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Assessment of the contamination by 2,4,6-tribromophenol of marine waters and organisms exposed to chlorination discharges

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Abstract

2,4,6-tribromophenol (TBP) is implied in the production of brominated flame retardants but is also a major chlorination by-product in seawater. A growing number of studies indicate that TBP is highly toxic to the marine biota, but the contribution of anthropogenic sources among natural production is still under question concerning its bioaccumulation in marine organisms. Here, several water sampling campaigns were carried out in the industrialized Gulf of Fos and clearly showed the predominant incidence of industrial chlorination discharges on the TBP levels in water, at the 1-10 ng L⁻¹ level in average and reaching up to 580 ng L⁻¹ near the outlets. The bioaccumulation of TBP was measured in 90 biota samples of the Gulf of Fos. The concentrations found in European conger muscle tissues (140 to 1000 ng g⁻¹ lipid weight, in average), purple sea urchin gonads (830 to 880 ng g⁻¹ lipid weight, in average), and Mediterranean mussel body (1500 to 2000 ng g⁻¹ lipid weight, in average) were above all published references. Significant correlations with fish length (European conger) and gonad somatic index (purple sea urchin) were also identified. Comparatively, fish, urchins and mussels from other Mediterranean sites analyzed within this study showed a lower bioaccumulation level of TBP, consistently with what found elsewhere. Industrial outflows were thus identified as hotspots for TBP in seawater and marine organisms. The environmental risk assessment indicated a high potential toxicity in the industrial Gulf of Fos, in particular near the outlets, and a limited threat to human but toxicological references are lacking.

Keywords

disinfection by-products; seawater; fish ; sea urchin ; mussel ; bioaccumulation

1. Introduction

Among other brominated flame retardants (BFRs), 2,4,6-tribromophenol (TBP) is considered as an emerging contaminant (Bidleman et al., 2019; Michalowicz et al., 2022). TBP can be used in pesticides, as a BFR or through the synthesis of other BFRs and is a degradation product of several BFRs. The industrial production tonnage of TBP appears unclear, for instance, ECHA (2016) indicated Europe registered tonnage above 1000 tons yr⁻¹ in 2013, but below 10 tons yr⁻¹ in 2014, and former worldwide estimations reported 9500 tons yr⁻¹ in 2001 (Michalowicz et al., 2022). TBP was found in all environmental compartments, ambient and indoor air, soil, sediment, marine and freshwater, as well as in their related biota (Koch and Sures, 2018; Bidleman et al., 2019; Jia et al., 2020; Michalowicz et al., 2022). The incidence of TBP produced by human activities is receiving a growing concern, as an increasing number of studies demonstrates its high toxicity to aquatic and terrestrial organisms, and potentially to humans, mainly resulting in neurological, reproductive, developmental effects and endocrine disruption (Deng et al., 2010; ECHA, 2016; Koch and Sures, 2018; Knudsen et al., 2019; Lebaron et al., 2019; Zheng et al., 2022; Zhou et al., 2023).

In the marine environment, TBP is naturally produced by algae (Koch and Sures, 2018; Bidleman et al., 2019), but was also identified as a chlorination by-product (CBP) for years in urban and industrial outlets (Khalanski and Jenner, 2012; Boudjellaba et al., 2016; Zhang et al., 2020). Among urban effluents, industrial chlorination systems are widely employed to prevent biological contamination in desalination plants and ship ballast waters (Agus and Sedlak, 2010; IMO, 2008), and to hinder the biofouling of waterpipes or heat exchangers in power plants, gas terminals, and other industries (Khalanski and Jenner, 2012; Quivet et al., 2022). After the quick conversion of HOCl/OCl⁻ to HOBr/OBr⁻ in seawater containing high levels of bromide, further reactions with organic matter produce various brominated CBPs among which bromophenols such as TBP (Heeb et al., 2014).

Nevertheless, the reporting of TBP measurements in marine waters, sediments and biota in exposed environments is still scarce in the literature. A few works reported TBP seawater levels, around 1.5 µg L⁻¹ (Boudjellaba et al., 2016) and 1.5-2.1 ng g⁻¹ in sediment (Manasfi et al., 2019) in the vicinity of industrial chlorination outlets, and still measured several km away from the source point (Korean nuclear power plant) at levels up to 0.020 µg L⁻¹ in water and 12 ng g⁻¹ in sediment (Sim et al., 2009). Among marine species, TBP was principally measured in fish with average levels from 12 ng g⁻¹ lipid weight (lw) in a remote area (Schlabach et al., 2011) to 1900 ng g⁻¹ lw in the industrialized Gulf of Fos (Boudjellaba et al., 2016) and mussel from 21 ng g⁻¹ lw away from sources (Lôfstrand et al., 2010) to 630 ng g⁻¹ lw in an urban environment (Schlabach et al., 2011). However, assessing the contribution of anthropogenic sources to the TBP levels measured in the marine environment and biota remains generally uncertain (Bidleman et al., 2019). On the other hand, an increasing number of studies reveals the acute and chronic toxicity of TBP to marine organisms, acting as an endocrine disruptor, affecting reproduction and larval growth among other (Schäfer et al., 2009; Li et al., 2009; Folle et al., 2020; Fu et al., 2020; Zhou et al., 2023). It has been also shown that cocktail effects may occur with accompanying CBPs (Lebaron et al., 2019), and that TBP may also be a precursor to other relevant contaminants (Arnoldsson et al., 2012).

Today, chlorination remains the predominant technique in seawater treatment, such as industrial biofouling or desalination (Boudjellaba et al., 2016; Grote et al., 2022), and could even expand in the future due to the guidelines in ship ballast water management favoring chlorination (IMO, 2008). Therefore, a better evaluation of the potential contamination of marine ecosystems by TBP anthropogenic sources appears essential. To our knowledge, the previous works investigating TBP in the marine environment were generally limited to one-shot measurements in water (Sim et al., 2009; Khalanski and Jenner, 2012; Boudjellaba et al., 2016; Manasfi et al., 2019), a single marine species analysis (Wu et al., 2019) or single geographical location (Dahlgren et al., 2016). It was also often a secondary result among other contaminants with limited interpretations and discussion (Dahlberg et al., 2016a; 2016b; Bidleman et al., 2019).

In the present study, we aimed at evaluating TBP concentrations in marine waters, as well as determining TBP in marine species of contrasted biological characteristics and potential exposure routes, to better assess the incidence of chlorination outlets on TBP diffusion in the marine ecosystem on the basis of statistically relevant data. Water was thus collected from the outlets to several km away during 7 campaigns covering all seasons and various meteorological conditions, also completed by the few previous punctual data (Boudjellaba et al., 2016; Manasfi et al., 2019). We also first report the determination of TBP by both spot and passive sampling together in an exposed area. TBP was also determined in three marine organisms of different trophic level and living habitat (Conger conger, *Paracentrotus lividus* and *Mytilus galloprovincialis*), originating from the Gulf of Fos hosting several chlorination discharges, as well as from unexposed locations in the French Mediterranean. The sampling design of biota, including a high number of samples of the three species in the exposed area, enabled to focus on the parameters driving the bioaccumulation of TBP in these marine organisms. These results, combined with a comparison to the concentrations recorded in unexposed places for the same species, permitted to further discuss the potential risks to marine life and human health.

