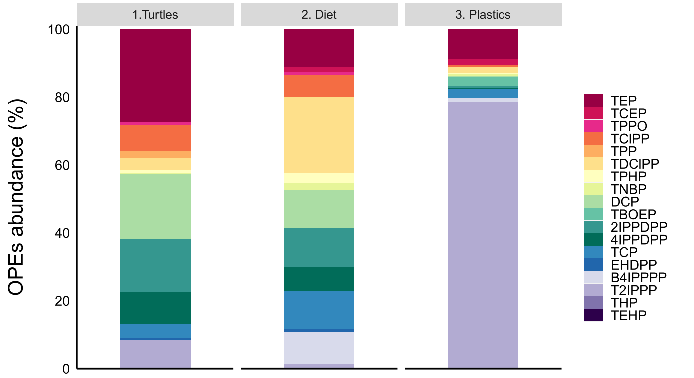

Fig. 2. Percentage contribution of detected OPEs to the total concentration levels in turtles, diet and plastics from Balearic Islands.

values between 5 and 9 and BCFs > 2000). Ear sticks were the only type of plastic debris were 2IPPDPP and 4IPPDPP were detected, but both compounds were present at high levels in most turtle samples and detected at high or intermediate levels in the three prey species. As ear sticks are uncommon in the stomach contents of loggerhead turtles from the Balearic Islands (Revelles et al., 2007a), diet is the most likely source of 2IPPDPP and 4IPPDPP for turtles. The potential source of TCP is less obvious. Fishing nets were the only type of plastic debris were TCP was detected, but this compound occurred in 41% of the turtle samples, as well as in squid and jellyfish samples. As fishing lines occur frequently in the stomach contents of turtles from the Balearic Islands (Revelles et al., 2007a) a mixed origin cannot be ruled out. On the other hand, T2IPPP was the main OPE in plastic bags and ear sticks. Occurring also at lower levels in pieces of plastic and plastic nets (Table 2, Fig. 2). T2IPPP was detected in 23% of the turtles, but was only detected at levels below LOQ in prey samples (Table 2, Fig. 2). Thus, the presence of T2IPPP in turtle muscles should basically come from the ingestion of plastic debris. Two other compounds, TPP and TBOEP, were only found in turtle and plastic debris, suggesting the latter as the source of contamination. However, TBOEP levels were much lower in turtle muscle than in plastic debris, which is consistent with previous studies showing evidences of their metabolization (Van den Eede et al., 2013). PCA on standardized data highlighted the differences between the OPE profiles of loggerhead turtles from the Balearic Island, their prey and plastic debris. PCA extracted five components with an eigenvalue >1, that explained 74.3% of the overall variability in the OPE profiles. The first component (PC1) explained 23.2% of the overall variability in the OPE profiles and positive values were strongly associated to high levels of TBOEP, TPHP, TCEP and B4IPPPP (Fig. 3). Samples of plastic debris and two prey species (sardine and squid) had positive values of PCA1, whereas jellyfish and loggerhead turtles had negative values (Table S5). The second component (PCA2) explained 19.4% of the overall variability in OPE profiles and opposed the abundance of 2IPPDPP, 4IPPDPP, TDCIPP, TEP, DCP and TPP (positive values), and that of EHDPP and TEHP (negative values) (Fig. 3). Only jellyfish clustered with turtles and bottle caps and fishing nets were the plastic debris with closer OPE profiles (Fig. 3). However, variability within turtles was broad and half of them largely departed from the profiles of jellyfish. Thus, the overall evidence suggests that loggerhead turtles derive some OPEs directly from the ingested plastic debris, although in most cases, the diet may also play a relevant role. The likely metabolization of TBOEP further differentiates the profiles of OPEs in turtles from those in plastic debris. It should be noted that diet, and not plastic debris, has been identified as the main source of PCBs, DDTs and chlordanes for sea turtles in the Pacific Ocean (Clukey et al., 2018), but none of those compounds were plastic additives.

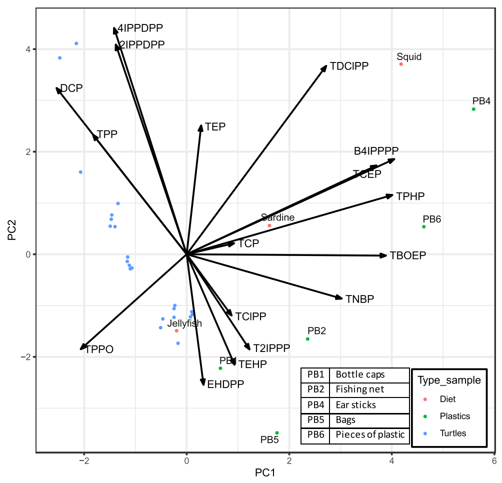

Fig. 3. PCA of turtles, prey and plastic debris from the Balearic Islands.

There are no studies showing the relationship between plastic ingestion and OPE levels in the marine biota. Cao et al. (2020) reported OPE levels in stranded foams from an island located in the South China Sea, and suggested that they can be potential sources of organic pollutants. Another recent study (Schmidt et al., 2021) analysed both microplastics and organic plastic additives, including OPEs, in surface seawater, sediment and zooplankton samples. The concentration of plastic additives in surface waters and the abundance of microplastic particles were not correlated, implying that they are not necessarily good indicators for each other in this compartment. Sea birds also ingest macroplastics voluntarily and Tanaka et al. (2013) have reported the occurrence of PBDEs in seabird tissues and in plastic debris, but not in the prey of the seabirds. In another study, Guo et al. (2019) demonstrated the transfer of flame retardants from FRs-incorporated recycled plastics to avian gastrointestinal tracts. Finally, levels of plastic additives in the liver and adipose tissue have been reported to be 10^1 - 10^5 times higher than the baseline (Tanaka et al., 2020), emphasizing the relevance of ingested plastic debris as a major source of plastic additives for sea birds.

Finally, it is necessary to point out that OPEs can be also bioconcentrated from ambient seawater to the turtles. However, taking in to account reported concentrations of OPEs in water samples, this would probably not be the main source of OPE contamination in turtles. A recent review (Wang et al., 2020) shows that OPE levels in surface waters ranged between 100 and 3000 ng/L. Seawater data are scarcer, but Schmidt et al. (2021) reported total OPE concentrations between 10 and 1000 ng/L. Therefore, OPEs in water were in the ppt level, whereas they increased to the ppb level in prey and plastic debris samples. OPEs can also bioconcentrated from suspended particle matter (SPM) to the turtles. In this case, (Yin et al., 2021) concluded that log K_{OC} between water and SPM was higher than that between water and sediment, indicating that OPEs in water were more easily adsorbed on SPM than on sediment.

3.3. *OPE biomagnification*

A compound biomagnifies when concentration levels in a predator were higher than those in its prey. As in this study we have data in predators (turtles) as well as in prey (jellyfish, squid and sardine), we can evaluate which OPEs were biomagnified. Five different compounds, TCP, DCP, 2IPPDPP, 4IPPDPP and T2IPPP seem to have biomagnification potential. Our study reveals increasing levels of ΣOPEs from jellyfish to sardine and squid, which agrees with their trophic positions (Revelles et al., 2007a; Cardona et al., 2012). Furthermore, prey species were broadly scattered along the second component in PCA, with increasing relevance of 2IPPDPP and 4IPPDPP, two compounds with high bio-accumulative potential, from jellyfish to squids (Fig. 3). The same is true for loggerhead turtles, with nine specimens clustered around jellyfish and the remaining individuals widely scattered along the second component. Although the specimens with the highest levels of 2IPPDPP and 4IPPDPP were larger than 50 cm CCL, there was no correlation between carapace length and position along PCA2 (p-value = 0.333), thus suggesting that individual differences in diet or plastic debris exposure are relevant.

Studies evaluating OPE biomagnification are limited and do not provide clear evidence to conclude whether or not these pollutants undergo the biomagnification process along trophic chains. Bekele et al. (2019) showed a biomagnification potential of OPEs in a marine food web, increasing this potential with the compound lipophilicity. In another study, Hallanger et al. (2015) found a weak biomagnification of OPEs from fish to their predators, whereas Strobel et al. (2018) found limited OPE biomagnification from ringed seals to polar bears. However, Brandsma et al. (2015) and Zhao et al. (2018) analysed the trophic transfer through entire marine food webs and found that the majority of OPEs exhibit trophic dilution instead biomagnification. Recently, Garcia-Garin et al. (2020a) reported similar OPE concentrations in fin whale (mean value of 985 ng/g lw) and their main prey, the krill (mean value of 949 ng/g lw), indicating no biomagnification. One explanation could be related with the rapid metabolism of some OPEs (Hou et al., 2016).

4. Conclusions

This is the first study showing the presence of OPEs in turtles. OPEs were detected in all the samples, with levels ranging from 6.18 to 100 ng/g ww. Levels were higher in the turtles from the Balearic Islands, but OPE profiles did not differ regionally. OPE levels were in the same order of magnitude than other legacy pollutants (PCBs and DDTs) in turtles.

Samples corresponding to the main turtle prey (jellyfish, squid and sardine), as well as different types of marine plastic debris were analysed, showing also the presence of OPEs at concentration levels between 5.21 and 90.5 ng/g ww and 10.9−868 ng/g, respectively. Some differences were observed in the OPE profiles of turtles, their prey and plastic debris. Moreover, it seems that turtles may have been contaminated by both prey and plastic debris. OPE biomagnification has been evaluated through data in predators and in preys, and some compounds (TEP, DCP, 2IPPDPP, 4IPPDPP and T2IPPP) seem to have biomagnification potential.

Further studies are needed to better understand the impact of OPEs in sea turtles, their relationship with marine plastic debris, and the potential toxicological effects that these contaminants can cause in sea turtles.

Author statement

Berta Sala: formal analysis; data treatment; methodology; validation; writing of the original manuscript draft and revision of the manuscript. **Aleix Balasch:** formal analysis; data treatment. **Ethel Eljarrat:** conceptualization and coordination of the study; supervision; methodology; validation; writing of the original manuscript draft and revision of the manuscript; resources; funding acquisition. **Luis Cardona:** sampling; data treatment; conceptualization of the study; writing of the original manuscript draft and revision of the manuscript. All authors have read and approved the final article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.117108.

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PUBLICACIÓ CIENTÍFICA #5

Supplementary information

"First study on the presence of plastic additives in loggerhead sea turtles (*Caretta caretta*) from the Mediterranean Sea."

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Table S1. Physico-chemical properties of the 19 OPEs analysed in this study, including molecular weight, solubility, log Kow and bioconcentration factor (BFC).

Acronym	Name	Cas number	Molecular wight (g/mol)	Solubility (mg/L)*	Log K_{OW}	BCF
TEP	Triethyl phosphate	78-40-0	182.5	$5x10^5$	0.80	1.4
TCEP	Tris(2-chloroethyl) phosphate	115-96-8	285.5	$7x10^3$	1.44	3.46
TPPO	Triphenylphosphine oxide	791-28-6	278.3	62.8	2.87	89.4
TClPP	Tris(2-chloroisopropyl) phosphate	13674-84-5	327.6	$1.6x10^3$	3.11	49.14
TPP	Tripropyl phosphate	513-08-6	224.23	827	1.87	5.317
TDClPP	Tris(1,3-dichloro-2-propyl) phosphate	13674-87-8	430.9	1.5	3.65	100.6
TPHP	Triphenyl phosphate	115-86-6	326.3	1.9	4.59	73.18
TNBP	Tri-n-butyl phosphate	126-73-8	266,31	$2.8x10^2$	4.00	69.65
DCP	Diphenyl cresyl phosphate	26444-49-5	340.3	0.24	5.25	213.5
TBOEP	Tris(2-butoxyethyl) phosphate	78-51-3	398.5	$1.2x10^3$	3.75	54.19
2IPPDPP	2-isopropylphenyl diphenyl phosphate	64532-94-1	368.4	-	5.31	$6.39x10^3$
4IPPDPP	4-isopropylphenyl diphenyl phosphate	55864-04-5	368.4	-	5.7	-
B4IPPP	Bis(4-isopropylphenyl)phenyl phosphate	55864-07-8	410.4	-	6.03	-
TCP	Tricresyl phosphate	1330-78-5	368.4	0.36	6.34	$8.56x10^3$
EHDPP	2-ethylhexyldiphenyl phosphate	1241-94-7	362.4	1.9	5.73	269.2
T2IPPP	Tris(2-isopropylphenyl) phosphate	64532-95-2	452.5	-	9.1	-
THP	Trihexyl phosphate	2528-39-4	350.5	$1.02x10^{-2}$	7.45	$2.72x10^5$
TEHP	Tris(2-ethylhexyl) phosphate	78-42-2	434.6	0.6	9.49	1.219
IDPP	Isodecyl diphenyl phosphate	29761-21-5	390.5	0.75	7.28	288.4

* Data obtained from Wei et al. (2015), Li et al. (2019), Blum et al. (2019), van der Veen and de Boer (2012), Phillips and Stapleton (2019), and WHO (1990, 1991, 1998 and 2000).

Table S2. Summary of turtle samples analysed, including sampling code, curved carapace length (CCL) and year of collection.

Catalan Coast			Balearic Coast		
Year	**Code**	**CCL (cm)**	**Year**	**Code**	**CCL (cm)**
2014	CC11	41	2014	CB2	77
2015	CC4	57	2014	CB3	62
2015	CC5	59	2014	CB18	66
2015	CC16	54	2014	CB21	26
2015	CC17	30.6	2014	CB22	43
2015	CC22	50	2014	CB23	61
2015	CC23	68	2015	CB12	16
2016	CC6	35	2015	CB19	52
2016	CC9	43	2016	CB1	57
2016	CC12	46	2016	CB7	69
2016	CC13	34	2016	CB10	38
2016	CC14	58.5	2016	CB11	39.5
2016	CC20	68	2016	CB13	70
2017	CC3	61	2016	CB20	60
2017	CC7	58	2016	CB16	65
2017	CC8	78	2017	CB5	24.5
2017	CC10	36	2017	CB6	52
2017	CC15	45	2017	CB8	24.5
2017	CC18	48	2017	CB9	48
-	CC21	44.7	2017	CB14	42
			2017	CB15	47
			2017	CB17	60

Table S3. Recoveries, relative standard deviations (RSDs) and limits of detection (LODs) and quantification (LOQs), expressed in ng/g wet weight (ww), of LC-MS-MS analysis of selected OPEs.

Analyte	Recovery	RSD (%)	mLOD	mLOQ
TEP	59	15	0.53	1.75
TCEP	68	3.7	0.05	0.10
TPPO	47	2.5	0.65	1.91
TClPP	64	3.0	0.07	0.20
TPP	40	16	0.06	0.19
TDClPP	56	8.1	0.04	0.09
TPHP	53	2.5	0.06	0.12
TNBP	72	3.2	0.02	0.06
DCP	73	4.0	0.05	0.08
TBOEP	65	12	0.02	0.04
2IPPDPP	42	2.9	0.08	0.30
4IPPDPP	41	6.7	0.09	0.30
B4IPPPP	61	5.1	0.21	0.69
TCP	78	13	0.07	0.11
EHDP	62	16	0.02	0.05
IDPP	85	4.5	0.04	0.14
T2IPPP	82	9.0	0.95	2.62
THP	81	10	0.05	0.17
TEHP	98	12	0.08	0.21

Table S4. Concentration levels (expressed in ng/g ww) of OPEs determined in Catalan and Balearic loggerhead sea turtles (*Caretta caretta*).

	TEP	TCEP	TPPO	TClPP	TPP	TDClPP	TPHP	TNBP	DCP	TBOEP	2IPPDPP	4IPPDPP	TCP	EHDPP	T2IPPP	ΣOPEs
Catalan Coast																
CC3	7.01	nq	nq	nd	0.71	124	50.5	nd	10.4	nd	48.8	143	nd	nd	nd	**384**
CC4	6.77	nd	nq	nd	0.25	nd	nd	nd	6.96	nd	nq	4.24	nd	nd	nd	**18.2**
CC5	4.59	nq	nq	nd	nd	nd	nd	0.73	8.44	nd	nd	nd	nq	nd	nd	**13.8**
CC6	5.09	nq	nd	nd	nd	nd	nd	nd	7.19	nq	nq	6.94	nd	nd	nd	**19.2**
CC7	6.06	nq	nq	nd	nd	nd	nd	nd	7.47	nd	nd	2.12	nd	nd	nd	**15.7**
CC8	nq	nq	nq	nd	nq	1.13	1.01	0.23	4.80	0.23	0.87	nd	nd	1.47	12	**21.7**
CC9	5.12	nq	nq	nd	2.83	nd	nd	nd	8.41	nd	nq	5.14	nd	nd	nd	**21.5**
CC10	4.13	nq	nq	nd	nd	nd	nd	nd	6.26	nd	nq	3.75	nq	nd	nd	**14.1**
CC11	5.26	nq	nq	nd	nd	0.36	nd	nd	8.14	nd	8.26	4.08	nd	nd	nd	**26.1**
CC12	6.44	nq	nd	nd	nd	nd	nd	nd	6.21	nd	3.73	4.61	nd	nd	nd	**21.0**
CC13	10.4	nq	nq	nd	nd	nd	nd	0.91	7.27	nd	nq	0.83	nq	nd	nd	**19.4**
CC14	5.13	nq	nq	nd	nd	nd	nd	0.25	7.84	nd	nd	0.34	nq	nd	nd	**13.6**
CC15	nd	nq	nq	3.43	nd	0.60	0.36	0.23	2.77	0.11	1.46	nq	nd	1.43	9.79	**20.2**
CC16	6.23	nq	nq	nd	nd	nd	nd	0.07	6.62	nd	nq	nd	nd	nd	nd	**12.9**
CC17	8.86	nq	nq	nd	nd	nd	nd	0.37	nd	nd	nq	3.25	nq	nd	nd	**12.5**
CC18	nd	nq	nq	8.54	nq	2.37	1.08	0.09	6.45	0.14	nd	0.72	nd	2.79	9.45	**31.6**
CC20	4.87	nd	nq	nd	1.35	nd	nd	nd	8.07	nd	nq	3.64	nd	nd	nd	**17.9**
CC21	10.5	nq	nq	nd	nq	0.57	nd	nd	6.28	nd	1.81	9.11	nq	nd	nd	**28.4**
CC22	9.23	nq	nq	nd	nd	nd	nd	0.89	6.98	nd	nq	7.07	nq	nd	nd	**24.2**
CC23	6.60	nd	nq	nd	0.23	nd	nd	0.42	7.93	nd	17.9	6.42	nq	nd	nd	**39.5**
Mean*	*5.58*	*nq*	*nq*	*0.65*	*0.27*	*0.28*	*0.15*	*0.23*	*6.53*	*0.03*	*1.83*	*3.29*	*nq*	*0.31*	*2.04*	***21.6***
% DF*	*74*	*85*	*85*	*10*	*30*	*30*	*20*	*50*	*95*	*15*	*80*	*85*	*40*	*15*	*15*	***100***
Balearic Islands																
CB01	4.28	nq	nq	nd	1.91	nd	nd	nd	10.2	nd	nd	5.51	23.8	nd	nd	**45.7**
CB02	9.58	nq	nd	nd	nd	nd	nd	0.48	8.64	nd	8.80	1.81	nd	nd	nd	**29.3**
CB03	1.74	nq	nq	5.87	nd	0.76	0.61	0.41	2.77	0.08	3.46	0.44	nd	0.08	8.95	**25.2**
CB05	42.7	nq	nq	nd	0.43	0.53	nd	nd	8.10	nq	3.88	6.35	nd	nd	nd	**62.0**
CB06	nq	nq	nq	4.76	nd	0.37	0.09	0.41	1.68	0.06	1.18	nq	nd	0.27	7.76	**16.6**
CB07	22.2	nq	nq	nd	2.70	nd	nd	nd	10.9	nq	2.51	9.28	3.83	nd	nd	**51.4**
CB08	2.93	nq	nq	8.65	nq	0.73	0.43	0.45	2.76	nd	nd	nd	nd	nq	nd	**16.0**
CB09	7.16	nq	nq	nd	nd	0.73	nd	nd	7.13	nd	5.69	9.58	nq	nd	nd	**30.3**
CB10	21.0	nq	nq	nd	nd	nd	nd	nd	6.85	nd	4.85	nd	nq	nd	nd	**32.7**
CB11	10.1	nq	nq	nd	nq	0.83	nd	nd	9.95	nd	nq	1.65	7.92	nd	nd	**30.5**
CB12	nd	nq	nq	22.9	nd	0.38	1.00	0.36	7.23	0.29	1.98	0.30	nd	nq	27.7	**62.1**
CB13	nq	nd	nq	6.52	nd	1.33	0.58	0.14	3.76	0.40	nd	nd	nd	nq	nd	**12.7**
CB14	2.48	nd	nq	nd	nd	1.83	0.90	0.07	6.42	0.20	0.09	nd	nd	2.52	11.0	**25.5**
CB15	18.0	nq	nq	nd	nd	nd	nd	nd	7.69	nd	4.28	1.60	nq	nd	nd	**31.6**
CB16	14.2	nq	nq	nd	nd	nd	nd	nd	6.18	nd	5.18	1.49	nd	nd	nd	**27.1**
CB17	nd	nq	nq	10.4	nq	1.56	1.34	0.25	8.50	0.07	nd	nd	nd	0.95	8.05	**31.1**
CB18	5.31	nq	nq	nd	nd	0.70	nd	nd	7.10	nd	16.1	6.60	nd	nd	nd	**35.8**
CB19	15.4	nq	nq	nd	nd	12.2	nd	nd	24.1	nd	40.7	7.85	nq	nd	nd	**100**
CB20	nd	nq	nq	1.95	nq	0.73	0.42	0.04	2.53	0.07	nd	nd	nd	0.34	nd	**6.08**
CB21	5.80	nd	nq	2.45	nq	1.20	1.00	0.69	4.81	0.30	nd	nd	nd	0.95	nd	**17.2**
CB22	17.6	nq	nq	nd	nd	nd	nd	nd	7.80	nd	10.9	7.45	nq	nd	nd	**43.8**
CB23	20.4	nq	nq	nd	3.56	5.11	nq	nd	7.79	nd	23.6	18.0	nq	nd	nd	**78.5**
Mean	*10.1*	*nq*	*nq*	*2.91*	*0.42*	*1.32*	*0.31*	*0.15*	*7.40*	*0.07*	*6.07*	*3.56*	*1.66*	*0.24*	*3.25*	***37.9***
% DF	*82*	*86*	*95*	*36*	*36*	*68*	*45*	*45*	*100*	*45*	*73*	*68*	*41*	*41*	*23*	***100***

*Mean value without the oulier CC3 and calculated with nd = ½ LOD; nq = LOD; ** DF: Detection frequency
nd: not detected, below LOD; nq: not quantifiable, below LOQ

Table S5. Component matrix for the two first principal components (PCs), which shows the correlation coefficients between the standardized values of the 19 OPEs selected for analysis and the tow first PCs.

