Bioaccumulation of organophosphorus flame retardants in the marine mussel *Mytilus galloprovincialis*

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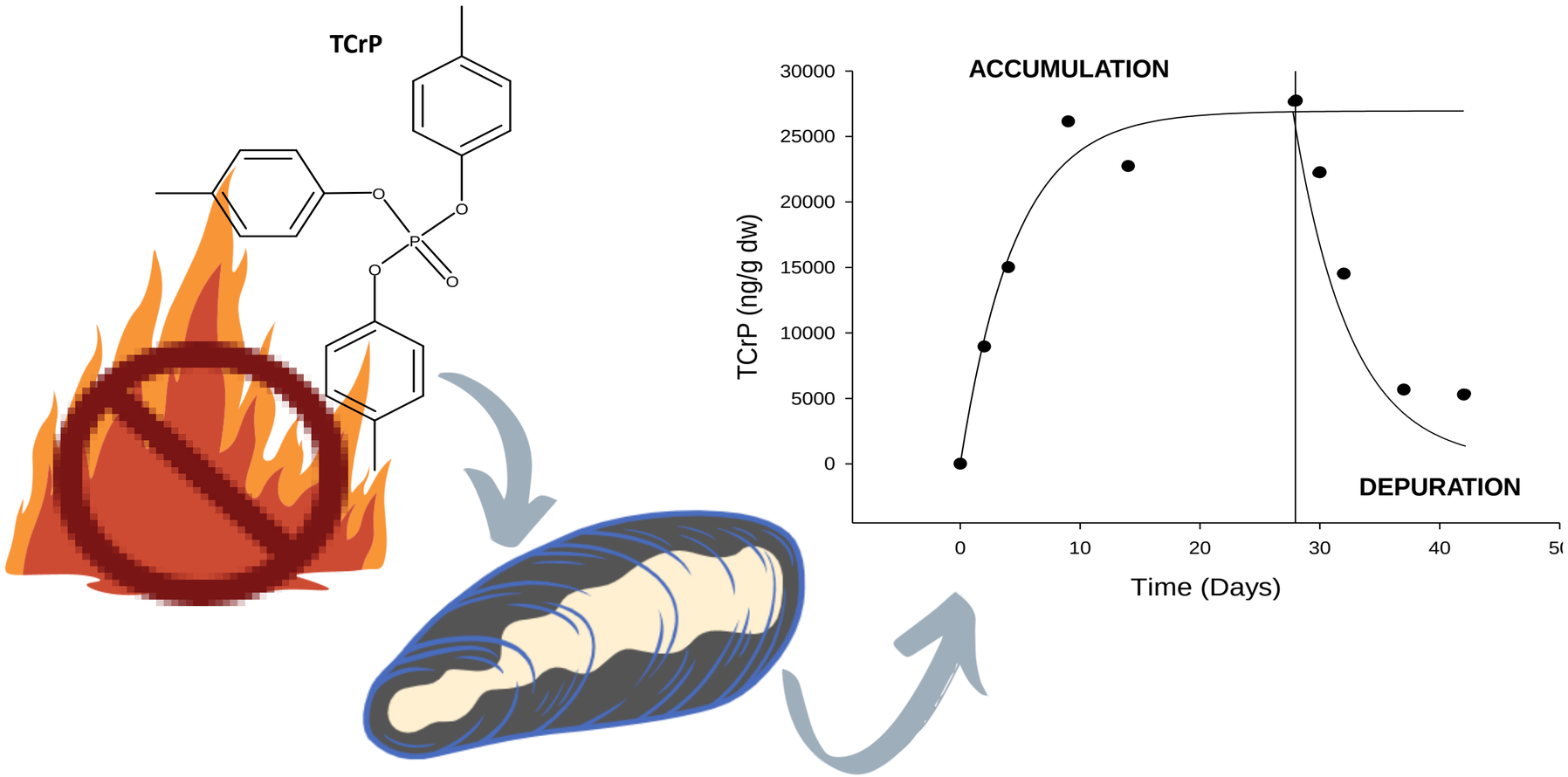
Graphical Abstract



Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)

- Bioaccumulation of 7 OPFRs in mussels at environmentally relevant concentrations was studied.
- OPFRs with aryl groups showed the highest BCF values.
- The log BCF was a linear direct function of log Kow and inverse function of log S.
- The tricresyl phosphate can be classified as an accumulative chemical.

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Keywords: OPFRs, MSPD, kinetics, K_{ow}

Abstract

The bioaccumulation and depuration of seven organophosphorus flame retardants (OPFRs) in marine mussel *Mytilus galloprovincialis* were studied. OPFRs showed to be bioavailable in aquatic environments. When mussels are exposed to environmentally relevant concentrations of OPFRs, uptake kinetics fit well to a first-order model with a single compartment; in contrast depuration rates were generally underestimated by that model, most likely because it does not take into account the biotransformation of OPFRs by the organisms. The highest bioaccumulation rates were observed for tricresyl phosphate (TCrP), triphenyl phosphate (TPhP) and 2-ethylhexyldiphenylphosphate (EHDPP). This could be due to the presence of aryl groups in these compounds, their low solubility in water, and their affinity for fat tissues. According to these findings TCrP, with a BCF value of 4042 L Kg^{-1} wet weight, should be classified in environmental regulations as an accumulative chemical.

1. Introduction

Flame retardants (FRs) are chemicals added to a wide range of materials used to prevent combustion and delay fire propagation (Kemmlein et al., 2003). Among the most used FRs are organophosphorus compounds, whose production increased due to the restrictions of polybrominated diphenyl ethers (PBDEs) (Wei et al., 2015). Organophosphorus flame retardants (OPFRs) are a growing environmental problem as they are considered "re-emerging" pollutants (Reemtsma et al., 2008), since they have been detected in all environmental compartments (Cristale et al., 2017), and because their high production volumes (Ramesh et al., 2020). OPFRs are commonly used as additives for plastics and textiles. They are not chemically bound to the polymer chains, and they can be easily released by volatilization, abrasion and/or leaching and reach the aquatic environment either directly or via deposition from the atmosphere (Bollmann et al., 2012; Wei et al., 2015).

These pollutants can be accumulated in organisms via the direct uptake from water by gills or body surfaces (bioconcentration) or by ingestion of particles/live foods associated with the pollutant; when the uptake is given by both routes it is termed bioaccumulation (Walker et al., 2012). The tendency of a chemical to accumulate in biological tissues can be quantified by the bioconcentration factor (BCF), determined as the ratio between the concentration in the organism tissues and that in the surrounding water at steady state (Beiras, 2018). The BCF depends on physicochemical properties of the chemical (solubility, hydrophobicity, persistence in water), and biological properties of the organism (uptake and depuration ability, body fat, metabolic transformation) (Hilvarsson et al., 2009).

First studies of OPFRs bioaccumulation date back to the 1970's, when Sheldon and Hites (1978) discovered a moderate potential for accumulation of substances such as tris (2-butoxyethyl) phosphate (TBEP) and triphenyl phosphate (TPhP) in aquatic organisms. Unlike the polybrominated diphenyl ethers they have replaced, OPFRs are not regarded as bioaccumulative in aquatic organisms. However, remarkable levels of these chemicals have been found recently in freshwater (Liu et al. 2019) and marine invertebrates (Castro et al. 2020, Choi et al. 2020a, Sundkvist et al. 2010).

Concentrations of OPFR reached 21 ng/g dw for TCPP (Choi et al. 2020a), or 291 ng/g dw for TPhP (Castro et al. 2020) in wild marine mussels, and remarkable bioaccumulation of tris(1,3-dichloro-2-propyl) phosphate in laboratory exposures has been recently reported (Sánchez-Marín et al. 2021). In addition, molecular effects of OPFRs on several freshwater organisms were frequently reported (Sasaki et al., 1981; Han et al., 2014; Du et al., 2015; Giraudo et al., 2015; Isales et al., 2015; Kim et al., 2015; Yuan et al., 2018), supporting a more exhaustive investigation on these emerging pollutants.

