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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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ABSTRACT

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.

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.

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

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, TClPP, 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

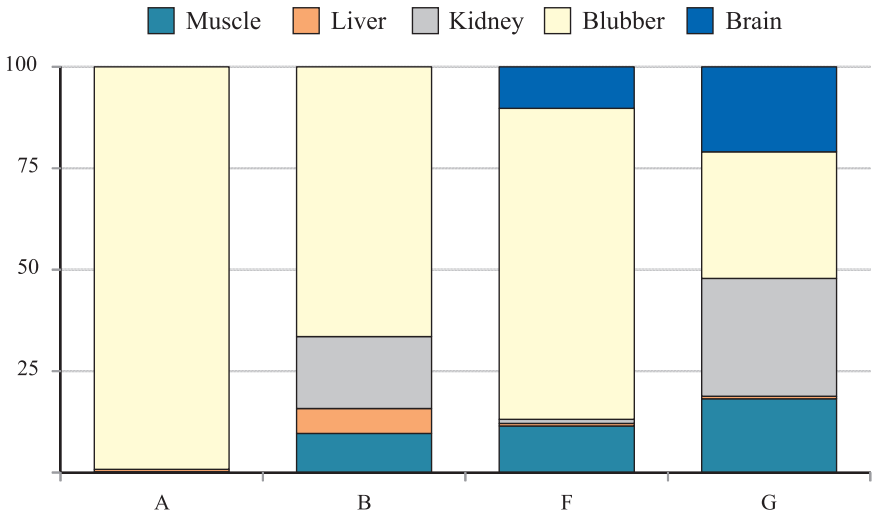

Fig. 1. Percentage contribution of OPFRs (expressed in ng/g dw) in each tissues, for individuals A, B, F and G.

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

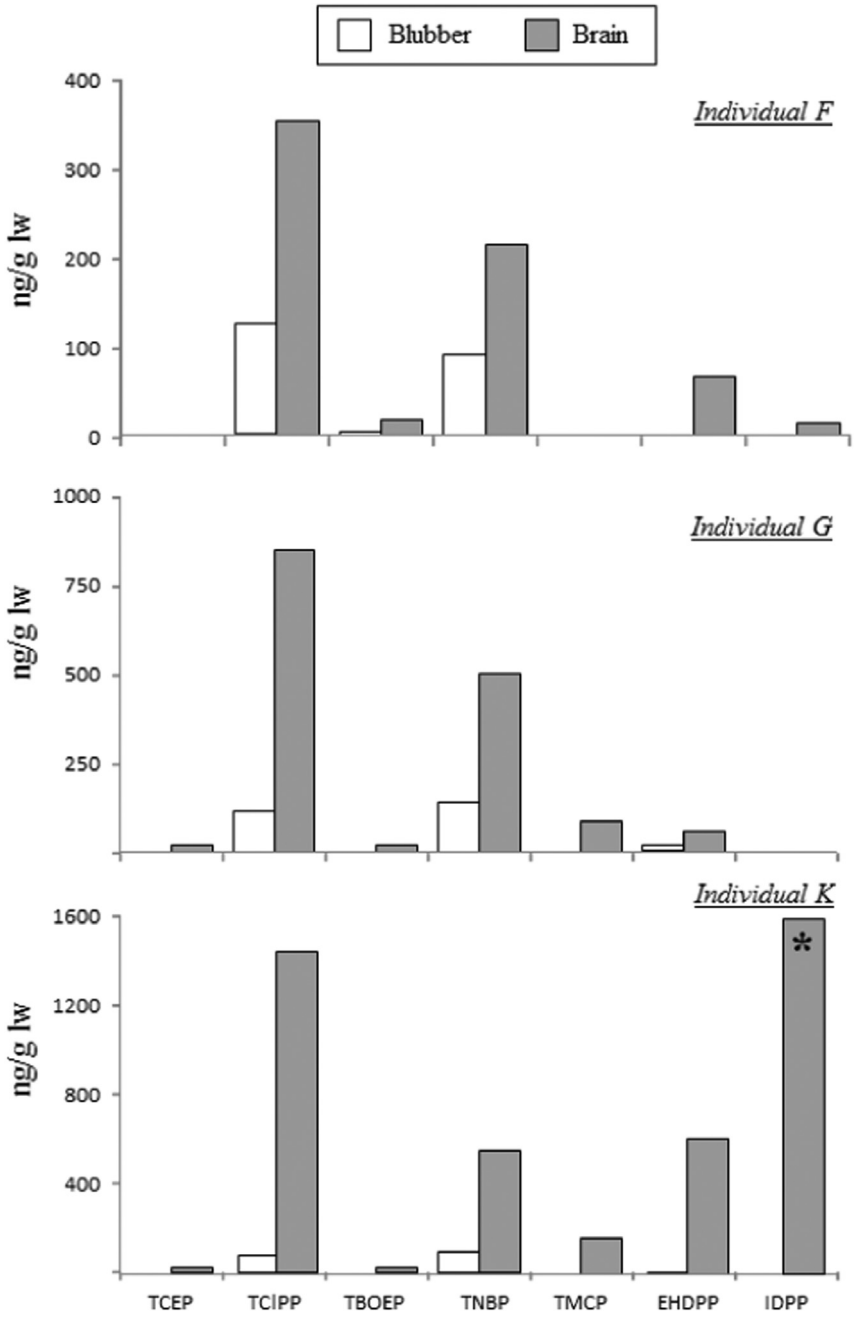

Fig. 2. Concentration levels of OPFRs in blubber and brain of individuals F, G and K. (* the real value is 18,285 ng/g lw; however it is not represented in scale to be able to observe the rest of compounds with very lower levels).