2. Materials and methods

2.1. Study area

The Gulf of Fos is a highly industrialized semi-enclosed bay located in the northwestern Mediterranean coast, subjected to freshwater inputs from the Rhône River and the Berre Lagoon. It hosts the greatest French harbor, where two liquefied natural gas (LNG) terminals, two power plants, petrochemical facilities and a steel production complex, employ chlorination techniques (hypochlorite injection or seawater electrochlorination) to maintain water pipes and heat exchangers effective (Boudjellaba et al., 2016). Typical chlorine operating targets are 1 mg Cl₂ L⁻¹ and are requested to be kept below 0.1 mg Cl₂ L⁻¹ in the outlets. During the studied period, the average cumulative flow of the chlorination discharges (of which data was available), was 39 144 m³ h⁻¹, with monthly averages varying between 15 000 and 70 000 m³ h⁻¹. It should be noted that we were unable to obtain any flow data for two major outlets (steel industry and petrochemical complex), but our observations during the field campaigns indicated that they were permanently active with estimated flows of approximately 20 000 m³ h⁻¹ each. Previous observations also showed that chlorinated waters were released in these outlets (Boudjellaba

et al., 2016; Manasfi et al., 2019). Compared to seawater, the water temperature in the outlets showed differences down to $\Delta T = -6\text{ }^{\circ}\text{C}$ in the LNG regasification releases, and up to $\Delta T = +8\text{ }^{\circ}\text{C}$ in the power plants discharges, and $+5\text{ }^{\circ}\text{C}$ in the steel and petrochemical complex outflows. A seasonal variability was noticed, with reduced outlet flows during summer due to the lower activity of power plants (Supporting Information S1).

2.2. Water sampling and analysis

The collection of water samples was realized in 26 sites, covering the known chlorination discharges ($N = 7$), the Rhône River and the Gulf of Fos. Sampling was carried out at the surface and 7 m deep (or 1 m above the bottom in shallower waters), for each of the 7 boat campaigns (April 2017, October 2017, February 2018, June 2018, November 2018, June 2019, June 2020, Supporting Information S1). A 5 L Niskin PVC bottle was employed to collect water. Bottle blank runs showed no detectable levels of TBP. As fully described by Manasfi et al. (2019), the seawater samples were transferred to 1 L amber glass bottles and ascorbic acid was immediately added. Back to laboratory, within a few hours from sampling, 0.5 g sodium carbonate and 0.5 mL acetic anhydride were added to 50 mL samples to derivatize TBP by acetylation. Samples were then extracted with MTBE and spiked with the internal standard 2,4,6-trichlorophenol (TCP). The organic phase was analyzed by GC/ECD (Clarus 580, Perkin Elmer, USA). Calibration was carried out in standard solution and also by mean of the standard addition method. The identification of TBP was regularly verified by GC/MS runs. The detection limit was 3 ng L⁻¹ of seawater, and the analytical relative standard deviation (RSD) was 1.6% in the range of quantification (10 – 1000 ng L⁻¹). Blank runs were realized between each sample and calibration was verified before, in the middle and after each analysis sequence.

2.3. Qualitative determination of TBP in passive samplers

Two Chemcatcher® (T.E Laboratories Ltd, Ireland) configurations were tested in PTFE holders, the Empore™ C18 receiving disk associated to a low-density polyethylene (LDPE) diffusive membrane, and the Atlantic™ HLB-L receiving disk to a polyethersulphone (PES) diffusive membrane. The PTFE holders were soaked in acetone for 12 h and rinsed with ultrapure water after ultrasonic cleaning. The C18 and HLB receiving phase, and the PES membrane were prepared by soaking for 12 h in methanol, and the LDPE membrane in n-hexane, according to recommendations (Vrana et al., 2006). All were rinsed and stored with ultrapure water. The field deployment of the Chemcatcher® was carried out for 28 and 35 days, around the spot sampling campaigns realized in June 2019 and June 2020, respectively. The devices were screwed on stainless steel disks, and hanged from navigation buoys, at approximately 2 m depth.

After exposure, an internal standard was added (2,4,6-TCP) and the receiving phases were rinsed with ultrapure water and extracted in an ultrasonic bath with 15 mL dichloromethane for 10 min and again with 21 mL of a hexane/acetone mixture (75/25 v/v) for 10 min. The organic phases were dried with sodium sulfate and filtered through 0.22 µm PTFE filters. The analysis was then carried out by GC-MS/MS (Trace GC Ultra coupled to a TSQ Quantum™ Triple Quadrupole, Thermo Scientific). The GC conditions temperature program were the same as for water analysis (Manasfi et al., 2019) and TBP was determined by electronic impact ionization and multiple reaction monitoring (MRM, collision energy 20 eV) with m/z 221.7 and m/z 141.2 as precursor and product ions, respectively. Full MS mass spectra

were also regularly recorded to ensure the identification of TBP. Calibration was carried out similarly to water spot samples analysis, using 2,4,5-TCP as internal standard, and repeated before and after each analysis sequence. Analytical RSD was 1.9% and limit of detection $0.3 \mu\text{g L}^{-1}$ in the extract. The extraction efficiency was measured after stirring for 7 days the devices in a synthetic seawater bath spiked with TBP at $200 \mu\text{g L}^{-1}$, with very satisfying yields for both HLB-L (100.9%) and C18 (79.4%) configurations. Blank tests showed that the prepared Chemcatcher® devices did not release detectable traces of TBP.

As the aim of this preliminary experiment was first qualitative, an approximate sampling rate (RS) value was deduced from the empirical model developed by Vrana et al. (2006) for the C18 configuration, considering $\log K_{OW}(\text{TBP}) = 4.13$ (NCBI, 2021). It resulted in $\text{RS} = 0.182 \text{ L d}^{-1}$, falling in the range of other phenolic compounds, 2,4-dichlorophenol (Nyomi et al., 2010) alkylphenols and bisphenol-A (Chen et al., 2020). Studies using the HLB/PES configuration report RS values in the range $0.010 - 0.100 \text{ L d}^{-1}$ for bisphenol-A and pesticides (Petrie et al., 2016 ; Grodtke et al., 2021), also an intermediate value $\text{RS} = 0.050 \text{ L d}^{-1}$ was arbitrary applied here.