Marine mussel is widely used to monitor chemical contamination due to its ubiquity, high water filtration rates, and a non-regulatory metabolism that favours the bioaccumulation of contaminants (Khadim, 1990; Kimbrough et al., 2008; Vidal-Liñán et al., 2018). As humans consume them widely as food, the study of the accumulation of contaminants in mussels is also of great relevance in food safety studies (Catarino et al., 2018). Therefore, this study aimed to investigate the bioaccumulation of 7 relevant OPFRs, viz. tris(1-chloro-2-propyl) phosphate (TCPP), TBEP, tri-iso-butyl phosphate (TiBP), tri-n-butyl phosphate (TnBP), TPhP, tricresyl phosphate (TCrP) and 2-ethylhexyldiphenylphosphate (EHDPP), in marine mussel, *Mytilus galloprovincialis*. Due to the large differences in OPFRs physicochemical properties, an attempt was further made to relate bioaccumulation to OPFRs properties.

2. Materials and methods

2.1. Chemicals and reagents

TCrP, TnBP, TBEP, TPhP and TCPP were supplied by Sigma-Aldrich (Milwaukee, WI, USA), EHDPP by Dr. Ehrenstorfer GmbH (Augsburg, Germany) and TiBP by Merck (Darmstadt, Germany). Purity of the compounds was 94 ± 5% except TCPP with 97.5% and EHDPP with 91.7%. Stock solutions were prepared at a concentration of 10 mg L^{-1} of each OPFR in dimethyl sulfoxide (DMSO) (99.9% purity

from Scharlau) for bioaccumulation experiments and in methanol for chemical analysis and stored in the dark at -20℃. Structures and physico-chemical properties of the studied OPFRs are shown in Table S1. During the exposure experiment, DMSO concentration did not exceed 0.1% (v / v), a concentration at which it presents low or no toxicity in cells (Jamalzadeh et al., 2016; Singh et al., 2017). For chemical analyses, three deuterium labelled OPFRs were used as internal standards (TnBP-d27, TPhP-d15 and TCCP-d18) purchased from Wellington Laboratories (Guelph, Ontario, Canada). A mixture with these three internal standards was prepared in methanol for experimental purposes.

Acetonitrile hypergrade for LC-MS and ethyl acetate for gas chromatography MS were supplied by Merck, methanol (≥ 99.9 %) for LC-MS grade by Thermo Fisher Scientific (Waltham, MA, USA) and formic acid (≥ 99.9 %) for LC-MS and dichloromethane (DCM) for pesticide residue analysis by VWR Chemicals (Radnor, PA, USA). Ultrapure water was obtained in the laboratory by purifying demineralized water in a Milli-Q GradientA-10 system (Merck-Millipore, Bedford, MA, USA).

Florisil (60–100 mesh) was provided by Supelco (Bellefonte, PA, USA) and silica gel 60 (0.040-0.063 mm) by Merck. Silica and Florisil were washed in a pressurized liquid extraction (PLE) system using an ASE 200 (Dionex, Idstein, Germany) apparatus, equipped with 33 mL stainless steel extraction cells, using first acetonitrile and then ethyl acetate at 60 °C. Subsequently, sorbents were dried into the oven at 120 °C for 24 h.

2.2. Samples and bioaccumulation experimental conditions

Individuals of *Mytilus galloprovincialis* with 40-50 mm shell length were collected from the intertidal zone of Toralla island (Galicia, NW Iberian Peninsula). Prior to experiments, mussels were cleaned from epibiont organisms and acclimated to incubation conditions for one week in the laboratory.

In the exposure experiment, a total of 160 mussels were exposed for 28 days to a mixed solution of the seven studied OPFRs with a nominal concentration of 1 μg L^{-1}, followed by a 14 days depuration period in clean seawater. To this aim, 16 mussels were distributed per glass tank containing 30 L of seawater with oceanic characteristics filtered at 1 μm and disinfected with UV radiation. Exposures

were conducted at a constant temperature of 16 ± 1°C in darkness, and tanks were continuously aerated with 0.22 µm filtered air. Water was renewed three times per week, prior to the addition of experimental solution. Mussels were fed before every water change for 2 h with a mixed diet of *Isochrysis galbana*, *Tetraselmis suecica* and *Chaetoceros gracilis* at a ratio of 2% of mussel dry weight (dw).

Samples of eight individuals were taken in duplicate after 0, 2, 4, 9, 14, 28 days of exposure, and after 2, 4, 9 and 14 days of depuration. The soft tissues of each sample were mixed and freeze-dried for 72 h. Water samples of 10 mL were taken in duplicate at different times before and after water renovation and addition of experimental solution, and frozen at -20ºC until further analyses.

2.3. Chemical analyses

2.3.1. Sample treatment

Mussels and water samples were shipped to IAQBUS, where they were subjected to analysis. Before extraction, freeze-dried mussel samples were homogenised using an electric grinder. Samples were processed using a matrix-solid phase dispersion (MSPD) method developed by (Castro et al., 2020), whereby 0.5 g freeze-dried mussel were mixed with 1.2 g activated silica into a glass mortar and spiked with 10 ng g^{-1} of a mixture of three IS (TnBP-d27, TPhP-d15 and TCCP-d18). The homogeneous mixture was transferred into a cartridge containing 3 g of deactivated (5% (w/w) H_2O) Florisil. Analytes were eluted with 10 mL of acetonitrile. Then, the extract was concentrated into a Turbovap II concentrator (Zymark, Hopkinton, MA, USA), evaporated to dryness under a purified nitrogen stream and reconstituted in 100 µL of methanol. The injection volume in the liquid chromatography-tandem mass spectrometry (LC-MS/MS) system was 5 µL of mussel extracts.

Water samples were defrosted, and aliquots of each sample were spiked with 5 ng mL^{-1} of a mixture of three IS (TnBP-d27, TPhP-d15 and TCCP-d18) and 45 µL were directly injected (large-volume injections) in the LC-MS/MS system (see section 2.3.2). For the analysis of EHDPP, a liquid-liquid extraction was carried out using 10 mL of sample and 4 mL of dichloromethane. An aliquot (3.5 mL)

of extraction solvent was transferred to a glass tube, evaporated to dryness under a purified nitrogen stream and reconstituted in 100 µL of methanol. Then, 5 µL of extracts were injected in the LC-MS/MS system.

2.3.2. Liquid chromatography-mass spectrometry analysis

Instrumental analysis was performed into a Waters Acquity UPLC® H-class system (Milford, MA, USA) equipped with a sample manager, a quaternary solvent pump, and a column oven. Chromatographic separation was carried out on a Luna 3 µm C18 column (50×2 mm) connected to a C18 (2×4 mm) guard cartridge, both supplied by Phenomenex (Torrance, CA, USA). As mobile phases, Milli-Q water (0.1% formic acid) (A) and methanol (0.1% formic acid) (B) were used at a flow rate of 0.2 mL min^{-1} and the column temperature was fixed at 35 °C. The gradient elution started at 65% B, increasing to 100% B in 24 min, hold for 2 min. Subsequently, it returned to initial conditions (65% B) in 0.1 min, hold for 5 min for column back-conditioning.

The LC system was coupled to a triple quadrupole mass spectrometer Xevo TQD (Waters) equipped with an electrospray ionization (ESI) source. Nitrogen, used as desolvation and cone gas, was provided by a nitrogen generator (Peak Scientific Spain, Barcelona, Spain) and argon (99.999%), for the collision induced dissociation (CID), was supplied from Nippon Gases (Madrid, Spain). Ionization was performed in positive mode using the following parameters: 3.4 kV (capillary voltage), 150◦C (source temperature), 450 ºC (desolvation temperature), 650 L h^{-1} (desolvation gas flow, N_2) and 0 L h^{-1} (cone gas flow, N_2). Analysis was performed in multiple reaction monitoring (MRM) mode, using two transitions per compound (Supplementary Material, Table S2) and a dwell time of 0.022 s per transition. Collision energy (CE) and cone voltage (CV) values were adjusted individually for each compound and IS (Supplementary Material, Table S2).

