Occurrence of Synthetic Musks in Marine Shellfish Along French Coasts

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Abstract The geographical distribution of synthetic musk (SM) contamination was investigated in filter-feeding molluscs collected along French coasts (English Channel, Atlantic and Mediterranean). Four SMs (tonalide - AHTN, galaxolide - HHCB, musk xylene – MX and musk ketone – MK) were analysed in samples collected between 2013 and 2015.

Musk patterns were dominated by the polycyclic musks AHTN and HHCB at all sites and in all study years, accounting for more than 90 % of the sum of the four studied musks. Over the 3-year period, median values at all sites were 0.007, 0.017, 0.18 and 0.83 ng/g ww for MX, MK, AHTN and HHCB respectively. Maximum concentrations were found for HHCB (14.1 ng/g ww) and AHTN (2.40 ng/g ww) in the Seine Bay (English Channel). Wide variations in contamination levels were found across the study sites, probably related to domestic activities. The Seine Bay, subjected to major anthropic pressure, was the most heavily-impacted site, while samples from the Atlantic coast exhibited the lowest levels. This study demonstrated the widespread occurrence of polycyclic musks on French coasts.

Keywords: Synthetic musks, shellfish, coastal marine environment

1. Introduction

Synthetic musk compounds (SMs) are extensively used as fragrances or additives in a wide range of consumer goods, such as personal care products (perfumes, soaps, deodorants, shampoo, etc.) and household products (air fresheners, cleaning agents, detergents, etc.). In 2004, four SMs, i.e. two nitromusks (musk ketone - MK and musk xylene - MX) and two polycyclic musks (galaxolide - HHCB and tonalide - AHTN) represented about 95 % of the European market for all nitro- and polycyclic musks, with HHCB accounting for around 79 % (OSPAR, 2008). Due to its high persistence and bioaccumulation capacity, MX has been banned under the European REACH regulation (Registration, Evaluation, Authorization and Restriction of Chemical substances) since 2011 (European Commission, 2011). This compound has also been included in the List of Chemicals for Priority Action of the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR Convention) since 1998.

SMs enter the aquatic environment mainly through effluents from waste water treatment plants and river discharges (Homem *et al*., 2015) and have been reported in coastal waters and sediments (OSPAR, 2004; Sumner *et al*., 2010; Lee *et al*., 2014). Due to their highly-lipophilic character, these substances tend to accumulate in marine biota and are transferred along the food chain to high trophic level organisms (Kannan *et al*., 2005; Rudel *et al*., 2006; Nakata *et al*., 2012; Picot Groz *et al*., 2014; Casatta *et al*., 2015; Cunha *et al*., 2015; Saraiva *et al*., 2016).

To our knowledge, data on SM contamination of the French coastal environment is very scarce. The aims of this study were to investigate SM contamination on French coasts and provide reference data for future monitoring of these substances.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals were carefully-selected to satisfy trace analysis requirements. Picograde® solvents (dichloromethane - DCM, n-hexane, isooctane, methanol, ethylacetate) were purchased from Promochem (Germany). Bio-Bead S-X3 polystyrene gel beads (200–400 Mesh) were supplied by Bio-Rad Laboratories Inc. (USA). Silica gel (100–200 Mesh), aluminium oxide (90 standardized), Florisil (60–200 Mesh) were supplied respectively by Sigma Aldrich (Germany), Merck (Germany) and Fluka (Germany).

All musk standards used to prepare the calibration solutions were purchased from Dr Ehrenstoffer (Germany): 1,3,4,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(□)-2-benzopyran (HHCB, 51 % purity) and 4-aceto-3,5-dimethyl-2,6-dinitrotert-butylbenzene (MK, 98 %) were purchased in powder form; 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene (AHTN) and 2,4,6 -trinitro-1,3-dimethyl-5-tert-butylbenzene (MX) were