Analyte	**PC 1**	**PC 2**
TEP	0.032	0.449
TCEP	0.647	0.292
TPPO	-0.487	-0.337
TClPP	0.094	-0.215
TPP	-0.308	0.429
TDClPP	0.544	0.658
TPHP	0.714	0.196
TNBP	0.632	-0.169
DCP	-0.479	0.603
TBOEP	0.743	-0.028
2IPPDPP	-0.222	0.749
4IPPDPP	-0.221	0.807
B4IPPPP	0.833	0.325
TCP	0.239	0.040
EHDP	0.055	-0.463
IDPP	0.910	-0.064
T2IPPP	0.297	-0.343
THP	0.919	0.079
TEHP	0.303	-0.396

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PUBLICACIÓ CIENTÍFICA #6

“Organophosphate ester plasticizers in edible fish from the Mediterranean Sea: Marine pollution and human exposure”

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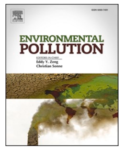

Organophosphate ester plasticizers in edible fish from the Mediterranean Sea: Marine pollution and human exposure[☆]

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ABSTRACT

Concentrations of organophosphate esters (OPEs) plasticizers were analysed in the present study. Fifty-five fish samples belonging to three highly commercial species, European sardine (*Sardina pilchardus*), European anchovy (*Engraulis encrasicolus*), and European hake (*Merluccius merluccius*), were taken from the Western Mediterranean Sea. OPEs were detected in all individuals, except for two hake samples, with concentrations between 0.38 and 73.4 ng/g wet weight (ww). Sardines presented the highest mean value with 20.5 ± 20.1 ng/g ww, followed by anchovies with 14.1 ± 8.91 ng/g ww and hake with 2.48 ± 1.76 ng/g ww. The lowest OPE concentrations found in hake, which is a partial predator of anchovy and sardine, and the higher δ^{15}N values (as a proxy of trophic position), may indicate the absence of OPEs biomagnification. Eleven out of thirteen tested OPEs compounds were detected, being diphenyl cresyl phosphate (DCP) one of the most frequently detected in all the species. The highest concentration values were obtained for tris(1,3-dichloro-2-propyl) phosphate (TDClPP), trihexyl phosphate (THP), and tris(2-butoxyethyl) phosphate (TBOEP), for sardines, anchovies, and hakes, respectively. The human health risk associated with the consumption of these fish species showing that their individual consumption would not pose a considerable threat to public health regarding OPE intake.

1. Introduction

Plastic pollution is an increasing threat for marine ecosystems globally (Eriksen et al., 2014; Worm et al., 2017) where the Mediterranean Sea stands out as a remarkably impacted area (García-Rivera et al., 2017; García-Rivera et al., 2018; De Haan et al., 2019; Compa et al., 2019) with estimates of up to 11.5 million items of floating marine macro-litter (Lambert et al., 2020) and a density of 16 items km^{-2} along the North Mediterranean Sea (Garcia-Garin et al., 2020a). Marine organisms are capable of ingesting plastics and microplastics with potential perturbations at different physiological levels (Gabriel et al., 2020). Beyond the ingestion of plastic itself, it is an increasing concern the accumulation of plastics additives and pollutants and the capacity of some of those to bioaccumulate and/or biomagnify (Bekele et al., 2019; Ding et al., 2020). Chemical additives associated with plastics, such as plasticizers and flame retardants like organophosphates esters (OPEs), deserve special attention due to their environmental widespread and their toxicological effects. OPEs are increasingly being used in several products (i.e. plastics, textile, furniture, etc.) and can be released into the environment through different processes (like abrasion and volatilization) resulting in a wide range of concentrations levels detected throughout the environment: indoor/outdoor air, water, soil, and sediment, as well as in animals and humans (Li et al., 2019).

Some OPEs are volatile and predominate in the air phase, while others are water soluble or sorb strongly to particulate matter (Reemtsma et al., 2008). Air deposition, river flux, air-water distribution and macro- and micro-plastics are different sources of OPEs in the marine environment. Due to their ubiquity and increasing environmental

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levels, OPEs have been the focus of recent studies in the marine biota (Sala et al., 2019; Garcia-Garin et al., 2020b; Aznar-Alemany et al., 2021), with increasing interest to investigate the potential derived toxicological effects in biota. For instance, some OPEs were proven to be neurotoxic and carcinogenic (van der Veen and de Boer, 2012) and *in vitro* studies also confirm OPEs to cause developmental neurotoxicity, as well as adverse transcriptomic, reproductive, endocrine, and carcinogenic effects (Frydrych, 2015; Du et al., 2019). Moreover, adverse effects on female reproduction and fetal development in humans have been described (Hu et al., 2017). Nevertheless, the information related to the occurrence of OPEs in marine organisms is still limited and the ultimate implications for human health are mostly unknown, with no reference doses for most OPEs (Bekele et al., 2021).

In the Northwestern Mediterranean Sea, European anchovy (*Engraulis encrasicolus*) and European sardine (*Sardina pilchardus*) are two of the most abundant and commercially important small pelagic fish (SPF) species (Cury et al., 2000). Beyond their economic importance, both species play a key ecological role at the intermediate levels of the food web (Coll et al., 2008; Palomera et al., 2007). Sardine and anchovy are planktivorous feeders, inhabiting the continental shelf and shelf-break, with sardine selecting shallower waters and anchovy occupying larger off-shore areas (Palomera et al., 2007). An important decline in sardine and anchovy populations in the Western Mediterranean Sea has been observed in the last decades, especially evident in the subareas GSA06 and GSA07 (as defined by the General Fisheries Commission for the Mediterranean (GFCM) (Coll et al., 2019; Coll and Bellido, 2019; Palomera et al., 2007; Saraux et al., 2019). This decline has not only implied a reduction of biomass and landings, but also a decrease in individual's total body size and condition (Albo-Puigserver et al., 2019; Van Beveren et al., 2014). Various hypotheses have been considered to explain these trends; for instance, an increase in fishing pressure, environmental changes (*i.e.* variations in temperature), changes in plankton composition, disease (including parasites), the impacts of recovering predators or competitors, or an increase in pollution (Albo-Puigserver et al., 2019; Coll et al., 2019; Coll and Bellido, 2019; Palomera et al., 2007; Pennino et al., 2020; Saraux et al., 2019). Precise knowledge of potential threats affecting SPF dynamics is important, as changes in their population may have consequences in higher trophic levels within marine ecosystems and in regional fisheries. In fact, a recent study expressed concern about the underlying effect for the stock of European hake (*Merluccius merluccius*), an important predator of anchovy and sardine (Sion et al., 2019).

In this context, a better understanding of the extent of plastic pollution, direct and/or indirect (such as through the herein analysed OPE plasticizers) in sardine and anchovy can help to identify the true extent of this threat. Previous studies have documented microplastics and fibres in the stomach contents of sardines and anchovies (Compa et al., 2018; Pennino et al., 2020), with values up to 58% and 60% recorded, respectively (Pennino et al., 2020) but their levels of OPEs in this areas had not been assessed before. We also included European hake in our analysis to compare the concentration of OPE plasticizers with those of sardine and anchovy and investigate potential bioaccumulation and/or biomagnification of these pollutants. European hake, with a wide bathymetric range occupying most of the shelf and shelf-break (Demestre et al., 2000; Fisher et al., 1987), is one the most important demersal target species for commercial fisheries in the Mediterranean Sea (Sánchez et al., 2007) and it is known to partially prey on sardine and anchovy with clear ontogenetic changes on their feeding patterns (Bozzano et al., 1997; Cartes et al., 2009, 2004; Ferraton et al., 2007; Lloret-Lloret et al., 2020).

In this study, our main objectives were: (i) to investigate the incidence of OPE plasticizers in three edible fish, two small pelagic fish and a higher trophic predator, along a latitudinal gradient in the Northwestern Mediterranean Sea; (ii) to relate the recorded contaminant values with potential biomagnification process; and (iii) to calculate the human daily intake of these pollutants based on estimates of fish consumption. The information generated here can provide insight towards the Sustainable Development Goals (SDG), covering not only SDG_{14}; the restoration and conservation of marine and coastal areas and ecosystems, but also SDG_3, aiming to improve both human and ecosystem health as well as animal well-being (Borja et al., 2020; United Nations, 2016).

2. Materials and methods

2.1. Sample collection and study area

Fish samples of European anchovy, European sardine, and European hake were collected in the Western Mediterranean area (Fig. 1), in Geographical Sub-Areas 6 (GSA06) during the MEDITS 2019 survey (MEDiterranean International bottom Trawl Survey (Bertrand et al., 2002a; Bertrand et al., 2002b). Samples were obtained through a bottom trawl GO73 with a 20 mm cod-end mesh size net (Bertrand et al., 2002). The GSA06 includes the Spanish continental waters of the Western Mediterranean Sea, from "Cape of Creus" in the north, to "Cape of Palos" in the south. It is an important fishing ground in the Mediterranean Sea (FAO, 2020; Papaconstantinou and Farrugio, 2000) characterized by topographic and hydrographic features with a latitudinal gradient (Estrada, 1996). The continental shelf broadens in the south, and it is widest around the Ebro Delta. It is considered a highly productive area due to the run-off from the Ebro River and the Liguro-Provençal-Catalan current which is the main current in the area, flowing south-westwards along the continental slope (Estrada, 1996).

Fifty-five fish samples were collected during May and June 2019 (Table S1) from the four sampling sites selected: Cape of Creus, Ebro Delta, Gulf of Valencia and Gulf of Alicante (Fig. 1). Samples were wrapped on aluminium foil, to prevent cross-contamination from plastic material, and were frozen onboard for further analysis (ICES-CIEM, 2015). Once in the laboratory, samples were defrozen and total length, sex and maturity stage were noted. Muscle samples of each fish were extracted for stable isotope determination and analysis of contaminant concentration. Throughout this process, samples were always handled over a non-plastic surface and only metal instruments were employed. European hake individual length ranged between 24.2 and 41.1 cm, being most of them in active mature stage; European sardine were between 13.5 and 16.3 cm and all of them were in the mature stage; and European anchovy individuals presented the smallest length, which ranged between 11.4 and 15.4 cm and they were mostly at mature stage (Table S1).

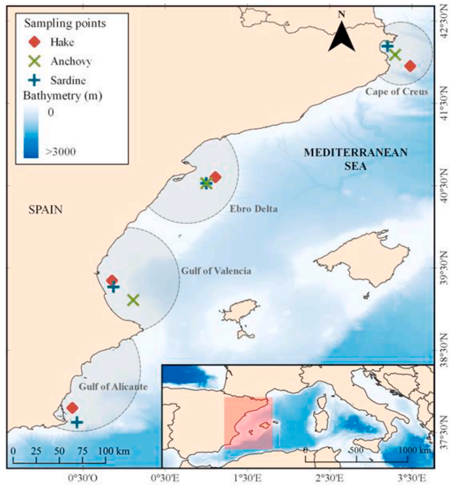

Fig. 1. Map of the Western Mediterranean Sea showing our four study areas (Cape of Creus, Ebro Delta, Gulf of Valencia and Gulf of Alicante) (in shaded grey) and the geographical locations where individuals of European hake (red rhombus), European anchovy (green cross) and European sardine (blue cross) were collected. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.2. Standards and reagents

Thirteen OPEs were included in this analytical study. Different analytical standards were used. Tris(2-chloroethyl)-phosphate (TCEP), TBOEP, THP, tris(2-ethylhexyl) phosphate (TEHP) and tris (chloroisopropyl)-phosphate (TClPP) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). 2-ethylhexyldiphenyl phosphate (EHDPP) and isodecyldiphenyl phosphate (IDPP) were purchased from AccuStandard (New Haven, CT, USA). Tri-cresyl phosphate (TCP) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Diphenyl cresyl phosphate (DCP), triphenyl phosphate (TPHP), TDClPP, tri-n-butyl phosphate (TNBP), and triphenylphosphine oxide (TPPO) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Labelled internal standards (IS) were also used. d_{15}-TPHP was obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA), whereas d_{27}-TNBP, d_{15}-TDClPP, $^{13}C_2$-TBOEP and d_{12}-TCEP were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada).

Acetone and hexane solvents for organic trace analysis were purchased from J.T. Baker (Center Valley, PA, USA). Methanol and water solvent were obtained from Merck (Darmstadt, Germany).

2.3. Sample preparation

Sample preparation was based on a previously developed method (Giulivo et al., 2016). All samples were lyophilized and homogenized and for every batch of samples, a laboratory blank with hydromatrix was done. To proceed with the ultrasounds extraction, 0.5 g of dry weight (dw) were transferred to a centrifuge tube with 15 mL of hexane:acetone (1:1). After 15 min in an ultrasound bath and 20 min of centrifugation at 22 °C and 4000 r.p.m, the liquid phase was collected. Extraction was carried out twice, and then both extracts were combined. Samples were concentrated to complete dryness under a gentle purified nitrogen source, and then reconstituted with 5 mL of hexane:methanol (1:3). Ten ng of each IS were added to an aliquot of 200 μL, before instrumental analysis (Giulivo et al., 2016). Additionally, another aliquot was used for the lipid content determination gravimetrically. The lipid content referred to wet weight (ww) was between 4.6 and 7.1% for European hake, 7.4 and 47% for European sardine, and 4.9 and 20% for European anchovy.

2.4. Instrumental analysis

Instrumental analysis of OPEs was carried out using a turbulent flow chromatography - high-pressure liquid chromatography - tandem mass spectrometry (TFC-HPLC-MS-MS), using the system Thermo Scientific TurboFlowTM, which allows performing an online sample purification and analysis. This system consisted of two quaternary pumps and three LC columns, two of them for the purification and the last one for separation. The purification columns used were CyclonTM-P (0.5 × 50 mm) and C18-XL (0.5 × 50 mm), and the chromatographic separation was done using the column Purosphere Star RP-18 (125 mm × 0.2 mm), which has a particle size of 5 μm. Detailed conditions used for purification and chromatographic separation were included in Table S2. The tandem MS of this system was a triple quadrupole (QqQ) that uses a heated electrospray ionisation source (H-ESI). Mass spectrometry detection parameters can be found in previous work (Giulivo et al., 2016). Selective reaction monitoring (SRM) mode was used for all compounds with two transitions monitored for each analyte. The most intense transition was used for quantification, while the second provided confirmation. Instrumental working parameters such as retention times, transitions, declustering potential, and collision energies were summarized in Table S3.

2.5. Quality assurance

When working with OPEs, there is an important issue to take into account: the blank contamination. Indoor air is contaminated with OPEs and therefore the contamination during the sample preparation process is an important factor. To solve this problem, the non-volumetric material was heated at 340 °C and wrapped with aluminum foil and lastly rinsed with an appropriate solvent just before use. The volumetric material was always rinsed before use with an appropriate solvent. Even taking these precautions, the blank signal was inevitable and uncontrollable, and was different from day to day. The blank signal can come from different places that cannot be controlled, like the ambient air or the nitrogen from the evaporator. A realistic goal is to minimize as much as possible the blank signal. Thus, for every batch of samples, a blank was included. Blank levels were subtracted from corresponding samples.

Instrumental parameters such as recoveries, limits of detection (LODs) and limits of quantification (LOQs) are summarized in Supporting information (Table S4). Recoveries ranged between 47% and 98%, always being within the range of acceptability (40–120%) (USEPA, 1994) for analytical methods based on quantification by isotopic dilution, with relative standard deviation always below 12%. LODs and LOQs ranged between 0.02 and 0.95 ng/g ww and 0.04–2.62 ng/g ww respectively.

2.6. Stable isotope analysis

In order to directly relate the OPE values detected in the three species with potential biomagnification processes, we used nitrogen stable isotopes analysis (SIA) as a proxy of the trophic position of the studied species (Post, 2002). All muscle samples were freeze-dried, powdered and 0.80–0.85 mg of each sample was packed into tin capsules. Isotopic analyses were performed at the Laboratory of Stable Isotopes of University of La Coruña, Galicia, Spain (*Servicio de Analisis Instrumental* (SAI)) through an elemental analyzer (Carlo Erba CHNSO 1108) coupled to an isotopic ratio mass spectrometer (Finnigan Matt Delta Plus). $\delta^{15}N$ (‰) values are reported relative to atmospheric nitrogen (Coplen, 2011). The accuracy (±SE) of the standards replicates and samples is $<0.3\%$.

2.7. Risk assessment of human exposure

Estimated daily intakes (EDIs), computed for high-exposure scenarios (95th percentile), have been calculated according to the following formula:

$$EDI = \frac{AC^{*}C}{BW}$$

where EDI is estimated daily intake of OPEs (ng/kg body weight (bw)/day), AC is the annual per capita fish consumption in Spain (94.4 g/person/day), C is the concentration detected of each OPE (ng/g ww) and BW is the mean body weight of the human consumers (68.5 kg) (AECOSAN and AESAN, 2006). The mean and 95th percentile concentrations of OPEs were used for the mean and high-exposure scenarios, respectively. It was assumed that all fish consumption corresponded to sardines, anchovies and hakes, and that 100% of the ingested OPEs were absorbed.

2.8. Statistical analysis

The concentration of OPEs in all samples was analysed using one-way analysis of variance (ANOVA) with Tukey's test employed to examine the statistical differences among fish species and sampling areas. The level of significance was set at a $p < 0.05$. All statistical analyses were carried out using R (Version February 1, 5042).

Multivariate statistical analysis on OPE compounds was conducted using principal components analysis (PCA) to investigate potential patterns in OPE data associated with spatial (i.e. Cape of Creus, Ebro Delta, Gulf of Valencia, and Gulf of Alicante) or species-specific differences (i.e. sardine, anchovy and hake) in analysed muscle samples. PCA analysis was performed in *FactorMineR* package (Lê et al., 2008) in R, where data was scaled (i.e. standardized) before PCA. *factoextraExtract* package (Kassambara and Mundt, 2020) in R was used to extract and visualize the results of the PCA.

Biomagnification was assessed using δ^{15}N values as a proxy of trophic position. A lineal model was developed between OPE values (ng/g ww) and δ^{15}N values. The significance level was set at $p = 0.05$.

3. Results and discussion

3.1. OPE levels in mediterranean fish

OPEs were detected in 96% of analysed samples with total OPE concentrations ranging between nd (not detected) and 73.4 ng/g ww. The obtained results are summarized in Table 1, indicating the minimum and maximum concentration levels, as well as mean and median values (for individual sample results see Supporting information, Table S5). Sardine presented the highest mean value with 20.5 ± 20.1 ng/g ww, followed by anchovy with 14.1 ± 8.91 ng/g ww and finally by hake with 2.48 ± 1.76 ng/g ww. Significant differences were observed between species (ANOVA, $F_{(2, 52)} = 9.76$, $p = 0.0002$): hake and sardine ($p < 0.001$) and hake and anchovy ($p = 0.05$). However, no significant differences were found between anchovy and sardine ($p > 0.05$).

The three species have different diet composition in the Western Mediterranean Sea. Sardine and anchovy are small pelagic fish and share diet similarities. Both are planktivorous feeders showing an opportunistic ingestion mainly of copepods, cladocerans, euphasiids, decapods and diatoms (Bachiller et al., 2020). In addition, both show particulate feeding capacity but sardines are greater filter-feeders. In the case of European hake, it is an ambush demersal predator with ontogenetic variation in its diet. This species mainly feeds on small crustaceans as well as in small fish, including small pelagic fish like sardine and anchovy (Mellon-duval et al., 2017; Lloret-Lloret et al., 2020). These variations in preying abilities and prey preferences may have resulted in some differences in OPEs ingestion. Nevertheless, OPEs absorption and accumulation does not only result from ingestion but also from other routes like water, sediments and organic matter through the gill and epithelial tissues (Wang et al., 2019).

If we compare concentration levels in the different species by sampling location, significant differences were found in Cape of Creus (ANOVA, $F_{(2, 12)} = 16.9$, $p = 0.0003$) between anchovy and hake ($p < 0.001$) and between anchovy and sardine ($p < 0.01$). In the Ebro Delta (ANOVA, $F_{(2, 12)} = 5.02$, $p = 0.026$), only significant differences between hakes and sardines ($p < 0.05$) were detected. Finally, significant differences were also observed between hakes and sardines in both Gulf of Valencia ($p < 0.05$) and Gulf of Alicante ($p < 0.05$).

The results showed that in all study areas hake was the species with the lowest $\sum$OPEs concentration and the highest δ^{15}N values (Figure S1). A significant negative relationship was found between δ^{15}N and total OPEs ($F_{(1,53)}$, $R^2 = 0.11$, p-value = 0.01, Figure S2), suggesting an absence of biomagnification. Similar results were presented by Brandsma et al. (2015) and Zhao et al. (2018) where they studied trophic transfer through entire marine food webs and found that most OPEs exhibit trophic dilution rather than biomagnification. This contrasts with results from Kim et al. (2011), which found demersal species accumulated more OPEs than pelagic species in Manila Bay (Philippines). They also found a positive correlation of OPEs with δ^{15}N only for one of the congeners (TPHP) but not for the rest. Both, Kim et al. (2011) results and our current results may indicate that the analysed OPEs are not biomagnified through the food web, but they are bioconcentrated by uptake from water and contact with sediments and organic matter through the gill and epithelial tissue.

Nevertheless, if we focus on congeners, TCP was the only one found in hake but absent in anchovy and sardine. This could indicate an uptake from another pathway, as previous research found that OPE accumulation could vary depending on whether congeners are acquired from the benthic or pelagic food web (Kim et al., 2011). In addition, hake feeds on several different prey sources (apart from sardine and anchovy) and with ontogenetic variation, so the TCP could have been acquired from other prey or from the environment itself. On the other hand, our TNBP concentration levels, expressed in lipid weight (lw) (Table S6), were higher in hake (mean value = 79.7 ± 123 ng/g lw) than in sardine and anchovy (22.5 ± 30.3 ng/g lw and 34.5 ± 35.8 ng/g lw respectively), indicating a possible biomagnification of TNBP. Bekele et al. (2019) also reported a significant positive relationship between TNBP concentrations (expressed in lw) and trophic levels. In addition, Garcia-Garin et al. (2020b) also reported results for fin whales and krill from North Atlantic, where TNBP was the most abundant OPE in both organisms, although OPE bioaccumulation in fin whales and biomagnification from krill to whales was not observed. Otherwise, Hallanger et al. (2015) found flimsy biomagnification of OPEs from fish to their predators from Svalbard (Norway) and TNBP was non detected in any sample. Significant negative correlations with lipid contents were found for OPEs concentration in ng/g ww ($r = -0.5$, $p < 0.05$) and with lipid-normalized concentrations of OPEs ($r = -0.61$, $p < 0.01$) in our study. Such results suggest that the bioaccumulation of these compounds is not related to lipid content. Numerous studies have reported that total OPEs were not basically associated with lipids (Sundkvist et al., 2010; Kim et al., 2011; Chen et al., 2012; Brandsma et al., 2015; Malarvannan et al., 2015; Hou et al., 2017). However, Liu et al. (2019b) suggest that chemical affinity of OPEs to lipids still plays a significant role in the deposition of OPEs in tissues. The same study found a relationship between the log K_{ow} values of OPEs and the correlation of OPEs concentration and lipid content. Compounds with a low log K_{ow} had a weak or no correlation, whereas compounds with a log $K_{ow} > 4$ had a positive correlation. Nevertheless, this correlation is not reflected in our study.