2.3.3. Quality assurance

MSPD procedural blanks were also prepared and submitted to the same protocol in order to check for any contamination during sample preparation. MSPD optimization and validation, as well as

material precleaning protocols in order to control blanks are detailed elsewhere (Castro et al., 2020). Briefly, recoveries between 85 and 115 % with relative standard deviation (RSD) below 24% were obtained (n=4). Since a different instrumentation was used, method quantification limits (mLOQ) were re-evaluated and ranged from 0.6 to 2.9 ng g^{-1} dw (Table S3), calculated as the highest value of those obtained from a signal-to-noise equal to 10 or 10 times the standard deviation of the blank. Blank concentrations remained below 0.2 ng g^{-1} dw in all cases.

In water samples, LOQs, with an injection volume of 45 µL, varied from 0.03 to 0.30 µg L^{-1}. Quantification was performed by injecting standards prepared in seawater in the mLOQ to 5 µg L^{-1} range. For EHDPP, a liquid-liquid extraction was necessary. Recovery, evaluated in seawater samples spiked at 1 µg L^{-1} of EHDPP (n=3), was of 85 % with RSD of 9% (Table S3) and mLOQ of 0.10 µg L^{-1}.

2.4. Bioaccumulation model

The accumulation and excretion of OPFRs was fitted to a first-order kinetic model, where the rates of uptake and excretion are directly related to the concentration of the substance in the compartment of origin, the organism is considered as a single compartment, and concentration in water is assumed constant, according to the expression (Landrum et al., 1992, Beiras, 2018):

$$C_a(t) = \frac{C_w K_u}{K_e}(1 - e^{-K_e t}) \quad (1)$$

where:

$C_a(t)$ is the accumulated concentration (µg Kg^{-1} dry weight) in mussels at time t

C_w is the OPFR concentration in water (µg mL^{-1})

K_u is the uptake rate constant (mL Kg^{-1} day^{-1})

K_e is the excretion rate constant (day^{-1})

t is the time (days)

Actual C_w values measured at t=0 were used for calculations (see Tab. S4). K_u and K_e were estimated by least squares fit of the accumulation data to equation (1) using DataFit software (Version 9.1.32). Concentrations below mLOQ were replaced with a value of 0. First-order kinetic model was represented using SigmaPlot software (Version 14.0).

The bioconcentration factor (BCF) can thus be calculated as the ratio between the uptake rate and excretion rate constants (Vidal-Liñán et al., 2018):

$BCF = K_u/K_e$ (L Kg^{-1}). (2)

The relationship between log BCF and phsyco-chemical properties (Supplementary Material, Table S1) was examined by a linear regression using R statistical software (Version 4.0.1).

3. Results and discussion

3.1. OPFRs concentrations in seawater

During the bioaccumulation experiment, concentrations of OPFRs in seawater at different times after adding a nominal concentration of 1 µg L^{-1} of each compound were measured (Table S4). After the first time of exposure (2 days), TCPP and TiBP showed a concentration significantly above the value at t_0 , this increase indicates an external source of contamination that could be explained by the presence of these compounds in seawater (Chen et al., 2019a; Hu et al., 2014), in the air and indoor dust (Ali et al., 2012; Fromme et al., 2014; García et al., 2007) and/or from leaching of the PVC pipes (Wang et al., 2017a). TnBP and TPhP do not shown significant differences between t_0 and t_{48}. On the other hand, EHDPP, TCrP and TBEP showed a reduction, between 23 and 45%, of their concentration in seawater after 48 h. However, during the following water renovations and addition of experimental solution after 24 h the concentration deemed stable with variations from the nominal concentration below 30% (Table S4).

3.2. Accumulation kinetics of OPFR in mussel tissues

Table S5 shows OPFRs concentrations measured in mussel samples taken at different exposure (up to 28 days) and depuration (additional 14 days) times. After acclimation (0 days exposure time), concentrations above mLOQ were found for TCPP, TPhP, TCrP and EHDPP, suggesting that mussels were exposed to these substances before collection from natural environment, or, as indicated above, for TCPP due to an external source of contamination. TCrP, TPhP and EHDPP showed a very rapid uptake, after only 48 h concentrations were 8964, 2401 and 862 ng g^{-1} (dw) respectively. On the other hand, TnBP, TiBP, TBEP and TCPP showed remarkably lower bioaccumulation, reaching a maximum concentration of 388, 167, 166 and 178 ng g^{-1} respectively after 14 days exposition. Depuration was very fast for aliphatic OPFRs (TiBP, TnBP, TBEP and TCPP) reaching concentrations bellow mLOQ, or in the case of TCPP at concentration level similar to t_0, after only 2 days (first depuration time collected). This fast depuration was also reported in the literature in lab experiment with zebrafish (Wang et al., 2017b) and carp (Tang et al., 2019). The depuration for TPhP and EHDPP was almost complete after 14 days, remaining only a 2% of the concentration at 28 days exposition. On the other hand, depuration of TCrP was slower with an 81% of depuration in 14 days. Slow depuration rate was also reported for TCrP and TPhP in zebrafish (Wang et al., 2017b).

The bioaccumulation kinetics fit well to the first-order asymptotic model described by equation (1) as presented in Fig. 1 and Table 1 (p-value < 0.05). Only for TnBP p-value was above 0.05 likely due to the higher concentration found at 14 days. K_u values ranged from 2.9×10^4 mL Kg^{-1} day^{-1} for TBEP to 1.3×10^7 mL Kg^{-1} day^{-1} for TCrP. In contrast, during the depuration phase, excretion rates are underestimated by the model for TiBP, TnBP, EHDPP and TBEP. We must bear in mind that the first-order bioaccumulation model does not take into account the potential metabolic transformation of the chemical in organisms, and biotransformation during both exposure and depuration periods would reduce accumulation and increase excretion rates above values predicted by the model (Arnot and Gobas, 2006). Choi et al. (2002b) reported biotransformation of TPhP in *Daphnia magna*, through several metabolic pathways involving phase I (hydrolases, hydroxylases, reductases) and phase II (transferases) enzymes that eventually lead to excretion of cysteine and sulphate-conjugated

metabolites. On the other hand, for TCPP, the depuration phase does not fit well to the model (p-value = 0.06) likely due to the background contamination.

Several studies have reported that OPFRs can be biotransformed by species such as *Cyprinus carpio* and small fish (Tang et al., 2019 ; Chen et al., 2019b). Within the exposure period, it was observed that the concentrations of some OPFRs (TBEP, TnBP, TiBP and TPhP) increased to a maximum and then decreased (see Fig. 1). This could indicate that mussels reaching a given threshold concentration in the tissues can trigger biotransformation pathways intended to eliminate these substances, as occurs in fish. In TCPP, TCrP and EHDPP this tendency was not seen, either because the threshold was not reached or because the mussels lack biotransformation mechanisms for these substances.

3.3. Bioaccumulation and molecular properties of the OPFRs

The BCFs (as dry weight basis) obtained are given in Table 1. Remarkable differences in BCF were found among the different OPFRs studied. The highest bioaccumulation potentials occur for the aryl chemicals, TCrP (59888 L Kg^{-1} dw), EHDPP (5696 L Kg^{-1} dw), and TPhP (3685 L Kg^{-1} dw). For comparison, these values were translated into a wet weight basis (Table 2) considering an average water content of mussel soft tissues of 85% (Castro et al., 2020). So, BCF ranged between 16 and 49 L Kg^{-1} ww for aliphatic OPFRs and between 553 and 8983 L Kg^{-1} ww for aryl OPFRs. The European Regulation of Registration, Evaluation, Authorization and Restriction of Chemical Substances (REACH) classifies as bioaccumulative those compounds with BCF values higher than 2000 L Kg^{-1} ww and very bioaccumulative those with BCF values above 5000 L Kg^{-1} ww. Considering the BCFs obtained in this study using marine mussels, TCrP could be classified as very bioaccumulative.