purchased as 10 and 100 ng/μL cyclohexane solutions respectively. The internal standard, deuterium labelled-musk xylene (*d15*-MX), was a 100 ng/μL acetone solution. *d10*-Phenanthrene (*d10*-Phe, 98 %) was ordered from Cambridge Isotope Laboratories Inc. (USA).
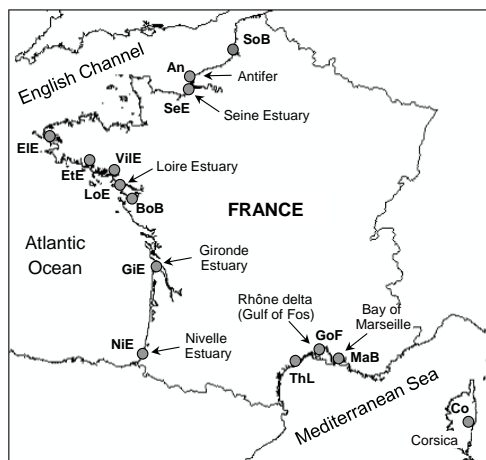

Figure 1. Sampling sites for studied shellfish collected in 2013 - 2015 from the English Channel, Atlantic and Mediterranean coasts

SoB: Somme Bay; An: Antifer; SeE: Seine Estuary; ElE: Elorn Estuary; EtE: Etel Estuary; VilE: Vilaine Estuary; LoE: Loire Estuary; BoB: Bourgneuf Bay; GiE: Gironde Estuary NiE: Nivelle Estuary; ThL: Thau Lagoon; GoF: Gulf of Fos; MaB: Marseille Bay; Co: Corsica

2.2. Sampling strategy

The shellfish used as contamination bioindicators were collected by the French Monitoring Network (Réseau National d'Observation de la Contamination Chimique - ROCCH), operated by Ifremer since 1979. Mussels (*Mytilus edulis* or *Mytilus galloprovincialis*) or oysters (*Crassostrea gigas*) were collected from selected coastal sites in the English Channel, Atlantic and Mediterranean in 2013 and 2014 (Fig. 1). Additional sites were sampled on the Atlantic coast in 2015 and no further samples on the Mediterranean coast.

The shellfish were collected outside the spawning season (from late November to early December) and handled in accordance with international guidelines for contaminant monitoring in biota (OSPAR, 2012). After 24-hr depuration in decanted water from the site, the shellfish were shelled and their flesh (whole body) was homogenized and stored at -20 °C prior to freeze-drying and storage in dry, cool and dark conditions pending further processing.

2.3. Sample preparation and analysis

From 2 to 5 grams of freeze-dried shellfish sample were spiked with internal standard *d15*-MX and extracted by Accelerated Solvent Extraction (ASE, Dionex) using DCM/Hexane (50:50, v:v). The extracts were cleaned on a gel permeation chromatography glass column (460 mm × 26 mm) filled with styrene–divinylbenzene (Bio-beads SX3) and eluted with DCM. Purification was further performed on both florisil and silica/alumina columns

eluted respectively with hexane/ethylacetate (98:2, v:v) and hexane/DCM (60:40, v:v). After concentration in isooctane, the extracts were spiked with *d10*-Phe solution, used as external standard.

SMs were analysed using an Agilent 7890 gas chromatograph coupled to an Agilent 5975 mass spectrometer (GC/MS). Quantification was performed in the selected ion-monitoring mode using electron impact ionization for the two polycyclic musks (HHCB and AHTN) and electron capture negative ionisation mode with methane as the reagent gas for the two nitro musks (MX and MK). The system was fitted with a DB-5 MS capillary column (40 m × 0.18 mm × 0.18 μm) with helium as the carrier gas (0.8 mL/min). Separation was carried out with the following GC oven temperature program: 1 min hold at 110 °C, increase to 180 °C at 10 °C/min, increase to 220 °C at 2 °C/min, increase to 320 °C at 25 °C/min and hold for 10 min. Relative response factors (RRFs) of target analytes relative to internal surrogate standard (*d15*-MX) were used for quantification. *d15*-MX recovery was calculated according to external standard (*d10*-Phe). Quantification (in bold type) and confirmation ions for target compounds were as follows: HHCB, m/z = **243**, 258, 213; AHTN, m/z = **243**, 258, 159; MX, m/z = **267**, 268, 251; MK, m/z = **264**, 265 294.).

