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Chlorination by-product concentration levels in seawater and fish of an industrialised bay (Gulf of Fos, France) exposed to multiple chlorinated effluents

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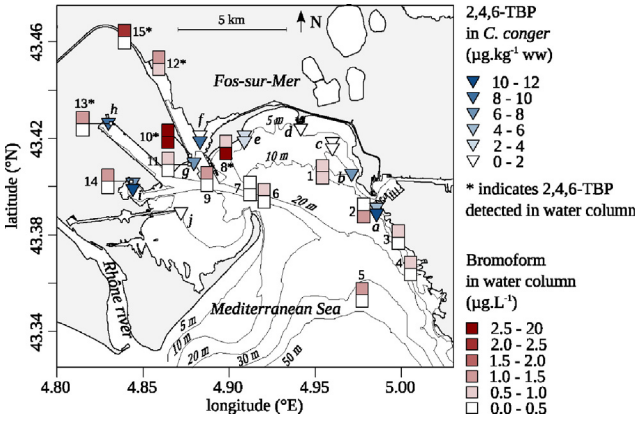
HIGHLIGHTS

- CTD profiles are a prerequisite to CBP analysis to avoid misleading interpretations.
- Different industrial activities lead to different discharge CBP patterns.
- Seasonal effect suggested an impact of temperature and other seawater parameters.
- A widespread contamination is observed, associated to an assessed environmental risk.
- Bioaccumulation levels of 2,4,6-TBP with a bioconcentration factor of 25 in conger eel.

GRAPHICAL ABSTRACT



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ABSTRACT

Chlorination is one of the most widely used techniques for biofouling control in large industrial units, leading to the formation of halogenated chlorination by-products (CBPs). This study was carried out to evaluate the distribution and the dispersion of these compounds within an industrialised bay hosting multiple chlorination discharges issued from various industrial processes. The water column was sampled at the surface and at 7 m depth (or bottom) in 24 stations for the analysis of CBPs, and muscle samples from 15 conger eel (*Conger conger*) were also investigated. Temperature and salinity profiles supported the identification of the chlorination releases, with potentially complex patterns. Chemical analyses showed that bromoform was the most abundant CBP, ranging from 0.5 to 2.2 µg L^{-1} away from outlets (up to 10 km distance), and up to 18.6 µg L^{-1} in a liquefied natural gas (LNG) regasification plume. However, CBP distributions were not homogeneous, halophenols being prominent in a power station outlet and dibromoacetonitrile in more remote stations. A seasonal effect was identified as fewer stations revealed CBPs in summer, probably due to the air and water temperatures increases favouring volatilisation and reactivity. A simple risk assessment of the 11 identified CBPs showed that 7 compounds concentrations were above the potential risk levels to the local marine environment. Finally, conger eel muscles

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presented relatively high levels of 2,4,6-tribromophenol, traducing a generalised impregnation of the Gulf of Fos to CBPs and a global bioconcentration factor of 25 was determined for this compound.

1. Introduction

Biofouling control is essential to industrial installations where large water volumes are used for cooling or heating purposes, and chlorination techniques are widely employed in order to maintain optimal operating conditions.

Chlorine is introduced either through the dissolution of chlorine gas or addition of a sodium hypochlorite solution, typically applying doses of 0.5–1.5 mg L^{-1} (expressed as Cl_2) (Allonier et al., 1999a, 1999b; Ma et al., 2011; Khalanski and Jenner, 2012). Bromine may also be directly produced by seawater electrolysis and in both cases form hypochlorous and hypobromous acid (HOCl and HOBr, respectively) species (Jenner et al., 1997; Taylor, 2006; Khalanski and Jenner, 2012). Once released in seawater, the products of this very quick reaction lead then to haloamines and various CBPs in presence of ammonia and organic matter (natural and anthropogenic). The nature and relative amounts of the CBPs in seawater may vary with the initial chlorine dose, pH, temperature, concentrations and composition of organic matter or inorganic species (Allonier et al., 1999a). The discharge of the chlorinated effluents is of main environmental concern, even if concentrations remain low, as the volumes released are generally very important. Along with residual free chlorine or bromine, the CBPs can constitute a threat to marine ecosystems (Taylor, 2006; Deng et al., 2010; Pignata et al., 2012; Khalanski and Jenner, 2012) and possibly to human health through atmospheric volatilisation and subsequent photolysis of brominated compounds into reactive oxidants (Quack and Wallace, 2003; Deng et al., 2010; Parinet et al., 2012).

In marine environments, much of the research on CBPs has focused on water desalination installations and thermal or nuclear power plants (Taylor, 2006; Agus and Sedlak, 2010; Khalanski and Jenner, 2012). These studies were concerned by cooling water releases, often sought for a limited number of compounds and more importantly by a single discharge point in open coast. Reactivity data of certain CBP classes, such as bromophenols (Sim et al., 2009), are particularly scarce, and rarely cover all the potential marine conditions of salinity, composition or temperature. In the same way, field impregnation data to CBPs are limited to a low number of compounds and species, and toxicological values based on few studies (Taylor, 2006; Khalanski and Jenner, 2012).

Industrialised embayments are found worldwide and the adjacent coasts and bays generally suffer from numerous aqueous discharges in a narrow area, inducing a particular stress on the local marine ecosystems and possibly more distant ones. The Gulf of Fos represents a semi-enclosed bay favouring water confinement in some of its more restricted inlets and docks and receives the plume of the second greatest Mediterranean river among other freshwater inputs, namely Rhône river (Ulses et al., 2005). It hosts the largest port of trade in France and in the Mediterranean Sea along with a major industrial zone mainly centred on steel and petrochemical industries but also waste incineration, cement works and other. Many of them use chlorination for biofouling control, principally for water cooling and LNG regasification purposes. The large volumes of chlorinated waters discharged in this coastal semi-enclosed system (several millions m^3 day^{-1}) can lead to a chronic exposure of the environment to CBPs, as well as a possibly significant atmospheric emission by the volatilisation of the semi-volatile CBPs from the seawater surface.

A better knowledge of the behaviour of CBPs in industrialised embayments is a prerequisite to evaluate their potential impact on the marine ecosystems and their transfer to the atmosphere. It is also essential for modelling and considering solutions with the industrial and local stakeholders. The present study aims to determine CBPs in the Gulf of Fos, taken as a whole with its multiple industrial releases, at a geographical scale which has not been documented in the literature. The measurements include outlet characterisation and distant seawater stations as well as fish bioconcentration. Water sampling was coupled to CTD measurements to identify the outflows and realised in winter and summer seasons to evaluate the influence of these parameters on CBP concentrations, while fish samples were conger eel muscles reflecting several months exposure.

2. Materials and methods

2.1. Study area

The Gulf of Fos is located in the North of the Gulf of Lion (Western Mediterranean), approximately 50 km west from Marseilles. It's flanked by the Berre lagoon to its east and the Rhône river delta to the west (Fig. 1).