Previous bioaccumulation studies of OPFRs in aquatic organisms are compiled in Table 2. Zhong et al. (2020) recently reported for TCPP an even lower BCF in mussel of 363 L Kg^{-1} fat weight, that assuming an average lipid content of 1.3% ww (Bellas et al., 2014) translates into a BCF of 4.7 L Kg^{-1} ww. The other studies are focussed mostly on freshwater fish and can be classified as field-based and laboratory-based studies. Field based-BCF values were calculated under natural OPFRs

concentrations between 0.6 and 264 ng L^{-1} (Hou et al., 2019, 2017; Liu et al., 2019). In laboratory based-BCF values the water concentrations considered were significantly higher between 0.8 and 50 µg $L^{-1.}$ (Hou et al., 2019; Tang et al., 2019; Wang et al., 2017b; Wang et al., 2016). BCF values reported in the literature were between 1.7 and 1008 L Kg^{-1} ww in function of the compound, the specie and the tissue considered (Table 2). In agreement with our study, the highest BCF were reported for aryl OPFRs (Hou et al., 2017; Liu et al., 2019; Tang et al., 2019; Wang et al., 2017b).

The most prominent OPFRs in molluscs and crustaceans reported in the literature are TPhP, TCPP and TCrP (Castro et al., 2020; Poma et al., 2018; Sundkvist et al., 2010). Although BCF of TCPP obtained in this study is one of the lowest, its high levels in biota could be explained since TCPP is the predominant OPFRs in sea water (Li et al., 2017; McDonough et al., 2018; Regnery and Püttmann, 2010; Schmidt et al., 2021; Zhong et al., 2020), because alkyl/aryl OPFRs are more readily degradable than chlorinated OPFRs.

Correlations between BCF and physico-chemical properties of OPFRs were evaluated. Hydrophobicity of chemicals can be described using the log K_{ow}, whose values ranging between 2.59 for TCCP and 5.73 for EHDPP (Table S1). The bioaccumulation and the log K_{ow} usually follow a linear relationship for most substances with log K_{ow}<6 (Hilvarsson et al., 2009). In the present study a significant correlation between log BCF and log K_{ow} was obtained described by the equation log BCF = -0.4006 + 0.8228 log K_{ow}. (r^2 = 0.729, p=0.0145; Fig.2). In a similar experiment carried out by Tang et al. (2019), where the substances were, among others, EHDPP, TPhP, TnBP and TBEP, this correlation was also observed (r = 0.933-0.975, p <0.01) in the investigated tissues, except serum in *Cyprinus* carp, which supports that the hydrophobicity of OPFRs plays an important role in accumulation in aquatic organisms (Geyer et al., 1982, 1991; Tang et al., 2019; Wang et al., 2019). The most hydrophobic OPFRs showed higher bioaccumulation values, in line with the literature (van der Veen and de Boer 2012, Hou et al. 2017, Liu et al. 2019). TCPP, the least hydrophobic OPFR tested, showed a substantially lower BCF than aryls, albeit higher than that expected from the K_{ow} value. On the other hand, in the EHDPP molecule an aryl group has been substituted by an alkyl group, compared with

TPhP and TCrP. It has been observed that the alkyl OPFRs have less potential for bioaccumulation among the studied compounds (Table 1); this could contribute to explain why EHDPP presents a BCF lower than expected according to its log K_{ow}.

The bioaccumulation is also related to the solubility of the compounds in water (S, mg L^{-1} 25 °C). In this case a significant inverse relationship between log BCF and log S (r^2=0.979; p<0.001; Fig. 2) was obtained (log BCF = 3.97 – 0.5988 log S).

It is well-known that for low and medium molecular-weight organic chemicals the log K_{ow} is a good predictor for bioaccumulation potential (MacKay 1982). Concerning OPFRs, van Der Veen and De Boer (2012) found that both K_{ow} and (except for chlorinated OPFRs) BCF generally increase with molecular weight. Although the present study was not designed to separate the effects of molecular volume and hydrophobicity, K_{ow} did predict experimental BCF values whereas we did not find any evidence of correlation between molecular weight -or molecular volume- and BCF (p>0.4).

4. Conclusions

OPFRs include a large variety of molecules with very different chemical properties. Although they are generally considered as non-bioaccumulative chemicals, their bioaccumulation potential increases as hydrophobicity increases. In quantitative terms, BCF of these substances in mussels was directly related to log K_{ow} according to the equation: log BCF = -0.4006 + 0.8228 log K_{ow}. However, other factors such as the presence of chlorine atoms or aryl groups increased BCF above values predicted on the basis of hydrophobicity alone. As a result, the highest BCFs values were found for TCrP and TPhP. In fact, TCrP exceeded the threshold of BCF>5000 L Kg^{-1} ww stated in the European legislation to be classified for regulatory purposes as a very bioaccumulative chemical. Therefore, regulatory limits for these chemicals in aquatic environments are advisable in order to protect consumer's health, and further research on their bioaccumulation and potential toxic effects of these increasingly used chemicals is necessary.

Accumulation kinetics were generally well described using a simple one-compartment, first-order kinetics model. However, excretion rates for some OPFRs were largely underestimated, most likely because this simple model does not take into account the ability of aquatic organisms to biotransform these substances.

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References

Ali, N., Dirtu, A.C., Van den Eede, N., Goosey, E., Harrad, S., Neels, H., Mannetje, A., Coakley, J., Douwes, J., Covaci, A., 2012. Occurrence of alternative flame retardants in indoor dust from New Zealand: Indoor sources and human exposure assessment. Chemosphere 88,1276-1282. https://doi.org/10.1016/j.chemosphere.2012.03.100

Arnot, J.A., Gobas, F.A.P.C., 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environ. Rev. 14,257-297. https://doi.org/10.1139/A06-005

Beiras, R., 2018. Bioaccumulation, in: Marine Pollution Sources, Fate and Effects of Pollutants in Coastal Ecosystems. Elsevier. 187–204.

Bellas, J., Albentosa, M., Vidal-Liñán, L., Besada, V., Franco, M.A., Fumega, J., González-Quijano, A., Viñas, L., Beiras, R., 2014. Combined use of chemical, biochemical and physiological variables in mussels for the assessment of marine pollution along the N-NW Spanish coast. Mar. Environ. Res. 96, 105-117. https://doi.org/10.1016/j.marenvres.2013.09.015

Bollmann, U.E., Möller, A., Xie, Z., Ebinghaus, R., Einax, W., 2012. Occurrence and fate of organophosphorus flame retardants and plasticizers in coastal and marine surface waters. Water Research 46, 531-538.https://doi.org/10.1016/j.watres.2011.11.028

Castro, V., Montes, R., Quintana, B., Rodil, R., Cela, R., 2020. Determination of 18 organophosphorus flame retardants/plasticizers in mussel samples by matrix solid-phase dispersion combined to liquid chromatography-tandem mass spectrometry. Talanta 208, 120470. https://doi.org/10.1016/j.talanta.2019.120470

Catarino, A.I., Macchia, V., Sanderson, W.G., Thompson, R.C., Henry, T.B., 2018. Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal. Environ. Pollut. 237, 675–684. https://doi.org/10.1016/j.envpol.2018.02.069

Chen, M., Gan, Z., Qu, B., Chen, S., Dai, Y., Bao, X., 2019a. Temporal and seasonal variation and ecological risk evaluation of flame retardants in seawater and sediments from Bohai Bay near Tianjin, China during 2014 to 2017. Mar. Pollut. Bull. 146, 874–883. https://doi.org/10.1016/j.marpolbul.2019.07.049

Chen, R., Hou, R., Hong, X., Yan, S., Zha, J., 2019b. Organophosphate flame retardants (OPFRs) induce genotoxicity in vivo: A survey on apoptosis, DNA methylation, DNA oxidative damage, liver metabolites, and transcriptomics. Environ. Int. 130, 104914. https://doi.org/10.1016/j.envint.2019.104914

Choi, W., Lee, S., Lee, H.K., Moon, H.B., 2020a. Organophosphate flame retardants and plasticizers in