2.4. QA/QC

Various quality assurance and quality control procedures were applied for each set of analyses: i) signal to noise ratio > 3 for the lower-intensity ion signal of each target analyte; ii) uncertainty of qualifier to quantifier ratio < 20 %; iii) relative standard deviation of RRF in calibration standards <10 %; iv) recovery of internal surrogate *d15*-MX > 60 %. In-house spiked reference material (0.2 ng/g ww) was included in each series of analyses to check reproducibility. Deviation from reference values ranged from 1 % (MX) to 8 % (MK). Method repeatability (n = 10) ranged from 1 % (MX) to 10 % (HHCB).

Procedural blanks were analysed with each set of 8 to 10 samples. Despite strict analytical procedures and the use of clean laboratories under positive pressure, all SMs were identified in procedural blanks. Concentrations in samples were therefore systematically corrected with regards to procedural blank values. Taking the blanks into account, the calculated limit of quantification (LOQ) mean values were 0.004, 0.007, 0.07 and 0.21 ng/g ww for MX, MK, AHTN, and HHCB respectively. For the calculation of arithmetic and geometric means or median value, a value equal to zero was used for levels below the LOQs.

3. Results and discussion

3.1. Concentrations along French coasts

AHTN and MK were quantified in all samples, while MX and HHCB were quantified in 94 % and 76 % of the 33 analysed samples, respectively. Over the 3-year period, HHCB and AHTN concentrations ranged from < 0.21 ng/g wet weight (ww) to 14.1 ng/g ww (median of 0.83 ng/g ww) and from 0.08 to 2.40 ng/g ww (median of 0.18 ng/g ww), respectively. The nitro-aromatic musks MX and MK

were detected at far lower concentrations (median value of 0.007 and 0.017 ng/g ww respectively). Summed concentrations of the four SMs ranged from 0.09 to 16.9 ng/g ww. Overall, polycyclic musk concentrations were significantly correlated with nitromusk concentrations (r = 0.92, p < 0.05), suggesting similar exposure sources and behaviours of these musks in molluscs.
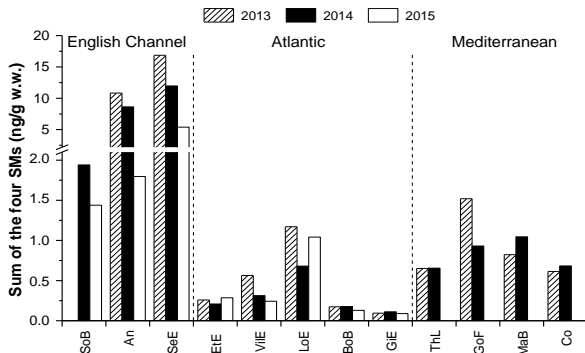

Figure 2. Musk concentration (sum of the four studied musks) in shellfish collected in 2013, 2014 and 2015 along French coasts

The geographical distribution of SM contamination levels in shellfish is shown in Figure 2 (sum of the four SMs) for samples collected along French coasts over the 3 study years. The levels determined for each musk were similar (mean factor of 1.3 ± 0.2) in all years at all sites except the Seine Bay in 2015, suggesting no evolution in musk use in France in recent years.

Wide variations in contamination were observed across the study sites, with the highest levels found on the coast of the English Channel (median value of 7.01 ng/g ww). Concentrations in samples from the Mediterranean coast were of 0.75 ng/g ww (median value), while samples from the Atlantic coast exhibited the lowest contamination levels (median value of 0.26 ng/g ww). However, on the Atlantic coast, contamination levels of around 1 ng/g ww were determined at the Loire estuary (Fig. 2) and at two additional sites (Elorn and La Nivelle estuaries, see Fig.1) studied in 2015 (results not shown).