The gulf has an average depth of about 20 m. It is characterised by several fresh-water inputs, the largest being the Rhône river (500 to 3500 $m^3 \cdot s^{-1}$) and a smaller being via the Berre lagoon brackish waters (100 to 200 $m^3 \cdot s^{-1}$). Additional fresh-water inputs from irrigation or navigation canals can also have some local incidence, mainly in the Dock 1 and the South Dock (estimated between 10 to 100 $m^3 \cdot s^{-1}$). Several sampling stations have been placed in these fresh water inputs (Fig. 1) to control potential CBP transport from non-local sources to the Gulf of Fos, even though THM were never detected for years in the last station before the Rhône river mouth (Arles, France), neither dissolved, associated to particulate or sediment materials (Eaufrance database, 2015). Tides are very limited in this part of the Mediterranean. The average tidal range is approximately 0.4 m, but it may still rule important water transports such as the exchanges between the Berre lagoon and the Gulf of Fos. Meteorological conditions are dominated by frequent and relatively strong north winds (around 40% per year) that can induce local upwelling phenomena within the gulf and, south-east winds (10 to 20% per year).

The Gulf of Fos undergoes a great anthropic pressure related to the major industrial activities in the area and to a lesser extent to agriculture and urbanisation. The industrial zone of Fos is the largest in Southern Europe. It includes two large liquefied natural gas (LNG) terminals (Fos-Cavaou by sampling station 8 and Fos-Tonkin by station 12) with maximum hourly regasification seawater flows of 30,000 $m^3 \cdot h^{-1}$ (electrochlorination) and 15,000 $m^3 \cdot h^{-1}$ (hypochlorite dosing), respectively. There is also four power plants with very irregular operating levels according to seasonal and economical fluctuations with maximum cooling water flows up to 45,000 $m^3 \cdot h^{-1}$, which is more than the estimated flow of the canal leading from Rhône river to Dock 1 in its northern end (Ulses et al., 2005). They are located by stations 4, 17, 10 and 100 m off station 21. The plant by station 4 does not use chlorination, but the plants by stations 10 and 21 which outlets are directed in Dock 1 employ electrochlorination. In addition, steel industry (main outlet by station 21) and oil refineries (outlets by station 17 and within South Dock) may also chlorinate sea water in volumes exceeding 10 000 $m^3 \cdot h^{-1}$ (Fig. 1).

2.2. Water sampling

Two sampling campaigns were realised, during winter (17 and 18 February 2014, 15 stations) and summer (23 and 24 June 2014, 21 stations). The sampling stations were located within the whole Gulf of Fos and by the major industrial outlets (Fig. 1), in order to evaluate sources

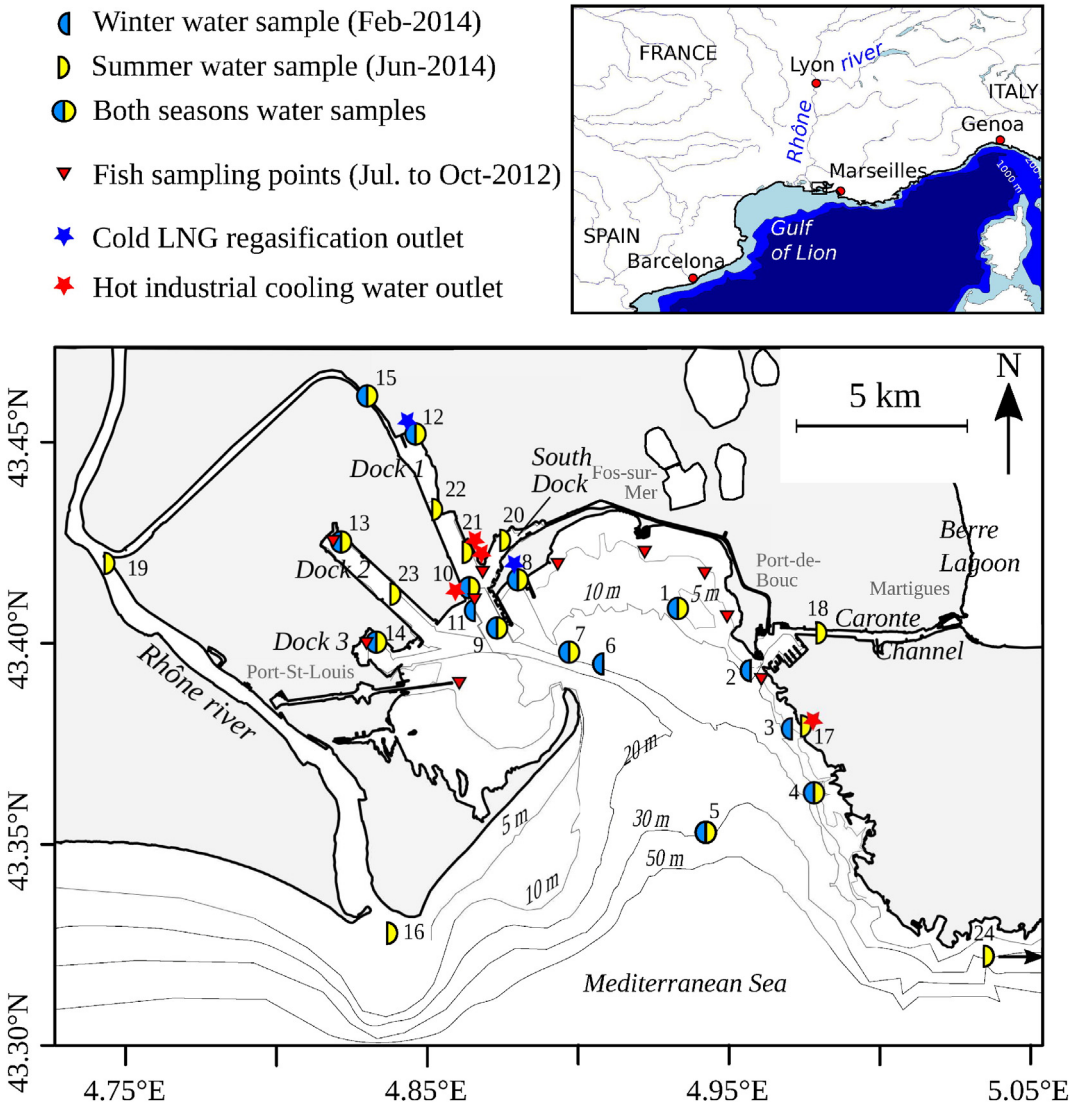

Fig. 1. Overview of the Gulf of Fos and localisation of the water and fish sampling stations, along with the main identified chlorination outlets.

and dispersion of CBPs in the gulf. For each station, two seawater samples were collected, at the surface and 7 m depth or bottom when above 7 m. In the most remote station (station 5), an additional sampling was performed at 20 m depth.

They were collected using a 5 L Niskin bottle (General Oceanics, USA). 1 L was placed in amber glass bottles for further CBPs analysis. Immediately after collection each CBP bottle was acidified with approximately 5 mL of ascorbic acid in order to stop any residual chlorine reaction and was kept at 4 °C in the dark until back to dock. They were then transported to the laboratory and stored at −80 °C until further analysis.

Temperature, pH, and salinity were determined on-site during water sampling using a CTD-type multi-parameter probe (MS5, OTT Hydrolab, Germany), throughout the water column.

2.3. Chemicals

Table 1 lists the CBPs investigated in the seawater samples with their abbreviations. The trihalomethanes (THMs), haloacetonitriles (HANs) and haloacetic acids (HAAs) were purchased from Supelco (USA) and were all above 98% purity except BCAN (95%), DBAN (90%) and CDBAA (95%). The halophenols (HPs) 2B4CP (98%) and 2,6-DBP (99%) were from Alfa Aesar (Germany), 2,4-DBP, (95%) and 2,4,6-TBP (99%) from Sigma-Aldrich (USA). Methyl tert-butyl ether (MTBE) was purchased from Merck, Germany (purity 99.8%).