343 sediment and bivalves along the Korean coast: Occurrence, geographical distribution, and a
344 potential for bioaccumulation. Mar. Pollut. Bull. 156, 111275.
345 https://doi.org/10.1016/j.marpolbul.2020.111275

346 Choi, Y., Jeon, J., Choi, Y., Kim, S.D., 2020b. Characterizing biotransformation products and pathways
347 of the flame retardant triphenyl phosphate in Daphnia magna using non-target screening. Sci.
348 Tot. Environ. 708, 135106. https://doi.org/10.1016/j.scitotenv.2019.135106

349 Cristale, J., Dantas, R.F., De Luca, A., Sans, C., Esplugas, S., Lacorte, S., 2017. Role of oxygen and DOM
350 in sunlight induced photodegradation of organophosphorous flame retardants in river water. J.
351 Hazard. Mater. 323, 242–249. https://doi.org/10.1016/j.jhazmat.2016.05.019

352 Du, Z., Wang, G., Gao, S., Wang, Z., 2015. Aryl organophosphate flame retardants induced
353 cardiotoxicity during zebrafish embryogenesis: By disturbing expression of the transcriptional
354 regulators. Aquat. Toxicol. 161, 25–32. https://doi.org/10.1016/j.aquatox.2015.01.027

355 Fromme, H., Lahrz, T., Kraft, M., Fembacher, L., Mach, C., Dietrich, S., Burkardt, R., Völkel, W., Göen,
356 T., 2014. Organophosphate flame retardants and plasticizers in the air and dust in German
357 daycare centers and human biomonitoring in visiting children (LUPE 3). Environ. Int. 71, 158–
358 163. https://doi.org/10.1016/j.envint.2014.06.016

359 García, M., Rodríguez, I., Cela, R., 2007. Microwave-assisted extraction of organophosphate flame
360 retardants and plasticizers from indoor dust samples. J. Chromatogr. A 1152, 280–286.
361 https://doi.org/10.1016/J.CHROMA.2006.11.046

362 Geyer, H., Sheehan, P., Kotzias, D., Freitag, D., Korte, F., 1982. Prediction of ecotoxicological
363 behaviour of chemicals: Relationship between physico-chemical properties and
364 bioaccumulation of organic chemicals in the mussel Mytilus edulis. Chemosphere 11, 1121–
365 1134. https://doi.org/10.1016/0045-6535(82)90122-9

366 Geyer, H.J., Scheunert, I., Brüggemann, R., Steinberg, C., Korte, F., Kettrup, A., 1991. QSAR for organic

chemical bioconcentration in Daphnia, algae, and mussels. Sci. Total Environ. 109–110, 387–394. https://doi.org/10.1016/0048-9697(91)90193-I

Giraudo, M., Douville, M., Houde, M., 2015. Chronic toxicity evaluation of the flame retardant tris (2-butoxyethyl) phosphate (TBOEP) using Daphnia magna transcriptomic response. Chemosphere 132, 159–165. https://doi.org/10.1016/j.chemosphere.2015.03.028

Han, Z., Wang, Q., Fu, J., Chen, H., Zhao, Y., Zhou, B., Gong, Z., Wei, S., Li, J., Liu, H., Zhang, X., Liu, C., Yu, H., 2014. Multiple bio-analytical methods to reveal possible molecular mechanisms of developmental toxicity in zebrafish embryos/larvae exposed to tris(2-butoxyethyl) phosphate. Aquat. Toxicol. 150, 175–181. https://doi.org/10.1016/j.aquatox.2014.03.013

Hilvarsson, A., Ohlauson, C., Blanck, H., Granmo, Å., 2009. Bioaccumulation of the new antifoulant medetomidine in marine organisms. Mar. Environ. Res. 68, 19–24. https://doi.org/10.1016/j.marenvres.2009.03.007

Hou, R., Liu, C., Gao, X., Xu, Y., Zha, J., Wang, Z., 2017. Accumulation and distribution of organophosphate flame retardants (PFRs) and their di-alkyl phosphates (DAPs) metabolites in different freshwater fish from locations around Beijing, China . Environ. Pollut. 229, 548-556. https://doi.org/10.1016/j.envpol.2017.06.097

Hou, R., Yuan, S., Feng, C., Xu, Y., Rao, K., Wang, Z., 2019. Toxicokinetic patterns, metabolites formation and distribution in various tissues of the Chinese rare minnow (Gobiocypris rarus) exposed to tri(2‐butoxyethyl) phosphate (TBOEP) and tri-n-butyl phosphate (TNBP). Sci. Total Environ. 668, 806–814. https://doi.org/10.1016/j.scitotenv.2019.03.038

Hu, M., Li, J., Zhang, B., Cui, Q., Wei, S., Yu, H., 2014. Regional distribution of halogenated organophosphate flame retardants in seawater samples from three coastal cities in China. Mar. Pollut. Bull. 86, 569–574. https://doi.org/10.1016/j.marpolbul.2014.06.009

Isales, G.M., Hipszer, R.A., Raftery, T.D., Chen, A., Stapleton, H.M., Volz, D.C., 2015. Triphenyl

391 phosphate-induced developmental toxicity in zebrafish: Potential role of the retinoic acid
392 receptor. Aquat. Toxicol. 161, 221–230. https://doi.org/10.1016/j.aquatox.2015.02.009

393 Jamalzadeh ,L., Ghafoori, H., Sariri, R., Rabuti, H., Nasirzade, J.,Hasani, H., Aghamaali, M. R., 2016.
394 Cytotoxic Effects of Some Common Organic Solvents on MCF-7, RAW-264.7 and Human
395 Umbilical Vein Endothelial Cells. Avicenna J Med Biochem. 4, e33453. https:// doi:
396 10.17795/ajmb-33453

397 Kemmlein, S., Hahn, O., Jann, O., 2003. Emissions of organophosphate and brominated flame
398 retardants from selected consumer products and building materials. Atmospheric Environment.
399 37, 5485–5493. https://doi.org/10.1016/j.atmosenv.2003.09.025

400 Khadim, M.A., 1990. Methodologies for monitoring the genetic effects of mutagens and carcinogens
401 accumulated in the body of marine mussels. Rev. Aquat. Sci. 2, 83–107.

402 Kim, S., Jung, J., Lee, I., Jung, D., Youn, H., Choi, K., 2015. Thyroid disruption by triphenyl phosphate,
403 an organophosphate flame retardant, in zebrafish (Danio rerio) embryos/larvae, and in GH3 and
404 FRTL-5 cell lines. Aquat. Toxicol. 160, 188–196. https://doi.org/10.1016/j.aquatox.2015.01.016

405 Kimbrough, K.L., Johnson, W.E., Lauenstein, G.G., Christensen, J.D., Apeti, D.A., 2008. An Assessment
406 of Two Decades of Contaminant Monitoring in the Nation’s Coastal Zone. Silver Spring,
407 MD.NOAATechnical Memorandum NOS NCCOS 74,105.

408 Landrum, P.F., Lee, H., Lydy, M.J., 1992. Toxicokinetics in aquatic systems: Model comparisons and
409 use in hazard assessment. Environ. Toxicol. Chem. 11, 1709-1725.
410 https://doi.org/10.1002/etc.5620111205

411 Li, J., Xie, Z., Mi, W., Lai, S., Tian, C., Emeis, K.C., Ebinghaus, R., 2017. Organophosphate Esters in Air,
412 Snow, and Seawater in the North Atlantic and the Arctic. Environ. Sci. Technol. 51, 6887–6896.
413 https://doi.org/10.1021/acs.est.7b01289

414 Liu, Y.-E., Luo, X.-J., Zapata Corella, P., Zeng, Y.-H., Mai, B.-X., 2019. Organophosphorus flame

retardants in a typical freshwater food web: Bioaccumulation factors, tissue distribution, and trophic transfer Environ. Pollut. 255, 113286. https://doi.org/10.1016/j.envpol.2019.113286

MacKay, D., 1982. Correlation of bioconcentration factors. Environ. Sci. Technol. 16, 274-278.