Various factors may explain contamination geographical distribution: proximity to sewage discharges, population densities, pressure due to tourism, tidal regimes, etc. Indeed, SMs mainly reach surface water through domestic sewage, due to only partial removal during wastewater treatment (Homen *et al*., 2015). Lu *et al*. (2015) also reported a direct correlation between concentrations observed in surface waters and city sizes along the Songhua River in China. Moreover, studies performed on coastal marine environments in the United Kingdom and Korea found the highest contamination levels in samples located adjacent to sewage discharges and determined that contamination distribution in coastal waters was governed by tidal regimes and dilution according to distance from the outfalls (Sumner *et al*., 2010; Lee *et al*., 2014).

3.2. Contamination patterns

Polycyclic musks prevailed over nitro musks at all sites, representing between 86 % and 99 % of total SM

concentrations (Fig. 3). Contamination patterns depend on species bioaccumulation and metabolisation capacity, SM use and the fate of SMs after discharge into the environment. Gatermann *et al*. (2002) reported similar bioaccumulation factors for both HHCB and AHTN in zebra mussels, i.e. slightly higher than that of MK and three times lower than that of MX. Bivalves are also known to have limited biotransformation capabilities. The observed contamination patterns reflect the higher volumes of polycyclic musks versus nitromusks used in Europe.

Nevertheless, differing HHCB / AHTN concentration ratios were observed across sites: HHCB was between < LOQ at 3 sites located on the Atlantic coast (Fig. 3) and 9 times higher than AHTN. Similar observations were made at all sites over the 3-year study period. However, three samples collected on additional sites from the Atlantic coast in 2015 revealed a different contamination pattern, with an HHCB/AHTN ratio lower than 0.5 (results not shown).

Several studies have demonstrated the potential use of HHCB / AHTN concentration ratios as anthropogenic wastewater markers in surface waters. Indeed, high ratios (> 3) were commonly reported in Europe for wastewater water treatment plant effluents (Homen *et al*., 2015) and could thus correspond to surface waters heavily impacted by wastewater discharges. Conversely, HHCB / AHTN ratios close to 1 were reported in freshly-fallen snow samples and meltwater from alpine glaciers (Ferrario *et al*., 2017) and in air samples collected along the North Sea (Xie *et al*., 2007), reflecting contamination due to regional atmospheric transport. In addition to increasing distances from point source, degradation and elimination processes (such as sorption on particles followed by sedimentation, phototransformation, etc.) must be taken into account to explain variations in HHCB / AHTN ratios (Buerge *et al*., 2003; Xie *et al*., 2007). In our study, the highest ratios (> 4) were observed in samples from the English Channel and Mediterranean coasts, suggesting contamination due to domestic sewage discharges.
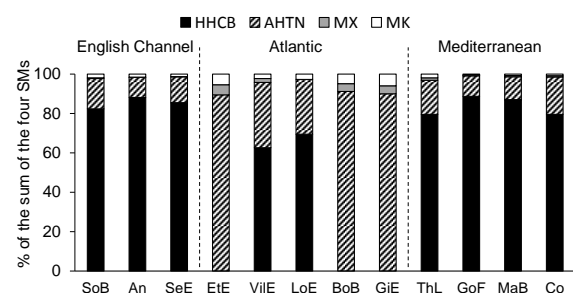

Figure 3. Contamination pattern (% of the sum of the four musks) of the four SMs (HHCB, AHTN, MX and MK) in shellfish collected in 2014 along French coasts

3.3. Comparison to European shellfish contamination

Table 1 shows the concentration ranges determined in our study, together with values reported in the literature for shellfish collected from various European coasts. Comparisons with data from the literature should be considered with caution due to differences in data calculation methods (dw or ww basis calculation), or lack of information on sample preparation. If depuration is not

Table 1. Synthetic musk concentration ranges (minimum - maximum or mean (± standard deviation) in ng/g ww) in shellfish from various coastal locations in Europe (including this study)