2.4. Marine organisms' samples

The marine organisms were collected throughout the Gulf of Fos, and for comparison purposes, away from industrial chlorination effluents (Figure 1), in the Port-Cros National Park (PC-NP, 100 km east), in the Sète region (80 km west) and in Corsica (STARESO scientific station). A summary of the main physiological parameters of marine biota is provided in Supporting Information S2, and full data in a separate CSV file (Supplementary data). The European conger (Conger Conger) is a benthic and sedentary fish, at the top of the trophic level in the Gulf of Fos. Its characteristics make it a reliable bioindicator for marine pollutants monitoring, including TBP, at fine spatial scales (Dron et al., 2019). The specimens were captured by means of bottom long-lines, measured and weighted, and immediately brought to the laboratory to remove muscle tissues which were stored at -40°C . Purple Sea urchin (*Paracentrotus lividus*) is a well-known model for marine ecotoxicological studies, recently employed to assess the toxicity of chlorination by-products including TBP (Lebaron et al., 2019). They were collected within 50 m from shore, measured and weighted. The Mediterranean mussel (*Mytilus galloprovincialis*) is a filter feeder extensively used for marine biomonitoring purposes (Andral et al., 2011). Mussels were collected on 5 navigation buoys throughout the Gulf of Fos, and within 50 from shore in the other locations, except in Sète (Thau Lagoon), where they were obtained from a local producer just after harvesting. They were immediately placed in 10 L of seawater sampled locally for 24h at room temperature (21°C), measured and weighted. In each site, at least 40 urchins and mussels of the largest dimensions were chosen and split into 2 to 4 sub-samples, equivalent in organism size distributions and number (10 to 20 depending on sites). After test or shell and byssus removal, urchin gonads and mussel soft tissues were weighted again and stored at -40°C .

All biota samples were freeze-dried, ground to fine powder with a ball mill equipped with ZrO_2 beads and capsules, and kept at -40°C . The lipid content was determined following the Folch method (Pérez-Palacios et al., 2008). The C and N elemental composition and the stable isotope ratios $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were calculated by determining in $400 \mu\text{g}$ aliquots packaged in tin microcapsules, with a continuous flow isotope ratio mass spectrometer coupled to an organic elemental analyzer (Thermo Scientific, DELTA V and Flash HT plus).

2.5. Extraction and analysis of TBP in marine organisms

All solvents were of analytical grade and standard stock solutions of TCP and TBP were prepared in dried acetonitrile. TBP was determined from 2 g of tissues spiked with 25 μL TCP solution (10 mg L⁻¹). The extraction method, inspired from Hong et al. (2004) and Ahn et al. (2007), was carried out by 30 min sonication in 50 mL dichloromethane/acetone (50:50, v/v). The extract was filtered (PTFE 0.45 μm) and evaporated under a gentle stream of nitrogen. 5 mL of n-hexane were added and loaded on a Florisil SPE cartridge (1 g/6 mL, Restek, USA, conditioned with 6 mL n-hexane) for further clean-up. The elution was realized with 2 \times 3 mL n-hexane/acetone (75:25, v/v). The solution was evaporated to 0.5 mL and finally diluted to 5 mL in n-hexane. The extraction recovery, calculated from spiked samples, was 101.7% (N = 5).

The sample extracts were analyzed by GC/ECD, equipped with a 30 m \times 0.25 mm \times 1 μm column (Elite-5MS, Perkin Elmer). Helium 5.0 was used as the carrier gas at a flow rate of 1 mL min⁻¹, and nitrogen as

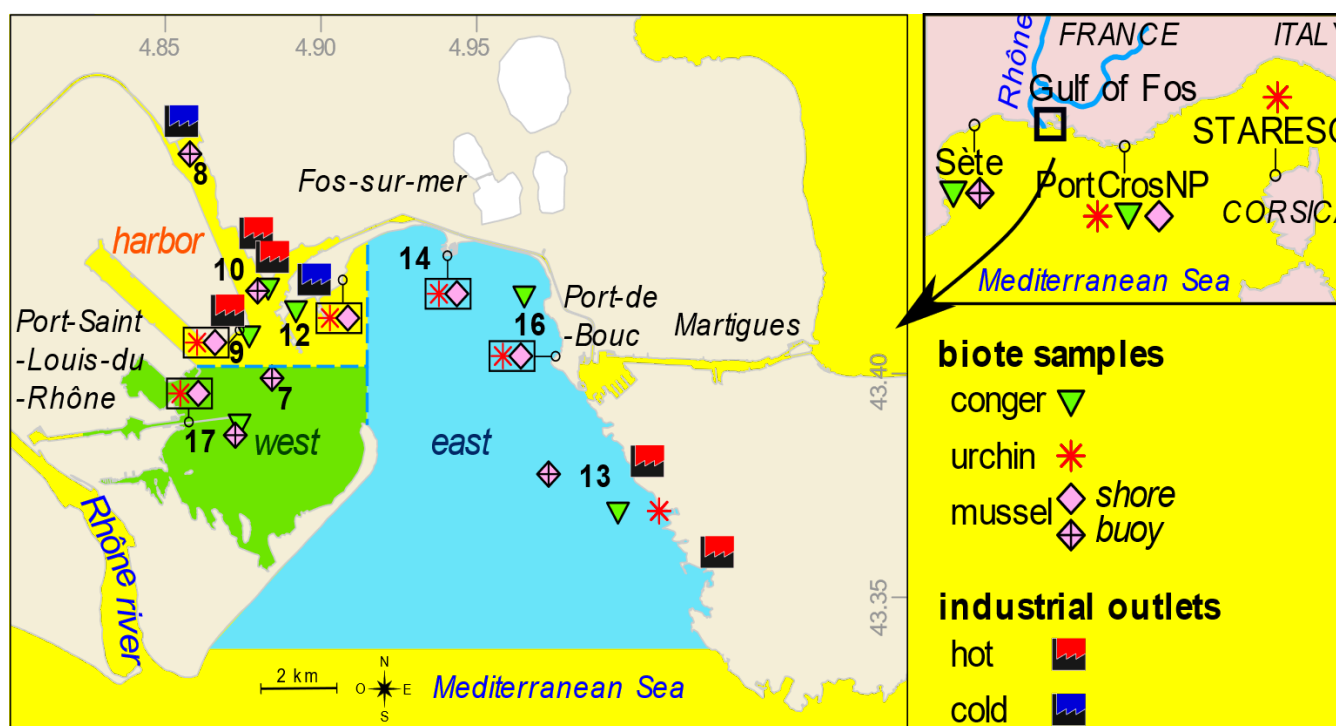


Figure 1. Locations identified by numbers of marine biota harvesting sites of European conger, purple sea urchins, and Mediterranean mussel.

make-up gas (30 mL min⁻¹). The injector temperature was 200°C and the program started at 65°C held for 5 min, increased at 2°C min⁻¹ to 240°C, finally increased at 40°C min⁻¹ to 310°C, held for 10 min. The calibration was defined using 8 concentration levels within a linearity range of 1 to 100 $\mu\text{g L}^{-1}$, resulting in $R^2 > 0.99$ and 0.97 in standard solution and spiked biota extract, respectively. The repeatability of the analytical method (N = 10) was 11% and 17%, respectively. The identification of TBP was regularly ensured through GC/MS runs. The detection and quantification limits (3 and 10 times the blank standard deviation, respectively, N = 10) determined at low concentration level were 0.27 ng g⁻¹ dry weight (dw) and 0.95 ng g⁻¹ dw of biota tissue, respectively. Blank runs were realized between

each sample and calibration was verified by analyzing the whole set of calibration levels, before, in the middle and at the end of each analysis sequence.