McDonough, C.A., De Silva, A.O., Sun, C., Cabrerizo, A., Adelman, D., Soltwedel, T., Bauerfeind, E., Muir, D.C.G., Lohmann, R., 2018. Dissolved Organophosphate Esters and Polybrominated Diphenyl Ethers in Remote Marine Environments: Arctic Surface Water Distributions and Net Transport through Fram Strait. Environ. Sci. Technol. 52, 6208–6216. https://doi.org/10.1021/acs.est.8b01127

Poma, G., Sales, C., Bruyland, B., Christia, C., Goscinny, S., Van Loco, J., Covaci, A., 2018. Occurrence of Organophosphorus Flame Retardants and Plasticizers (PFRs) in Belgian Foodstuffs and Estimation of the Dietary Exposure of the Adult Population. Environ. Sci. Technol. 52, 2331–2338. https://doi.org/10.1021/acs.est.7b06395

Ramesh, M., Angitha, S., Haritha, S., Poopal, R.K., Ren, Z., Umamaheswari, S., 2020. Organophosphorus flame retardant induced hepatotoxicity and brain AChE inhibition on zebrafish (Danio rerio). Neurotoxicol. Teratol. 82. https://doi.org/10.1016/j.ntt.2020.106919

REACH, 2020. https://echa.europa.eu/es/regulations/reach/understanding-reach (accessed 10.16.20).

Reemtsma, T., Quintana, J.B., Rodil, R., García-López, M., Rodríguez, I., 2008. Organophosphorus flame retardants and plasticizers in water and air I. Occurrence and fate. TrAC - Trends Anal. Chem. 27, 727–737. https://doi.org/10.1016/j.trac.2008.07.002

Regnery, J., Püttmann, W., 2010. Occurrence and fate of organophosphorus flame retardants and plasticizers in urban and remote surface waters in Germany. Water Res. 44, 4097–4104. https://doi.org/10.1016/j.watres.2010.05.024

Sánchez-Marín, P., Vidal-Liñán, Fernández-González, L.E., Montes, R., Rodil, R., Quintana, J.B.,

439 Carrera, M., Mateos, J., Diz, A.P., Beiras, R., 2021. Proteomic analysis and biochemical
440 alterations in marine mussel gills after exposure to the organophosphate flame retardant
441 TDCPP. Aquat Toxicol.230,105688. https://doi.org/10.1016/J.AQUATOX.2020.105688

442 Sasaki, K., Takeda, M., Uchiyama, M., 1981. Toxicity, absorption and elimination of phosphoric acid
443 triesters by killifish and goldfish. Bull. Environ. Contam. Toxicol. 27, 775–782.
444 https://doi.org/10.1007/BF01611095

445 Schmidt, N., Castro-Jiménez, J., Oursel, B., Sempéré, R., 2021. Phthalates and organophosphate
446 esters in surface water, sediments and zooplankton of the NW Mediterranean Sea: Exploring
447 links with microplastic abundance and accumulation in the marine food web. Environ. Pollut.
448 272, 115970. https://doi.org/10.1016/j.envpol.2020.115970

449 Sheldon, L.S., Hites, R.A., 1978. Organic Compounds in the Delaware River. Environ. Sci. Technol. 12,
450 1188–1194. https://doi.org/10.1021/es60146a006

451 Singh, M., McKenzie, K., Xiaoling, M., 2017. Effect of dimethyl sulfoxide on in vitro proliferation of
452 skin fibroblast cells. J. Biotech. Res. 8, 78-82.

453 Sundkvist, A.M., Olofsson, U., Haglund, P., 2010. Organophosphorus flame retardants and plasticizers
454 in marine and fresh water biota and in human milk. J. Environ. Monit. 12, 943–951.
455 https://doi.org/10.1039/b921910b

456 Tang, B., Poma, G., Bastiaensen, M., Yin, S.S., Luo, X.J., Mai, B.X., Covaci, A., 2019. Bioconcentration
457 and biotransformation of organophosphorus flame retardants (PFRs) in common carp (Cyprinus
458 carpio). Environ. Int. 126, 512–522. https://doi.org/10.1016/j.envint.2019.02.063

459 Van der Veen, I., de Boer, J., 2012. Phosphorus flame retardants: Properties, production,
460 environmental occurrence, toxicity and analysis. Chemosphere 88,1119-1153.
461 https://doi.org/10.1016/j.chemosphere.2012.03.067

462 Vidal-Liñán, L., Villaverde-de-Saa, E., Rodil, R., Benito Quintana, J., Beiras, R., 2018. Bioaccumulation

of UV filters in Mytilus galloprovincialis mussel. Chemosphere 190,267-271. https://doi.org/10.1016/j.chemosphere.2017.09.144

Walker, C.H., Sibly, R.M., Hopkin, S.P., Peakall, D.B., 2012. Principles of Ecotoxicology, CRC Press.

Wang, G., Du, Z., Chen, H., Su, Y., Gao, S., Mao, L., 2016. Tissue-specific accumulation, depuration, and transformation of triphenyl phosphate (TPHP) in adult zebrafish (Danio rerio). Environ. Sci. Technol. 50, 13555–13564. https://doi.org/10.1021/acs.est.6b04697

Wang, Y., Hou, M., Zhang, Q., Wu, X., Zhao, H., Xie, Q., Chen, J., 2017a. Organophosphorus Flame Retardants and Plasticizers in Building and Decoration Materials and Their Potential Burdens in Newly Decorated Houses in China. Environ. Sci. Technol. 51, 10991–10999. https://doi.org/10.1021/acs.est.7b03367

Wang, G., Shi, H., Du, Z., Chen, H., Peng, J., Gao, S., 2017b. Bioaccumulation mechanism of organophosphate esters in adult zebrafish (Danio rerio). Environ. Pollut. 229, 177–187. https://doi.org/10.1016/j.envpol.2017.05.075

Wang, X., Zhong, W., Xiao, B., Liu, Q., Yang, L., Covaci, A., Zhu, L., 2019. Bioavailability and biomagnification of organophosphate esters in the food web of Taihu Lake, China: Impacts of chemical properties and metabolism. Environ. Int. 125, 25–32. https://doi.org/10.1016/j.envint.2019.01.018

Wei, G.L., Li, D.Q., Zhuo, M.N., Liao, Y.S., Xie, Z.Y., Guo, T.L., Li, J.J., Zhang, S.Y., Liang, Z.Q., 2015. Organophosphorus flame retardants and plasticizers: Sources, occurrence, toxicity and human exposure. Environ. Pollut. 196,29-46. https://doi.org/10.1016/j.envpol.2014.09.012

Yuan, S., Li, H., Dang, Y., Liu, C., 2018. Effects of triphenyl phosphate on growth, reproduction and transcription of genes of Daphnia magna. Aquat. Toxicol. 195, 58–66. https://doi.org/10.1016/j.aquatox.2017.12.009

Zhong, M., Wu, H., Li, F., Shan, X., Ji, C., 2020. Proteomic analysis revealed gender-specific responses

of mussels (Mytilus galloprovincialis) to trichloropropyl phosphate (TCPP) exposure. Environ. Pollut. 267, 115537. https://doi.org/10.1016/j.envpol.2020.115537

Fig. 1. Concentration (ng g^{-1} dw) of TBEP (a), TiBP (b), TnBP (c), TPhP (d), TCrP(e), EHDPP (f) and TCPP (g) in the soft tissues of exposed mussels for 28 days to 1 µg L^{-1} and subsequently in clean seawater for 14 further days. Lines describe values predicted by the first order kinetic model. Note the different scales on the Y axis.

Fig. 2. Relationship between the bioconcentration factor (BCF) of seven OPFRs in *M. galloprovincialis* mussels and (a) the octanol-water partition coefficient (K_{ow}) (log BCF = -0.4006+0.8228 log K_{ow}. (r^2 = 0.729, p=0.0145)) and (b) the solubility (log BCF = 3.97 – 0.5988 log (Water solubility) ((r^2=0.979; p<0.001).