what extent do plastics transfer additives to organisms upon ingestion. 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

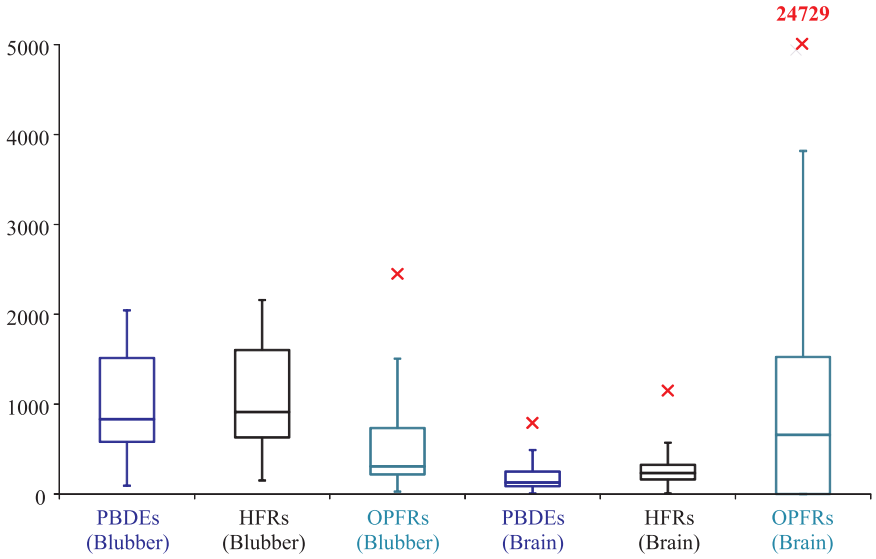

Fig. 3. Box plot of concentration levels (expressed in ng/g lw) by families of FRs and tissues (24,729 ng/g lw, the outlayer value in OPFRs in brain, it's not in scale).

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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Table S1. Recoveries, relative standard deviations (RSDs) and limits of detection (LODs) and quantification (LOQs) of LC-MS-MS analysis of selected OPFRs in dolphin samples.

Analyte	R* (%)	RSD (%)	LOD (ng/g lw)	LOQ (ng/g lw)
TCEP	63	4.7	1.39	4.64
TPPO	52	5.5	0.46	1.53
V6	65	6.5	4.65	15.5
TCIPP	62	7.4	1.69	5.62
TDClPP	48	4.5	0.34	1.12
TPHP	54	1.4	6.37	21.2
TNBP	78	1.9	0.82	2.74
DCP	73	2.9	11.6	38.8
TBOEP	69	2.1	0.35	1.16
TMCP	70	3.5	3.65	12.2
EHDPP	71	3.0	0.39	1.29
IDPP	69	3.4	2.13	7.12
TBNPP	76	0.5	37.4	125
IPPP	92	0.4	51.6	172
THP	84	1.7	0.40	1.32
TEHP	102	3.6	1.76	5.87

*Recoveries and RSDs were determined by spiking 0.25 g dw of sample with 20 ng of each OPFRs. Three replicates were made.

Table S2. Concentration levels (expressed in ng/g lw) of OPFRs in common dolphin individuals (A to K) (*Delphinus Delphis*) from the Alboran Sea.