2.6. Data analyses

All the statistical analyses and graphics were realized with the R software (R Core Team, 2020) and Inkscape (www.inkscape.org).

As in previous works (Dron et al., 2019), the Gulf of Fos was divided into three areas (harbor, east and west), on the basis of isotope ratios $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ adjusted to fish length in European conger muscle tissues (Figure 1). The geographical discrimination was very comparable to what obtained in European conger fished 5 years earlier (Dron et al., 2019), corroborating the specificity of the 3 areas, in particular among freshwater inputs (Supporting Information S3).

The influence of biological parameters on TBP concentrations in marine organisms was evaluated through multilinear regressions. They were restricted to the Gulf of Fos in order to avoid geographical influences as expected from the very different isotope ratios observed in the other locations studied (Supporting Information S2). Stepwise multilinear regression was applied using the backward elimination of the parameter with the lowest Student t-value to minimize the global p-value, and a new analysis carried out until 3 variables remained. Then, cross-product terms were included. The best models were qualified considering global and individual p-values, after verification that the residuals fulfilled the Shapiro-Wilk normality test.

Due to the limited number of samples in reference zones, Mann-Whitney rank tests (non-parametric Wilcoxon median comparison tests) were realized to evaluate significant differences between locations ($p < 0.05$).

3. Results and discussion

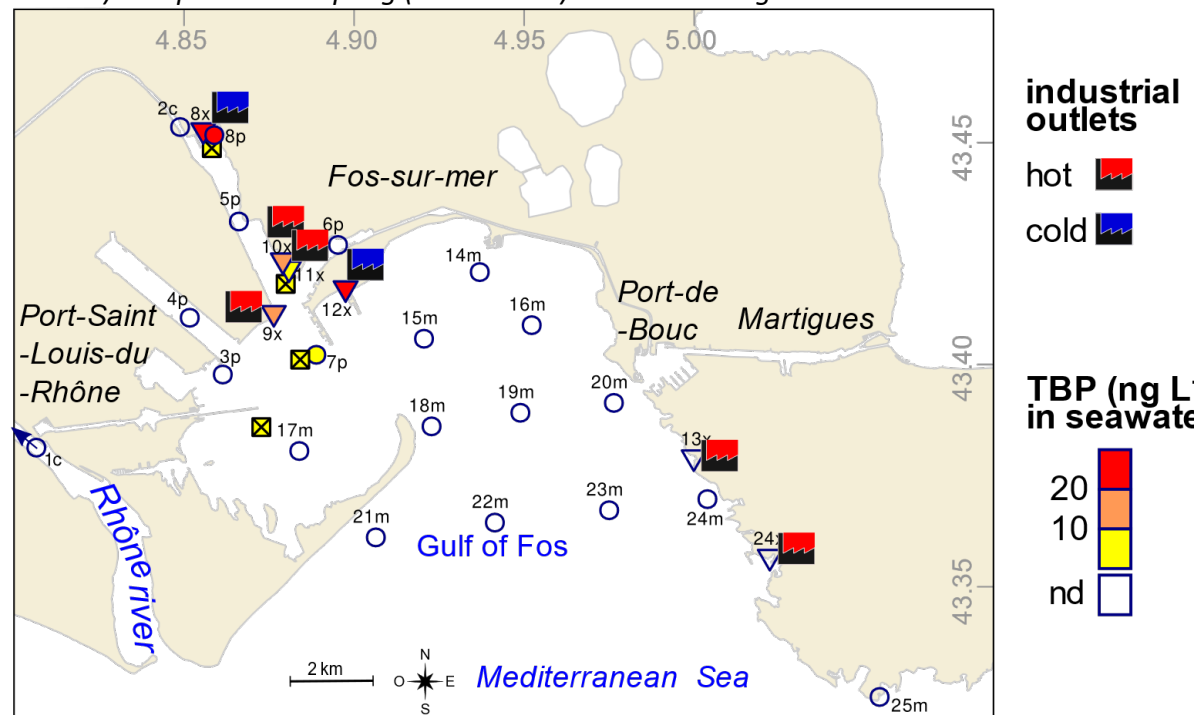
3.1. TBP in industrial outlets and marine waters

Quantifiable levels of TBP were found for each campaign except October 2017 (Supporting Information S4), in at least one of the industrial outlets and two harbor sites nearby (8p and 7p, Figure 2). TBP was detected once in the harbor entrance (site 7p, 41 ng L⁻¹), in the steel complex outlet (site 10x, always active, i.e. visible outflow) and in the power plants releases in site 9x (active in 5 out of 7 campaigns) and in site 11x (active in 6 campaigns). On the other hand, in the LNG outlets, it was found twice in both sites 8x and 8p (active in 3 campaigns), and three times in site 12x (active in 6 campaigns). The TBP levels reached up to 580, 220 and 430 ng L⁻¹ in sites 8x, 8p and 12x, respectively, but only 48, 88 and 140 ng L⁻¹ in the warmed waters of sites 11x, 9x and 10x, respectively. The higher concentrations in the LNG outlets suggested that the cooled chlorinated waters produced through the regasification process could favor the formation of TBP. Unlike Boudjellaba et al. (2016) which reported higher TBP levels during winter but no detectable concentrations during summer, no seasonality was observed here. Comparable levels were found in the chlorinated waters outflows of nuclear power plants (Allonier et al., 1999; Sim et al., 2009) and ship ballast (Delacroix et al., 2013). Away from discharge outlets, the concentrations found in the literature dropped below 5 ng L⁻¹ (Reineke et al., 2006; Sim et al., 2009) or

even below 0.3 ng L^{-1} in remote sites (Vetter et al., 2018), more than one order of magnitude below the concentrations measured punctually in sites 8p and 7p.

As reported in previous works (Boudjellaba et al., 2016; Manasfi et al., 2019), TBP was never detected in the Rhône River (site 1c), thus not representing a significant input to the Gulf of Fos. TBP was neither detected in the sites located offshore (Figure 2). Consequently, it clearly appeared that the local industrial

Figure 2. Locations identified by numbers of water spot sampling (triangles = industrial outlets, circles = other) and passive sampling (cross boxes) colored among TBP mean concentrations.



chlorination discharges were the very predominant source of TBP in the Gulf of Fos, further diluting or degrading below detection limits.