Table 1. Fit parameters of the bioaccumulation model (± 95% confidence interval) and bioconcentration factors calculated with measured concentrations in water (t_0).

Compound	K_u (mL Kg^{-1} day^{-1})	P values for K_u	K_e (day^{-1})	P values for K_e	BCF (L Kg^{-1} dw)
TCPP	40502 ± 3239	0.04	0.38± 0.40 [a]	0.06	107
TiBP	76827 ± 43916	0.008	0.36 ± 0.24	0.01	212
TnBP	173946 ± 189425[a]	0.06	0.54 ± 0.65 [a]	0.08	324
TPhP	1735207 ± 1314163	0.02	0.47 ± 0.40	0.03	3685
EHDPP	679297 ± 215530	0.0009	0.12 ± 0.05	0.004	5696
TCrP	13076099 ± 6071935	0.004	0.22 ± 0.13	0.009	59888
TBEP	28586 ± 24687	0.03	0.14 ± 0.16 [a]	0.08	207

[a] **P values >0.05**

Table 2. Comparison of BCFs (L Kg^{-1} ww) obtained in this work to those from the literature for river and marine species considering both field and laboratory samples at different water concentrations (μg L^{-1}).

Reference	Type of study	Organism		TCPP	TnBP	TiBP	TBEP	TPhP	TCrP	EHDPP
This work	Laboratory	*Mytilus galloprovincialis*	Water concentration	1.51	0.95	0.71	0.73	1.11	0.45	0.53
			BCF	16	49	32	31	553	8983	854
Zhong et al. (2020)	Laboratory	*Mytilus galloprovincialis*	Water concentration	33	NS	NS	NS	NS	NS	NS
			BCF	4.7	NS	NS	NS	NS	NS	NS
Liu et al. (2019)	Field	*Ophiocephalus argus, Clarias batrachus, Cirrhinus molitorella* and *Carassius auratus*	Water concentration	0.076	0.051	NS	NS	0.013	0.00062	0.0017
			BCF	20-45	20-228	NS	NS	18-162	58-382	48-289
Hou et al. (2017)	Field	*Pseudorasbora parva*	Water concentration	0.055 – 0.264	0.0257-0.233	NS	0.0135-0.158	0.0027-0.0093	NS	0.0054-0.0114
		Carassius auratus	BCF	250	173	NS	38.5	1008	NS	163

		Misgurnus anguillicaudatus							
Hou et al.,(2019)	Laboratory	*Gobiocypris rarus*	Water concentration	NS	10	NS	20	NS	NS	NS
			BCF	NS	3.94-27.76	NS	2.20-16.67	NS	NS	NS
Tang et al. (2019)	Laboratory	*Cyprinus carpio*	Water concentration	7.3	5.9	NS	5.4	5.2	NS	5.3
			BCF	2.7-5.6	1.7-31.4	NS	4.8-14.8	2.3-60.5	NS	3.5-78.5
Wang et al. (2017b)	Laboratory	*Danio rerio*	Water concentration	NS	35	NS	50	7.3	0.8	NS
			BCF	NS	6.7-25.0	NS	4.8-17.3	32.1 - 157.4	76-364	NS

NS: not studied.

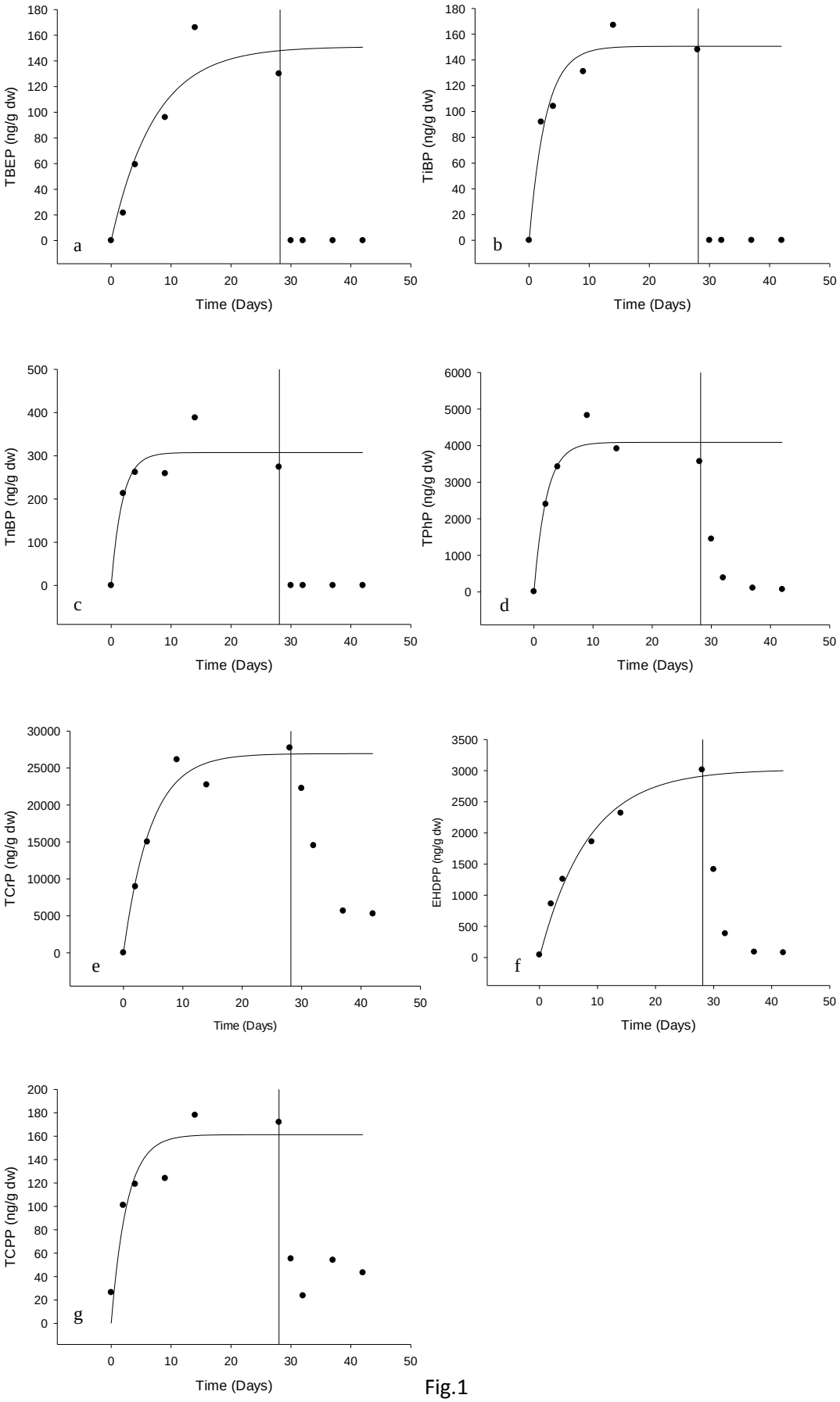

Fig.1

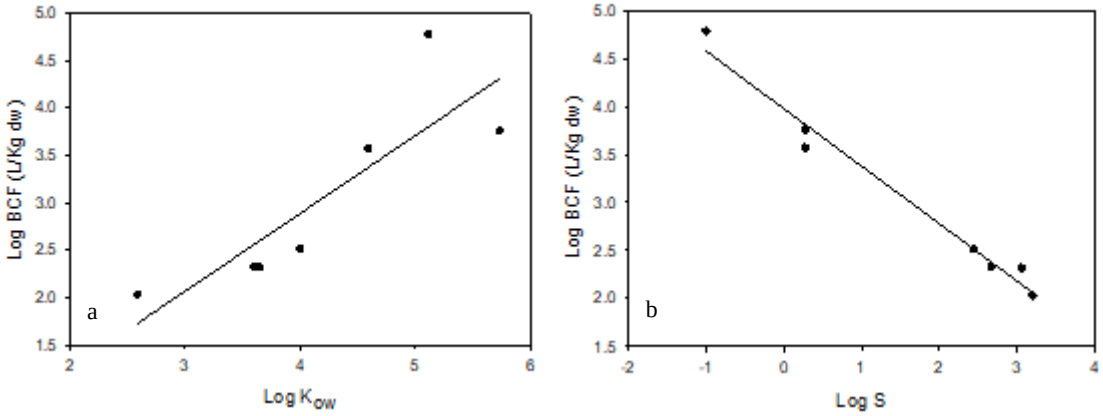
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Fig.2

CRediT author statement

María del Carmen Mata: Sampling, Methodology, Investigation, Writing – original draft. **Verónica Castro:** Methodology, Investigation, Writing – original draft. **José Benito Quintana:** Resources, Writing - review & editing, Supervision, Funding acquisition. **Rosario Rodil:** Resources, Writing - review & editing, Supervision, Funding acquisition. **Ricardo Beiras:** Resources, Writing - review & editing, Supervision, Funding acquisition. **Leticia Vidal-Liñán:** Methodology, Investigation, Resources, Writing - review & editing, Supervision.