Table 1
OPE levels (expressed in ng/g ww) in muscles of European sardine (*Sardina pilchardus*), European anchovy (*Engraulis encrasicolus*) and European hake (*Merluccius merluccius*) from the Western Mediterranean Sea.

	Sardine					Anchovy					Hake				
	Min.	Max.	Mean[a]	Median[a]	DF (%)	Min.	Max.	Mean	Median	DF (%)	Min.	Max.	Mean	Median	DF (%)
TCEP	nd	8.36	2.38	1.87	80	nd	1.64	0.16	0.03	21	nd	nd	nd	nd	0
TPPO	nd	3.64	1.14	0.65	65	nd	3.26	1.21	0.33	36	nd	4.49	0.63	0.33	10
TClPP	nd	6.64	0.54	0.04	10	nd	2.92	0.40	0.03	29	nd	nd	nd	nd	0
TDClPP	nd	62.9	10.1	1.72	65	nd	3.06	0.66	0.03	43	nd	nd	nd	nd	0
TPHP	nd	9.39	2.42	1.30	75	nd	2.28	0.66	0.46	50	nd	nd	nd	nd	0
TNBP	nd	0.70	0.26	0.14	60	nd	0.49	0.17	0.11	64	nd	0.98	0.20	0.01	35
DCP	nd	4.53	2.09	2.14	80	nd	6.05	3.84	3.95	100	nd	1.45	0.31	0.03	35
TBOEP	nd	19.5	1.45	0.01	10	nd	nd	nd	nd	0	nd	3.84	0.98	0.01	35
TCP	nd	nd	nd	nd	0	nd	nd	nd	nd	0	nd	2.34	0.15	0.04	10
EHDPP	nd	nd	nd	nd	0	nd	nq	nd	nd	7	nd	nd	nd	nd	0
IDPP	nd	nd	nd	nd	0	nd	nd	nd	nd	0	nd	nd	nd	nd	0
THP	nd	nd	nd	nd	0	nd	25.1	6.91	4.82	50	nd	nd	nd	nd	0
TEHP	nd	nd	nd	nd	0	nd	nd	nd	nd	0	nd	nd	nd	nd	0
ΣOPEs	1.39	73.4	20.5	14.9	100	3.82	34.7	14.1	12.4	100	nd	6.97	2.48	1.73	90

nd = not detected, below LOD; nq = not quantifiable, below LOQ.

[a] Mean and median values were calculated assigning nd values to ½ LOD, and nq values to LOD.

In vivo toxicological laboratory tests have shown that TNBP was lethal for certain animal species, mainly rats, chick, rabbits, zebrafish and mice, with a median lethal dose (LD_{50}) of over 1.40*10^6 ng/g (van der Veen and de Boer, 2012). But, their concentrations in our muscle samples of hake, sardine and anchovy were five orders of magnitude lower (between nd and 0.98 ng/g ww).

Comparing the different sampling sites, significant differences were detected in OPE levels for anchovy (ANOVA, $F_{(2, 12)} = 7.19$, $p = 0.009$) (Fig. 2a), being the levels in the Ebro Delta significantly lower (mean value of 6.96 ± 1.47 ng/g ww) than those in Cape of Creus (mean value of 21.6 ± 9.27 ng/g ww) ($p < 0.01$). Regarding data on sardine (Fig. 2b), no significant differences were observed between the 4 sampling areas (ANOVA, $F_{(3, 16)} = 2.40$, $p = 0.105$). Finally, the concentrations obtained for hake samples presented significant differences between sampling areas (ANOVA, $F_{(3, 16)} = 6.23$, $p = 0.005$) (Fig. 2c), with lower levels in Cape of Creus ($p < 0.05$) and Gulf of Alicante ($p < 0.05$) when compared to those in the Ebro Delta.

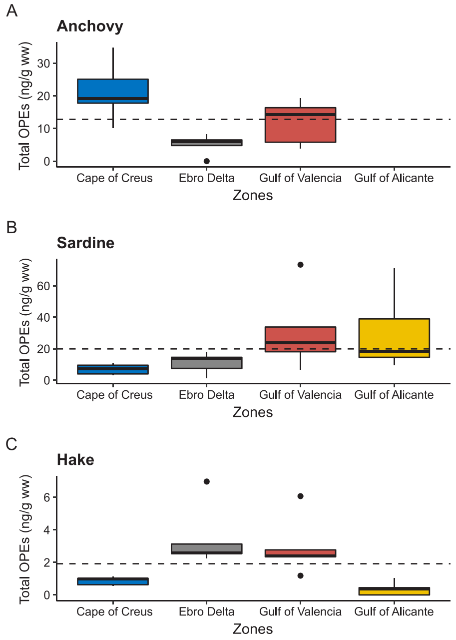

Fig. 2. Boxplot of the total OPEs detected in each of the zones for each species. A: European anchovy, B: European sardine, C: European hake (dotted lines correspond to mean values).

In general, the four studied areas presented OPE contamination levels within a similar range of concentration and no significant differences have been observed with each other (ANOVA, $F_{(3, 51)} = 1.07$, $p = 0.369$). In a recent study, Garcia-Garin et al. (2020a) reported results of the floating marine macro-litter (FMML) observed with drone surveys along the Northwestern Mediterranean Sea. The authors reported that FMML densities were significantly different throughout the surveyed area, where higher densities were found close to the coastline in the Ebro Delta, while Cape of Creus showed the lowest values. It should be noted that the drone surveys in Cape of Creus were conducted one year before those conducted in the other two areas, and during a different season, thus a proper comparison is not feasible (Garcia-Garin et al., 2020a).

Recent research in the same area reported higher concentrations of microplastic ingestion in the Gulf of Alicante for sardines and in the Ebro Delta for anchovies (Pennino et al., 2020). Authors showed higher microplastic ingestion was found in individuals with lower body condition; a similar correlation could occur in the case of contaminant concentrations and should be further investigated. Despite spatial variation was not always significant for OPE concentrations, sardine also showed the highest OPE levels in the Gulf of Alicante; however, anchovy showed an opposite pattern with lower OPE levels in the Ebro Delta. In fact, no relationship was detected between OPE levels and microplastic ingestion for another species (bogue, *Boops boops*) in the same area (Garcia-Garin et al., 2020c). Further analysis should focus on the direct relationship, if any, between ingestion of microplastics and concentration of OPE from the same individual samples. In another recently published study of OPEs in sea turtles (*Caretta caretta*) from the Balearic coast, 10 compounds (TEP, TClPP, TDClPP, TPHP, TNBP, DCP, 2IPPDPP, 4IPPDPP, TCP and EHDPP) were detected in plastic debris, turtle muscle and prey, demonstrating that turtles could acquire them from plastic waste, diet or both (Sala et al., 2021).

Some studies are available on the occurrence of OPEs in marine fish and there is evidence of its occurrence for different marine and freshwater species worldwide, such as in Pearl River Delta, China (Liu et al., 2018; Zhang et al., 2018; Liu et al., 2019a, 2019b), Swedish lakes and coastal areas (Sundkvist et al., 2010) and also in the Mediterranean Sea (Castro et al., 2020; Garcia-Garin et al., 2020c). In fact, recent research proved the presence of OPEs in the muscle of bogue from Barcelona and Cape of Creus, and reported levels of 10.6 ± 4.9 and 7.9 ± 6.5 ng/g ww, respectively (Garcia-Garin et al., 2020c). These results were similar to our OPE levels in hake (1.45 ± 0.24 ng/g ww) and sardine (7.65 ± 3.29 ng/g ww) from Cape of Creus, whereas anchovy presented levels of one order of magnitude higher (21.6 ± 9.27 ng/g ww). Kim et al. (2011) detected mean concentrations of TBOEP, TNBP and TCP in fishes collected from Manila Bay (Philippines) similar to concentrations found in our study. Notwithstanding, OPEs accumulation and elimination may be compound specific and may vary between fish species, and even the different observed OPE patterns may also reflect the different OPEs usage per region.

3.2. OPE patterns in mediterranean fish

Eleven out of thirteen tested OPEs were detected. Only IDPP and TEHP were not detected in any sample. For sardine, compounds with the highest detection frequencies were DCP and TCEP with 80% of positive samples, followed by TPHP (75%) and TPPO and TDClPP (65%). For anchovy, DCP was detected in all samples, followed by TNBP (64%) and TPHP and THP (50%). Finally, the most frequent OPEs in hake samples were TNBP, DCP and TBOEP (35%). As regards concentration levels, the highest values in sardine were obtained for TDClPP (10.1 ± 17.8 ng/g ww); for anchovy, THP was the predominant compound (6.91 ± 7.97 ng/g ww), and for hake, TBOEP (0.98 ± 1.39 ng/g ww). The OPE profiles in all species by sampling area are presented in Fig. 3. As can be seen, OPE profiles exhibited several differences between species. However, within each species, OPE distribution profile was similar among different sampling areas, except for Ebro Delta for anchovy and Cape of Creus for hake.

Samples were displayed in a biplot using PC1 (23.8%) vs. PC2 (16.7%) and PC2 vs. PC3 (11.6%) scores to investigate potential patterns in OPE data associated with spatial or species-specific differences. The original variables (i.e. OPE compounds) were also displayed (Fig. 4). OPE compounds with high (positive or negative) values along a PC axes in a biplot have high importance for that PC axis. PCA is showing that the separation is bigger between species (Fig. 4a-d) rather than between areas where the overlap is higher (Fig. 4b-e). These results would indicate a similar use of the different OPEs throughout the sampled area. And the differences in the OPE profiles may be related to different capacities of acquisition, bioaccumulation, and metabolism, for each species. Differences in feeding behavior and metabolic efficiency among different individuals or species could greatly influence the accumulation potential of OPEs. Due to the lack of research on metabolites of OPEs, the understanding of the complete phenomenon of OPEs in the aquatic organisms tested is still insufficient, considering that the biomagnification potential of some OPEs could be affected due to their high metabolization potential. Liu et al. (2019b) calculated that the ratios of the concentrations of OPEs in other tissues to the liver (OLR) of wild aquatic species from a closed e-waste contaminated pond in Qingyuan, southern China, were larger than 0.5, indicating the effect of metabolism on the deposition of OPEs in the liver. In two of the studied species (i.e. snakehead (*Ophiocephalus argus*) and mud carp (*Cirrhinus molitorella*)), the OLRs of kidney were significantly lower than those in other tissues ($p < 0.05$), suggesting that the kidney may also be involved in the metabolism of OPEs. Snakehead showed higher OLR values than mud carp, which could be due to the higher metabolism potential of the former, considering that snakehead occupied a higher trophic level (Liu et al., 2019b). It is necessary to take in to account the different physiological strategies of each species. Sardines and anchovies have opposite reproductive strategies (capital-breeder *vs* income-breeder, respectively), presenting different reproductive periods in this area (Palomera et al., 2007; Albo-Puigserver et al., 2020). Sardine accumulates mesenteric fat during the spring-summer season prior to the reproduction period (capital breeder behaviour), while anchovy present a lower seasonal variability on fat reserves, as expected for an income breeder. Hake is considered an income breeder (Domínguez-Petit et al., 2010), i.e. the acquired energy is immediately used for reproductive investment, meaning there is no need for related, extensive storage (Rijnsdorp, 1990). The liver is an important organ for energy storage and is usually the first site for lipid (energy) storage in a number of benthic and demersal species such as gadoids (e.g., Kjesbu et al., 1991; Lambert and Dutil, 1997; Lloret et al., 2008). Lipids normally constitute nearly 70% of the dry liver of hake (Lloret et al., 2008), confirming the important role of the liver in energy storage in these species. In contrast, lipids constituted on average only 3% of the dry muscle of hake (Lloret et al., 2008).

3.3. Risk assessment of human exposure

Fish species analysed in the present study are commonly consumed by humans. Therefore, obtained OPE levels can provide reliable information for human exposure assessment to these contaminants through fish ingestion. Table 2 showed the different EDI values obtained for individual OPEs as well as for ∑OPEs. The primary contributors to the total OPE exposure through fish consumption (calculated mean and 95th percentile) were TDClPP (4.94 and 12.6 ng/kg bw/day respectively), followed by THP (3.20 and 8.57 ng/kg bw/day respectively) and by DCP (2.87 and 5.05 ng/kg bw/day respectively), and an EDI of 16.9 ng/kg bw/day for mean concentration and 37.1 ng/kg bw/day for 95th percentile was obtained based on the ∑OPEs. In addition, the EDI values of each congener of the OPEs analysed in this study were compared with the EDI values calculated from existing studies (Table S8). Our EDI values were well below the oral reference dose (RfD) values proposed by USEPA (EPA, 2018), which ranged between 600 ng/kg bw/day for EHDPP to 100,000 ng/kg bw/day for TEHP.

Hazard quotients (HQ) were calculated by dividing the obtained EDI values by the corresponding RfDs (Table 2). It was reported that if the HQ was higher than 1, then a potential risk to humans might occur (Ding et al., 2018). The obtained HQ were between $5.00 * 10^{-6}$ and $7.33 * 10^{-4}$ (Table 2), being much lower than the threshold value of 1. Thus, if fish consumption was only coming from those species, the consumption of fish from the Northwestern Mediterranean Sea would not pose a considerable threat to public health regarding OPE intake. Similar results, with a low level of risk of OPE associated with fish consumption, were found by Bekele et al. (2021) for ten fish species from coastal area of Laizhou Bay, North China (EDI ranged between 3.1-22.1 and 1.7–12 ng/kg bw/day for urban and rural residents, respectively).

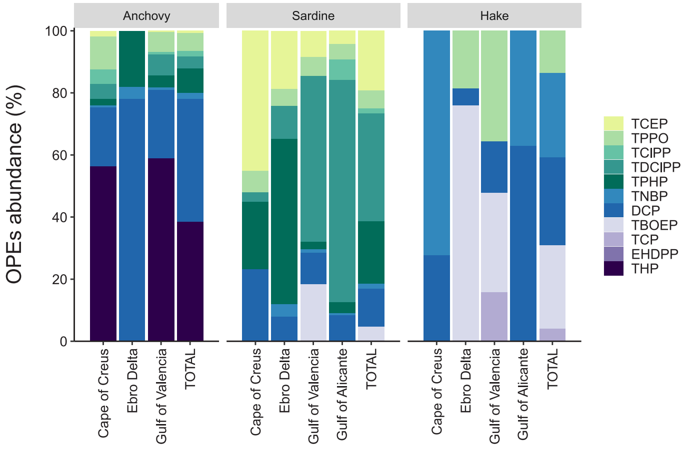

Fig. 3. Percentage contribution of detected OPEs to the total concentration levels in European anchovy, European sardine and European hake from Cape of Creus, Ebro Delta, Gulf of Valencia and Gulf of Alicante.

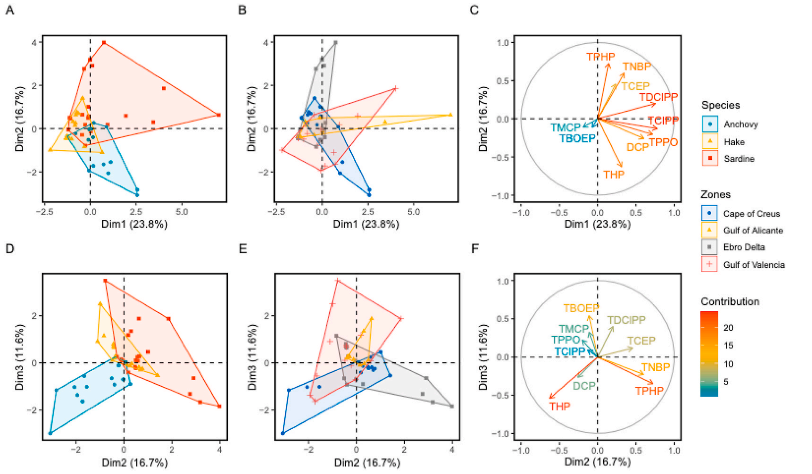

Fig. 4. Principal component analysis (PCA) of OPEs congeners. PC1: A,B,C; PC2: D,E,F. Convex hulls for species (A and D) and zones (B and E) are delimitated in different colours. Loadings are shown in subplot C for PC1-PC2 and in subplot F for PC2-PC3. The further away from the origin an OPE congener is located in the plot, horizontally or vertically, the more important it is for the direction of the first, second or third component of the PCA (*i.e.* more contribution). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Estimated daily intakes (EDIs) of OPEs via fish ingestion (ng/kg bw/day) and associated hazard quotients (HQ).

	RfD*	C** (Mean)	EDI (Mean)	HQ (Mean)	C** (95th percentile)	EDI (95th percentile)	HQ (95th percentile)
TCEP	7000	0.86	1.18	$1.68 * 10^{-4}$	2.16	2.98	$4.25 * 10^{-4}$
TPPO	20,000	0.99	1.37	$6.85 * 10^{-5}$	1.20	1.65	$8.25 * 10^{-5}$
TClPP	10,000	0.33	0.45	$4.50 * 10^{-5}$	0.52	0.72	$7.20 * 10^{-5}$
TDClPP	20,000	3.58	4.94	$2.47 * 10^{-4}$	9.13	12.6	$6.30 * 10^{-4}$
TPHP	7000	1.04	1.43	$2.04 * 10^{-4}$	2.25	3.10	$4.43 * 10^{-4}$
TNBP	10,000	0.21	0.29	$2.90 * 10^{-5}$	0.26	0.35	$3.50 * 10^{-5}$
DCP	–	2.08	2.87	–	3.67	5.05	–
TBOEP	1500	0.81	1.1	$7.33 * 10^{-4}$	1.40	1.93	$1.29 * 10^{-3}$
TCP	20,000	0.07	0.10	$5.00 * 10^{-6}$	0.14	0.20	$9.82 * 10^{-6}$
EHDPP	600	nq	–	–	nq	–	–
IDPP	–	nd	–	–	nd	–	–
THP	–	2.32	3.20	–	6.22	8.57	–
TEHP	100,000	nd	–		nd	–	–
∑OPEs	–	12.3	16.9	–	27.0	37.1	–

*Oral reference dose (RfD) expressed in ng/kg bw/day, and recommended by the USEPA (EPA, 2018).
**OPEs concentration, expressed in ng/g ww.
nd = not detected, below LOD; nq = not quantifiable, below LOQ.

However, it is important to note that OPEs can be ingested through the migration of plasticizers in packaging plastics to food (Ma et al., 2019), as well as through other food items. Some studies reported EDI values via ingestion of different food groups, with values of 85 ng/kg bw/day for the sum of five OPEs in Sweden (Poma et al., 2017) or of 103 ng/kg bw/day for the sum of seven OPEs in Belgium (Poma et al., 2018). Other similar studies, such as those of Zhang et al. (2016), Xu et al. (2017), Ding et al. (2018) and Wang and Kannan (2018) presented EDI values for the sum of OPEs between 9 and 52 times higher than those computed for this study (Table S8). In all studies TCEP and TPHP were detected and presented the lowest EDI mean value in Spain (1.18 and 1.43 ng/kg/bw/day respectively) and highest in China (182 and 77 ng/kg/bw/day respectively).

Moreover, human OPE exposure also occurs by other routes, such as indoor/outdoor inhalation (Wong et al., 2018), dermal absorption (He et al., 2018) and dust ingestion (Kim et al., 2019). The sum of all these exposures can bring the EDI values closer to the established safety limits.

4. Conclusions

OPEs were detected in almost all the fish samples with concentration levels ranging from not detected to 73.4 ng/g ww. Small pelagic fish had higher amounts of OPE compounds in their muscle tissue than their predator, hake. Even if there is no evidence of OPE biomagnification, certain congeners such as TCP and TNBP showed higher concentration levels in hake than in their prey. It must be considered that there are metabolic differences between hake and small pelagic fish, which affect the bioaccumulation and metabolization capacity of OPEs. These compounds can also bioconcentrate through different sources, through water and/or contact with sediments and organic matter.

Sardine was the most contaminated fish with a mean OPE level of 20.5 ± 20.1 ng/g ww, and concentrations differing significantly by location and with the highest value in the Gulf of Valencia. Further research is needed to explore correlations between human activities around studied areas and OPE concentrations.

Estimated daily intake of OPEs through fish consumption was assessed, showing no considerable threat to public health. However, OPEs can arise human body through diverse pathways such as food ingestion, indoor/outdoor inhalation, dermal absorption and dust ingestion. The sum of all these exposure sources can pose certain risks to human health. Therefore, it is recommended to minimize human exposure to OPEs, reducing pollution in marine ecosystems, and preventing an increase in OPE levels in marine fish.

Author statement

Berta Sala: formal analysis; data treatment; methodology; validation; writing of the original manuscript draft and revision of the manuscript. Joan Giménez: sampling; formal analysis; data treatment; writing of the original manuscript draft and revision of the manuscript. Julio Fernández-Arribas: formal analysis; data treatment; methodology. Carlota Bravo: formal analysis; data treatment; methodology. Elena Lloret-Lloret: sampling; formal analysis; data treatment; writing of the original manuscript draft and revision of the manuscript. Antonio Esteban: sampling; revision of the manuscript. José María Bellido: sampling; revision of the manuscript. Marta Coll: conceptualization and coordination of the study; supervision; writing of the original manuscript draft and revision of the manuscript; resources; funding acquisition. Ethel Eljarrat: conceptualization and coordination of the study; supervision; methodology; validation; writing of the original manuscript draft and revision of the manuscript; resources; funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.118377.

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PUBLICACIÓ CIENTÍFICA #6

Supplementary information

"Organophosphate ester plasticizers in edible fish from the Mediterranean Sea: Marine pollution and human exposure"

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Table S1. General information about European sardine (*Sardina pilchardus*), European anchovy (*Engraulis encrasicolus*) and European hake (*Merluccius merluccius*) samples collected from the Western Mediterranean area. Sex: Male (M), Female (F), Indeterminate (I). Mature stage for hake: A: active; I: Inactive. Mature stage for sardine and anchovy: 1 to 6 (1: Immature, 2: Maturing, 3: Mature, 4: Ripe and running, 5: Spent, 6: Resting).