A standard stock solution of each compound was prepared in MTBE. Intermediate standard solutions were obtained by dilution of the standard stock solution in artificial seawater (ASW) reconstituted according ASTM International standard practise for the preparation of substitute ocean water (method D1141-98, 2013). Table S1 in the Supplementary Material summarises the ASW content.

2.4. Seawater sample preparation

2.4.1. Extraction of THMs, HPs, HANs and HKs

50 mL of seawater were first adjusted to a pH value between 4.5 and 5.5 using a phosphate buffer (10 μM). Subsequently, the sample was extracted by adding 5 mL MTBE containing 20 μL of 1,2,3-trichloropropane (99%, Supelco, USA) as an internal standard. The flask then was sealed, shaken manually for 1 min and allowed to stand for 6 min. Finally, 1 mL of the supernatant organic phase was sampled for chromatographic analysis.

2.4.2. Extraction of HAAs

HAAs in seawater samples were analysed according to the USEPA 552.3 method (USEPA, 2003), as their methyl esters derivatives. 40 mL

Table 1
List of abbreviations used for analysed chlorinated by-products (CBPs).

CBP full name	Abbrev.
Trihalomethanes (*THMs*)	
Chloroform	–
Bromodichloroform[a]	BDCM[a]
Dibromochloroform	DBCM
Bromoform	–
Haloacetonitriles (*HANs*)	
Dichloroacetonitrile[a]	DCAN[a]
Trichloroacetonitrile[a]	TCAN[a]
Bromochloroacetonitrile	BCAN
Bromodichloroacetonitrile[a]	BDCAN[a]
Dibromoacetonitrile	DBAN
Haloketones (*HKs*)	
1,1-Dichloro-2-propanone[a]	DCP[a]
1,1,1-Trichloropropanone[a]	TCP[a]
Haloacetic acids (*HAAs*)	
Chloroacetic acid[a]	MCAA[a]
Dichloroacetic acid[a]	DCAA[a]
Trichloroacetic acid[a]	TCAA[a]
Bromochloroacetic acid[a]	BCAA[a]
Bromodichloroacetic acid[a]	BDCAA[a]
Dibromochloroacetic acid	DBCAA
Bromoacetic acid[a]	MBAA[a]
Dibromoacetic acid	DBAA
Tribromoacetic acid	TBAA
Halophenols (*HPs*)	
2-Bromo-4-chlorophenol	2B4CP
2,4-Dibromophenol	2,4-DBP
2,6-Dibromophenol	2,6-DBP
2,4,6-Tribromophenol	2,4,6-TPB

[a] Never detected compounds.

of seawater sample was acidified with 1.5 mL of concentrated sulfuric acid (H_2SO_4) in an amber glass vial. 20 μl of the internal standard 2,3-dibromopropionic acid (99.9%, Supelco, USA, 10 mg L^{-1} in MTBE). MTBE (4 mL) and sodium sulphate – salting out reagent – (16 g) were added to the solution. The flask was shaken manually during a few minutes and allowed to stand for 5 min. 3 mL of the organic phase were extracted and transferred into a 15 mL vial to which 1 mL of sulphuric acid in methanol were added (esterification step). The vial was shaken and placed in a water bath at 50 °C for 2 h. After cooling and cleaning with 4 mL of saturated sodium bicarbonate, the HAA extract was stored in amber glass vial at 4 °C prior to chromatographic analysis.

2.5. Analytical methods for seawater samples

The analysis of the organic phases containing the CBPs were carried out using a gas chromatograph equipped with an Elite 5MS capillary column and coupled to a ^{63}Ni electron capture detector (GC-ECD model Clarus 580, Perkin Elmer, Norwalk, CT, USA). Carrier and makeup gases were helium 5.0 (1 mL min^{-1}) and nitrogen (30 mL min^{-1}), respectively. The temperature programme for the determination of the THMs, HPs, HANs and HKs was as follows: initially 35 °C increasing to 145 °C at a rate of 10°C min^{-1}, than at a rate of 20 °C min^{-1} up to 225 °C and finally at 10 °C min^{-1} to 260 °C, which temperature was hold for 2 min. For the HAAs, temperature was initially set to 40 °C, then increased to 75 °C at a rate of 15 °C min^{-1}, to 100 °C at 5 °C min^{-1}, and finally up to 135 °C at 10 °C min^{-1} which was hold for 2 min.

Calibrations were performed with 13 levels of concentrations with standard solutions prepared in ASW. The correlation coefficients obtained for THMs, HANs, HPs, HKs and HAAs were above $R^2 = 0.98$, except for 2,4,6-TBP ($R^2 = 0.96$). Blank runs were also realised regularly to ensure the reliability of the analytical methods. The detection limits (LD) and quantification limits (LQ) were estimated using the classical 3σ and 10σ approaches respectively, i.e. calculation of LD and LQ through analysis of the standard deviation of blank measurements (n = 10). Samples were analysed twice and in case of variation exceeding 5%, analysed three times. All the analytical features are presented in Table S2 of the Supplementary Material.

All the seawater analysis results are presented in detail in the Supplementary Material Table S3 (winter campaign) and Table S4 (summer campaign).

2.6. Fish sampling and analysis

European conger eel (*Conger conger*, Linnaeus 1758) samples were collected from July to October 2012 in 11 fishing spots (1 to 2 congers caught in each spot) named *a* to *j* from east to west (Fig. 1). A total of 15 conger eels were caught, with body sizes ranging from 100 to 140 cm and weights from 2000 to 6000 g (see details in Supplementary Material Table S5). Conger eel offers several advantages regarding contaminant bioaccumulation purposes, mainly as being sedentary and at a high trophic level. It is also understood that individuals measuring more than 100 cm are exclusively immature females of at least 5-years old (Flores-Hernandez, 1990; O'Sullivan et al., 2003; Filiz and Bilge, 2004; Correia et al., 2009). Thus, contaminant exposure of the sampled conger eels can be considered as local, although noting that freshwater inputs (Rhône river plume) and dietary inputs (non-sedentary preys) may still bring to them some contaminants originating away from the Gulf of Fos. The bioaccumulation also reflects a time relevant exposure (several years) and the variations due to sex or breeding are avoided.

The muscle tissues were removed directly on dock using sterile single-use scalpels, placed in aluminium foil and frozen for storage at −32 °C. The samples were then sent to the Wessling laboratories (Saint-Quentin-Fallavier, France) for the analysis of THMs (chloroform, bromoform, BDCM and DBCM), HAAs (DCAA, TCAA, DBAA, BCAA, chloroacetic and bromoacetic acids) and HPs (2,4-DBP, 2,4,6-TBP, 2-, 3-, and 4-bromophenols).

They were extracted using a weak alkaline tetramethylammonium hydroxide solution and centrifuged. Cold acetonitrile was added to precipitate proteins, and after a second centrifugation the alkaline solution was degreased with n-hexane. The aqueous extract was used to determine HPs and HAAs. The measurement of HP was adapted from the normalised method EN-12,673, involving acetylation with acetic anhydride and analysis by GC/MS (gas chromatography coupled to mass spectrometry detection). The analysis of HAAs was adapted from the normalised method DIN-38,407-F25, which implies diazomethane alkylation and GC/MS analysis.