	A	B	C	D	E	F	G	H	I	J	K
Muscle											
TCEP	nq	nd	nq	nq	nq	nd	nq	32,0	nd	nd	na
TClPP	nd	nq	nd	nd	nd	nd	nd	nq	nd	nd	na
TPPO	nq	nq	nd	nd	nq	nq	nd	nd	358	94,7	na
TBOEP	69,5	26,3	70,2	nq	58,8	64,4	219	nq	30,5	320	na
TNBP	nd	nq	nq	399	nq	nq	2720	1309	nd	nd	na
DCP	nd	210	376	nd	nd	nd	nd	nd	nd	nd	na
TPHP	nd	nd	nd	nd	nd	nq	nd	nd	nq	nd	na
TMCP	nq	nq	258	nq	nd	nd	nq	358	nq	nq	na
EHDPP	nq	nq	nq	nd	nq	688	nd	189	nq	nd	na
IDPP	nd	334	nq	nd	118	7,71	nd	53,7	9,74	168	na
IPPP	nd	nd	nd	nd	nd	nd	nd	nd	1390	nd	na
THP	nd	nd	nd	nd	nd	nd	nd	nd	nd	3,74	na
ΣOPFRs	69,5	571	704	399	177	760	2939	1943	1788	587	na
Kidney											
TCEP	nq	nd	nq	nd	nd	nq	nd	nd	nq	nd	na
TClPP	nd	nd	nd	nd	nq	nd	nd	nd	nq	nd	na
TPPO	nq	nd	19,5	nd	nq	nq	nd	nd	nq	nd	na
TBOEP	nq	nd	34,1	nd	32,6	16,3	nq	nq	7,88	nd	na
TNBP	nd	nd	nd	nd	73,1	nd	nd	nd	nd	nd	na
DCP	nd	nd	nd	nd	132	nd	nd	nd	nd	nd	na
TPHP	nd	nd	nd	nd	nd	nd	306	nd	nd	nd	na
TMCP	nd	nq	nq	31,3	61,5	nd	nq	nq	nq	nq	na
EHDPP	nd	nq	nq	nd	8,16	nd	2,71	nd	nq	nd	na

IDPP	nd	732	nd	19,0	254	nq	480	361	nq	127	na
IPPP	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	na
THP	nd	nd	nd	nd	nd	nq	nq	nd	nq	nq	na
ΣOPFRs	nd	732	53,6	50,3	561	16,3	789	361	7,88	127	na
Liver											
TCEP	nq	91,2	nq	na	nq	nd	18,1	nq	32,4	115	na
TClPP	nq	nd	nd	na	nd	nd	nd	nd	nq	529	na
TPPO	nq	nd	nd	na	nq	nd	nd	nd	nq	nq	na
TBOEP	26,0	37,9	51,2	na	17,9	9,66	12,0	nq	33,6	47,6	na
TNBP	nq	nd	nq	na	nq	nq	nd	98,4	nq	nq	na
DCP	nd	nd	nd	na	nd	nd	nd	nd	nd	nd	na
TPHP	nd	nd	nd	na	nd	nd	nd	nd	nq	nd	na
TMCP	78,5	nd	nq	na	nq	nq	nd	nq	nd	nd	na
EHDPP	nq	nd	nq	na	nq	nq	nd	nq	nd	nd	na
IDPP	nd	nd	nd	na	nq	nd	nd	nq	nd	20,0	na
IPPP	nd	nd	nd	na	nd	nd	nd	nd	nd	nd	na
THP	nd	nd	nd	na	nq	nd	nd	nd	nd	nd	na
ΣOPFRs	105	129	51,2	na	17,9	9,66	30,1	98,4	66,0	712	na
Blubber											
TCEP	38,1	nq	nd	na	nd	nq	nd	nd	nd	na	nd
TClPP	1258	142	387	na	94	124	114	nq	62,2	na	80,7
TPPO	nq	nq	nd	na	nd	nd	nd	nd	nd	na	nq
TBOEP	25,3	8,39	2,49	na	3,51	4,38	nq	nq	1,56	na	nq
TNBP	595	127	264	na	73,9	91,0	136	27,2	110	na	92,0
DCP	nd	nd	nd	na	nd	nd	nd	nd	nd	na	nd
TPHP	nd	nd	nd	na	nd	nd	nd	nd	nd	na	nd
TMCP	158	nd	27,1	na	nd	nd	nd	nq	nd	na	nd
EHDPP	349	29,9	40,5	na	17,0	nd	17,4	nd	33,2	na	3,42

IDPP	26,5	nq	13,0	na	nd	nd	nd	nd	1123	na	nd
IPPP	nd	nd	nd	na	nd	nd	nd	nd	nq	na	nd
THP	nd	nd	nd	na	nd	nd	nd	nd	nd	na	nd
ΣOPFRs	2450	308	734	na	189	219	267	27,2	1330	na	176
Brain											
TCEP	nd	nd	na	na	na	nq	20,2	na	na	na	23,0
TClPP	nd	nd	na	na	na	351	847	na	na	na	1438
TPPO	nd	nd	na	na	na	nd	nq	na	na	na	nd
TBOEP	nd	nd	na	na	na	16,3	19,9	na	na	na	21,3
TNBP	nq	nq	na	na	na	213	501	na	na	na	551
DCP	nd	nd	na	na	na	nd	nd	na	na	na	nd
TPHP	nd	nd	na	na	na	nd	nd	na	na	na	nd
TMCP	nd	nd	na	na	na	nd	83,1	na	na	na	161
EHDPP	nq	nd	na	na	na	66,1	56,9	na	na	na	601
IDPP	nd	nd	na	na	na	12,3	nd	na	na	na	18285
IPPP	nd	nd	na	na	na	nd	nd	na	na	na	3649
THP	nd	nd	na	na	na	nd	nd	na	na	na	nd
ΣOPFRs	nd	nd	na	na	na	659	1527	na	na	na	24729

na – sample not available; nd – below limit of detection; nq – below limit of quantification.