SPECIE	SAMPLES	TOTAL LENGTH (cm)	SEX	MATURITY STAGE
European hake	L150_n1	29.2	F	I
	L150_n2	32.8	F	A
	L150_n3	39.1	F	A
	L150_n4	29.6	M	A
	L150_n5	27.2	M	I
	L144_n1	33.9	F	A
	L144_n2	32.9	M	A
	L144_n3	30.8	F	A
	L144_n4	31	F	A
	L144_n5	27.6	M	A
	L116_n1	35.9	F	A
	L116_n2	31.1	F	A
	L116_n3	30	F	A
	L116_n4	25.2	F	A
	L116_n5	24.2	M	A
	L73_n1	41.1	F	A
	L73_n2	37.4	F	A
	L73_n3	26.1	M	A
	L73_n4	37.8	F	A
	L73_n5	37.1	F	A
European sardine	L71_n6	15.2	F	6
	L71_n7	15.2	F	6
	L71_n8	15.6	M	6
	L71_n9	16.0	M	6
	L71_n10	16.3	F	6
	L115_n1	14.2	F	6
	L115_n2	14.2	F	6
	L115_n3	15.0	F	6
	L115_n4	13.6	M	6
	L115_n5	14.0	M	6
	L148_n1	14.3	F	6
	L148_n2	13.6	F	6
	L148_n3	13.5	M	6
	L148_n4	13.7	F	6
	L148_n5	13.8	F	6
	L143_n1	13.7	F	6
	L143_n2	14.1	F	6
	L143_n3	15.0	F	6
	L143_n4	14.3	M	6
	L143_n5	14.4	M	6
European anchovy	L143_n1	11.6	F	5
	L143_n2	11.5	M	5
	L143_n3	12.9	-	-
	L143_n4	11.4	M	5
	L143_n5	11.4	M	5
	L114_n1	15.2	F	5
	L114_n2	15.4	F	5
	L114_n3	14	F	5
	L114_n4	13.4	F	5
	L114_n5	13.5	F	5
	PW_RS_n1	13.9	F	5
	PW_RS_n2	13.6	F	5
	PW_RS_n3	14	F	5
	PW_RS_n4	13.5	M	3
	PW_RS_n5	13.4	M	3

Table S2. TFC-LC conditions for purification and chromatographic separation of OPEs.

			Pump1: Load Pump (Turboflow)					**Pump2: Elute Pump (Chromatographic)**				
			Injection volume: 20µl									
			Purification columns: PI: CycloneTM-P (0.5x50mm) PI: C18-XL (0.5x50mm)					Analytical column: PI: Purosphere STAR RP-18 (125mmx0.2mm)				
			Solvent A: PI: Water + 0.1% formic acid Solvent B: PI: Methanol+ 0.1% formic acid					Solvent A: PI: Water + 0.1% formic acid Solvent B: PI: Methanol+ ammonium acetate				
Step	Loop	Start (min)	Flow (ml/min)	Gradient	A%	B%	Description	Flow	Gradient	A%	B%	Description
1	out	0.00	0.75	Step	98	2	Sample loading into the TF column	0.25	Step	50	50	Analytical column conditioning
2	in	2.00	0.13	Step	-	100	Analyte transfer to analytical column	0.12	Step	100	-	Analyte transfer to analytical column
3	in	4.00	0.75	Step	-	100	TurboFlow ^{TM} column cleaning	0.25	Step	50	50	LC separation
4	in	5.00	0.75	Step	-	100	TurboFlow ^{TM} column and loop cleaning	0.25	Ramp	20	80	LC separation
5	in	7.00	0.75	Step	-	100	TurboFlow ^{TM} column and loop cleaning	0.25	Step	20	80	LC separation
6	in	8.00	0.75	Step	-	100	TurboFlow ^{TM} column and loop cleaning	0.25	Ramp	10	90	LC separation
7	out	13.00	0.75	Step	-	100	TurboFlow ^{TM} column conditioning	0.25	Step	10	90	Analytical column cleaning
8	out	21.00	0.40	Step	-	100	TurboFlow TM column conditioning	0.25	Ramp	-	100	Analytical column cleaning
9	out	26.00	0.40	Step	-	100	TurboFlow TM column conditioning	0.25	Step	-	100	Analytical column cleaning
10	out	35.00	0.40	Step	-	100	TurboFlow TM column conditioning	0.25	Ramp	50	50	Analytical column conditioning
11	out	36.00	0.75	Step	100	-	TurboFlow TM column conditioning	0.25	Step	50	50	Analytical column conditioning

Table S3. Instrumental TFC-LC-MS-MS parameters: retention times (Rt), transitions (SRM_1 and SRM_2), declustering potential (DP) and collision energy (CE_1 and CE_2) for each OPE analyte.

	Rt (min)	SRM_1/SRM_2	DP (V)	CE1/CE_2 (V)		Rt (min)	SRM_1/SRM_2	DP (V)	CE1/CE_2 (V)
TCEP	4.9	287→224	58	16	TBOEP	13.1	399→299	69	13
		287→98		39			399→199		17
TPPO	9.2	279→201	108	28	TCP	15.4	369→165	54	59
		279→173		35			369→91		41
TCIPP	9.5	327→99	62	24	EHDPP	15.9	363→251	66	15
		327→250		10			363→151		45
TDClPP	11.3	431→99	78	27	IDPP	18.4	391→251	80	26
		431→320		16			391→152		53
TPHP	11.7	327→152	83	39	THP	20.6	351→99	71	22
		327→215		27			351→267		12
TNBP	12.6	267→99	47	20	TEHP	29.2	435→99	65	36
		267→210		8			435→80		59
DCP	12.8	341→151	80	36					
		341→228		27					

Table S4. Recoveries, relative standard deviations (RSDs) and limits of detection (LODs) and quantification (LOQs), expressed in ng/g wet weight (ww), of LC-MS-MS analysis of selected OPEs.

Analyte	**Recovery (%)**	**RSD (%)**	**LOD**	**LOQ**
TCEP	68	3.7	0.05	0.10
TPPO	47	2.5	0.65	1.91
TClPP	64	3.0	0.07	0.20
TDClPP	56	8.1	0.04	0.09
TPHP	53	2.5	0.06	0.12
TNBP	72	3.2	0.02	0.06
DCP	73	4.0	0.05	0.08
TBOEP	65	12	0.02	0.04
TCP	78	13	0.07	0.11
EHDP	62	16	0.02	0.05
IDPP	85	4.5	0.04	0.14
THP	81	10	0.05	0.17
TEHP	98	12	0.08	0.21

Table S5. OPE concentration levels (expressed in ng/g wet weight) in European sardine (*Sardina pilchardus*), European anchovy (*Engraulis encrasicolus*) and European hake (*Merluccius merluccius*) from the Western Mediterranean area.

Location	Code	TCEP	TPPO	TClPP	TDClPP	TPHP	TNBP	DCP	TBOEP	TCP	EHDPP	IDPP	THP	TEHP	∑OPEs
Sardine															
Cape of Creus	L148_n1	nd	nq	nd	1.06	2.94	nd	nd	nd	nd	nd	nd	nd	nd	3.99
	L148_n2	nd	nd	nd	nd	3.10	nd	nd	nd	nd	nd	nd	nd	nd	3.10
	L148_n3	6.54	nq	nd	nd	nd	nd	2.98	nd	nd	nd	nd	nd	nd	9.51
	L148_n4	1.88	nq	nd	nd	2.01	nd	3.43	nd	nd	nd	nd	nd	nd	7.32
	L148_n5	8.36	nd	nd	nd	nd	nd	2.18	nd	nd	nd	nd	nd	nd	10.5
Ebro Delta	L143_n1	4.99	nq	nd	nq	9.39	0.63	2.89	nd	nd	nd	nd	nd	nd	17.9
	L143_n2	5.62	nq	nd	2.39	5.29	0.45	0.86	nd	nd	nd	nd	nd	nd	14.6
	L143_n3	0.37	nq	nd	0.02	6.48	0.70	0.03	nd	nd	nd	nd	nd	nd	7.60
	L143_n4	nd	nq	nd	3.52	9.03	0.61	0.81	nd	nd	nd	nd	nd	nd	14.0
	L143_n5	nd	nq	nd	0.30	1.09	nd	nd	nd	nd	nd	nd	nd	nd	1.39
Gulf of Valencia	L115_n1	0.94	2.58	nd	nd	1.04	0.59	3.59	9.33	nd	nd	nd	nd	nd	18.1
	L115_n2	2.50	2.88	nd	12.2	1.21	0.46	4.53	nd	nd	nd	nd	nd	nd	23.7
	L115_n3	2.62	3.28	nd	62.9	1.38	0.65	2.55	nd	nd	nd	nd	nd	nd	73.4
	L115_n4	2.09	nd	nd	8.72	nd	nd	3.52	19.5	nd	nd	nd	nd	nd	33.8
	L115_n5	5.11	nd	nd	nd	nd	0.10	1.50	nd	nd	nd	nd	nd	nd	6.70
Gulf of Alicante	L71_n6	1.08	nd	nd	6.56	nd	nd	2.10	nd	nd	nd	nd	nd	nd	9.74
	L71_n7	1.58	nd	nd	9.89	1.07	0.18	1.84	nd	nd	nd	nd	nd	nd	14.6
	L71_n8	0.11	nd	nd	14.2	1.08	nq	2.84	nd	nd	nd	nd	nd	nd	18.2
	L71_n9	1.86	3.03	3.47	27.2	1.41	0.24	1.80	nd	nd	nd	nd	nd	nd	39.0
	L71_n10	1.92	3.64	6.64	52.4	1.84	0.52	4.24	nd	nd	nd	nd	nd	nd	71.1
Anchovy															
Cape of Creus	PW_RS_n1	nd	2.53	0.21	1.69	1.02	0.12	3.26	nd	nd	nd	nd	10.3	nd	19.1
	PW_RS_n2	nd	2.65	nd	nd	nd	0.08	3.35	nd	nd	nd	nd	11.7	nd	17.8
	PW_RS_n3	1.64	2.80	nd	1.26	1.15	0.26	3.05	nd	nd	nd	nd	nd	nd	10.2
	PW_RS_n4	nd	nd	2.92	1.27	nd	0.33	5.10	nd	nd	nq	nd	25.1	nd	34.7
	PW_RS_n5	0.19	3.26	1.81	0.85	nd	nd	5.53	nd	nd	nd	nd	13.5	nd	25.1
Ebro Delta	L143_n1	nd	nd	nd	nd	nd	0.46	4.33	nd	nd	nd	nd	nd	nd	4.78
	L143_n3	nd	nd	nd	nd	0.90	0.49	4.57	nd	nd	nd	nd	nd	nd	5.95
	L143_n4	nd	nd	nd	nd	1.45	0.01	5.07	nd	nd	nd	nd	nd	nd	6.53
	L143_n5	nd	nd	nd	nd	2.28	0.01	6.05	nd	nd	nd	nd	nd	nd	8.34
Gulf of Valencia	L114_n1	0.03	2.70	0.37	nd	nd	0.11	1.55	nd	nd	nd	nd	9.62	nd	14.4
	L114_n2	0.10	nd	nd	nd	1.12	0.26	4.30	nd	nd	nd	nd	nd	nd	5.78
	L114_n3	nd	nd	nd	3.06	nd	nd	2.33	nd	nd	nd	nd	13.8	nd	19.2
	L114_n4	nd	nd	nd	1.01	1.10	nd	1.71	nd	nd	nd	nd	nd	nd	3.82
	L114_n5	nd	nd	nd	nd	nd	0.15	3.60	nd	nd	nd	nd	12.6	nd	16.4
Hake															
Cape of Creus	L150_n1	nd	nd	nd	nd	nd	0.38	0.63	nd	nd	nd	nd	nd	nd	1.01
	L150_n2	nd	nd	nd	nd	nd	0.62	0.50	nd	nd	nd	nd	nd	nd	1.12
	L150_n3	nd	nd	nd	nd	nd	0.55	nd	nd	nd	nd	nd	nd	nd	0.55
	L150_n4	nd	nd	nd	nd	nd	0.63	nd	nd	nd	nd	nd	nd	nd	0.63
	L150_n5	nd	nd	nd	nd	nd	0.98	nd	nd	nd	nd	nd	nd	nd	0.98
Ebro Delta	L144_n1	nd	2.20	nd	nd	nd	nd	0.92	3.84	nd	nd	nd	nd	nd	6.97
	L144_n2	nd	nd	nd	nd	nd	nd	nd	3.12	nd	nd	nd	nd	nd	3.12
	L144_n3	nd	nd	nd	nd	nd	nd	nd	2.54	nd	nd	nd	nd	nd	2.54
	L144_n4	nd	nd	nd	nd	nd	nd	nd	2.57	nd	nd	nd	nd	nd	2.57
	L144_n5	nd	nd	nd	nd	nd	nd	nd	2.24	nd	nd	nd	nd	nd	2.24
Gulf of Valencia	L116_n1	nd	nd	nd	nd	nd	nd	nd	2.77	nd	nd	nd	nd	nd	2.77
	L116_n2	nd	nd	nd	nd	nd	nd	1.18	nd	nd	nd	nd	nd	nd	1.18
	L116_n3	nd	4.49	nd	nd	nd	nd	1.46	nd	0.12	nd	nd	nd	nd	6.07
	L116_n4	nd	nd	nd	nd	nd	nd	nd	nd	2.34	nd	nd	nd	nd	2.34
	L116_n5	nd	nd	nd	nd	nd	nd	nd	2.40	nd	nd	nd	nd	nd	2.40
Gulf of Alicante	L73_n1	nd	nd	nd	nd	nd	0.01	0.44	nd	nd	nd	nd	nd	nd	0.45
	L73_n2	nd	nd	nd	nd	nd	0.38	nd	nd	nd	nd	nd	nd	nd	0.38
	L73_n3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	L73_n4	nd	nd	nd	nd	nd	0.32	0.73	nd	nd	nd	nd	nd	nd	1.05
	L73_n5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

nd: not detected, below LOD; nq = not quantifiable, below LOQ

Table S6. OPE concentration levels (expressed in ng/g lipid weight) in European sardine (*Sardina pilchardus*), European anchovy (*Engraulis encrasicolus*) and European hake (*Merluccius merluccius*) from the Western Mediterranean area.

Location	Code	TCEP	TPPO	TClPP	TDClPP	TPHP	TNBP	DCP	TBOEP	TCP	EHDPP	IDPP	THP	TEHP	∑OPEs
Sardine															
Cape of Creus	L148_n1	nd	nq	nd	64.7	180	nd	nd	nd	nd	nd	nd	nd	nd	245
	L148_n2	nd	nd	nd	nd	202	nd	nd	nd	nd	nd	nd	nd	nd	202
	L148_n3	265	nq	nd	nd	nd	nd	121	nd	nd	nd	nd	nd	nd	386
	L148_n4	94.4	nq	nd	nd	101	nd	172	nd	nd	nd	nd	nd	nd	367
	L148_n5	304	nd	nd	nd	nd	nd	79.1	nd	nd	nd	nd	nd	nd	383
Ebro Delta	L143_n1	295	nq	nd	nq	556	37.2	173	nd	nd	nd	nd	nd	nd	1061
	L143_n2	238	nq	nd	101	224	18.8	36.4	nd	nd	nd	nd	nd	nd	618
	L143_n3	13.7	nq	nd	nd	238	25.9	nd	nd	nd	nd	nd	nd	nd	278
	L143_n4	nd	nq	nd	362	930	63.1	83.2	nd	nd	nd	nd	nd	nd	1438
	L143_n5	nd	nq	nd	15.4	56.2	nd	nd	nd	nd	nd	nd	nd	nd	71.6
Gulf of Valencia	L115_n1	97.2	268	nd	nd	107	61.3	372	966	nd	nd	nd	nd	nd	1872
	L115_n2	141	162	nd	685	68.3	25.9	255	nd	nd	nd	nd	nd	nd	1337
	L115_n3	205	257	nd	4921	108	51.1	199	nd	nd	nd	nd	nd	nd	5741
	L115_n4	98.5	nd	nd	412	nd	nd	166	920	nd	nd	nd	nd	nd	1597
	L115_n5	158	nd	nd	nd	nd	3.14	46.3	nd	nd	nd	nd	nd	nd	207
Gulf of Alicante	L71_n6	74.5	nd	nd	452	nd	nd	144	nd	nd	nd	nd	nd	nd	671
	L71_n7	119	nd	nd	745	80.2	13.5	139	nd	nd	nd	nd	nd	nd	1097
	L71_n8	9.31	nd	nd	1156	87.6	nq	232	nd	nd	nd	nd	nd	nd	1485
	L71_n9	272	443	507	3972	206	35.6	263	nd	nd	nd	nd	nd	nd	5699
	L71_n10	413	784	1430	11284	396	113	915	nd	nd	nd	nd	nd	nd	15335
Anchovy															
Cape of Creus	PW_RS_n1	nd	755	61.7	505	304	35.7	973	nd	nd	nd	nd	3072	nd	5706
	PW_RS_n2	nd	832	nd	nd	nd	25.6	1052	nd	nd	nd	nd	3660	nd	5570
	PW_RS_n3	165	283	nd	127	117	26.7	308	nd	nd	nd	nd	nd	nd	1027
	PW_RS_n4	nd	nd	814	353	nd	91.0	1422	nd	nd	nq	nd	6995	nd	9675
	PW_RS_n5	35.8	623	346	162	nd	nd	1056	nd	nd	nd	nd	2569	nd	4792
Ebro Delta	L143_n1	nd	nd	nd	nd	nd	107	1019	nd	nd	nd	nd	nd	nd	1126
	L143_n3	nd	nd	nd	nd	125	68.6	637	nd	nd	nd	nd	nd	nd	831
	L143_n4	nd	nd	nd	nd	130	nd	455	nd	nd	nd	nd	nd	nd	585
	L143_n5	nd	nd	nd	nd	159	nd	423	nd	nd	nd	nd	nd	nd	582
Gulf of Valencia	L114_n1	nd	907	125	nd	nd	36.1	520	nd	nd	nd	nd	3226	nd	4814
	L114_n2	24.8	nd	nd	nd	278	64.3	1064	nd	nd	nd	nd	nd	nd	1431
	L114_n3	nd	nd	nd	652	nd	nd	496	nd	nd	nd	nd	2946	nd	4094
	L114_n4	nd	nd	nd	195	212	nd	329	nd	nd	nd	nd	nd	nd	736
	L114_n5	nd	nd	nd	nd	nd	27.6	642	nd	nd	nd	nd	2246	nd	2916
Hake															
Cape of Creus	L150_n1	nd	nd	nd	nd	nd	161	268	nd	nd	nd	nd	nd	nd	429
	L150_n2	nd	nd	nd	nd	nd	202	165	nd	nd	nd	nd	nd	nd	367
	L150_n3	nd	nd	nd	nd	nd	226	nd	nd	nd	nd	nd	nd	nd	226
	L150_n4	nd	nd	nd	nd	nd	298	nd	nd	nd	nd	nd	nd	nd	298
	L150_n5	nd	nd	nd	nd	nd	397	nd	nd	nd	nd	nd	nd	nd	397
Ebro Delta	L144_n1	nd	1002	nd	nd	nd	nd	420	1751	nd	nd	nd	nd	nd	3173
	L144_n2	nd	nd	nd	nd	nd	nd	nd	1640	nd	nd	nd	nd	nd	1640
	L144_n3	nd	nd	nd	nd	nd	nd	nd	1534	nd	nd	nd	nd	nd	1534
	L144_n4	nd	nd	nd	nd	nd	nd	nd	1342	nd	nd	nd	nd	nd	1342
	L144_n5	nd	nd	nd	nd	nd	nd	nd	2778	nd	nd	nd	nd	nd	2778
Gulf of Valencia	L116_n1	nd	nd	nd	nd	nd	nd	nd	1887	nd	nd	nd	nd	nd	1887
	L116_n2	nd	nd	nd	nd	nd	nd	433	nd	nd	nd	nd	nd	nd	433
	L116_n3	nd	1979	nd	nd	nd	nd	641	nd	53.2	nd	nd	nd	nd	2673
	L116_n4	nd	nd	nd	nd	nd	nd	nd	nd	1721	nd	nd	nd	nd	1721
	L116_n5	nd	nd	nd	nd	nd	nd	nd	933	nd	nd	nd	nd	nd	933
Gulf of Alicante	L73_n1	nd	nd	nd	nd	nd	nd	318	nd	nd	nd	nd	nd	nd	318
	L73_n2	nd	nd	nd	nd	nd	193	nd	nd	nd	nd	nd	nd	nd	193
	L73_n3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	L73_n4	nd	nd	nd	nd	nd	117	263	nd	nd	nd	nd	nd	nd	380
	L73_n5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

nd: not detected, below LOD; nq = not quantifiable, below LOQ

Table S7. Physico-chemical properties of the OPEs analysed in this study, including molecular weight, solubility, log Kow and bioconcentration factor (BFC).

Acronym	Name	Cas number	Molecular weight (g/mol)	Solubility (mg/L)*	Log K_{ow} *	BCF*
TCEP	Tris(2-chloroethyl) phosphate	115-96-8	285.5	$7x10^3$	1.44	3.46
TPPO	Triphenylphosphine oxide	791-28-6	278.3	62.8	2.87	89.4
TClPP	Tris(2-chloroisopropyl) phosphate	13674-84-5	327.6	$1.6x10^3$	3.11	49.14
TDClPP	Tris(1,3-dichloro-2-propyl) phosphate	13674-87-8	430.9	1.5	3.65	100.6
TPHP	Triphenyl phosphate	115-86-6	326.3	1.9	4.59	73.18
TNBP	Tri-n-butyl phosphate	126-73-8	266.3	$2.8x10^2$	4.00	69.65
DCP	Diphenyl cresyl phosphate	26444-49-5	340.3	0.24	5.25	213.5
TBOEP	Tris(2-butoxyethyl) phosphate	78-51-3	398.5	$1.2x10^3$	3.75	54.19
TCP	Tricresyl phosphate	1330-78-5	368.4	0.36	6.34	$8.56x10^3$
EHDPP	2-ethylhexyldiphenyl phosphate	1241-94-7	362.4	1.9	5.73	269.2
THP	Trihexyl phosphate	2528-39-4	350.5	$1.02x10^{-2}$	7.45	$2.72x10^5$
TEHP	Tris(2-ethylhexyl) phosphate	78-42-2	434.6	0.6	9.49	1.219
IDPP	Isodecyl diphenyl phosphate	29761-21-5	390.5	0.75	7.28	288.4

*Data obtained from Wei et al. (2015), Li et al. (2019), Blum et al. (2019), van der Veen and de Boer (2012), Phillips and Stapleton (2019), and WHO (1990, 1991, 1998 and 2000).