The THMs were analysed following the normalised method ISO-16,035 with GC/MS detection.

2.7. Environmental risk assessment (ERA)

A simple environmental risk assessment (ERA) of identified CBPs in the Gulf of Fos was carried out by equating punctual real levels of CBPs found during the present study ("site-specific" concentrations) to predicted environmental concentrations (PEC) and by comparing these PEC with predicted no effect concentrations (PNEC). The PNEC values for the individual CBPs were estimated using available toxicity data and by applying correction factors (AF) according to EU TGD. (2003). The AF considers the reliability of toxicity tests according to the nature and number of data (among other, freshwater or salt water, long-term toxicity tests, multiple trophic levels tests). When toxicity data were not available for a specific brominated compound, toxicity data for the closest chlorinated analogue were chosen. In this latter case, risk assessment will be minimised because it is well known that brominated byproducts are more cytotoxic and genotoxic than their resembling chlorinated compound (Richardson et al., 2007; Escobar-Hoyos et al., 2013).

Statistical analyses were realised using the R software, and all the artwork was elaborated with the R and Inkscape softwares (R Core Team, 2015; Inkscape, 2015).

3. Results and discussion

3.1. General air and sea conditions

Both winter and summer campaigns were carried out under very similar calm sea and wind conditions, following overall southerly wind episodes. Tidal ranges were below 0.2 m and the sampling campaigns were held during ebbing tides. Minimum and maximum air temperatures during sampling were 5 to 15 °C in winter and 18 to 31 °C in summer.

In winter, off shore stations presented slightly lower surface temperatures (12–12.5 °C) than bottom (13.8 °C at 10 m depth) temperatures. On the contrary, summer seawater temperature was higher at the surface (22–23 °C) than deeper (20 °C at 10 m depth, and 18 °C at 25 m depth). Water temperatures in specific depth ranges were homogeneous across the whole study area, and Rhône river freshwaters also being in the same range.

The salinity in the Gulf of Fos waters presented relatively elevated values and was higher in winter than in summer (39.7 and 38.7 at the bottom of the off shore stations, respectively). The surface salinity pattern clearly reveals a common Rhône river freshwaters plume intrusion inside the gulf (Ulses et al., 2005) with values dropping down to 35 in the enclosed part of the gulf (stations 1, 6, 7, 9), but not expanding towards the southern part, as shown for the summer campaign in Fig. 2. This phenomenon is possibly accentuated by the Berre Lagoon inputs. On the other hand, bottom salinities were all above 37, except for the Rhône river and navigation canal stations (stations 19 and 15 salinities are 10 and 35, respectively at the bottom).

Common values are observed for pH across the whole Gulf of Fos, between 8.15 and 8.20 in winter, and between 8.05 and 8.15 in summer. The pH is also homogeneous within the whole water column in all stations, except where waters are mainly constituted of fresh water and present logically values between 7.50 and 8.00 (stations 15, 16, 19).

3.2. Industrial outlets

The nature of the biofouling process may influence the diffusion, the quantity and the chemistry of the CBPs released, also several outlets were investigated here (3 in winter, 5 in summer) presenting different operational characteristics and discharge temperatures. Usual industrial cooling water systems such as thermal power plants release warmer waters than at intake which remains at the surface, as in this example due to a slight ΔT of + 1.0 °C (winter campaign, station 10). In contrast, an electro-chlorination output from a LNG regasification system releases cold waters that drop down to the bottom, as in this case due to a ΔT of − 1.5 °C (winter campaign, station 8) illustrated in Fig. 3a.

The numerous outputs in the Dock 1 of the Fos harbour illustrates here a particularly complex pattern in the mixing of different water layers. The regasification system employed in the LNG plant in the north of Dock 1 (station 12), uses fresh or brackish waters from the navigation canal nearby, to which sodium hypochlorite is added. Due to its weak salinity, the cold output (ΔT = − 2.0 °C) remains at the surface as shown on Fig. 3b. On the other hand, the other major output in Dock 1 at the time of sampling (station 21, south of Dock 1) issues from a steel industry complex which cooling waters are at ΔT = + 4.5 °C compared to the nearby harbour entrance (station 9). As a result, the centre of Dock 1 temperature profile (station 22, approximately 2 km midway from stations 12 and 21) is somewhat difficult to interpret, with a comparable salinity pattern as the LNG outlet station 12 but with much warmer waters at the surface. The cold LNG outlet waters possibly sunk to an intermediate depth, 1 to 3 m depth, with a remaining ΔT = − 1.0 °C in the Dock 1 centre station 22 (Fig. 3), and then back flowed to the northern part of the dock (station 12) explaining its low temperature at the bottom. At the surface of station 22, the low salinity along with calm meteorological conditions suggest supernatant waters flowing down from the navigation canal, the latter also presenting temperatures of 21 to 25 °C from bottom to surface in the upstream station 15. However, particularly complex water movements occur within Dock 1, and CTD profiles are a prerequisite to identify the outflows in the water column, even in the more simple cases as for stations 8 and 10.

Among all investigated CBPs, the chlorination outlets presented detectable levels of the THMs $CHBr_3$ and DBCM, the 4 HPs and the 2 HANs. The 3 HAAs, measured only during the summer campaign, were also all found in the steel industry outlet.

Bromoform is generally found predominantly in outlets discharging in marine environments and represents in the outflows of the present study 95 to 100% of the THMs. Its levels measured here in the outlet flows, identified from temperature and salinity profiles, are in the same range as observed for thermal or nuclear power stations (Khalanski and Jenner, 2012), from 2.5 μg L^{-1} (power plant, station 10) to 18.5 μg L^{-1} (LNG terminal, station 8). Both surface and bottom concentrations are indicated in Table 2. They show little or no difference in the case of warm releases (power plant and steel industry), while a factor 3 to 25 is reached between the bottom and surface waters bromoform levels of cold LNG discharges. On the other hand, the Dock

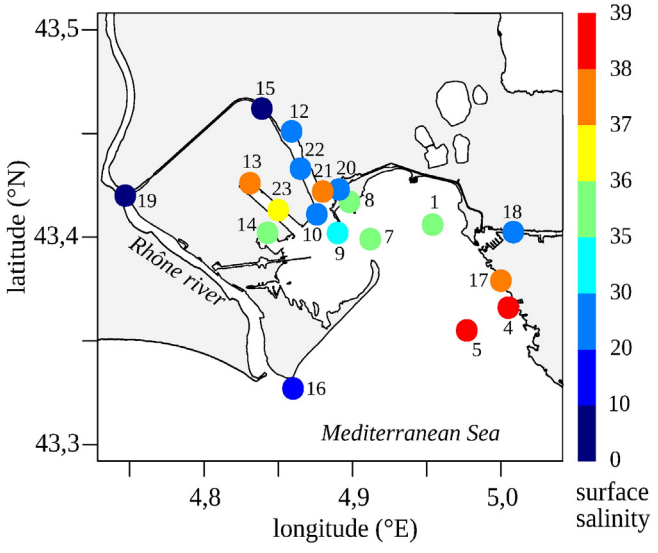

Fig. 2. Surface salinity measured in summer (mean of first 20 cm depth).