Location	Biota type[a]	Sampling year	Concentration (ng/g ww) Min - max; mean (± standard deviation)				References
			HHCB	**AHTN**	**MX**	**MK**	
France							
English Channel	*M*	*2013-2015*	*1.00 - 14.1*	*0.30 - 2.40*	*0.007 - 0.026*	*0.019 - 0.378*	*This study*
Atlantic	*M, O*	*2013-2015*	*< 0.21 - 0.91*	*0.08 - 1.03*	*< 0.004 - 0.015*	*0.003 - 0.056*	*This study*
Mediterranean	*M*	*2013, 2014*	*0.47 - 1.26*	*0.10 - 0.24*	*0.004 - 0.008*	*0.005 - 0.020*	*This study*
Atlantic (La Rochelle)	O	2013	0.634 ± 0.002[b]	0.408 ± 0.002[b]	0.006 ± 0.0002 [b]	0.002 ± 0.0002[b]	Saraiva *et al.*, 2016
Atlantic (Arcachon Bay)	O	2013	< 0.388[b]	0.428 ± 0.008[b]	< 0.002[b]	< 0.0004[b]	Saraiva *et al.*, 2016
	M	2013	< 0.388[b]	0.246 ± 0.006[b]	< 0.002[b]	0.003 ± 0.0002[b]	Saraiva *et al.*, 2016
Other European Countries							
Portugal, Sado	O	2013	0.960 ± 0.002[b]	1.370 ± 0.004[b]	0.008 ± 0.0002[b]	< 0.0004 [b]	Saraiva *et al.*, 2016
Spain, Urdaibai	O	2013	0.530 ± 0.002[b]	0.612 ± 0.006[b]	< 0.002[b]	0.002 ± 0.0002[b]	Saraiva *et al.*, 2016
Italy, North Adriatic Sea	C		< 0.6 - <0.7	4.6 - 11.1	na	na	Casatta *et al.*, 2015
Italy, Po Est.	M	2013	5.87	2.21	nd	nd	Cunha *et al.*, 2015
Spain, Ebro Delta	M	2013	1.21	0.97	nd	nd	Cunha *et al.*, 2015
Portugal, Tagus Est.	M	2013	2.88	1.82	nd	nd	Cunha *et al.*, 2015
South Portugal	M	2011	< 0.1 - 2.2[b]	na	na	< 10[b]	Picot Groz *et al.* 2014
Germany, North Sea	M	1992-2000	0.5 - 1.7	0.4 - 2.5	<0.1 - 0.3	< 0.1 - 0.6	Rüdel *et al.*, 2006
Germany, Baltic Sea	M	1992-2000	< 0.2 - 0.3	< 0.2 - 0.4	< 0.1	< 0.1	Rüdel *et al.*, 2006

[a] M: mussels; O: oysters; C: clams; [b] Recalculated under assumption of a humidity percentage of 80 %

nd: not detected; na: not analysed

performed, ingested particles could contribute to reported quantified concentrations (Booij *et al.*, 2002). Moreover, results are rarely associated with sampling periods, despite the significant influence of shellfish sexual maturity (spawning season and lipid content) on contamination by hydrophobic organic contaminants.

Saraiva *et al.* (2016) recently reported data on the contamination of wild mussels and oysters collected from two Atlantic sites in 2013. The reported levels were in a similar range to those found in our study for samples from the Atlantic coast. In Northern Europe, Rüdel *et al.* (2006) performed a retrospective analysis of mussel samples from the German Environmental Specimen Bank collected between 1986 and 2000. The levels determined for the North Sea, ranging from 0.5 – 1.7 ng/g ww for HHCB and 0.4 – 2.5 ng/g ww for AHTN, were fairly similar to those obtained in most of our samples, while far lower levels were reported for the Baltic Sea. The highest levels found in our study were also similar to those reported for sites located at the mouth of three main European rivers in Italy, Spain and Portugal (Cunha *et al.*, 2015; Saraiva *et al.*, 2016), or Southern Portugal beaches subjected to high recreational and tourism pressures (Picot Groz *et al.*, 2014). Globally, levels determined in the French coastal environment appears to be in a similar range to those reported in other European countries.

4   Conclusion

Our study showed evidence of widespread contamination of the French coastal environment by SMs, with predominant polycyclic musks versus nitro musks. Levels determined in the French coastal environment were in a similar range to those reported in other European countries.

The data obtained in this study are the first reported on a national scale. Globally, contamination levels were of the same order of magnitude from one year to the next. Additional studies are required to further assess the temporal trends of this particular source of coastal contamination.

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