Table S8. Comparison of OPEs congeners estimated daily intake (EDI) values (ng/kg/ bw/day) for human adults around the world.

Countries	OPE concentrations	TCEP	TPPO	TClPP	TDClPP	TPHP	TNBP	DCP	TBOEP	TCP	EHDPP	IDPP	THP	TEHP	∑OPEs
Spain [a]	Mean (95th percentile)	1.18 (2.98)	1.37 (1.65)	0.45 (0.72)	4.94 (12.6)	1.43 (3.10)	0.29 (0.35)	2.87 (5.05)	1.12 (1.93)	0.10 (0.20)	-	-	3.2 (8.57)	-	17.0 (37.1)
Norway [b]	95th percentile	28	-	-	0	34	-	-	0		280		-	-	340
Swedish [c]	Mean	6	-	8.5	11.9	9.7	-	-	-	-	48.6	-	-	-	85
Belgium [d]	Mean	2.8	-	18.5	9.6	46.6	5.5	-	-		15		-	4.9	103
China [e]	95th percentile	13.9[f] (7.5)[g]	3.2 (1.7)	12.8 (6.9)	3.1 (1.7)	15.8 (8.5)	17.6 (9.5)	9.7 (5.2)	18.5 (10.0)	-	13.9 (7.5)	-	7.9 (4.3)	22.1 (12.0)	-
China [h]	Median	8.7	-	6.4	0.0	2.3	3.0	-	5.8	-	-	-	-	26.5	55.0
China [i]	Mean	162[j] (182)[k]	-	72 (82)	37 (42)	68 (77)	-	-	39 [f] (44)[g]	-	-	-	-	160 (174)	539 (880)
USA [l]	95th percentile	5.6	-	15.4	7.9	4.0	13	-	102	-	6.9	-	-	9.5	266

[a]This study; [b](Xu et al. 2017) ; [c](Poma et al. 2017) ; [d](Poma et al. 2018); [e](Bekele, Zhao, and Wang 2021);[f] urban residents; [g] rural residents; [h](Ding et al. 2018); [i](Zhang et al. 2016); [j]EDI values for male; [k]EDI values for female; [l](Wang and Kannan 2018).

Figure S1. Boxplot of δ^{15}N for each species in each zone.

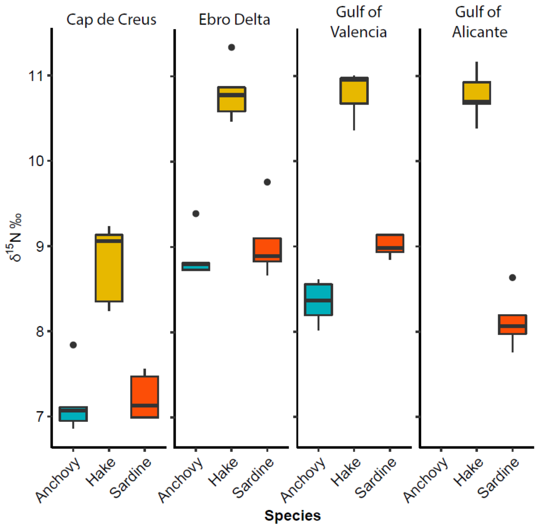
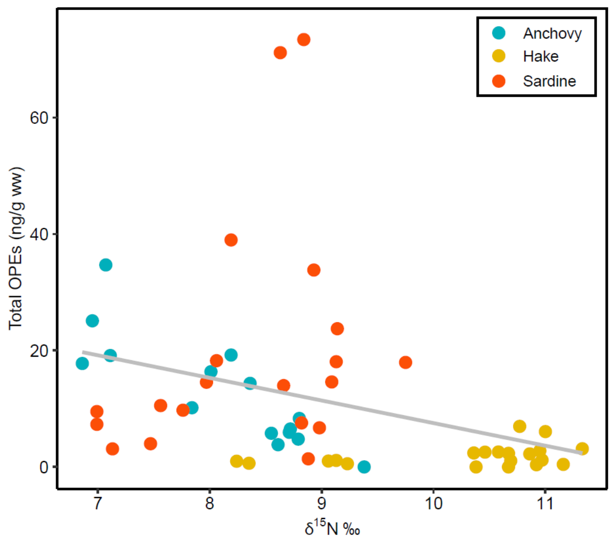


Figure S2. Relationship between total OPEs (ng/g ww) and δ^{15}N. Species are coloured with different colours.



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“Transplacental transfer of plasticizers and flame retardants in fin whales (*Balaenoptera physalus*) from the North Atlantic Ocean”

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Transplacental transfer of plasticizers and flame retardants in fin whales (*Balaenoptera physalus*) from the North Atlantic Ocean

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Keywords

Halogenated flame retardants; Marine mammals; Maternal transfer; Organophosphate esters; Short chain chlorinated paraffins.

Abstract

Marine mammals are one of the groups of animals most affected by marine pollution including that by organic compounds which, besides bearing recognised harmful effects to adults, they may also affect foetuses through placental transfer. In this study we analysed samples of fin whale *(Balaenoptera physalus)* females and their foetuses collected in the western Iceland summer feeding grounds in 2018. Three different families of organic pollutants (halogenated flame retardants (HFRs), organophosphate esters (OPEs) and short chain chlorinated paraffins (SCCPs)) were analysed and their placental transfer investigated. HFRs were detected in 87.5% of females and 100% of foetus samples with concentrations that ranged between nd-15.4 and 6.37-101 ng/g lw, respectively. OPEs were detected in all samples, both from females (85.8-567 ng/g lw) and foetuses (nq-1130 ng/g lw). SCCPs were detected in 87.5% of female samples and 100% of foetal samples with concentrations that ranged between nd-30.9 nq-574 ng/g lw, respectively. For OPE compounds, a significant negative correlation was observed between the logarithm maternal transfer ratio and their log $K_{ow,}$ indicating that a high lipophilicity reduced placental transfer rate. Interestingly, DBDPE was the compound with the highest log K_{ow} but also the one that was transferred the most from mothers to foetuses, calling for in-depth research on this pollutant. These results constitute the first evidence of mother-calf transfer of plasticizers and flame retardants in fin whales. Further investigations are needed to determine their potential effects on this species and other groups of animals.

1. Introduction

Flame retardants (FRs) are a group of compounds of persistent organic pollutants (POPs) that are added to materials to improve their fire resistance (Alaee et al., 2003). For decades, the most widely used FRs were polybrominated diphenyl ethers (PBDEs). Once in the environment they are persistent, have bioaccumulative and long-range transport potential, and may bear adverse effects on both wildlife and human health (Ohoro et al., 2021). Because of their ability to affect hormonal and neuronal regulation, as well as thyroid and liver activity, some of them were banned and alternative compounds developed (Stockholm Convention, 2017). The latter include new halogenated FRs (HFRs), organophosphate ester compounds (OPEs) and short-chain chlorinated paraffins (SCCPs) which are used as FRs, plasticizers, lubricants and metal working fluids. All of them can reach the ocean. However, a number of studies have shown that these alternative

FRs also have toxic effects. One of the emerging HFRs, the decabromodiphenyl ethane (DBDPE), has been shown to cause liver damage in fish and mice (Feng et al., 2013). It also induced alterations in different enzymatic processes, causing for example a decrease in glucose levels (Sun et al., 2014). OPEs are associated with altered hormone levels, decreased reproductive function, anomalous development of the nervous system, abnormal growth in humans and animal models, and may be carcinogenic (Dishaw et al., 2011; Van der Veen and de Boer, 2012; European Commission, 2014; Kojima et al., 2013; Liu et al., 2012). Finally, SCCPs affect liver and thyroid involvement (Jin et al., 2019) and also appear to be carcinogenic (Wang et al., 2019).

Marine mammals are long-lived, occupy high trophic levels in aquatic food webs, and have a thick subcutaneous lipid layer (i.e. blubber), all which makes them choice species for monitoring POP contamination and its long-term potential risk to marine ecosystems (e.g. Aznar-Alemany et al., 2021; Borrell and Aguilar, 2007; Garcia-Garin et al., 2022; Garcia-Garin et al., 2020). Transfer to offspring of pollutants that bioaccumulate in the maternal body is of particular concern as it may represent a risk to offspring due to early stage exposure (Borrell and Aguilar, 2005).

Among marine mammals, fin whales (*Balaenoptera physalus*) are particularly useful to assess pollutant prevalence in the ocean because they are relatively abundant mysticetes, are long-range migrants, and forage over large water masses (Aguilar and García Vernet, 2018). In the northern hemisphere, fin whale females attain sexual maturity at an approximate body length of 18.5 m and an age of 6-10 years (Aguilar and García-Vernet, 2018; Sigurjónsson, 1995). The gestation period lasts about 11 months, at the end of which a calf of about 1-1.5 metric tons and 6-7 m long is born (Aguilar and García-Vernet, 2018). Most mother-foetus transfer of pollutants occurs around birth because the placental membranes are thinner, the umbilical circulation is greater and the concentration of neutral lipids (with chemical affinity for POPs) in the foetus is higher than in early stages of pregnancy (Aguilar and Borrell, 1994).

There are different studies reporting data of PBDE maternal transfer in marine mammals (Frouin et al., 2012; Hoydal et al., 2015; Park et al., 2010; Zaccaroni et al., 2018). However, to our knowledge, the only published information on maternal transfer of SCCPs is on frogs and water snakes (Du et al., 2019; Guan et al., 2020), and of DBDPE in water snakes and chicken (Liu et al., 2018; Zheng et al., 2014). No data appear to be available for maternal transfer of OPEs. Here we aim to fill this research gap by assessing the occurrence of the aforementioned compounds (HFRs, OPEs and SCCPs) and their transfer from mother to foetuses in fin whales from the North Atlantic Ocean.

2. Materials and methods

2.1. Sample collection

Samples were collected from whales caught off West Iceland by the Hvalur H/F whaling company in August 2018 (Figure 1). Blubber was collected from 8 pregnant females and the entire dorsal fin from their corresponding foetuses (Table S1). The female body length ranged from 17.4 to 20.4 m, and that of foetuses from 1.50 to 4.12 m (corresponding to the early-mid stage of the gestation period). Samples were wrapped in aluminium foil and stored frozen (-20ºC) until analysis.

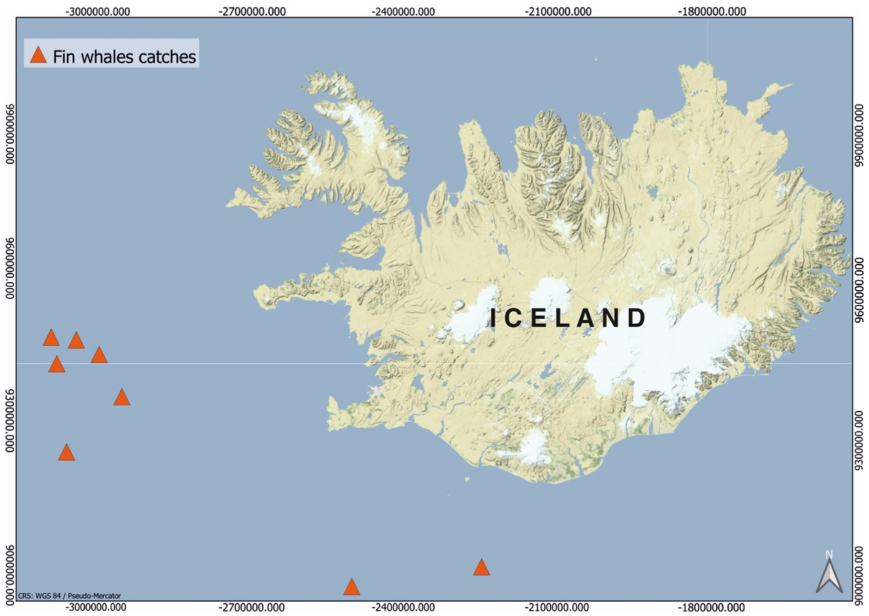

Figure 1. Map showing the sampling location.

2.2. Standards and reagents

Native and internal standards (IS) of OPEs, HFRs and SCCPs were included in this analytical study. For OPEs, tris(2-chloroethyl)-phosphate (TCEP), tris(2-butoxyethyl) phosphate (TBOEP), trihexyl phosphate (THP), tris(2-ethylhexyl) phosphate (TEHP) and tris(chloroisopropyl)-phosphate (TClPP) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Tris(2-isopropylphenyl) phosphate (T2IPPP) was purchased from Chiron (Trondheim, Norway). 2-ethylhexyldiphenyl phosphate (EHDPP) and isodecyldiphenyl phosphate (IDPP) were purchased from AccuStandard (New Haven, CT, USA). Diphenylcresyl phosphate (DCP), triphenyl phosphate (TPHP), tris(1,3-dichloro-2-propyl) phosphate (TDClPP), tri-n-butyl phosphate (TNBP), triphenylphosphine oxide (TPPO), triethyl phosphate (TEP) and tripropyl phosphate (TPP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2-isopropylphenyl diphenyl phosphate (2IPPDPP), 4-isopropylphenyl diphenyl phosphate (4IPPDPP) and bis(4-isopropylphenyl) phenyl phosphate (B4IPPPP) were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). Regarding ISs, labelled d$_{15}$-TPHP was obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA), whereas d$_{27}$-TNBP, d$_{15}$-TDClPP, ^{13}C$_2$-TBOEP and d$_{12}$-TCEP were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada).

For PBDEs, native and ^{13}C-labelled standard mixtures containing BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183 and BDE-209, were used and purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). The EFRs DBDPE, pentabromoethyl benzene (PBEB), hexabromobenzene (HBB), 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EHTBB) and bis(2-ethylhexyl)tetrabromophthalate (BEHTBP) were bought from Wellington Laboratories Inc. (Guelph, ON, Canada).

Three SCCP standard mixtures, C$_{10}$-C$_{13}$ Mix 1 (SCCP, 49% Cl: Hordalub 17), C$_{10}$-C$_{13}$ Mix 3 (SCCP, 66.7% Cl: Cereclor 70) and C$_{10}$-C$_{13}$ Mix 4 (SCCP, 56% Cl: Hordalub 80), as well as the IS 1,1,1,3,10,11-hexachloroundecane, were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

Dichloromethane (DCM), isooctane, methanol and sulphuric acid were purchased from Merck (Darmstadt, Germany). Acetone and hexane for organic trace analysis were purchased from J.T. Baker (Centre Valley, PA, USA). Cyclohexane was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Sample preparation

All samples were lyophilized and then grounded, homogenized and stored in sealed containers at -20ºC until analysis. For OPE analyses, sample extraction was based on a previous optimized and validated method (Giulivo et al., 2016). An ultrasound extraction with 0.5 g dry weight (dw) of sample was carried out with 15 mL of hexane:acetone (1:1). After 15 min of sonication and 20 min of centrifugation at 22ºC and 4000 rpm, the liquid phase was transferred to a vial. The extraction was repeated, and extracts combined. Then, extracts were dried thoroughly with purified nitrogen and reconstituted with 5mL of hexane:methanol (1:3). Finally, a 200 µL aliquot of the extract was transferred to a vial and ISs were added prior to instrumental analysis.

The extraction of HFRs and SCCPs was performed according to previous work (De La Cal et al., 2003; Labandeira et al., 2007). Lyophilized sample (1.5 g dw) was spiked with ^{13}C-labeled standards for HFRs and with 1,1,1,1,3,10,11-hexachloroundecane for SCCPs. Pressurized liquid extraction (PLE) with hexane:dichloromethane (1:1) was carried out, working at 100ºC and 1500 psi. The extract was then subjected to an acid treatment to remove the fat content. The organic phase was cleaned by solid phase extraction (SPE) using Al-N cartridges (5 g) and eluting with hexane:dichloromethane (1:2). Extracts were finally reconstituted with 40 µL of toluene for instrumental analysis of HFRs. Then, extracts were brought to dryness under a continuous stream of nitrogen and reconstituted with 200 µL of cyclohexane for the instrumental determination of SCCPs.

Additionally, the lipid content of samples was gravimetrically determined. Obtained extracts were evaporated to dryness, then left into the oven at 90ºC, and weighted until constant weight.

2.4. Instrumental analysis

Instrumental analysis of OPEs was carried out using a turbulent flow chromatography - high pressure liquid chromatography - tandem mass spectrometry (TFC-HPLC-MS-MS), using the system Thermo Scientific TurboFlowTM, which allows to perform an online sample purification and analysis. This system consisted of two C quaternary pumps and three LC columns, two of them for the purification and the last one for separation. The purification columns used were CyclonTM-P (0.5 x 50 mm) and C18-XL (0.5 x 50 mm), and the chromatographic separation was done using the column Purosphere Star RP-18 (125 mm x 0.2 mm), which has a particle size of 5 µm. The tandem MS of this system was a triple quadrupole (QqQ) that uses heated- electrospray ionisation source (H-ESI). Chromatographic separation and mass spectrometry detection parameters can be found in a previous published work (Giulivo et al., 2016).

HFRs were analysed by GC-MS-MS using an Agilent 7890A gas chromatograph (GC) coupled to an Agilent 7000B triple quadrupole mass spectrometer and a DB-5ms capillary column. Brominated compounds were analysed using electronic ionization (EI). The instrumental conditions and the spectrometric determination are described in a previous publication (Barón et al., 2014).

SCCPs were analysed on an Agilent 6890 GC coupled with a 5973 MS working in electron-capture-negative-ion (ECNI) (Agilent Technologies, Santa Clara, USA) in the selective ion monitoring (SIM) mode. The separation was conducted in a DB-5-ms capillary column (15 m length, 0.25 mm I.D, 0.1 µm film thickness) (Agilent Technologies, Santa Clara, USA) with methane as the reagent gas. The instrumental conditions and the spectrometric determination are based on Geiß et al. (2010). The quantification method was by multiple linear regression calibration with two selected *m/z* signals, and verified with two other mass combinations. The weighted sum of the peak areas of two *m/z* values (*m/z* 327 and 423) led to a sum determination which was applicable to the different SCCP mixtures most likely to be found in the aquatic environment (Geiß et al., 2010).

2.5. Quality assurance

Contamination by FRs and plasticizers may come from different sources. To minimize the potential contamination during the sampling and analysis processes, plastic material was not used and all the non-volumetric material was brought to 340°C and rinsed with acetone just before their use. For each batch of samples, a laboratory blank with hydromatrix was included. Laboratory blank levels were then subtracted from corresponding samples.

Instrumental parameters such as recovery rates, limits of detection (LODs) and limits of quantification (LOQ) are summarized in Table S2. Recovery rates ranged from 40 to 98%, with relative standard deviation always below 20%, with the only exceptions for BEHTBP (32%) and DBDPE (24%). LODs and LOQs ranged between 0.01-19.3 ng/g lipid weight (lw) and 0.04-53.0 ng/g lw, respectively.

2.6. Statistical analysis

The normality and homoscedasticity of the data were tested using the Shapiro–Wilk and Levene's test, respectively. All compounds, except DBDPE, deviated from a normal distribution.

To examine the statistical differences in concentrations among different compounds (OPEs, HFRs and SCCPs), the non-parametric Kruskal-Wallis rank sum test was used. To compare concentrations of females with their respective foetuses, the Wilcoxon signed rank test was used for OPEs, PBDEs and SCCPs, and the parametric Paired t-test was used for DBDPE.

For those samples in which the same compound was detected in the mother-foetus pair, the ratio between these concentrations ($R_{foetus/mother}$) was calculated (Table 2 and Figure S1). Furthermore, the logarithmic transformation of this ratio for each pollutant (log $R_{foetus/mother}$) was used to examine the potential transfer of individual compounds between females and their offspring with respect to the lipophilicity of each contaminant, using the logarithmic octanol-water coefficient (log K_{ow}) (Figure 4). The maternal transfer ratio of OPE compounds was evaluated using the Pearson's rank correlation coefficient test. The level of significance was set at a $p < 0.05$. For mean and standard deviation calculations, values for nd were taken as ½ LOD, and for nq were taken as LOD.

All statistical analyses were performed using software RStudio (Version 2022.02.0 Build 443).

3. Results and discussion

FR and plasticizer concentrations in blubber of fin whale females and dorsal fin of their foetuses are summarized in Table 1 (for results of individual samples see Supporting information, Table S3).

Table 1. OPE, HFR and SCCP mean concentrations (expressed in ng/g lw), associated standard deviation, concentration range, and detection frequency in maternal and foetal fin whale samples.

	Maternal blubber				**Foetal skin**			
	Mean	**SD**	**Range**	**DF (%)**	**Mean**	**SD**	**Range**	**DF (%)**
OPEs								
TEP	36.2	48.6	nd - 149	87.5	346	443	nq - 1083	100
TCEP	3.59	1.95	nd - 5.38	87.5	57.2	160	nd - 454	12.5
TPPO	11.5	3.00	nd - nq	75.0	35.0	80.6	nd - 235	12.5
TClPP	9.80	20.5	nd - 60.2	62.5	1.27	1.64	nd - 5.33	12.5
TDClPP	40.2	9.47	22.9 - 50.4	100	35.3	98.7	nd - 280	12.5
TPHP	48.7	22.9	18.1 - 88.7	100	9.29	24.5	nd – 70.0	12.5
TNBP	2.14	2.00	nq - 6.00	100	nd	-	nd	0
2IPPDPP	53.5	22.5	nq - 76.9	100	nd	-	nd	0
4IPPDPP	14.0	5.66	nq - 20.1	100	6.49	16.4	nq - 47.0	25
EHDPP	4.47	3.72	nd - 7.64	75	nd	-	nd	0
B4IPPPP	11.6	7.05	nd - 21.1	87.5	nd	-	nd	0
T2IPPP	119	46.7	nq - 179	100	17.8	19.5	nd - 65.2	25
∑OPEs	317	141	85.8 - 567	100	488	731	nq - 2046	100
HFRs								

BDE-28	0.08	0.08	nd – 0.18	75	0.12	0.14	nd - 0.39	50
BDE-47	2.88	3.31	nd - 8.54	62.5	2.79	2.37	0.47 - 5.98	100
BDE-100	0.33	0.35	nd - 0.78	62.5	0.11	0.24	nd - 0.70	12.5
BDE-99	0.77	0.96	nd - 2.67	62.5	0.40	0.60	nd - 1.47	37.5
BDE-154	0.78	0.79	nd - 2.06	62.5	0.10	0.25	nd - 0.72	12.5
BDE-153	0.19	0.23	nd - 0.58	37.5	nd	-	nd	0
DBDPE	0.81	0.69	nd - 1.61	62.5	33.9	30.7	nd - 97.1	87.5
∑HFRs	5.77	6.06	nd - 15.4	87.5	37.3	29.8	6.37 - 101	100
SCCPs								
SCCPs	6.89	10.1	nd - 30.9	87.5	84.0	199	nq - 574	100

nd: not detected, below LOD; nq = not quantifiable, below LOQ.