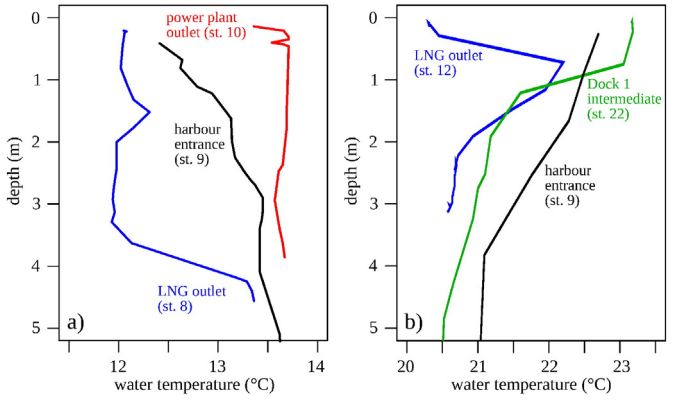

Fig. 3. Water temperature profiles a) in a cold outlet -station 8, LNG regasification circuit- and in a hot outlet -station 10, power plant cooling circuit- compared with the nearby off shore station 9, during the winter campaign, and b) in a cold LNG regasification system outlet using fresh or brackish waters – station 12 – during the summer campaign along with the other Dock 1 stations 9 and 22, during the summer campaign.

Table 2
Bromoform concentrations (μg L^{-1}) in identified industrial outlets, and corresponding outlet flows (m^3 h^{-1}) when known. Deep waters were sampled 3 m depth, except station 8 (4 m) and station 21 (1 m).

# Station. activity	Winter surface/deep	Summer surface/deep	Outlet flows winter|summer
8. LNG	0.76/18.57	nd/nd	9860|0[a]
12. LNG	1.14/0.72	3.27/1.00	0[a]|4300
10. pwr plant	2.51/3.27	1.19/1.17	Yes|no[b]
17. petroch, pwr p.	–	nd/nd	–|yes[b]
21. steel industry	–	7.55/7.33	–|yes[b]

"nd" not detected, "–" not measured.
Underlined concentrations indicate the position of the outflows as identified from CTD profiles.
[a] Units stopped or at minimal rate, involving no chlorination during sampling.
[b] Flows observed or not at outlet (yes or no, respectively), but values not communicated.

1 outlets not operating still presented bromoform levels ranging from 0.7 to 1.2 μg L^{-1} (station 12 in winter, station 10 in summer) due to diffusion from other nearby discharges in Dock 1. It can finally be noted that, surprisingly, no bromoform (nor other CBP) was measured by the outlet of the eastern Lavéra petrochemical complex (station 17). It presented a $\Delta T = +4.5$ °C compared to the nearby marine station 4, possibly indicating a cooling water system outlet, but no information could have been gathered concerning the process used for biofouling control in the corresponding industrial units.

As shown in Fig. 4 other CBPs were in minority in the LNG chlorination outlets, where bromoform represented 80 to 100% among THM + HP + HAN. In the LNG outlet station 8, other contributions were HANs, 2,4,6-TBP and DBCM (9.5%, 6.7%, and 3.5%, respectively). Hot outflows exhibited much higher contributions from HPs representing 28.8% (steel industry outflow) to 69.8% (power plant outflow). In both cases, 2-B-4-CP is the most represented. The HAAs, which benefited the summer campaign, were not detected in the only operating LNG terminal cold outflow (station 12), but concentrations of 2.2 μg L^{-1}, 2.4 μg L^{-1} and 1.2 μg L^{-1} for DBAA, CDBAA and TBAA, respectively, were recorded in the steel industry hot water discharge (station 21). In the latter, the relative contribution of HAAs to CBPs is relatively high, between HPs and THMs. These distributions are relatively comparable with what observed by Allonier et al. (1999a) in nuclear power plants cooling waters, except for HPs which were below 1%. The variability of the CBP contributions shows the complexity of the reactivity involved in chlorination discharges, depending on numerous parameters such as operational conditions, seasonal variations and organic compounds present, and requiring much more sampling and studying to be properly characterised and better understood.

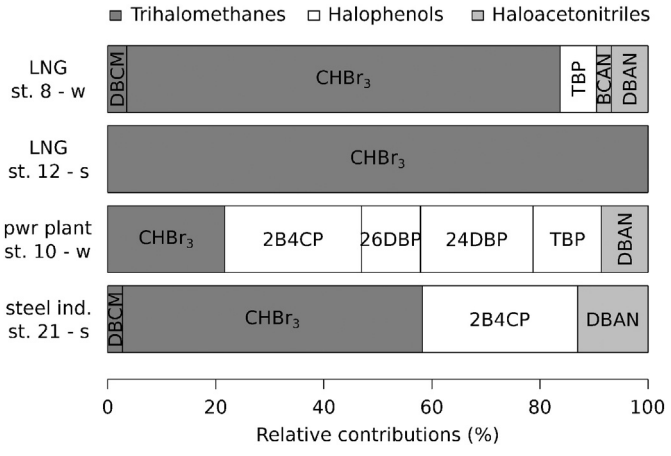

Fig. 4. Relative contributions of CBPs from the THM, HP and HAN classes, in the industrial outflows identified from CTD profiles, i.e. LNG stations 8 and 12 (deep/winter and surface/summer, respectively), power plant station 10 (surface/winter) and steel industry (surface/summer). "s" and "w" correspond to summer and winter campaigns, respectively.

3.3. CBP diffusion in the Gulf of Fos

Various CBPs are released to the Gulf of Fos, originating from numerous outlets which are principally located within Dock 1. Among the latter, the south LNG outlet (station 8) was also identified as a major CBP cold release, and in a lower extent, Dock 2 probably receives chlorination discharges from the several chemical industries that it hosts. On the east shore, the present study did not reveal detectable CBP levels at the investigated stations, but the major petrochemical complex (stations 2, 3 and 17) is still suspected of releasing chlorination effluents. Considering the outlets where the flows were known at sampling time (LNG outlets by stations 8 and 12 in winter and summer, respectively) it represented 183 g h^{-1} and 14 g h^{-1} of bromoform, respectively, discharged into the sea (i.e. 1.7 t $year^{-1}$). As these LNG units were both operating at one third of their capacity and that releases from other industrial activities were not taken into account, these values can be considered as lower bound (Table 2). In addition, it is considered that no dilution occurs in the first metres of the release to the sea between the release and sampling points (less than 5 m for station 8, 200 m for station 12), which is another lower bound hypothesis. Consequently, a rough estimation results in a global production of 5 to 20 t $year^{-1}$ of bromoform within the whole Gulf of Fos, which represents up to 10% of the estimated annual production of all the French coastal power plants (Khalanski and Jenner, 2012).