To our knowledge, passive samplers were employed here for the first time in an exposed area to determine TBP in water, and similarly to a previous study in remote ocean (Vetter et al., 2018), the aim of this primary approach was to evaluate the capabilities of the Chemcatcher® device and limited to qualitative measurements. The Chemcatcher® devices were located in the vicinity (150 to 300 m distance) of industrial outlets, by sites 8p, 10x, and 12x, as well as in the more distant site 17m (Figure 2). TBP was detected in all samples extracted from HLB-L membranes, with higher levels found in June 2019 than in June 2020 ($1.0\text{-}1.5$ and $0.2\text{-}0.7 \text{ ng L}^{-1}$, respectively, and considering $R_s = 0.050 \text{ L d}^{-1}$). It was also detected in all samples extracted from C18 membranes in June 2019 ($0.2\text{-}0.4 \text{ ng L}^{-1}$, considering $R_s = 0.182 \text{ L d}^{-1}$), but not in June 2020 (Supporting Information S5). These levels were homogeneous among the deployment sites, corroborating that TBP may diffuse at levels that were not detectable by spot sampling, supporting further development of this technique.

3.2. TBP levels in the marine biota from the Gulf of Fos and relations with biological parameters

TBP was detected in all the marine organisms sampled in the Gulf of Fos industrial bay (full data is provided in Supplementary data), and the mean concentration levels (Table 1) referred to wet weight (ww) were higher in purple sea urchin gonads ($28 \pm 10 \text{ ng g}^{-1} \text{ ww}$), than in Mediterranean mussel soft tissues ($16 \pm 9 \text{ ng g}^{-1} \text{ ww}$) and European conger muscles ($6.2 \pm 3.9 \text{ ng g}^{-1} \text{ ww}$). Their mean variability calculated between sub-samples (each containing 10-20 pooled individuals) were 22.6% and 31.8% for sea urchin gonads and mussels, indicating a satisfying reproducibility of the whole methodology including sampling, sample preparation, extraction steps, and chemical analysis. The RSDs between conger specimens from single sites were slightly higher (36.3% in average) but were determined at the individual level.

As expected from the log Kow(TBP) and previous works (Löfstrand, 2011 ; Bidleman et al., 2019), the concentration of TBP in tissues of marine biota was globally well correlated with lipid content ($R^2 = 0.41$, $p < 0.001$, Figure 3a). Consequently, the TBP levels in the different marine organisms sampled in the Gulf of Fos were tightened when expressed relative to lipid weight, i.e. $840 \text{ ng g}^{-1} \text{ lw}$ in sea urchin gonads, $1800 \text{ ng g}^{-1} \text{ lw}$ in mussel soft tissues, and $820 \text{ ng g}^{-1} \text{ lw}$ in conger muscle (Table 1). Thus, further comparisons and interpretations will be discussed on a lipid weight (lw) basis, except when otherwise indicated. These levels were globally much higher than what reported by other studies in fish and mussel from the Baltic Sea (Table 1) where the highest levels were found in the vicinity of a urban area (Schlabach et al., 2011). Even fewer data were available from other seas but those were also below what measured here in the Gulf of Fos (Chung et al., 2003; Wu et al., 2019). The results obtained in the industrialized Gulf of Fos corroborated the higher levels found by urban centers, and more globally that anthropic pressures result in a higher exposure of the marine environment to TBP.

A multilinear regression restricted to European conger and including available biological data (fish length, weight, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, water content), revealed that the TBP concentrations relative to lipid weight were negatively correlated to fish length ($R^2 = 0.50$, $p < 0.001$, Figure 3b). As fish length can be

assimilated to age (Dron et al. 2019), this implied that younger fish accumulated more TBP than older ones. As no significant correlation was found with stable isotopes $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values ($p > 0.1$), biomagnification did not seem to occur through the food chain, at least for European conger. This is corroborated by the comparable or lower TBP levels measured in European conger compared to purple sea urchin and Mediterranean mussel, respectively, leading to biomagnification factors, $\text{BMF} < 1$ ($\text{BMF} = c_{\text{predator}} / c_{\text{prey}}$), assuming that sea urchin and mussel are potential preys (Lu et al., 2018). European conger feeds principally of small fish, but also cephalopods, crustacean and mollusca (Cau and Manconi, 1984). In European conger from the Gulf of Fos, we observed that $\delta^{15}\text{N}$ values stabilize when total length exceeds 100 cm (Dron et al., 2019), and attributed it to a shift towards settled feeding habits with preys of higher trophic level, or to a physiological change related to a transition from pre-