3.1. Concentrations in females blubber

HFRs were detected in 7 out of the 8 female samples analysed. Total HFR concentration levels ranged from nd (not detected) to 15.4 ng/g lw. All the PBDE congeners included in our analytical methodology, with the exception of BDE-209, were detected. Congener BDE-28 was the most frequently detected, with a 75% of detection frequency followed by BDE-47, BDE-100, BDE-99 and BDE-154 with 62.5%. Regarding the emerging HFRs, only DBDPE was detected with a 62.5% of detection frequency, whereas EHTBB, BEHTBP, PBEB and HBB were not detected in any sample. The HFR with the highest concentration was BDE-47 with a mean value of 2.88±3.31 ng/g lw, followed by DBDPE with 0.81±0.69 ng/g lw (Table S3, Figure 2).

Rotander et al. (2012) also reported ∑PBDE concentrations in fin whales from the same region for samples collected during 1986-89 and 2006-09. Concentrations obtained for the first period ranged from 5 to 12 ng/g lw, and those for the second presented slightly higher levels, ranging from 20 to 31 ng/g lw. The concentration in the samples collected during 2018 for the present study (5.77 ng/g lw), were 4-5 times lower than those found during 2006-2009, reflecting the EU ban of PBDEs in 2004 (Betts, 2008) and their inclusion in the POPs Stockholm Convention in 2009 (UNEP, 2009). A similar temporal trend was observed in a recent study in which PBDE levels in striped dolphins (*Stenella coeruleoalba*) from the Mediterranean Sea decreased about 60% along three decades (from 1990 to 2018) (Aznar-Alemany et al., 2021). Besides the observed temporal variation differences between areas may also be large. Thus, Taniguchi et al. (2019) found mean ∑PBDE concentrations of 0.82 ng/g lw in blubber of fin whales collected in 2013 near the Antarctic Peninsula (Southern Ocean), values that are much lower than those for Icelandic whales in any period and confirm the lower pollution levels characteristic of the Southern Hemisphere (Reijnders et al., 2018).

OPEs were detected in all maternal blubber samples analysed, with total concentrations ranging between 85.8 and 567 ng/g lw. Twelve out of the 19 analysed OPEs were detected, but TPP, DCP, TBOEP, IDPP, THP and TEHP were not detected in any sample. TDClPP, TPHP, 2IPPDPP and 4IPPDPP were the most frequently detected compounds being found in all analysed samples. T2IPPP was detected in 7 out of the 8 females and presented the highest concentration, with a mean value of 119±46.7 ng/g lw. This is followed by 2IPPDPP and TPHP with 53.5±22.5 ng/g lw and 48.7±22.9 ng/g lw, respectively (Table S3, Figure 2). In a recent study, Garcia-Garin et al. (2020) analysed fin whale muscle samples from individuals caught in 2015 from the same population. Although they found higher mean ∑OPE concentrations in their individuals, mature females showed a mean ∑OPE concentrations of 435±261 ng/g lw. *i.e.* quite similar concentrations to those obtained in the present study. Concurrently, they also found that the most abundant compound was T2IPPP.

SCCPs were detected in 5 out of the 8 of analysed samples. Concentrations ranged between nd and 30.9 ng/g lw (Table S3) . Casà et al. (2019) reported SCCP concentrations in the blubber of humpback whales (*Megaptera novaeangliae*) sampled off the Australian coast between 2007 and 2015. SCCPs were detected in all adult and juvenile individuals, with levels ranging from 10 to 46.0 ng/g lw, similar to concentrations found in the present study. Other sources have reported considerably higher SCCP concentrations in cetaceans although the concentrations were determined in odontocetes, which have a higher trophic level and are therefore more likely to accumulate these compounds. Thus, Tomy et al. (2000) analysed SCCP compounds in the blubber of beluga whales (*Delphinapterus leucas*) from three different areas. They found concentrations between 130-290 ng/g lw in northwest Greenland, between 150-320 ng/g lw in the Mackenzie Delta, and between 490-1600 ng/g lw in the St Lawrence estuary, a region heavily contaminated by SCCPs. Moreover, Zeng et al. (2015) reported concentrations between 570 and 5800 ng/g lw in the blubber of stranded finless porpoises (*Neophocaena phocaenoides*), and between 920-24000 ng/g lw in the blubber of Indo-Pacific humpback dolphins (*Sousa chinensis*) from the South China Sea. Undoubtedly, such high levels are related to the fact that China is the world largest SCCP producer (Zhou et al., 2018).

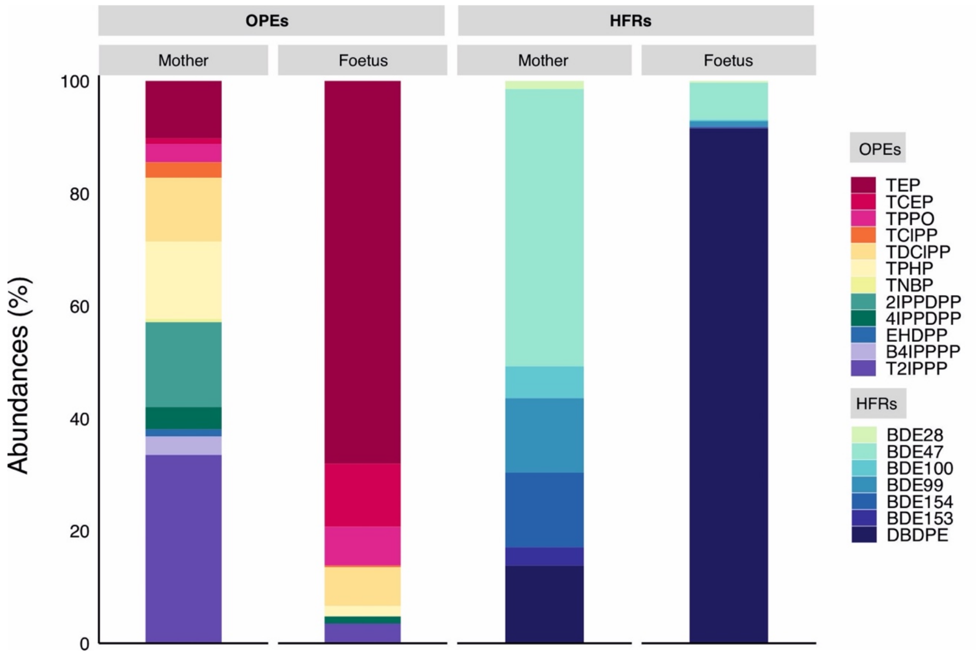

Figure 2. Percentage contribution of detected (A) HFRs and (B) OPEs to the total concentration levels in mothers and foetuses.

contaminants in fin whale females. The highest concentrations corresponded to OPEs with a mean level of 317±141 ng/g lw, while HFR and SCCP, with mean concentrations of 5.77±6.06 ng/g lw and 6.89±10.1 ng/g lw, respectively, were not significantly different between them (Kruskal-Wallis, $p>0.05$). A higher concentrations of OPEs *versus* HFRs was also observed in dolphins from the southwestern Indian Ocean (Aznar-Alemany et al., 2019), all which suggest a greater input of OPEs to the environment, arguably as a result of OPEs not being used only as FRs, but also as plasticisers.

3.2. Concentrations in foetal samples

The foetal samples consisted of a homogenate of skin and hypodermis because the blubber was almost non-existent. However, the germinal epithelium of the skin contains prominent lipid droplets (Geraci et al., 1986) that are potentially capable of retaining measurable amounts of lipophilic contaminants. Aguilar and Borrell (1994) showed that the concentrations (expressed on a lipid basis) of organochlorine compounds in the skin and blubber of striped dolphins were significantly correlated and the distribution factors of the lipid-based concentrations (calculated, as defined by Massé et al. (1986)), as the ratio between the concentration of organochlorines in the blubber and that measured in the skin from the same individual) were between 0.9 and 1.1, which means that both tissues are suitable for calculating organochlorine concentrations. This suggests that the concentration of lipophilic compounds, as those analysed in this study, can be reliably compared between female blubber and foetal hypodermic tissue as long as they are expressed on a lipid basis.

HFRs were detected in all foetuses, with concentrations ranging from 6.37 to 101 ng/g lw (Table S3). The mean value for foetuses (37.3±29.8 ng/g lw) was 6.5 times higher than that found in maternal samples (5.77±6.06 ng/g lw), although non-significant differences were found (Wilcoxon signed rank test, p-value=0.1). It is intriguing that for three paired samples (106, 124 and 144), PBDEs were not detected in females whereas they were detected in their corresponding foetuses. All the six PBDE congeners detected in female samples were also detected in at least one foetus, with the exception of BDE-153, which was not detected in any foetus. Contrarily, BDE-47 was the only HFR detected in all foetuses (Figure 3B). Similar results were reported by Desforges et al. (2012) and Zaccaroni et al. (2018), who also analyzed the maternal transfer of PBDEs in beluga whales and sperm whales (*Physeter macrocephalus*), respectively. Their studies revealed that BDE-47 was the most frequently PBDE detected, as well as the congener with highest concentrations.

Regarding the emerging HFRs, DBDPE was detected in 7 out the 8 analysed foetuses at concentrations ranging from nd to 97.1 ng/g lw, with a mean value of 33.9±30.7 ng/g lw. This mean value for foetuses was 42 times higher than the mean value found in maternal samples (0.81±0.69 ng/g lw), being both significantly different (Paired t-test, p-value=0.018). It is interesting to note that for two paired samples (124 and 144) DBDPE was not detected in maternal samples but was found in their foetuses.

OPEs were detected in all foetal samples, with total concentrations ranging between not quantifiable (nq) and 2046 ng/g lw (Table S3), and a mean concentration of 488±731 ng/g lw. This mean value for foetuses is strongly influenced by the concentration of foetus 144F, which was unusually high (2046 ng/g lw). The Wilcoxon signed rank test showed that females had statistically higher concentrations that their corresponding foetus (p-value <0.0001). Only 8 of the 12 OPEs detected in maternal samples were found in foetal samples, being TEP the compound most frequently detected (75% of cases) (Table S3, Figure 3B).

SCCPs were also detected in all foetal samples at concentration levels between nq and 574 ng/g lw, and a mean concentration of 84.±199 ng/g lw. This mean value for foetuses was 12 times higher than the mean value found in mother samples (6.89±10.1 ng/g lw) a difference that proved to be significant (Wilcoxon signed rank test, p-value=0.022).

Figure 3B shows a comparison between the concentration of each group of contaminants in the foetal samples. The pattern of variation was similar to that of maternal samples, with the highest concentrations corresponding to OPEs, followed by SCCPs and HFRs but, in this case, none of the concentrations showed significant differences among compounds.

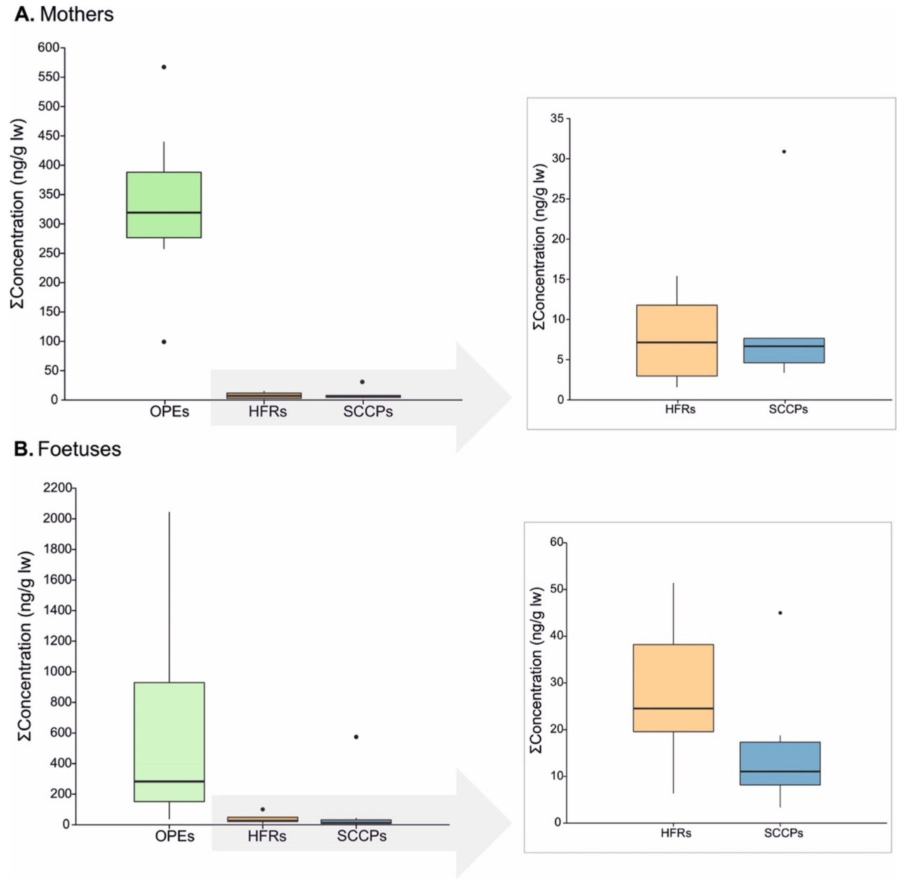

Figure 3. Box plot of the total OPE, HFR and SCCP concentrations detected in (A) mothers and (B) foetuses.

3.3. Transplacental transfer of studied contaminants

For many FRs, maternal transfer is known to be a primary source of exposure to foetuses, neonates and calves of different species (Du et al., 2019; Kajiwara et al., 2008; Zaccaroni et al., 2018). In this study, transplacental transfer of HFRs, OPEs and SCCPs was evaluated by analysing eight paired mother-foetus samples. Sample size was necessarily limited due to the difficulty of obtaining samples of this nature. The results from the foetal samples indicate that several compounds were transferred transplacentally, including 5 PBDEs, DBDPE, 8 OPEs and SCCPs. However, looking compound by compound, only 4 of them were detected in the majority of foetuses: BDE-47, DBDPE, TEP and SCCPs. To the best or our knowledge, this is the first study reporting transplacental transfer of DBDPE, OPEs and SCCPs in marine mammals.

The relationship between the logarithm maternal transfer ratio of all pairs and the log K_{ow} of all compounds studied is represented in Figure 4. OPEs are the only group of compounds showing a moderately significant negative linear correlation (Pearson, r = -0.62, p-value = 0.018), as it has been already observed in previous marine mammal studies for polychlorinated biphenyls (PCBs), PBDEs, organochlorine pesticides (OCPs), DDX and MeO-PBDEs (Brown et al., 2016; Kajiwara et al., 2008; Weijs et al., 2013). In general, high lipophilicity and large molecular size and weight of the compound tends to decrease its chemical's mobility, uptake and bioaccumulation, and thus reduce its transplacental transfer rate. This has been observed in a previous study on mother-calf transfer of organochlorine compounds in common dolphins (*Delphinus delphis*) from the south-western Mediterranean sea (Borrell and Aguilar, 2005) and in another one on the transplacental transfer of PCBs and PBDEs in Arctic beluga whales from Hendrickson Island (Canada) (Desforges et al., 2012). However, in the present study, transplacental transfer of PBDEs versus log K_{ow} showed no significant correlation.

Amongst PBDEs, BDE-99 and BDE-154 showed $R_{foetus/mother} > 1$ in all paired samples, whereas BDE-28 and BDE-47 presented ratios between 0.79-3.26 and 0.28-3.00, respectively (Table 2). PBDEs presented a very similar ratio independently of their K_{ow} value (Figure 4 and Figure S1). DBDPE also presented $R_{foetus/mother} > 1$ in all paired samples, with values ranging from 12.7 to 137, showing a high maternal transfer of this compound. DBDPE, which has a log K_{ow} value of 11.1 (see Table 2), showed the highest mother-foetus ratios. We have found no satisfactory explanation to this result and, indeed, previous studies have shown that chemicals with high log K_{ow} values, generally with large molecular weight and/or size, less readily crossed cell membranes in utero (Mull et al., 2013).

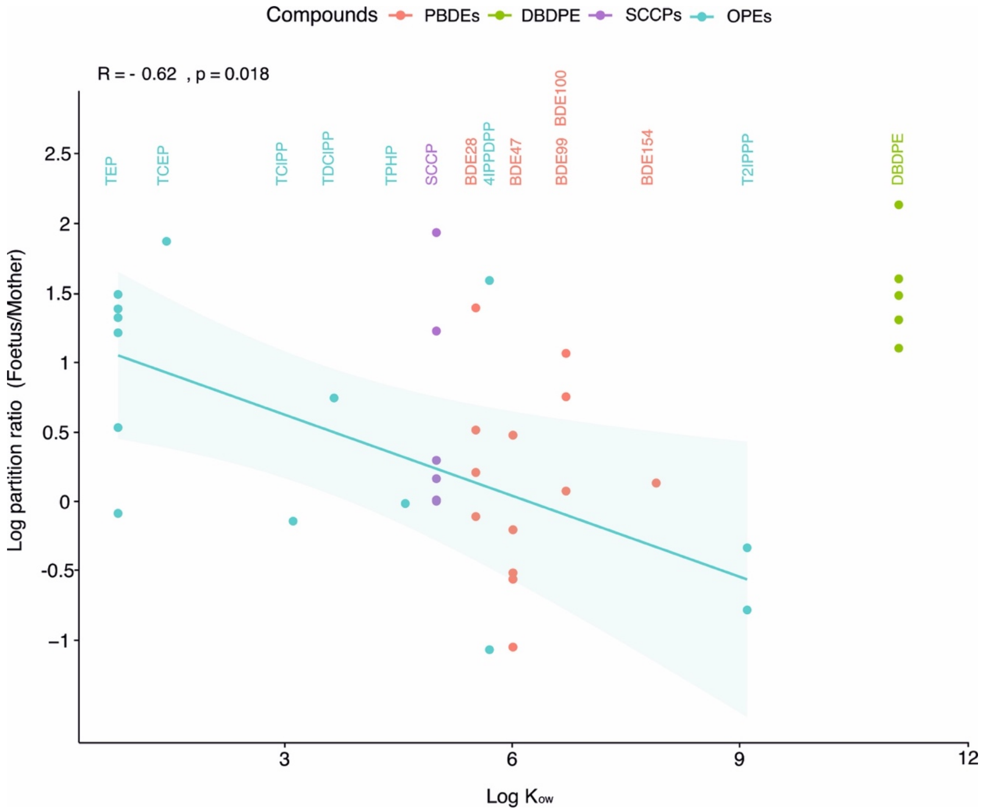

Figure 4. Relationship between logarithmic $R_{foetus/mother}$ and log K_{OW} for PBDEs (red), emerging HFRs (green), OPEs (blue) and SCCPs (purple). Log K_{OW} values are detailed in Table S4. $R_{foetus/mother}$ values were calculated assuming that nq values = LOD. The regression line of the OPEs is shown with its confidence intervals at 95% (-0.867-0.137). The Pearson's correlation coefficient (R) for OPEs is also depicted.

Regarding OPEs, only TEP, TCEP and TDClPP showed $R_{foetus/mother} > 1$, whereas TClPP, TPHP and T2IPPP showed $R_{foetus/mother} < 1$. TEP was the OPE with the highest maternal transfer being detected in all foetal samples and with $R_{foetus/mother}$ values up to 24. It should be noted that OPEs are compounds with very different physicochemical characteristics and their K_{ow} ranges from 0.80 for TEP to 9.1 for T2IPPP (Table 2). Also, it is important to mention that several OPEs are known to be easily metabolized (Greaves et al., 2016; Yao et al., 2021) and the metabolic processes may affect the maternal transfer ratio. Wang et al. (2017) reported metabolic transformation of TCEP, TDClPP and TNBP (among other OPEs) in adult zebrafish under laboratory conditions. TEP is the OPE with the highest concentrations as well as the one with the highest detection frequency in fin whale foetal samples, a finding likely related to its low metabolization as demonstrated in previous studies (Strobel et al., 2018).

Finally, SCCPs are pollutants with log K_{ow} values ranging between 5.09 and 6.43 (EC, 1999; Sun et al., 2017); they showed $R_{foetus/mother}$ values very close to 1, with the exception of one paired sample (144) in which $R_{foetus/mother}$ was 86 (Table 2).

The current study has confirmed that the maternal transfer of HFRs, OPEs and SCCPs occurs in fin whales. These compounds are considered to be toxic to aquatic organisms. HFRs have been related to disruption of thyroid function, neurobiological development and foetal toxicity/teratogenicity in mammals (Alonso et al., 2014; Hall et al., 2003). OPEs are potential endocrine disruptors and have neurotoxic effects, while the Cl-containing OPEs are carcinogenic (Meeker and Stapleton, 2010; Van der Veen and de Boer, 2012; Wang et al., 2015; Wei et al., 2015). In their turn, SCCPs effects are mainly related to oxidative stress, metabolic disturbance, endocrine disruption and binding to biomacromolecules (Wang et al., 2019). Prenatal exposure to this mixture of plasticizers and FRs may result in adverse effects on foetus and calves, as in these age-segments the organ growth and development is at its maximum rate and the ability to cope with the toxic impact is limited.

The fin whale is listed as *Vulnerable* by the UICN Red List (Cooke, 2018). As this species, including the western Icelandic population, has been shown to be exposed to multiple contaminants (Borrell, 1993; Garcia-Garin et al., 2021, 2020; Rotander et al., 2012), monitoring of its pollutant levels should continue. Moreover, further research is needed to establish the actual toxicological implications of prenatal HFRs, OPEs and SCCPs exposure in these animals, as well as in the development of calves.

Table 2. $R_{foetus/mother}$ of mother-foetus pairs for OPEs, HFRs and SCCPs. nq = LOD was used to calculate the $R_{foetus/mother}$

		50F/50	**54F/54**	**89F/89**	**106F/106**	**114F/104**	**124F/124**	**133F/133**	**144F/144**	**Mean ****	**SD *****
OPEs	TEP	0.82	16.3	21.0	30.9	3.40			24.3	12.2	12.4
	TCEP								74.5	9.31	26.3
	TClPP								0.72	0.09	0.25
	TDClPP								5.55	0.69	1.96
	TPHP								0.97	0.12	0.24
	4IPPDPP			38.9				0.09		4.87	13.8
	T2IPPP		0.46			0.16				0.08	0.17
HFRs	BDE-28	3.26	0.79				24.7	1.62		3.80	8.53
	BDE-47	0.28	0.31	0.63		0.09		3.00		0.86	1.21
	BDE-99			5.67				1.19		0.98	2.11
	BDE-100			11.62						1.45	4.11
	BDE-154			1.36						0.17	0.48
	DBDPE	12.7	40.0	136	30.4	20.3				39.9	49.2
SCCPs	SCCPs		16.8	1.46		1.02	1.00	1.98	86.1	13.5	29.8

* For $R_{foetus/mother}$ calculations, nq values were assumed as LOD values.
**Mean and SD calculated using 0 in the empty spaces.
***SD = Standard deviation

4. Conclusions

FRs and plasticizers were analysed in fin whale maternal and foetal samples. HFRs, OPEs and SCCPs were detected in maternal blubber, with the highest concentrations corresponding to OPEs (mean level of 317±141 ng/g lw), followed by SCCPs (6.89±10.1 ng/g lw) and HFRs (5.77±6.06 ng/g lw). The high OPE concentrations observed suggest a greater input of these pollutants into the environment, as a result of these compounds being used both as FRs and plasticisers.