These multiple CBP discharges lead to an overall contamination of the Gulf of Fos during the winter campaign with bromoform detected in 14 of the 15 sampled stations (Fig. 5), at a level of 0.53 to 1.05 μg L^{-1} in the south and east parts of the gulf (stations 1 to 7), and of 0.95 to 2.20 μg L^{-1} within the harbour but away from outlets (stations 9, 11, 13, 14, 15). Additionally, DBAN was also detected in nearly all stations (except 5 and 6) during the winter campaign at a concentration of 0.9 to 1.0 μg L^{-1}. In winter, several other CBPs were detected away from the chlorination outlets, namely chloroform, DBCM, and 2,4,6-TBP in some confined parts of the harbour (stations 13 and 15). Away from outlets, globally less stations presented detectable levels of CBPs in summer, in particular in the south and east parts of the Gulf of Fos (no CBPs detected in stations 4 and 5). However, bromoform was still measured in most of the harbour stations away from outlets at levels ranging from 0.5 to 1.0 μg L^{-1}. Halophenols were again detected in harbour stations 13 and 15, reaching 0.41 μg L^{-1} (2,4-DBP) and 3.7 μg L^{-1} (2-Br-4-CP + 2,6-DBP + 2,4-DBP), respectively. Finally, HAAs were present in 3 stations (1, 20 and 22), as CDBAA and TBAA. It should be noted that off gulf stations 16 (Rhône river mouth), 19 (Rhône river) and 24 (midway from Marseilles) did not present any detectable level of the investigated CBPs. Thus, it could be considered that no external input brought significant levels of CBPs into the Gulf of Fos during the summer campaign. Detailed results can be found in the Supplementary Material Tables S3 and S4 (winter and summer campaigns, respectively).

Compared to literature data, bromoform concentrations are clearly above background levels. The latter are about 0.025 μg L^{-1}, and rarely exceed 0.1 μg L^{-1} except in extensive beds of macro-algae, which is not the case in the Gulf of Fos (Quack and Wallace, 2003). On the other hand, HAA and HAN levels are comparable to published outlet data and HPs are higher when detected (Allonier et al., 1999b; Taylor, 2006). The results show a global contamination of the Gulf of Fos with detectable CBPs several km away from outlets. The station 5 for example is located 10 km away from outlets and its bromoform concentration of 1.1 μg L^{-1} shows a moderate decrease from outlets. This demonstrates a limited dilution or some accumulation phenomenon, while other works, dealing with open coast releases, reveal a faster decrease of the bromoform and DBAN concentrations (Jenner et al., 1997; Sam, 2001; Khalanski and Jenner, 2012).

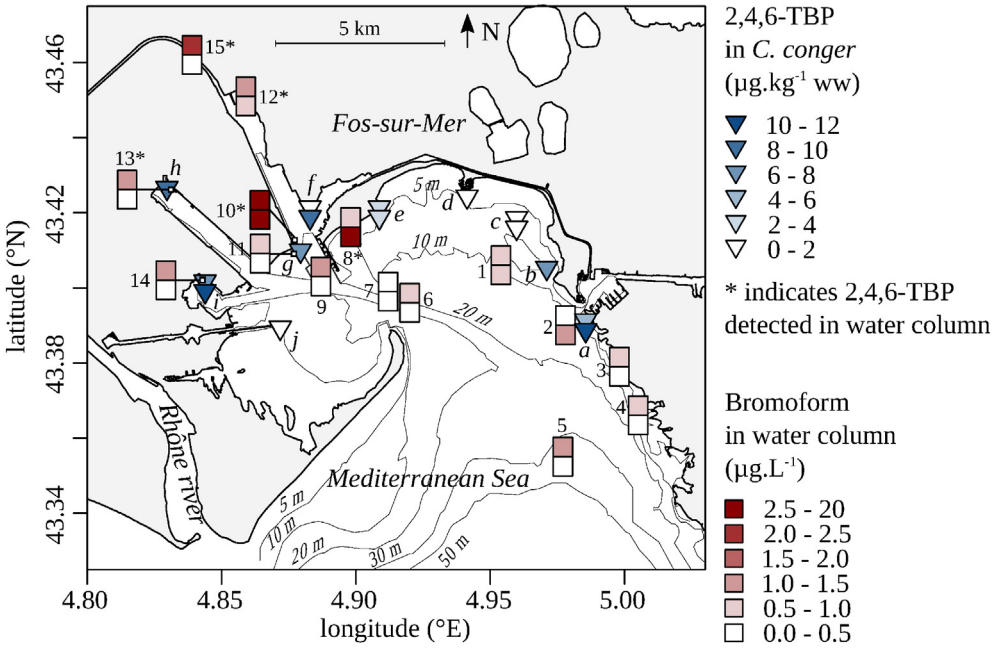

Fig. 5. Distribution of 2,4,6-TBP in conger eel muscle samples (2012 fishing campaign, sampling points *a* to *j*) and of bromoform in the water column samples (winter campaign stations 1 to 15 : top and bottom squares represent surface and 7 m depth or bottom, respectively) within the whole Gulf of Fos. Detectable levels of 2,4,6-TBP in water samples (surface or deep, winter campaign) are indicated by "*" next to the corresponding station number.

Bromoform is almost only distributed at the surface in winter (Fig. 5), when the seawater is colder at the surface, while it is observed only in a few stations in summer. The colder temperature of the surface layer compared to deep waters in winter may act as a thermal barrier to volatilisation. In addition, seawater and air temperatures were approximately twice lower in winter than in summer showing a probable influence of temperature on bromoform volatilisation or reactivity. Bromoform concentrations also drop from outlets (see Table 2) to harbour and gulf waters. On the other hand, DBAN in winter presents comparable concentrations in the outlets (1.0 to 1.6 µg L^{-1}) compared to the other stations (0.9 to 1.0 µg L^{-1}), which is consistent with the longer persistence of DBAN compared to bromoform reported in other studies (Khalanski and Jenner, 2012). During the summer campaign, DBAN could have reacted to form HAAs, justifying the absence of DBAN in harbour and gulf stations but detectable levels of HAAs in a few stations (1, 20, 22).

Except for the chlorination outlets, it appears that stations 13, 14 and 15 present the highest CBP levels (mainly bromoform and HPs), probably due to their confined situation at the end points of Docks 2, 3, and 1, respectively. In winter, 2,4,6-TBP was the only detected HP in these stations, while is was completely absent in summer, replaced by dibromophenols and 2-Br-4-CP as of HPs, suggesting a possible effect of temperature. This is supported by the scarce studies dealing with halophenols reactivity that mention a higher volatilisation rate of trichlorophenols compared to dichlorophenols, but clearly too little is known concerning the reactivity and fate of bromophenols in the environment (Sim et al., 2009; Khalanski and Jenner, 2012).

The results of the simplified environmental risk assessment of the individual CBPs detected in the Gulf of Fos are summarised in Table 3. Seven of the eleven CBP found during this study were found at concentrations that may induce a risk to the aquatic environment of the Gulf of Fos. Indeed, with PEC/PNEC values superior to unity, HAAs, THMs and more specifically HPs (Table 3) present toxicity for aquatic organisms already reported (Taylor, 2006; Tsolaki et al., 2010; Mazik et al., 2013). However, several of these compounds are affected by a high AF indicating that limited toxicity data are available. Also, because of lacking toxicity data on brominated compounds, some PNEC indicated in Table 3 have been taken from their chlorinated analogues. Notwithstanding,

Table 3
PEC/PNEC values and the corresponding literature toxicity and PNEC data for CBPs identified in the Gulf of Fos.