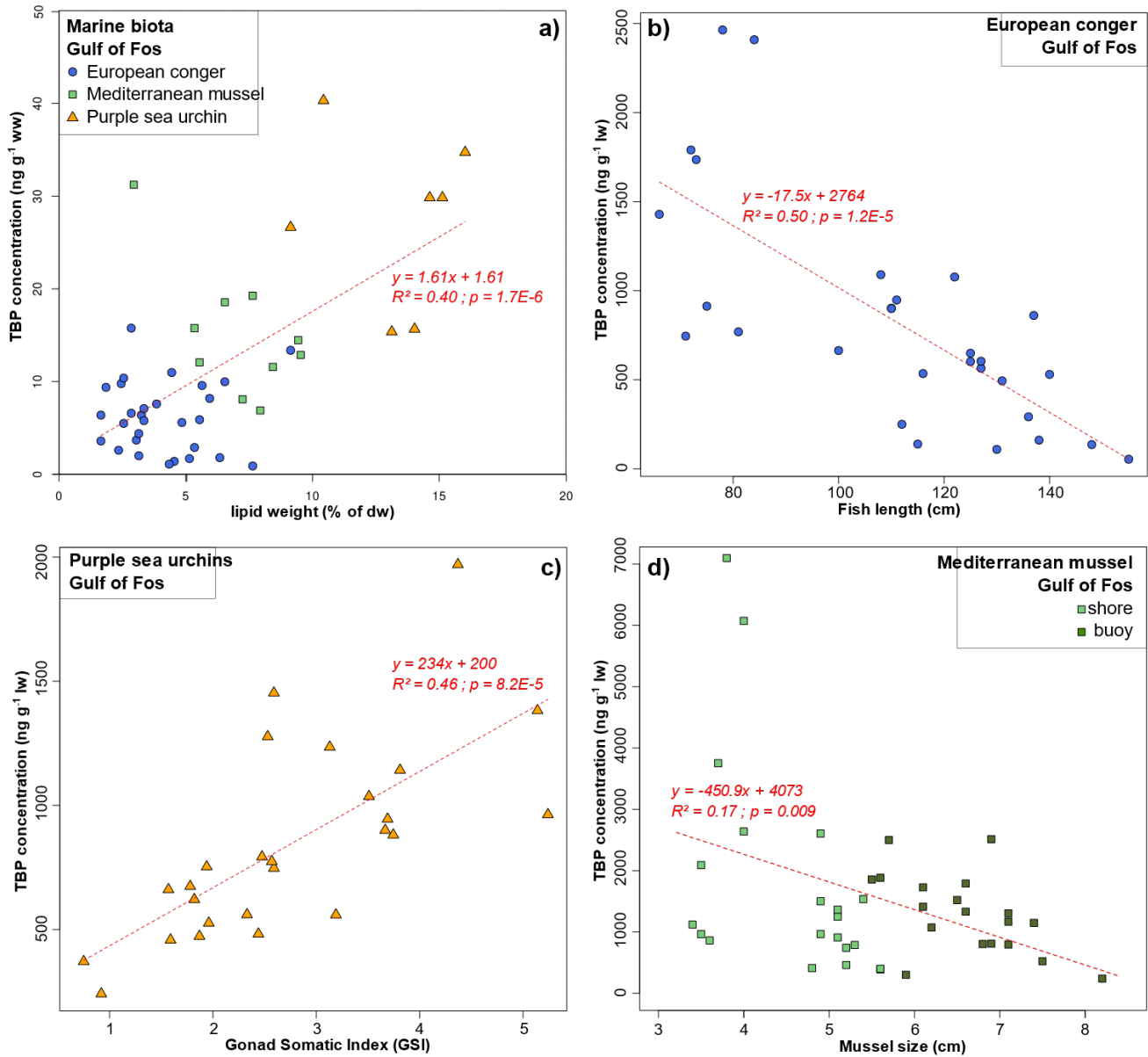


Figure 3. Correlations between a) all species TBP levels ($\text{ng g}^{-1} \text{ww}$) and lipid weight (% of dw), b) European conger TBP levels ($\text{ng g}^{-1} \text{lw}$) and fish length (cm), c) purple sea urchins TBP levels ($\text{ng g}^{-1} \text{lw}$) and gonad somatic index (GSI), and d) Mediterranean mussel TBP levels ($\text{ng g}^{-1} \text{lw}$) and shell size (cm). Urchin and mussel concentrations in a) are pooled, in c) and d) include all sub-samples concentrations.

vitellogenic to early vitellogenic stage. From these observations, the higher accumulation levels of TBP in younger fish suggested the influence of a metabolic change with age, or a biodilution effect due to lower TBP levels in higher trophic level preys. Assuming the preferential accumulation of TBP in fat tissues, it should be also considered that a majority of congers fished were older than three major chlorination installations operating since 2010 only (LNG terminal in 12x and power plants in 9x and 11x). In 2017, 61% were born before 2010 (up to 12 yr. old) and 67% in 2019 (up to 15 yr. old) (Supplementary data). Further monitoring should be restricted by fish of similar exposure and verify this point and determining TBP in a larger number of species of the local food web should precise the incidence of biodilution.

Similarly to European conger, the TBP concentrations relative to lipid weight in Mediterranean mussel showed a negative correlation with the size of the shell, even though less significant (Figure 3d). Mediterranean mussel collected from buoys and shore sites had comparable concentration levels, except from site 20 (East shore), where mussel shell size was below 4 cm while it was above 5 cm in most other sites. As reported for PCBs and organochlorine pesticides (Milun et al., 2020), the accumulation of contaminants decreases in larger in mussel. This was very probably due to the changing metabolism and physiology which is associated to a slower growth rate in larger organisms, generally occurring from a 4-6 cm size for this species (Andrisoa et al., 2019). The slower growth rate is associated to a reduced metabolism, resulting in slower accumulation rates for larger individuals, which may also possibly possess higher elimination capacities, both leading to decreasing TBP levels with shell size.

On the other hand, the TBP concentrations were not correlated with the test diameter in purple sea urchins, but positively correlated with the gonad somatic index (GSI) at a significant level ($R^2 = 0.46$, $p < 0.001$, Figure 3c). This was consistent with the lipophilic character of TBP, as higher GSI and lipid contents are generally associated (Rocha et al., 2019). Similarly, higher nonylphenol levels were measured in spring, also corresponding to higher GSI values, in purple sea urchin from South-East Mediterranean (Amri et al., 2017).

Table 1. TBP concentration ranges (mean) relative to wet (ww) and lipid weight (lw) in marine biota.

Specie	Sampling location and date	TBP (ng g ⁻¹ ww)	TBP (ng g ⁻¹ lw)	Ref.
FISH (muscle)				
Pelagic carnivores	East Australia (1994-95)	nd-0.9 (0.3)		Whitfield (1998)
Benthic carnivores	East Australia (1994-95)	nd-12 (1.5)		Whitfield (1998)
Omnivores	East Australia (1994-95)	nd-4.3 (1.0)		Whitfield (1998)
<i>S. canaliculatus</i>	Hong-Kong (2000)	1.4-11 (5.8)		Chung (2003)
<i>E. areolatus</i>	Hong-Kong (2000)	0.6-6.4 (3.3)		Chung (2003)
<i>G. aculeatus</i> a,b	East Sweden (2013)	nd-27 (6.9)	nd-1200 (290)	Dahlgren (2016)
			c	
<i>P. fluviatilis</i> a,b	East Sweden (2013)	0.3-2.0 (1.3)	6.3-69 (38)	c Dahlgren (2016)
<i>S. alpinus</i> b	Faroe Islands (remote, 2009)	(0.1)	(12)	Schlabach (2011)
<i>P. fluviatilis</i> b	Finland (urban, 2009)	0.1-3.5 (1.1)	8.2-250 (90)	Schlabach (2011)
<i>C. harengus</i> a	East Sweden (2013)	0.2-0.9	3.9-12	Dahlberg

C. conger	FR Med. (industrial, Fos 2012)	nd-10 (4.6)	nd-8100 (1900)	(2016b) Boudjellaba (2016)
C. conger	FR Med. (industrial, Fos 2017)	2.0-16 (7.4)	290-2500 (1000)	This study
C. conger	FR Med. (industrial, Fos 2019)	0.9-2.9 (1.6)	54-250 (140)	This study
C. conger	FR Med. (Sète 2019)	1.0-1.2 (1.1)	65-95 (82)	This study
C. conger	FR Med. (PC-NP 2020)	2.0-4.4 (3.2)	260-610 (460)	This study
BIVALVES (soft tissues)				
Oyster O. rivularis	Hong-Kong (2000)	0.2-1.9 (1.0)		Chung (2003)
Clam T. philippinarum	Hong-Kong (2000)	1.1-23 (9.5)		Chung (2003)
M. edulis	South Sweden (2003-06)	0.1-0.4 (0.3)	7.5-35 (21)	c Lõfstrand (2010)
M. edulis	Norway (urban, 2009)	11-13 (12)	500-770 (630)	Schlabach (2011)
M. edulis	Iceland (remote, 2009)	(3.2)	(460)	Schlabach (2011)
M. edulis	Southeast Sweden (2011-12)	0.4-2.6 (1.0)	17-240 (63)	Dahlberg (2016a)
M. galloprovincialis	FR Med. buoys (industrial, Fos 2017)	11-26 (16)	800-2500 (1500)	This study
M. galloprovincialis	FR Med. shore (industrial, Fos 2017)	6.3-47 (19)	410-7100 (2000)	This study
M. galloprovincialis	FR Med. lagoon (farm, Sète 2020)	6.6-9.9 (8.0)	420-770 (610)	This study
M. galloprovincialis	FR Med. shore (PC-NP 2020)	1.5-3.3 (2.1)	220-510 (340)	This study
SEA URCHIN (gonads)				
P. lividus	FR Med. (industrial, Fos 2017)	9.0-54 (27)	240-200 (830)	This study
P. lividus	FR Med. (industrial, Fos 2019)	27-41 (35)	790-960 (880)	This study
P. lividus	Corsica (remote, STARESO 2019)		(490)	This study
P. lividus	FR Med. (PC-NP 2019)		130-360 (240)	This study
OTHER				
Squid L. reynaudii	South Africa (2017)		1.7-47 (20)	Wu (2019)
Prawns (tails)	East Australia wild (1993-96)	nd-130 (8.8)		Whifield (1997)
Prawns (tails)	East Australia farm (1993-96)	nd-0.2 (0.1)		Whifield (1997)
Gammarus sp.	East Sweden (2013)	9.4-15 (11)	1100-1500 (1300)	Dahlgren (2016)
Alga C. tenuicorne	East Sweden (2013)	0.2-1.2 (0.6)	420-1400 (720)	Dahlgren (2016)
Alga D. foeniculaceus	South Sweden (2003-06)	(0.5)	(180)	c Lõfstrand (2010)
Bacteria N. spumigena	South Sweden (2003-06)	(0.0004)	(0.2)	c Lõfstrand (2010)