For the first time, emerging HFRs, OPEs and SCCPs were detected in marine mammal foetuses, thus demonstrating their transplacental transfer. In OPEs, the log $R_{foetus/mother}$ and log K_{ow} showed a significant negative correlation, seems to indicate that, in such compounds, the higher the K_{ow}, the lower the transmission from mother to foetus. Previous ecotoxicological studies have demonstrated the toxicity of the analysed compounds, with the potential for synergistic effects. Further investigations are required to determine the actual effects of the exposure to plasticizers and FRs on fin whale foetuses, as well as their potential impact on calves.

Acknowledgements

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PUBLICACIÓ CIENTÍFICA #7

Supplementary information

“Transplacental transfer of plasticizers and flame retardants in fin whales (*Balaenoptera physalus*) from the North Atlantic Ocean”

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Environmental Pollution
(Enviat l’11 d’abril del 2022)

Transplacental transfer of plasticizers and flame retardants in fin whales (Balaenoptera physalus) from the North Atlantic Ocean

Table S1. General information about fin whale (*Balaenoptera physalus*) samples collected from the North Atlantic Sea.

Sample	**Code**	**Length (m)**	**Sex**
Mother Blubber	50	20.1	Female
	54	19.5	Female
	89	19.5	Female
	106	20.4	Female
	114	19.5	Female
	124	18.3	Female
	133	19.8	Female
	144	17.4	Female
Foetus Skin	50F	1.50	Male
	54F	1.68	Male
	89F	3.24	Male
	106F	2.13	Male
	114F	3.20	Female
	124F	3.48	Female
	133F	4.12	Male
	144F	3.54	Female

Table S2. Recoveries, relative standard deviations (RSDs) and limits of detection (LODs) and quantification (LOQs), expressed in ng/g lipid weight (lw), of OPEs, HFRs and SCCPs.

Analyte	**Recovery (%)**	**RSD (%)**	**mLOD**	**mLOQ**
OPEs				
TEP	59	15	10.6	35.4
TCEP	68	3.7	1.08	3.14
TPPO	47	2.5	13.1	38.7
TClPP	64	3.0	1.39	4.01
TPP	40	16	1.16	3.85
TDClPP	56	8.1	0.77	2.57
TPHP	53	2.5	1.23	4.10
TNBP	72	3.2	0.46	1.23
DCP	73	4.0	0.92	1.69
TBOEP	65	12	0.46	1.54
2IPPDPP	42	2.9	0.69	2.30
4IPPDPP	41	6.7	1.21	4.04
TCP	78	13	1.39	0.69
EHDPP	62	16	0.31	1.08
B4IPPPP	61	5.1	4.16	13.9
IDPP	85	4.5	0.77	2.93
T2IPPP	82	9.0	19.3	53.0
THP	81	10	0.92	3.39
TEHP	98	12	1.69	4.16
HFRs				
BDE-28	87	1.5	0.01	0.04
BDE-47	81	2.9	0.01	0.05
BDE-100	77	3.5	0.06	0.19
BDE-99	81	3.3	0.03	0.09
BDE-154	75	2.6	0.01	0.04
BDE-153	71	1.4	0.06	0.19
BDE-183	56	3.4	0.17	0.58
BDE-209	72	16	0.23	0.77
EHTBB	56	9	0.07	0.24
BEHTBP	89	32	0.13	0.42
PBEB	71	7.4	0.01	0.04
HBB	76	6.4	0.06	0.21
DBDPE	87	24	0.09	0.29
SCCPs				
SCCPs	74	10	0.73	2.45

Table S3. OPE, HFR and SCCP concentration levels (expressed in ng/g lipid weight) in pregnancy fin whales from the North Atlantic Sea.

		Mother								**Foetus**							
		50	54	89	106	114	124	133	144	50F	54F	89F	106F	114F	124F	133F	144F
	% lipid	61 %	81 %	87 %	86 %	79 %	85 %	83 %	67 %	7 %	7 %	4 %	4 %	15 %	24 %	17 %	10 %
HFRs	BDE-28	0.12	0.19	0.05	nd	0.18	nq	0.11	nd	0.39	0.15	nd	nd	nd	0.25	0.18	nd
	BDE-47	8.54	4.92	1.27	nd	6.38	nd	1.89	nd	2.35	1.51	0.80	0.47	0.57	4.96	5.67	5.98
	BDE-100	0.69	0.78	nq	nd	0.78	nd	0.25	nd	nd	nd	0.70	nd	nd	nd	nd	nd
	BDE-99	2.67	1.28	0.26	nd	1.50	nd	0.43	nd	nd	nd	1.47	nd	nd	1.18	0.51	nd
	BDE-154	1.42	2.06	0.53	nd	1.46	nd	0.70	nd	nd	nd	0.72	nd	nd	nd	nd	nd
	BDE-153	0.37	0.42	nd	nd	0.58	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	BDE-209	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	∑PBDEs	**13.8**	**9.65**	**2.11**	**nd**	**10.9**	**nq**	**3.38**	**nd**	**2.74**	**1.66**	**3.69**	**0.47**	**0.57**	**6.39**	**6.36**	**5.98**
	DBDPE	1.61	1.24	0.71	1.57	1.18	nd	nd	nd	20.4	49.7	97.1	47.7	23.9	9.66	nd	22.3
	∑HFRs	**15.4**	**10.9**	**2.82**	**1.57**	**12.1**	**nq**	**3.38**	**nd**	**23.1**	**51.4**	**101**	**48.2**	**24.5**	**16.0**	**6.36**	**28.2**
OPEs	TEP	149	nq	51.5	nq	nq	nq	nd	41.2	122	174	1083	328	36.1	nq	nq	1003
	TCEP	nd	5.38	nq	3.14	3.79	3.94	4.73	6.09	nd	nd	nd	nd	nd	nd	nd	454
	TPPO	nq	nq	nd	nq	nq	nq	nq	nd	nd	nd	nd	nd	nd	nd	nd	235
	TClPP	nd	5.61	nq	Nd	nd	nq	60.2	7.39	nd	nd	nd	nd	nd	nd	nd	5.33
	TDClPP	42.2	49.3	22.9	31.5	41.7	36.7	47.0	50.4	nd	nd	nd	nd	nd	nd	nd	280
	TPHP	88.7	46.9	18.1	36.0	32.9	38.5	56.0	72.5	nd	nd	nd	nd	nd	nd	nd	70.0
	TNBP	6.00	2.71	3.81	1.65	1.56	nq	nq	nq	nd	nd	nd	nd	nd	nd	nd	nd
	2IPPDPP	76.9	57.0	2.85	54.0	48.7	56.5	60.7	71.6	nd	nd	nd	nd	nd	nd	nd	nd
	4IPPDPP	18.1	16.3	nq	13.1	14.8	14.6	14.1	20.1	nd	nd	47.0	nd	nd	nd	nq	nd
	EHDPP	7.64	nd	nd	8.24	8.44	5.77	5.06	nq	nd	nd	nd	nd	nd	nd	nd	nd
	B4IPPPP	nq	14.8	nd	nq	14.5	15.0	16.7	21.1	nd	nd	nd	nd	nd	nd	nd	nd
	T2IPPP	179	141	nq	110	116	129	106	150	nd	65.2	nd	nd	nq	nd	nd	nd
	∑OPEs	**567**	**339**	**85.8**	**237**	**262**	**280**	**348**	**417**	**122**	**239**	**1130**	**328**	**36.1**	**nq**	**nq**	**2046**
SCCPs	**SCCPs**	**nq**	**nq**	**30.9**	**nd**	**7.66**	**3.38**	**4.61**	**6.67**	**nq**	**13.0**	**45.0**	**18.8**	**7.85**	**3.39**	**9.10**	**574**

nd: not detected, below LOD; nq = not quantifiable, below LOQ.

Table S4. Physico-chemical properties of OPEs, HFRs and SCCPs analysed in this study.

Acronym	**Name**	**Cas number**	**Molecular weight (g/mol)**	**Solubility (mg/L)***	**Log K_{OW}***
OPEs					
TEP	Triethyl phosphate	78-40-0	182.5	$5x10^5$	0.80
TCEP	Tris(2-chloroethyl) phosphate	115-96-8	285.5	$7x10^3$	1.44
TPPO	Triphenylphosphine oxide	791-28-6	278.3	62.8	2.87
TClPP	Tris(2-chloroisopropyl) phosphate	13674-84-5	327.6	$1.6x10^3$	3.11
TPP	Tripropyl phosphate	513-08-6	224.2	827	1.87
TDClPP	Tris(1,3-dichloro-2-propyl) phosphate	13674-87-8	430.9	1.5	3.65
TPHP	Triphenyl phosphate	115-86-6	326.3	1.9	4.59
TNBP	Tri-n-butyl phosphate	126-73-8	266.3	$2.8x10^2$	4.00
DCP	Diphenyl cresyl phosphate	26444-49-5	340.3	0.24	5.25
TBOEP	Tris(2-butoxyethyl) phosphate	78-51-3	398.5	$1.2x10^3$	3.75
2IPPDPP	2-isopropylphenyl diphenyl phosphate	64532-94-1	368.4	-	5.30
4IPPDPP	4-isopropylphenyl diphenyl phosphate	55864-04-5	368.4	$2.65x10^{-2}$	5.70
TCP	Tricresyl phosphate	1330-78-5	368.4	0.36	6.34
EHDPP	2-ethylhexyldiphenyl phosphate	1241-94-7	362.4	1.9	5.73
B4IPPPP	Bis(4-isopropylphenyl)phenyl phosphate	55864-07-8	410.4	-	6.03
IDPP	Isodecyl diphenyl phosphate	29761-21-5	390.5	0.75	7.28
T2IPPP	Tris(2-isopropylphenyl) phosphate	64532-95-2	452.5	-	9.10
THP	Trihexyl phosphate	2528-39-4	350.5	$1.02x10^{-2}$	7.45
TEHP	Tris(2-ethylhexyl) phosphate	78-42-2	434.6	0.6	9.49
HFRs					
BDE-28	2,4,4-TriBDE	41318-75-6	407	0.33	5.52
BDE-47	2,2,4,4-TetraBDE	5436-41-1	486	0.095	6.01
BDE-99	2,2,4,4,5-PentaBDE	60348-60-9	565	0.039	6.71
BDE-100	2,2,4,4,6-PentaBDE	189084-64-8	565	0.054	6.71
BDE-153	2,2,4,4,5,5-HexaBDE	67774-32-7	644	0.017	7.90
BDE-154	2,2,4,4,5,6-HexaBDE	207122-15-4	644	$8.7x10^{-4}$	7.92
DBDPE	Decabromodiphenyl ethane	1163-19-5	971.2	$9.5x10^{-5}$	11.1
SCCPs					
SCCPs	Short-chain chlorinated paraffins	85535-84-8	320-500	0.15-0.47	5.09-6.43

* OPE data obtained from Blum et al. (2019), van der Veen and de Boer (2012), Phillips and Stapleton (2019), and WHO (1990, 1991, 1998 and 2000); HFR data obtained from Watanabe and Tatsukawa (1990), and He et al. (2018); and, SCCPs data obtained from EC (1999) and Sun et al. (2017).

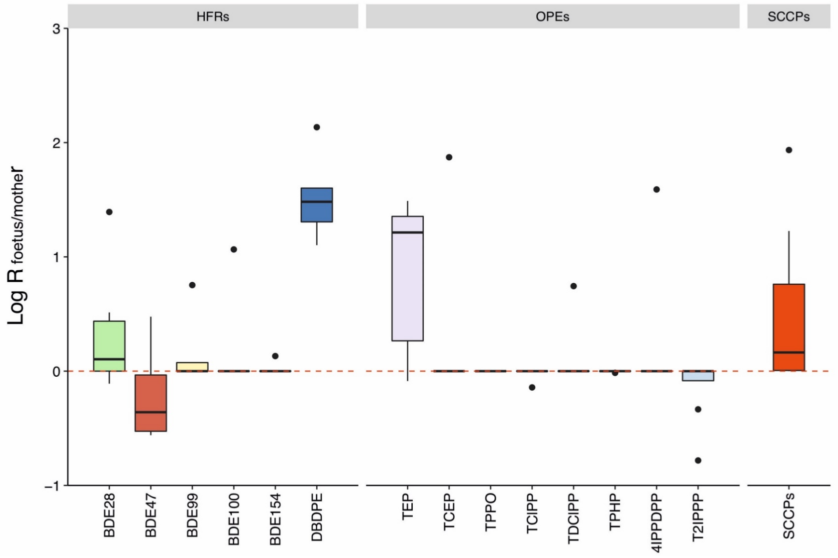

Figure S1. Logarithmic $R_{foetus/mother}$ obtained for HFRs, OPEs and SCCPs, in paired samples of mother-foetus.

Transplacental transfer of plasticizers and flame retardants in fin whales (Balaenoptera physalus) from the North Atlantic Ocean

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4.4 Discussió

En aquest apartat s'hi discuteixen i es comparen els resultats que s'han obtingut de les espècies estudiades dels diferents ecosistemes, així com el comportament dels OPEs en les diferents cadenes tròfiques i els diferents perfils de contaminació. Tenint en compte que alguns dels OPEs es metabolitzen, s'espera que les espècies que es troben en els nivells tròfics superiors puguin metabolitzar millor els OPEs i per tant, presentin concentracions inferiors. Així doncs, les diferències que hi puguem trobar vindran condicionades tant per la contaminació dels punts de mostreig, per l'espècie com pel nivell tròfic.

Aquesta tesi aporta per primera vegada resultats d'OPEs en diferents especies de biota marina, incloent mostres de preses i predadors. Així que disposem de mostres de diferents nivells tròfics. El krill té un nivell tròfic 2 a l'estiu ja que és quan menja fitoplàncton i de 3 a l'hivern, quan s'alimenta de zooplàncton (Cardona et al., 2012). A l'igual que la medusa que també es troba entre els nivells 2-3. La sardina i l'anxova, tenen un nivell tròfic de 3-3,5, depenent de la talla i la zona ja que s'alimenten bàsicament de zooplàncton carnívor (Cardona et al., 2015). Pel que fa a les tortugues babaues, tenen un nivell tròfic molt variable, entre 3 i 4, en funció de la quantitat de meduses i peixos de la seva dieta (Revelles et al., 2007). El lluç també té un dieta variada segons la seva mida, els individus més petits s'alimenten de crustacis i de peixos els individus més grans (Ferraton et al., 2007), per tant també es mouen a un nivell tròfic d'entre 3 i 4. Els calamars ocupen un nivell tròfic superior, al voltant de 4 perquè s'alimenten bàsicament de peixos. El nivell tròfic del rorqual comú a Islàndia també és de 4, a on s'alimenta de krill i de peixos (Borrell et al., 2021). A més, algunes de les mostres analitzades, com els peixos, són comestibles i per tant serveixen com aliment dels humans, els quals encapçalen la xarxa tròfica. Analitzant i comparant els resultats obtinguts en cadascun dels estudis podem arribar a tenir una visió global del comportament dels OPEs dins la xarxa tròfica marina, podent comprovar el seu potencial de bioacumulació i biomagnificació.

Per tal de poder comparar totes les espècies analitzades en aquesta tesi, s'ha fet un diagrama de caixes amb totes les concentracions d'OPEs trobades en ng/g ww. L'ordre que s'ha seguit per ordenar les espècies ha estat el seu nivell tròfic. A més, també hi hem afegit les dades de dofí llistat recol·lectades al Mar Mediterrani entre l'any 2017 i 2018 (les més actuals), per poder tenir una visió de les diferents espècies mostrejades en un mateix període de temps. Aquesta espècie de dofí es troba en els nivells superiors de la cadena tròfica marina, a un nivell de 4 (Borrell et al., 2021). Val a dir que aquesta comparació no és del tot correcte ja que es tracten de diferents ecosistemes, llocs amb graus de contaminació diferent i diferent nombre d'individus mostrejats, però a falta de dades, hem optat per dur a terme la comparativa d'aquesta manera.

Mirant la Figura 24 s'observa que l'espècie amb una concentració més elevada de OPEs és el calamar, però cal tenir en compte que en aquest cas es va fer un *pool* de diferents individus i per tant, només disposem d'un únic valor (90,5 ng/g lw). En segon lloc, hi trobem la tortuga babaua (30,3±18,9 ng/g ww), que inclou les tortugues del Mediterrani català i baleàric, la diferència entre les dues regions no és significativa (*Publicació #5*) i per tant, podem dir que es comporten de manera similar. Tal i com s'explica a la *Publicació #5*, hi ha estudis que evidencien la gran presència de plàstics flotants en aquella zona (Ruiz-Orejón et al., 2018). Seguidament, amb valors força similars hi trobem la sardina i el dofí llistat, amb unes mitjanes de 20,0±19,8 ng/g ww i 18,7±14,0 ng/g ww respectivament. L'anxova i el krill també veiem que no s'allunyen d'aquestes concentracions amb valors respectius de 13,7±8,98 i 12,6±9,46 ng/g ww. El rorqual comú, és considerat un depredador molt actiu ja que consumeix gran quantitat d'aliment gràcies a la seva estratègia per captar-lo, filtrant una gran quantitat d'aigua i retenint l'aliment amb les seves barbes. Aquesta estratègia però, també li juga en contra a l'hora d'empassar residus plàstics ja que no és gens selectiva, tot i que la concentració mitjana trobada (13,2±27,8 ng/g ww) no és superior a la de les espècies que són més selectives a l'hora de menjar, com és el cas del dofí llistat. La medusa té una concentració un ordre de magnitud inferior (4,55 ng/g ww) i a l'igual que el calamar, només es disposa d'un sol *pool* de mostres. Per últim hi tenim el lluç, alimentant-se de diferents espècies de peixos més petits. Els valors del lluç són els més baixos, fins un ordre de magnitud inferiors (2,48 ±1,76 ng/g ww) que el de les espècies més contaminades. Per veure si hi havia diferències entre els nivells tròfics analitzats, s'ha dut a terme un test no paramètric Kruskal-Wallis, al tractar-se de dades no normals. Per fer-lo s'han eliminat tots aquelles dades considerades atípiques (Figura 24). Veiem doncs que no existeixen diferències significatives (Kruskal-Wallis, p-valor=0,6204) entre els diferents nivells de la cadena tròfica, indicant que no hi hauria tendència a la biomagnificació dels OPEs. A més, els individus de les espècies que es troben a un nivell tròfic més alt no són les que presenten concentracions totals d'OPEs més elevades, pel que segurament metabolitzen més aquests contaminants.

Com bé ja s'ha explicat en el capítol 1 de la introducció, els OPEs són compostos amb característiques fisico-químiques molt diverses entre ells i per tant es comporten de manera molt diferent i fins i tot alguns d'ells s'arriben a metabolitzar. Per aquest motiu, hem avaluat la biomagnificació fixant-nos no en la totalitat d'OPEs, sinó en cada compost en particular.

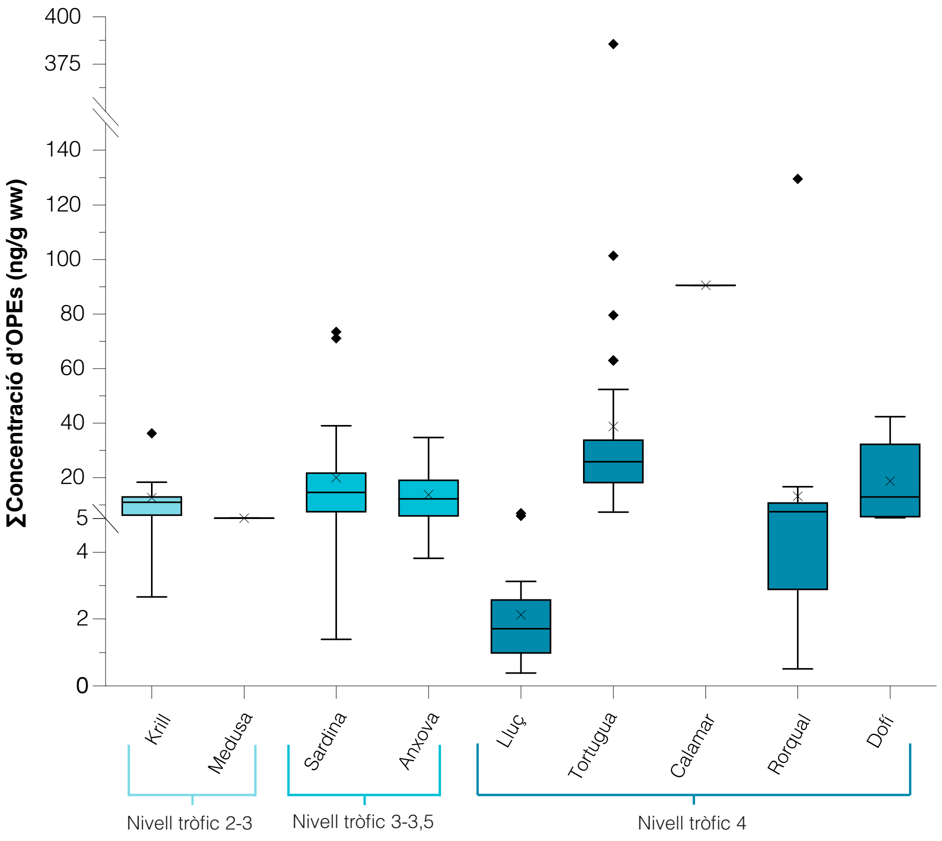

Figura 24. Diagrama de caixes de la suma de la concentració d'OPEs de cada espècie estudiada

Per tal de veure si hi ha transferència dels OPEs de les preses cap els seus predadors, s'ha calculat el BMF de la manera següent:

$$BMF = \frac{C_{predador}}{C_{presa}}$$

On $C_{predador}$ és la concentració mitjana del predador i C_{presa} és la concentració mitjana de la presa. Així doncs, tots aquells valors de BFM que siguin superiors a 1 significarà que hi ha biomagnificació (Taula 16).

Per tant, si un compost es biomagnifica o no, s'ha mirat segons la concentració mitjana d'aquell compost per espècie i no per la freqüència de detecció en cada individu analitzat.