CBP	Toxicity data				AF	PNEC µg L^{-1}	PEC* µg L^{-1}	PEC/PNEC
	Test organism	End point	Conc. mg L^{-1}	ref				
Bromoform	*C. variegatus* (fish)	NOEC, 96 h mortality saltwater	2.9	a	50	58	18.5	0.3
DBAA	*P. promelas* (fish)	LC_{50}, 4 days	69	a	10,000	6.9	3.0	0.4
CDBAA¶	*C. pyrenoidosa* (green algae)	NOEC, 14 day growth freshwater	0.3	a	500	0.6	2.4	4
TBAA¶	*C. pyrenoidosa* (green algae)	NOEC, 14 day growth freshwater	0.3	a	500	0.6	1.2	2
DBAN	*P. promelas* (fish)	EC_{50}	550	a	10,000	55	1.6	0.03
Chloroform	*C. dubia* (crustacea)	NOEC, 10 day mortality	3.4	a	100	34	1.8	0.05
DBCM	*D. magna* (crustacea)	NOEC, 21 days	0.06	a	500	0.13	0.8	6.2
TBP	Bacciliariaphycea (diatoms)	NOEC, photosynthesis	0.5	a	1000	0.5	1.5	3
24DBP¶	*O. mykiss* (fish)	NOEC, 21 day growth saltwater	0.21	b	500	0.42	2.4	5.7
26DBP	*T. marina* (algae CCMP898)	EC_{50}	5	c	10,000	0.5	2.0	4.0
2B4CP	*T. marina* (algae CCMP898)	EC_{50}	4.2	c	10,000	0.42	4.0	9.6

References: a (Delacroix et al., 2013), b (Johnson et al., 2012), c (Liu and Zhang, 2014).
* PEC has been assimilated as the maximum concentration detected in this study.
¶ For brominated compounds lacking toxicity data, the PNEC values were considered as of their closest resembling chlorinated compound.

and because the toxicity of brominated compounds is generally known to be higher than of chlorinated ones (Richardson et al., 2010), these high instantaneous levels should be considered also not only in terms of individual fluxes but also in terms of cocktail effects (Delacroix et al., 2013). Further studies are thus needed to take into account the combined effect of all CBP compounds.

Very few studies have been undertaken to assess the environmental risk linked to the impact of CBPs to the marine environment and most of them compare the levels detected to their Maximum Contaminant Levels set by national environmental protection agencies (Kim et al., 2015). Our results are consistent with those obtained by Delacroix et al. (2013) who had studied the impacts of various chlorination treatments of ballast waters. Among the 22 CBPs identified, four presented a ratio (PEC/PNEC) higher than unity, i.e. TBAA (4.0), DBCM (2.5), MBAA (1.5) and chlorates (1.9). In the present study, we have used the same toxicity data set (PNEC) and higher ratios are found (Table 3). This can be explained by the highest CBP concentrations found and possibly by the post-treatment applied to chlorinated ballast waters (neutralisation of residual chlorine to a maximum of 2.0 mg L^{-1}). This post-treatment, applied in desalination plants and in ships to ballast waters, may serve as an example to reduce formation of unintended release of CBPs within the Gulf of Fos (Werschkun et al., 2014).

3.4. CBPs in conger eel muscle samples

Among the 15 CBPs (4 THMs, 5 HPs and 6 HAAs) investigated in conger eel muscle samples, only 2,4,6-TBP was detected. This is consistent with the bioconcentration factors (BCF) calculated by QSAR (quantitative structure-activity relationship) evaluation, presenting 2,4,6-TBP as the most susceptible to be bioaccumulated among the different CBPs (Khalanski and Jenner, 2012). CBPs, and particularly bromophenols should accumulate much more in fats rather than in muscle tissues (Whitfield et al., 1998; Chung et al., 2003), and Grove et al. (1985) indicated a bioconcentration factor of only 1.4 in edible parts of aquatic organisms.

Detectable levels of 2,4,6-TBP were found in 10 of the 15 fish muscle samples (detailed concentrations in Supplementary Material Table S5), with concentrations ranging from 2.80 to 10.39 μg kg^{-1} wet weight (ww). As can be observed on Fig. 5, they were located within Docks 1, 2 and 3, as well as in the vicinity of stations 8 (LNG outlet), and 2 (petrochemical complex outlet). As conger eels are known to be sedentary in their coastal life (Flores-Hernandez, 1990; O'Sullivan et al., 2003; Correia et al., 2009), this geographic distribution is consistent with a greater exposure of the congers living by the chlorination outlets.

The seawater stations 8, 10 and 13 presented 2,4,6-TBP levels during the winter campaign of 1.56, 1.47 and 1.44 μg L^{-1}, respectively. They were also located by the conger eel stations *e*, *g* and *h*, respectively, for which muscle samples concentrations were 2.92, 7.74 and 8.13, respectively. As 2,4,6-TBP was not detected at each depth nor in summer, bioconcentration factors (BCF) were deduced from these matching stations averaging water concentrations to surface, deep and summer values, with not detected levels considered as 0. The resulting BCF were 8, 21 and 23. In another manner, the Gulf of Fos can be considered as one whole site, and a global BCF calculated. Therefore, 8 seawater stations were considered (1, 2 and 8 to 15), the other being relatively distant from fishing points. Again not detected values were considered as 0 and for conger eel muscle tissues, and the global BCF = 25 for 2,4,6-TBP in conger eel muscle tissues.

Literature data indicate 2,4,6-TBP mean values in fish muscle samples ranging from 2.7 to 4.2 μg kg^{-1} ww in brown-spotted groupers (*Epinephelus areolatus*) and rabbitfish (*Siganus canaliculatus*), respectively (Chung et al., 2003), and from 0.26 to 1.49 μg kg^{-1} ww in pelagic and benthic carnivores, respectively (Whitfield et al., 1998). Cetaceans presented comparable concentrations in blood, ranging from 0.1 to 2.0 μg kg^{-1} ww depending on the considered specie (Nomiyama et al., 2011). The authors propose natural (algae production) as well as anthropogenic sources (flame-retardant and wood fungicide) to 2,4,6-TBP in marine species, but they reveal an incapacity of favouring one or another, especially as 2,4,6-TBP is also highly suspected of resulting from some PBDE metabolism in fish (Chung et al., 2003; Nomiyama et al., 2011).

As the present work reveals a higher mean level in conger eel muscle samples (4.80 μg kg^{-1} ww) and a geographical distribution in accordance with chlorination outlets positions, the latter may be considered as a supplementary source, at least at a local scale in industrialised embayments. It is likely that chlorination water discharges participates at a significant level in the sampled conger eel 2,4,6-TBP concentrations, and demonstrates a continuous exposure of the ecosystem to CBPs. This is a matter of concern since 2,4,6-TBP has a potential toxicity on marine biota and significant adverse effects on fish populations (Deng et al., 2010).

4. Conclusions

Several industrial activities discharge chlorination by-products (CBPs) in the Gulf of Fos. Bromoform levels at the outlets could differ from a factor of 7, LNG regasification being the most elevated at the sampling time in winter (18.6 μg L^{-1}), and steel industry during the summer campaign (7.6 μg L^{-1}). The position of the effluent fluxes in the water column were confirmed by CTD measurements, which revealed to be essential in chlorination impact studies. The relative distributions of CBPs in chlorination outlets were generally dominated by bromoform, but the patterns differed strongly. This variability in concentrations and relative distributions suggest different reactivity, which is worth to be explored with a higher time-resolved monitoring.