a whole fish. b Anadromous fish or potentially mixed fresh/marine influence. C expressed as ng g⁻¹ EOM (extractable organic matter). FR Med. : French Mediterranean

3.3. Impact of chlorination discharges on TBP bioaccumulation

Within the Gulf of Fos, TBP mean levels (ng g⁻¹ lw) showed some significant differences among the between TBP levels and physiological parameters, it was interesting to compare the results adjusted to fish length, mussel size and sea urchin GSI, according to the regression equations obtained previously (Figure 3). Thus, the residuals issued from the linear models applied to TBP concentrations toward these parameters showed no significant geographical difference across the Gulf of Fos for European conger and Mediterranean mussel. Only sea urchins from the west and most enclosed part had significantly higher concentrations (Supporting Information S6).

On the other hand, the marine organisms sampled from other locations, supposed away from major chlorination discharges in the NW Mediterranean (Figure 1), systematically had concentration levels below those found in the Gulf of Fos (Figure 4). Despite the smaller number of samples, the dispersion of the concentrations in these remote areas were also much lower than in the Gulf of Fos. A high heterogeneity is often characteristic of industrial or at least anthropogenic influence and supports here the hypothesis of the impact of industrial chlorination discharges. Finally, adjusting the TBP levels from remote areas based on the regression equations obtained in the Gulf of Fos (Figure 3) resulted in median residuals for conger and mussel below those of the Gulf of Fos (Supporting Information S6). The GSI values were not available in reference sites for sea urchins, and their TBP levels could not be adjusted. Even though the linear models from the Gulf of Fos may not be appropriate in other locations, due to the variability in growth and development rates, at least for mussels (Steffani and Branch, 2003), these calculations are in line with the hypothesis that the vicinity of industrial chlorination discharges induce a greater accumulation of TBP in marine organisms.

To the best of our knowledge, the TBP levels recorded in marine biota collected in the Gulf of Fos were superior to what has been previously reported in the literature, on the contrary to the remote sites studied here (Sète, Corsica, Port-Cros NP) which were globally in the range of most values encountered in other seas (Table 1). Other relatively high levels were reported in blue mussel (*M. edulis*) from an urban area of Norway (Schlabach et al., 2011), as in fish and bivalves from Hong-Kong (Chung et al., 2003), highlighting the potential incidence of urban associated outflows.

Even though international literature remains scarce for TBP in marine biota, the results obtained here indicated a significant influence of chlorination industrial discharges on TBP bioaccumulation in marine species. Further research and data are needed to improve comparisons and interpretations, in contaminated areas, as the data collected here are still insufficient to study TBP pathways from water to bioaccumulation in biota, for instance.

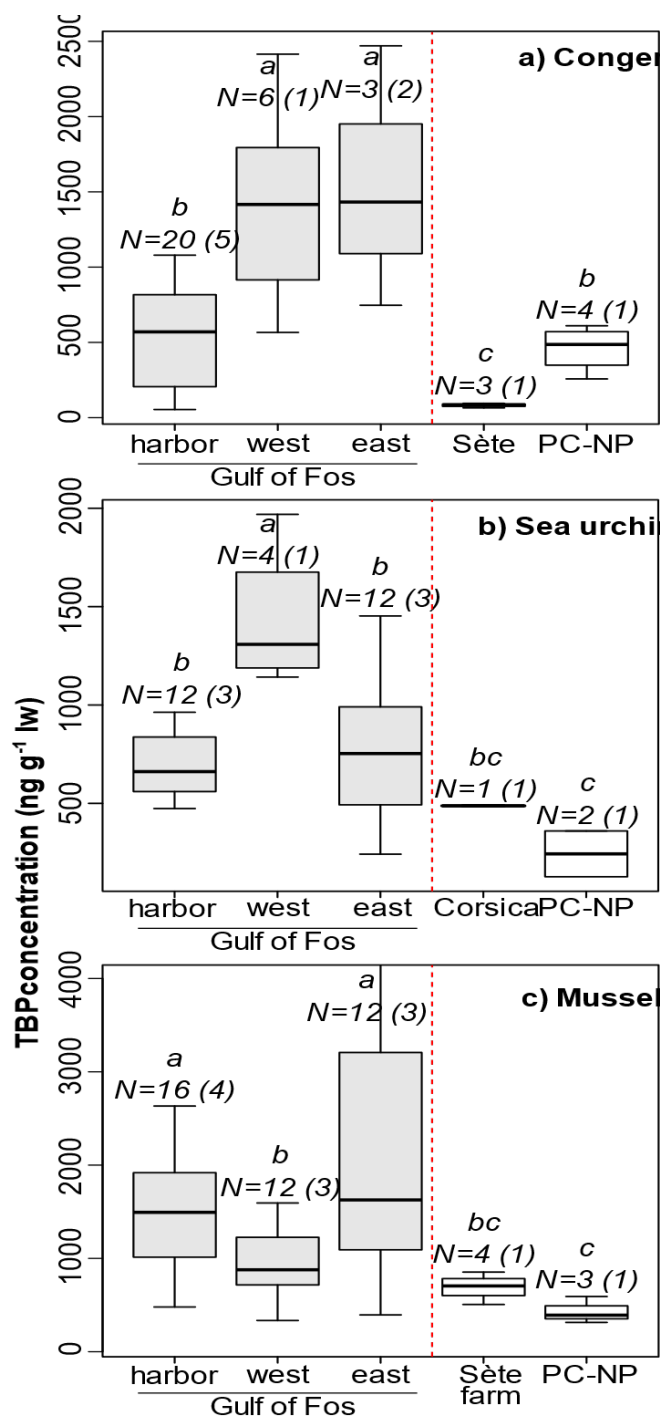


Figure 4. TBP concentration levels (medians, quartiles and 1.5*interquartile ranges) in the defined zones of the Gulf of Fos (grey), and more remote locations along the French Mediterranean (white) for A) European conger, B) sea urchin, and C) mussel.