La representació gràfica del BFM de la Figura 25 s'ha fet mitjançant la transformació logarítmica en base 10 de cada BMF i per tant, ens indicarà que hi ha biomagnificació per a tots aquells compostos que tinguin un logBMF més gran que 0.

Els resultats ens mostren que el predador que presenta uns BMF més alts és la tortuga babaua, on sembla que el TEP, DCP, 2IPPDPP, 4IPPDPP i el T2IPPP es biomagnifiquen amb valors de BMF>1 (Taula 16). El comportament del T2IPPP es repeteix en el rorqual comú a on també presenta una BMF de 1,09, tot i que en aquest cas la biomagnificació és més baixa. Aquest contaminant també es va detectar en els plàstics analitzats recollits en la costa de les Illes Balears (*Publicació #5*), a on presentava una concentració mitjana de 198±371 ng/g. Per tant, els nivells en tortuga es poden deure tant a l'afecte de la biomagnificació a través de la dieta com per la bioacumulació d'OPEs de l'impacte directe del consum de residus plàstics. Aquest pot ser el motiu pel qual el valor de BMF de 6,85 no sigui del tot real i estigui sobreestimat. El TPPO i el TNBP també es biomagnifiquen en el cas del rorqual comú i en canvi, pel lluç veiem que només es biomagnifica el TBOEP. El fet de que els compostos que es bioagnifiquen per a cada presa-predador siguin diferents, pot ser degut a la contaminació de cada àrea d'estudi.

El BMF del la ΣOPEs pel nostre estudi oscil·la entre 0,14-0,94, mentre que en estudis semblants per ΣPBDEs els valors de BMF són un ordre de magnitud superiors anant de 1,95 a 3,67 (Y. X. Sun et al., 2015).

Taula 16. BMF de concentració d'OPEs dels predadors dividit entre preses

	BMF		
Compost	Tortuga babaua / preses	Lluç / preses	Rorqual comú - krill
TEP	2,24	-	-
TCEP	0,09	-	-
TPPO	0,94	0,54	1,36
TCIPP	1,08	-	-
TPrP	-	-	-
TDCIPP	0,15	-	-
TPHP	0,25	-	-
TNBP	0,19	0,90	1,18
DCP	1,67	0,11	-
TBOEP	-	1,14	-
2IPPDPP	1,30	-	0,23
4IPPDPP	1,30	-	0,58
TCP	0,36	-	-
EHDPP	0,83	-	-
B4IPPPP	-	-	-
IDPP	-	-	-
T2IPPP	6,85	-	1,09
THP	-	-	-
TEHP	-	-	-
ΣOPEs	0,94	0,14	1,05

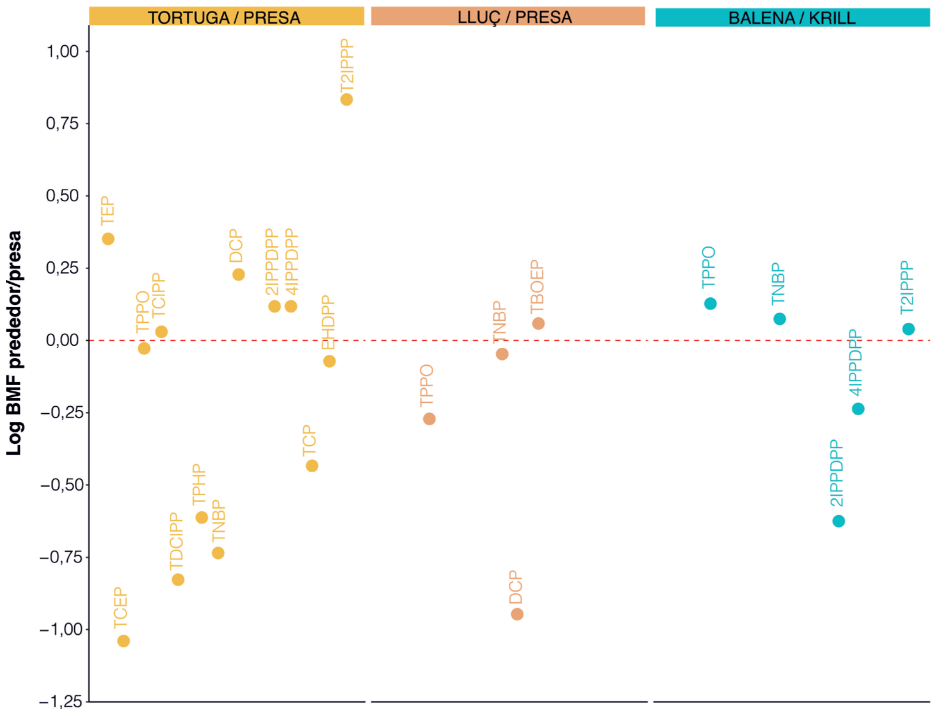

Figura 25. Representació del BMF entre predadors i preses, transformació logarítmica

A més, al tenir les dades de les concentracions de balenes i fetus vam poder calcular quins compostos es transferien a través de la placenta. Pel que fa a la transferència, en el cas de les mares es va veure que el TEP era el compost que més es transferia als fetus. Aquest mateix contaminant és el segon que més es biomagnifica per la tortuga babaua amb un BMF de 2,24. Els altres dos contaminants que també es van transferir, tot i que en menor mesura, van ser el TCEP i el TCIPP, aquest darrer s'ha vist que també es biomagnifica per la totuga babaua (BMF>1).

En definitiva, no podem dir que els OPEs es biomagnifiquen de manera general. Al ser una família que inclou compostos molt diferents entre sí, comportant-se cadascun de manera totalment diferent, ja que veiem que uns es mentabolitzen, mentre que els altres queden acumulats als teixits. A l'hora de parlar de la biomagnificació d'aquest grup s'hauria de mirar per a cada compost per poder tenir una visió real del seu comportament. En canvi, altres compostos amb estructures i propietats molt més semblants com és el cas dels PBDEs, si que es parla de biomagnificació a nivell global.

Perfil dels OPEs i metabolització

Una vegada s'ha estudiat la biomagnificació de cadascun dels OPEs, s'ha volgut mirar la influència en cadascuna de les espècies d'aquells OPEs que s'ha vist que més es biomagnificaven (TEP, TPPO, TNBP, DCP, TBOEP, 2IPPDPP, 4IPPDPP i T2IPPP) i també dels que en teoria tenen més facilitat per metabolitzar-se en els organismes vius (TDCIPP, TPHP, TNBP, TBOEP) (Yao et al., 2021). Per fer-ho, s'ha representat en un gràfic de barres la mitjana de la concentració d'aquests OPEs detectats en cadascuna de les espècies estudiades, ordenades per odre tròfic (Figura 26). En aquest cas doncs, només comentarem els resultats obtinguts pels compostos esmentats anteriorment ja que els altres no ens aporten informació rellevant en aquest estudi.

En l'estudi publicat recentment per Yao et al. (2021), s'ha vist que el TNBP i el TBOEP es metabolitzen millor en el fetge que en l'intestí dels peixos i que els metabòlits trobats a l'intestí poden ser transportats fins aquest a través de la bilis. Per una altre banda, Wang et al. (2017) i Van den Eede et al. (2013) van observar que les vies de reacció per a metabòlits d'OPEs dels peixos zebra eren principalment la ruptura dels enllaços èster (o hidròlisi), la decloració (TCEP i TDCIPP), l'escissió dels enllaços èter (TBOEP), l'hidroxilació de l'oxigen i l'àcid glucurònic conjugat. (Strobel et al., 2018) també va estudiar la metabolització dels OPEs però en aquest cas, en ós polar (*Ursus maritimus*) i foca ocel·lada (*Pusa hispida*), on va observar que qualsevol esgotament dut a terme per l'enzim dels OPEs va ser més eficient pels microsomes dels ossos polars que pel de les foques. L'estudi va revelar que per ambdues espècies hi havia un esgotament complet del TBOEP, però en el cas dels ossos polars també es va observar pel TNBP i el TPHP, mentre que per les foques no es van metabolitzar per complert. Un comportament similar va tenir el TDCIPP que es va metabolitzar parcialment en els ossos i marginalment en les foques. Aquest fet ve donat a que els ossos polars tenen una activitat enzimàtica més elevada que les foques i per tant poden metabolitzar millor els OPEs. Observant els nostres resultats (Figura 26), veiem que el TNBP, el qual esperaríem que es metabolitzés, es troba present en totes les espècies estudiades i en concentracions més elevades en les que es troben més amunt de la cadena tròfica i per tant, podríem dir que té tendència a la biomagnificació. El TBOEP i el TDCIPP, dos dels altres OPEs que tendeixen a metabolitzar-se, s'han trobat en individus dels diferents nivells tròfics. En el cas del TBOEP trobem concentracions similars en la sardina (1,45 ng/g ww), el lluç (0,98 ng/g ww) i el dofí llistat (0,87 ng/g ww). Pel TDCIPP, el calamar i la sardina son les dues espècies amb concentracions més elevades i ambdues pertanyen a nivells tròfics diferents (3 i 4), per tant podem dir que quest compost en aquest cas només es bioacumula ja que el calamar s'alimenta de peixos com la sardina. Per últim, veiem que el TEP, que ja s'ha vist que no es metabolitza (Strobel et al., 2018), si que es biomagnifica al llarg de la xarxa tròfica marina. Cal tenir en compte, que el TEP no es va poder analitzar en totes les espècies estudiades.

Pels compostos estudiats a l'apartat anterior que tendien a biomagnificar-se en els predadors, observem que el DCP mostra un clar augment de la concentració per les espècies dels nivells tròfics més alts, passant de 2,00 ng/g ww per la sardina (nivell 3) a 11,5 ng/g ww al calamar (nivell 4). A més, cal tenir en compte que tant el calamar com a la tortuga babaua, que són els que tenen concentracions més altes de DCP, s'alimenten directament de sardines i anxoves. De manera similar es comporta el TPPO, que també augmenta la seva concentració al llarg de la xarxa tròfica. Per altre banda, el 2IPPDPP, 4IPPDPP i el T2IPPP no mostren un patró tant clar de biomagnificació tot i que també es troben en diferents nivells però en concentracions més variades en individus d'un nivell a l'altre.

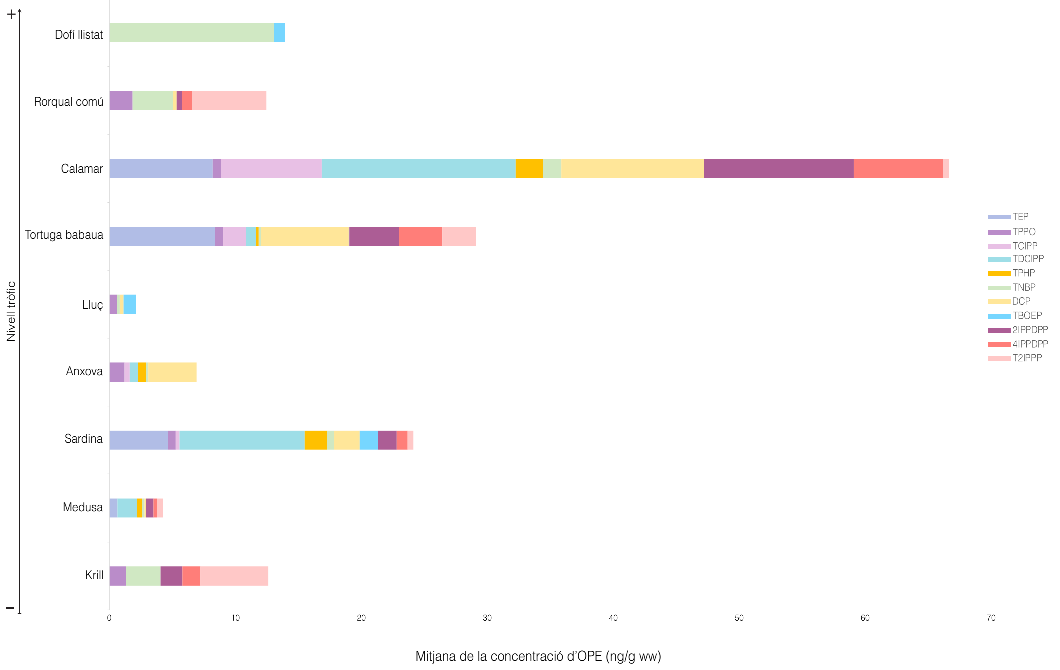

Figura 26. Concentració dels OPEs en les diferents espècies estudiade

A més, la majoria d'OPEs presenten efectes toxicològics, tal i com està explicat a l'apartat *1.9 Avaluació del risc a l'exposició dels OPEs* d'aquesta tesi. Com per exemple, El TPPO que pot ser disruptor del sistema immunològic i el TDCIPP que pot causar neurotoxicitat, a més d'estrès oxidatiu i interferències en el sistema endocrí (Yao et al., 2021).

Alguns metabòlits dels OPEs es consideren més tòxics que els seus compostos pare, com és el cas del DPHP, que va ser capaç de desregular més del doble dels gens aviaris relacionats amb el metabolisme del lípids/colesterol que el seu precursor, el TPHP (Su et al., 2014) i també el cas del OH-EHDPP, que va provocar una activitat antagonista del receptor d'andrògens 3,1 vegades major que el seu compost pare, el EHDPP (Li et al., 2020). Així doncs, això suggereix que els OPEs pateixen un metabolisme específic dels teixits, on els seus residus poden arribar a tenir una influència important en els organismes (Fu et al., 2021).

En conclusió, s'ha observat que el TEP, el DCP, el TNBP i el TPPO presenten una tendència a la biomagnificació a través de la cadena tròfica marina, mentre que el TDCIPP, 2IPPDPP, 4IPPDPP i el T2IPPP no presenten un augment tan clar. Tot i així, s'ha trobat concentracions d'aquests contaminants en espècies de diferents nivells i per tant, si que s'observaria un comportament de bioacumulació en els predadors, com seria el cas de la tortuga babaua i el rorqual comú. A més, pel que fan el TNBP i el TDCIPP, els estudis publicats demostren que són compostos que tendeix a metabolitzar-se i per contra, en el nostre estudi sembla que es biomagnifiquen. En canvi, el TEP si que es comporta com s'esperaria ja que s'ha vist que no es metabolitza. Finalment, com ja hem dit, els OPEs són compostos molt diferents entre si, i es comporten de manera molt diversa en el medi ambient i sobretot, en els organismes vius. Per aquest i altres motius, és necessari seguir investigant en la bioacumulació i biomagnificació dels OPEs.

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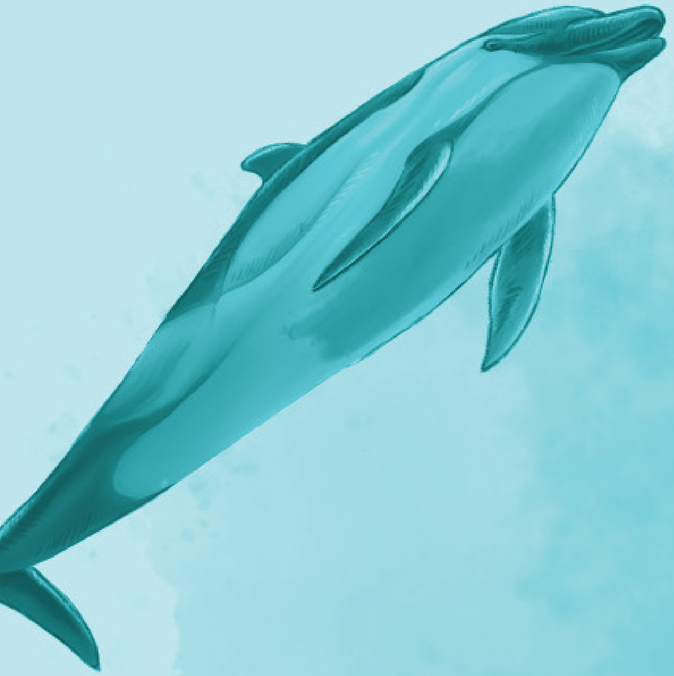

CAPÍTOL V

CONCLUSIONS

CONCLUSIONS

5.1 Conclusions generals

Després d'haver analitzat els OPEs en diferents espècies de biota marina i haver pogut estudiar amb més profunditat el seu comportament en els organismes i en la xarxa tròfica marina, els resultats obtinguts en aquesta tesi, exposats i discutits en els capítols anteriors, donen lloc a les conclusions següents:

1. S'ha realitzat un estudi de distribució dels OPEs en diferents teixits, a partir de mostres de dofins, i s'ha observat que aquests contaminants s'acumulen més en el greix que a teixits amb una activitat metabòlica més elevada com és al fetge. Tot i així, s'ha escollit el múscul com a teixit idoni per analitzar els OPEs, ja que la matriu és més neta que el greix i la freqüència de detecció també és del 100%.

2. Per altre banda, s'han analitzat els nivells de contaminació per OPEs en tres zones diferents: Mar d'Alboran, Mar Mediterrani (per la costa catalana) i l'Oceà Índic (per la costa de Sudàfrica). Els resultats obtinguts mostren nivells d'OPEs superiors per l'Oceà Índic. No obstant, en aquests estudis també es va poder estudiar la concentració de PBDEs en les mateixes zones d'estudi, observant que eren molt més baixes que les trobades pels OPEs. Es va destacar que pel cas de la costa de Sudàfrica els OPEs eren significativa-

ment superiors als FRs clàssics, fins a dos ordres de magnitud per sobre. Aquest fet, fa pensar que la contaminació d'OPEs, a banda de venir del seu ús com a FRs, també hi té un gran impacte la seva funció com a plastificant ja que es tracta d'una zona molt contaminada pels residus plàstics. Els OPEs són utilitzats com a FRs i plastificants i una de les possibles fonts d'entrada a les aigües marines podria ser aquesta gran quantitat de residus plàstics que acaben al mar i oceans. A més, alguns articles publicats mostren nivells d'aquests residus superiors a l'Índic. La gestió de residus de zones amb menys recursos fa que una quantitat més gran d'aquests acabin al medi ambient i en conseqüència als rius, mars i oceans. Tot i així, les fonts d'entrada dels OPEs en poden ser moltes altres, com és el cas del transport atmosfèric i oceànic.

3. També s'ha dut a terme un estudi temporal dels nivells de contaminació dels OPEs a partir de mostres de múscul de dofí des dels anys 1990 al 2018. L'evolució de les concentracions d'aquests contaminants al llarg del temps s'ha mantingut constant, demostrant una entrada continua dels OPEs al medi marí. En canvi, els PBDEs ja prohibits des del 2009 han disminuït la seva concentració.

4. S'han detectat OPEs per primera vegada en mostres de diferents espècies de biota marina, així com krill, meduses, tortugues, calamars, rorquals i dofins. La presència en totes aquestes espècies mostra la ubiqüitat d'aquests contaminants en el medi marí.

5. Tot i no tractar-se d'estudis en un mateix ecosistema, s'ha intentat fer una comparativa dels nivells d'OPEs trobats en cadascuna de les espècies estudiades amb el seu nivell tròfic. S'ha vist que els nivells totals d'OPEs no mostren biomagnificació al llarg de la cadena tròfica. No obstant, com que els OPEs són una família de compostos de característiques molt diferents, és preferible realitzar l'estudi de manera individualitzada per cada compost per separat. A més, també s'ha dut a terme l'estudi de la contaminació dels OPEs en diferents parelles de presa-predador d'un mateix ecosistema, com el rorqual-krill; tortuga-medusa/calamar/sardina i lluç-anxova/sardina. L'anàlisi d'aquests contaminants en els predadors i les preses ens ha permès calcular el BMF, el qual ens permet veure quins OPEs poden tenir un potencial de biomagnificació. Els OPEs que presenten un BMF>1 són: el TEP, TCIPP, DCP, 2IPPDPP, 4IPPDPP en el cas de les tortugues babaues; el TBOEP pel lluç; i el TPPO, TNBP i el T2IPPP en el rorqual comú. A més, en el cas de les tortugues es va poder veure que les concentracions d'OPEs podien venir tant de la dieta, com de la ingesta directa de plàstics. S'ha de tenir en compte que aquests contaminants també es poden bioconcentrar passivament als organismes vius a través de l'aigua i/o del contacte amb els sediments o la matèria orgànica. A més a més, pel cas del lluç, al ser un peix de consum humà, també es va poder avaluar la ingesta diària estimada d'OPEs a través del consum de peix. Es va veure que no existeix una amenaça considerable per a la salut pública, però que tot i així s'ha de tenir en compte que els OPEs poden arribar al cos humà per altres vies com la inhalació, la ingesta, etc. I que la suma d'aquesta exposició sí que podria comportar riscos per la salut humana. Alguns dels compostos que hem vist que tendeixen a biomagnificar-se, també estan descrits a la literatura com a compostos que es metabolitzen, com és el cas, per exemple del TNBP. Està clar que la metabolització dependrà del sistema metabòlic de cada espècie i individu. Tanmateix, són necessaris estudis que determinin quines espècies són capaces de metabolitzar cadascun dels OPEs i quines els bioacumulen en els seus teixits.

6. Un altre estudi realitzat en aquesta tesi va ser el de transferència placentària dels OPEs i altres contaminants com les SCCPs i el DBDPE en mares de rorqual comú i mostres de fetus, essent la primera vegada que es mostra la transferència d'aquests compostos per aquesta via. En aquest cas, es va veure que pels OPEs, la relació de concentració de fetus i mares mantenia una correlació negativa estadísticament significativa amb el log K_{ow}, indicant que en aquests contaminants, com major és el K_{ow}, menor és la transmissió de mare a fetus. Així doncs, és necessari posar atenció especialment a compostos com el TEP (amb un log K_{ow} de 0,80), el qual en el nostre estudi en tortugues babaues mostra una tendència a la biomagnificació, i a més també és l'OPE que es transfereix de mares a fetus en major grau. Aquest fet podria ser degut a que s'ha vist que no es metabolitza i al tenir un pes molecular més petit, li podria facilitar el pas entre els teixits. Tot i així, cal destacar que aquest compost no es va poder analitzar en totes les espècies.

Finalment, de tots els treballs realitzats en el transcurs d'aquesta tesi podem concloure que els OPEs són uns contaminants que es troben altament presents en el medi marí, cosa que comporta a la contaminació dels teixits de les espècies de biota marina. Tot i que les seves propietats no s'ajusten a les descrites pels POPs -com per exemple la seva persistència al medi-, degut al seu ampli ús, estan presents de manera contínua al medi ambient i fins i tot són capaços d'acumular-se i alguns d'ells biomagnificar-se. Aquest fet, juntament amb les seves propietats toxicològiques, els converteixen en contaminants de gran interès i que mereixen un seguiment exhaustiu per tal de poder avaluar el risc que suposa la ubiqüitat d'aquests contaminants al medi marí, i així poder preveure els diferents destins del reservori d'aquests compostos un cop entren a la columna d'aigua i interaccionen amb la biota present.