A widespread contamination of the Gulf of Fos was identified, with relatively high levels of bromoform (0.6 to 2.2 μg L^{-1}) and DBAN in winter (0.9 to 1.0 μg L^{-1}). Haloacetic acids and halophenols were also measured at high concentrations, in particular when waters are confined in some more restricted inlets and docks of the bay. A seasonal effect was suspected to lower the CBP concentration in summer, through an accelerated transfer to the atmosphere due to warmer air and sea temperatures. The temperature profiles of the water column are likely to influence the volatilisation of CBPs, which may be of human health concern for the surrounding populations. However, significant improvement in the knowledge of water to air transfer of CBPs is required in order to better evaluate the potential risks for human health.

The chronic exposure of the aquatic ecosystem was very likely to cause a significant impregnation of fish in the bay. High 2,4,6-TBP levels were measured in conger eel muscle samples (10 out of 15 samples, 2.8 to 10.4 μg kg^{-1} ww), which cannot be only attributed to natural exposure. The global bioconcentration factor calculated for this compound was 25 in conger eel muscles. The 2,4,6-TBP, among other CBPs, revealed PEC/PNEC ratios above unity indicating potential toxicological issues to aquatic organisms. A better knowledge of detailed CBP fluxes and fate are required to better evaluate their environmental impact, as well as their associated toxicity and effect on populations.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.09.046.

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Supplementary material

“Chlorination by-product concentration levels in seawater and fish of an industrialized bay (Gulf of Fos, France) exposed to multiple chlorinated effluents”

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Table S1.Contents of the artificial seawater (amounts of salts expressed in g L^{-1}) used for the preparation of the analytical standards, according to ASTM International standard practice for the substitute ocean water (method D1141-98, 2013).

NaCl	24.53
$MgCl_2$, 7 H_2O	5.2
Na_2SO_4	4.09
$CaCl_2$	1.16
KCl	0.695
$NaHCO_3$	0.201
H_3BO_3	0.027
$SrCl_2$	0.025
NaF	0.003
KBr	0.101

Table S2. Features of CBP analysis in seawater by GC-ECD : ranges (µg L^{-1}), coefficients of correlation R^2, limits of detection (LD, µg L^{-1}), limits of quantification (LQ, µg L^{-1}), relative standard deviations (RSD, %) and recoveries (%), for THM, HAN, HK and HP.

	THM				HAN					HK		HP			
	CHCl3	BDCM	DBCM	CHBr3	DCAN	TCAN	BCAN	BDCAN	DBAN	DCP	TCP	2B4CP	24DBP	26DBP	TBP
range	2.0-100	2.7-100	0.1-100	0.1-200	0.9-100	1.3-100	0.6-100	1.2-100	0.6-100	1.0-100	1.3-100	0.4-100	0.4-100	0.5-100	0.2-100
R^2	0.998	0.994	0.989	0.998	0.992	0.994	0.992	0.991	0.990	0.992	0.990	0.997	0.995	0.982	0.967
LD	1.30	1.02	0.06	0.11	0.98	1.32	0.60	1.20	0.32	0.90	0.90	0.26	0.22	0.32	0.11
LQ	2.00	2.68	0.09	0.12	0.98	1.35	0.62	1.20	0.60	1.00	1.27	0.36	0.42	0.49	0.19
RSD	5	20	11	17	6	8	4	8	12	12	15	8	8	10	12
Recovery	100	100	100	100	100	60	80	100	100	86	82	84	67	65	58

Table S2 (continued). Features of CBP analysis in seawater by GC-ECD : ranges (µg L^{-1}), R^2, limits of detection (LD, µg L^{-1}), limits of quantification (LQ, µg L^{-1}), relative standard deviations (RSD, %) and recoveries (%), for HAA analysed as their methyl-ester derivatives extracted in MTBE.

	HAA								
	MCAA	DCAA	TCAA	BCAA	BDCAA	DBCAA	MBAA	DBAA	TBAA
range			0.4-50	0.5-50	0.5-20	0.8-50	1.3-100	0.3-50	0.8-10
R^2	0.992	0.995	0.992	0.997	0.983	0.994	0.998	0.997	0.988
LD	1.0	0.7	0.3	0.3	0.5	0.8	1.0	0.2	0.7
LQ	1.2	0.8	0.4	0.5	0.6	0.8	1.3	0.3	0.8
RSD	19	13	3	6	12	7	13	5	20
Recovery	80	100	77	100	25	30	80	86	20

Table S3. CBP concentrations (µg L^{-1}) in seawater samples collected during the winter campaign.

#	depth	CHCl3	DBCM	CHBr3	2B4CP	26DB	24DB	TBP	BCAN	DBAN
1	surface			0.57						0.90
	7 m			0.89						
2	surface			1.05						0.90
	7 m									
3	surface			0.53						0.90
	5.5 m									
4	surface			0.87						0.90
	7 m									
5	surface			1.05						
	7-20 m									
6	surface			0.64						
	7 m									
7	surface									0.91
	7 m									
8	surface			0.76						
	4 m		0.81	18.57				1.56	0.63	1.57
9	surface			1.18					1.73	0.91
	7 m									
10	surface			2.51	2.95	1.26	2.41	1.47		1.00
	3 m		0.79	3.27	2.93					1.24
11	surface			0.95						0.91
	3 m									0.90
12	surface			1.14				1.43		0.90
	3 m			0.72						1.01
13	surface		0.79	1.19				1.44		
	7 m									0.97
14	surface			1.35						
	7 m									0.90
15	surface	1.80						1.54		0.90
	3 m			2.20						0.89

Empty spaces : not detected.
CBPs which were not detected in any station do not figure in the table.
HAAs were not measured during the winter campaign.

Table S4. CBP concentrations (µg L^{-1}) in the seawater samples collected during the summer campaign.

#	depth	DBCM	BDCM	CHBr3	2B4CP	26DB	24DB	DBAN	DBAA	CDBAA	TBAA
1	surface										
	7 m									1.8	
8	surface									1.5	
	4 m										
9	surface			0.89							
	7 m										
10	surface			1.19	3.99	1.98					
	3 m			1.17	2.30	0.60					
12	surface			3.27							
	3 m			1.00							
13	surface										
	7 m						0.41				
14	surface			0.52							
	7 m										
15	surface		1.11		3.68						
	3 m			0.93	2.35	0.53	0.55				
20	surface										
	7 m									4.60	
21	surface	0.37		7.55				1.77	2.20	2.40	1.24
	1 m	0.38		7.33			0.91	1.74	3.00		
22	surface			0.98							0.77
	7 m										

Empty spaces : not detected.
CBPs which were not detected in any station do not figure in the table.
Stations were any CBP was detected do not figure in the table (*i.e.* stations 4, 5, 7, 16, 17, 18, 19, 23, 24)

Table S5. Concentrations of 2,4,6-TBP (µg kg^{-1} wet weight) in conger eel (Conger conger) muscle samples, total length and weight of the individual fishes.

Sample	Total length (cm)	Total weight (kg)	2,4,6-TBP
a1	133	4.55	5.03
a2	126	4.25	10.25
b	135	5.14	7.80
c1	116	3.00	nd
c2	122	3.70	nd
d	120	3.19	nd
e1	137	5.60	2.80
e2	108	2.20	3.03
f1	100	2.40	9.01
f2	108	2.50	nd
g	125	3.50	7.74
h	117	3.30	8.13
i1	133	4.90	8.24
i2	140	5.70	10.39
j	127	3.40	nd

nd : not detected