N is the total number of samples and between brackets the number of harvesting locations. Different letters above boxes indicate significant differences.

3.4. Environmental risk assessment

As the TBP levels in biota were homogeneous across the Gulf of Fos, the risk assessment was evaluated from the average (0.83 ng L⁻¹ considering not-detected = LD/2) and maximum (580 ng L⁻¹) water concentrations over all sampling sites and campaigns (excluding Rhône River and control). A Predicted No-Effect Concentration (PNEC) was calculated using the endpoint values listed in Table 2. According to the ECHA guidance (ECHA, 2008), an assessment factor AF = 10 was applied to the lowest endpoint, resulting in PNEC = 30 ng L⁻¹. This was in accordance with the latest ECHA (2016) estimation (25 ng L⁻¹) and confirms that previous calculations around 500 ng L⁻¹ were overestimated due to lacking toxicological references (Schlabah et al., 2011; Delacroix et al., 2013). The corresponding ratios with the Predicted Environmental Concentration (PEC) and Maximal Concentration (PEC_{max}) in the Gulf of Fos were PEC/PNEC = 0.03 and PEC_{max}/PNEC = 19. Beside a high contrast, directly proportional to the difference between average and maximal water concentrations, it suggested that the organisms living in the Gulf of Fos undergo a significant toxicological risk that may be a threat in the long-term, in particular around to chlorination outlets. Water concentrations above PNEC occurred in marine water samples for each of the 7 campaigns except October 2017. Several studies pointed out that TBP may be toxic at low levels (Table 2), supporting those efforts in TBP monitoring should be achieved to improve ecotoxicological evaluations.

Table 2. Endpoints considered to estimate the PNEC value in seawater.

Organism	Endpoint	Level (ng L ⁻¹)	Ref.
Diatoms Bacciliariophyceae sp.	NOEC, photosynthesis	500 000	Delacroix (2013)
Abalone H. discus hannai larvae	NOEC development 24h	1000	Li (2009)
Sea urchin S. nudus larvae	NOEC development 1h	1000	Agatsuma (2008)
Sea urchin P. miliaris	NOEC, fertility	33 000	Schäfer (2009)
Sea urchin P. lividus larvae	NOEC, development 48h	381 000	Lebaron (2019)
Fish D. rerio	LOEC, reproduction 120d	300	Deng (2010)
Fish R. quelen larvae	LOEC, survival 96h	300	Folle (2020)
Fish D. rerio	LOEC, endocrine disruption	300	Fu (2020)

Considering Gulf of Fos averaged wet weight concentrations in biota (ww) and the mean TBP level in seawater, the calculation of field bioaccumulation factors (BAF) resulted in elevated values, from 11,000 L kg⁻¹ in European conger muscle, to 28,000 L kg⁻¹ in Mediterranean mussel, and 51,000 L kg⁻¹ in purple sea urchins. They are much higher than what calculated in a previous study in the Gulf of Fos (BAF = 25 L kg⁻¹), due to that this earlier study mainly focused on exposed sites with TBP water concentrations 100-1000 times higher (Boudjellaba et al., 2016), as observed here. The TBP field BAF values calculated were also much higher than bioconcentration factors (BCF) resulting from laboratory studies and model results (BCF = 20 to 600) (ECHA, 2016; Khalanski and Jenner, 2012). On the other hand, estimations

based on Arnot and Gobas (2006) equations were closer, with BAF = 2000 to 4700 L kg⁻¹ in fish and 220 to 3000 L kg⁻¹ in invertebrates. As well, a recent study indicated that the BCF increased significantly at lower concentrations, up to BCF = 1260 after a 144h exposure of zebrafish larvae to a water TBP level of 300 ng L⁻¹ (Fu et al., 2020). The results showed that TBP could possibly have an unexpected high bioaccumulation potential in marine organisms at environmental levels. If such values were confirmed, TBP would be classified as very bioaccumulative (Arnot and Gobas, 2006; ECHA, 2017).

Recent studies revealed that TBP induced acute and chronic toxic effects to freshwater adult fish *O. niloticus* after oral exposures down to 5 ng g⁻¹ (Folle et al., 2021) and even 0.5 ng g⁻¹ (Oliveira Ribeiro et al., 2021). In the present study, average levels were much higher in the tissues of marine biota from the Gulf of Fos (Table 1), thus consisting in a significant environmental risk to other organisms, and suggesting that TBP contamination could extend through the whole food chain in the Gulf of Fos. On the other hand, mean levels from reference sites (Sète, Corsica, Port-Cros NP) were below 5 ng g⁻¹ (Table 1). The incidence of TBP exposure on human health is still poorly documented, and a provisional oral reference dose p-RfD = 9 µg kg⁻¹ d⁻¹ was derived from only a single study establishing a sub-chronic exposure p-RfD based on increased serum creatinine in rats (USEPA, 2009). Considering the most contaminated organisms in the Gulf of Fos, this p-RfD value would be reached for a 70 kg adult for the consumption of 39.4 kg d⁻¹ of European conger, or 13.4 kg d⁻¹ of Mediterranean mussel, or 11.7 kg d⁻¹ of purple sea urchin. This indicated that human health should not be affected by TBP contamination of marine biota in the industrial Gulf of Fos. However, some recent studies revealed its strong endocrine disrupting potential (Leonetti et al., 2016 ; Zheng et al., 2022), and cocktail effects should be considered (Lebaron et al., 2019). Therefore, further research is clearly required to refine the human toxicological values for TBP and to evaluate potential impacts to human health.

4. Conclusion

TBP was measured in marine waters, with a decreasing gradient from chlorination outlets to more distant sites, confirming the large contribution of industrial chlorination processes to the TBP levels recorded in the industrialized Gulf of Fos. The development of passive sampling was a promising method to better evaluate the diffusion of TBP. While often below detection limits in spot water analyses, the qualitative data gathered by passive sampling showed that TBP may diffuse at low levels over larger areas than previously expected.

The bioaccumulation was elevated in marine organisms from the Gulf of Fos, significantly higher than in other West Mediterranean sites, supporting the potentially harmful impacts of the overexposure to TBP induced by chlorination discharges. While interesting relations between TBP levels and physiological parameters were highlighted, the exposure and accumulation pathways remained unclear and need to be further explored. TBP appeared as potentially very bioaccumulative and its accumulation in marine biota clearly associated to the chlorination discharges in the industrial bay.

The levels recorded in European conger, purple sea urchin, and Mediterranean mussel of the Gulf of Fos were also consistent with oral toxic effects to some marine organisms, but on the basis of current knowledge, not to humans. However, the evaluation of the toxicity to humans is particularly scarce and should be reevaluated in the light of the potential risk induced by fish consumption where potential TBP sources such as chlorination discharges are expected.

Finally, further cocktail effects induced by other chlorination by-products, but also other organic and metallic pollutants, should be explored to accurately assess the risks induced by industrial discharges to ecosystems and human health in such environments.

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