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

SUPPORTING INFORMATION TO:

Bioaccumulation of organophosphorus flame retardants in the marine mussel *Mytilus galloprovincialis*

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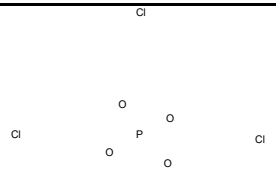
Table S1. Physico-chemical properties of the OPFRs studied. MW: molecular weight. K_{ow} octanol:water partition coefficient. Data compiled from ChemSpider (2020), REACH (2020), van der Veen & de Boer (2012) and Jchem for Excel (ChemAxon).

Structure	Name	Formula	MW (g mol^{-1})	Molecular Volume ($Å^3$)	Water solubility (mg L^{-1} 25 °C)	log K_{ow}
	Tris(1-chloro-2-propyl) phosphate (TCPP)	$C_9H_{18}C_{l3}O_4P$	327.6	261	1600	2.59
	Tris (2-butoxyethyl) phosphate (TBEP)	$C_{18}H_{39}O_7P$	398.5	400	1100 -1200	3.65
	Triisobutyl phosphate (TiBP)	$C_{12}H_{27}O_4P$	266.3	270	475.6	3.6

	Tri-n-butyl phosphate (TnBP)	$C_{12}H_{27}O_4P$	266.3	270	280	4.00
	Triphenyl phosphate (TPhP)	$C_{18}H_{15}O_4P$	326.3	278	1.9	4.59
	Tricresyl phosphate (TCrP)	$C_{21}H_{21}O_4P$	368.4	329	0.1-0.3	5.11
	2-Ethylhexyldiphenylphosphate (EHDPP)	$C_{20}H_{27}O_4P$	362.4	343	1.9	5.73

Table S2. Studied compounds, labeled compounds used as IS, retention time (RT), cone voltage (CV), quantifier (Q1) and qualifier (Q2) m/z transition (precursor > product ion), and collision energy (CE) selected in every case.

Compound	IS	RT (min)	CV (V)	Q1	CE (V)	Q2	CE (V)
TCPP	TCPP-d18	3.15	25	327>99	30	327>251	10
TPhP	TPhP-d15	5.88	50	327>152	30	327>215	25
TiBP	TnBP-d27	7.40	25	267>99	15	267>155	15
TnBP	TnBP-d27	7.79	25	267>99	15	267>155	10
TBEP	TnBP-d27	9.20	35	399<199	15	399<299	10
TCrP	TPhP-d15	11.57	50	369<165	40	369<243	25
EHDPP	TnBP-d27	13.22	20	363<251	5	363<152	50
TCPP-d18	-	3.10	25	347<102	25	345<102	25
TPhP-d15	-	5.77	50	342<160	30	342<223	20
TnBP-d27	-	7.46	25	294>102	15	294>106	10

Table S3. Recoveries, repeatability (as %RSD, in brackets), and quantification limits (mLOQs) of the analytical methods.

Compound	Water			Mussels			
	Linearity (R^2)[b]	% Recovery (RSD) (n=3) 1 ng mL^{-1}	mLOQ (ng mL^{-1})	Linearity (R^2)[c]	% Recovery (RSD) (n=4) [a] 10 ng g^{-1}	100 ng g^{-1}	mLOQ (ng g^{1} dw)
TCPP	0.9966	-	0.096	0.9998	109 (1)	100 (4)	0.97
TPhP	0.9998	-	0.279	0.9967	100 (5)	103 (5)	1.4
TiBP	0.9992	-	0.292	0.9988	109 (6)	98 (6)	1.1
TnBP	0.9996	-	0.229	0.9988	104 (5)	100 (3)	0.58
TBEP	0.9989	-	0.028	0.9970	85 (24)	93 (4)	1.9
TCrP	0.9948	-	0.035	0.9934	101 (7)	115 (6)	1.4
EHDPP	0.9989	85 (9)[d]	0.099[d]	0.9958	85 (12)	98 (8)	2.9

[a] Values of a previous study (Castro et al., 2020). [b] Linear range mLOQ-10 µg L^{-1}, except to EHDPP (mLOQ-1 µg L^{-1})

[c] Linear range mLOQ-950 µg L^{-1}, except to EHDPP (mLOQ-5000 µg L^{-1}), TPhP (mLOQ-9500 µg L^{-1}) and TCrP (mLOQ-50000 µg L^{-1})

[d]Results obtained by liquid-liquid extraction

Table S4. Concentration (µg L^{-1}) of OPFRs measured in seawater with mussels, at the beginning (t_0), after 48 h (t_{48}) (before water renovation) and 24h after water renovation (t'_{24}).

	Measured concentration (µg L^{-1})		
Compound	t_0	t_{48}	t'_{24}
TiBP	0.71	1.66	2.15
TnBP	0.95	0.94	1.30
TCPP	1.51	1.88	2.67
TPhP	1.11	1.02	1.39
EHDPP	0.53	0.29	0.72
TCrP	0.45	0.29	1.10
TBEP	0.73	0.56	0.71

Table S5. Concentrations (ng g^{-1} dw) of OPFRs measured in *M. galloprovincialis* mussel *soft* tissues upon exposure for 28 days to a nominal concentration of 1 µg L^{-1} and further depuration in clean seawater for 14 days.

	Concentration measured in mussel (ng g^{-1} dw)						
Exposure time (Days)	TCPP	TPhP	TiBP	TnBP	TBEP	TCrP	EHDPP
0	26.4	11.2	<mLOQ	<mLOQ	<mLOQ	9.5	43.1
2	101	2401	91.9	213	21.5	8964	862
4	119	3425	104	262	59.2	15021	1258
9	124	4827	131	259	96.0	26148	1861
14	178	3918	167	388	166	22740	2320
28	172	3568	148	274	130	27733	3014
30	55.2	1449	<mLOQ	<mLOQ	<mLOQ	22265	1415
32	23.6	389	<mLOQ	<mLOQ	<mLOQ	14526	383
37	54.1	107	<mLOQ	<mLOQ	<mLOQ	5669	89.3
42	43.3	71.2	<mLOQ	<mLOQ	<mLOQ	5295	77.7

References

Castro, V., Montes, R., Quintana, J. B., Rodil, R., & Cela, R. (2020). Determination of 18 organophosphorus flame retardants/plasticizers in mussel samples by matrix solid-phase dispersion combined to liquid chromatography-tandem mass spectrometry. *Talanta*, *208*, 120470. https://doi.org/10.1016/j.talanta.2019.120470

ChemSpider (2020). Retrieved from http://www.chemspider.com

JChem for Excel, ChemAxon. (2020). Retrieved from https://chemaxon.com

Van der Veen, I., & de Boer, J. (2012). Phosphorus flame retardants: Properties, production, environmental occurrence, toxicity and analysis. In Chemosphere (Vol. 88, Issue 10, pp. 1119–1153). Elsevier Ltd. https://doi.org/10.1016/j.chemosphere.2012.03.067