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Exposure of the population of Catalonia (Spain) to musk fragrances through seafood consumption: Risk assessment

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ABSTRACT

The occurrence of ten synthetic musks in samples of 10 widely consumed fish and shellfish species from Tarragona (Catalonia, Spain) was determined. The most used nitro and polycyclic musks, as well as a well-known transformation product in tissues, were analyzed. Furthermore, the human health risks derived from the musk exposure through seafood consumption were characterized. None of the nitro musks were detected in any of the analyzed samples. In contrast, most of the polycyclic musks were found, being galaxolide (HHCB) and tonalide (AHTN) present in all the samples. HHCB was the greatest contributor, with maximum levels in sardine and mackerel (367 and 304 ng g^{-1} (d.w.) (dry weight), respectively). The highest exposure to individual musks was estimated for HHCB and HHCB-Lactone, with average values of 19.7 and 6.8 ng kg^{-1} bw day^{-1}, respectively, in adults. A notably lower mean exposure was calculated for AHTN, cashmeran (DPMI) and traseolide (ATII), being ranged between 1.1 and 3.7 ng kg^{-1} bw day^{-1}. The current concentrations of musks in fish and shellfish should not mean human health risks for the adult population living in Tarragona. However, a continuous monitoring would be desirable to assure that the exposure does not follow increasing temporal trends.

1. Introduction

In recent years, the environmental fate of emerging organic contaminants (EOCs) has been an issue of increasing interest for the scientific community. EOCs may be released to the environment by a number of natural and anthropogenic sources, being potentially toxic to the humans and the ecosystems. Among the broad variety of EOCs, much attention has been paid to synthetic musks. These are cyclic EOCs used not only as fragrances in a wide variety of daily products such as personal care products (e.g., perfumes, skin creams, soaps) and household products (e.g., detergents, softeners), but also as food additives (Bester, 2009; Budd, 2013).

Synthetic musks also comprise a broad range of different compounds which can be divided according to their chemical structure, including nitro, polycyclic, macrocyclic and alicyclic musks. Although nitro musks have been traditionally the predominant compounds in the commercial market, their use has been decreasing due to their bio-accumulative and toxicological properties. These are related to the presence of a nitro-aromatic group in their structures, as well as their high stability against chemical and biological degradation (Homem et al., 2013). In this respect, the European Union banned the use of some nitro musks (musk ambrette, musk moskene and musk tibetene) in cosmetic products, while others (musk xylene and musk ketone) have been restricted (Commission Directive 98/62/EEC; Regulation 1223/2009/EC). Contrasting with the decreasing use of nitro musks from 1990s, polycyclic musks have been increasingly utilised. Galaxolide and tonalide are the two predominant polycyclic musks, being also identified in the high production list of some environmental protection agencies. Although there is no consensus yet regarding the potential endocrine disrupting activity of polycyclic musks, the use of tonalide in the cosmetic industry has been recently regulated by the European Union (Commission Directive 2008/42/EC). Since they smell more intensely and degrade more easily in the environment, macrocyclic musks have emerged as a potentially good alternative to polycyclic compounds. Notwithstanding, their synthesis price is still too high to be considered as a viable option (Bester, 2009; Vallecillos et al., 2013). Finally, the use of alicyclic

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Abbreviations: dSPE, Dispersive solid-phase extraction; d.w., dry weight; EOCs, emerging organic contaminants; GC–IT-MS/MS, gas chromatography-ion trap-tandem mass spectrometry; QuEChERS, Quick, Easy, Cheap, Effective, Rugged and Safe; l.w, lipid weight.; ME, matrix effect; MDLs;, method detection limits; MQLs, method quantification limits; RSD, relative standard deviation; STPs, sewage treatment plants; w.w., wet weight

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musks, as well as the investigations focused on their toxicological properties, is still very scarce. Although alicyclic musks have been suggested to biodegrade (Seyfried et al., 2014), more studies are necessary to confirm it.

The widespread use of synthetic musks in consumer products means that they are flushed down into sewer systems after their usage, entering sewage treatment plants (STPs). Although partial elimination in STPs has been reported for nitro as well as for polycyclic musks, little information is available for macrocyclic musks. Moreover, no data have been published for alicyclic musks (Ligon et al., 2008; Lopez-Nogueroles et al., 2013; Vallecillos et al., 2015a). Moreover, some synthetic musks can be transformed in STPs as well as in biota, being transformation products even more problematic than the parent musks (Rimkus et al., 1999), in terms of potential toxicity. Due to the lipophilic characteristics of synthetic musks and their slow biodegradation, a bioconcentration trend can be expected in surface water (Posada-Ureta et al., 2012), sludge (Vallecillos et al., 2012), sediments (Hu et al., 2011) and living species, such as fish (Subedi et al., 2011; Subedi et al., 2012; Zhang et al., 2013). Although dermal absorption from personal care products applications is reported as one of the major sources of exposure to musk fragrances, other exposure pathways such as dietary intake or inhalation cannot be disregarded (Roosens et al., 2007). Some studies have confirmed a relatively high presence of musks in fatty fish (Kannan et al., 2005). The consumption of seafood in Spain is notorious, contributing to the intake of up to 27% of protein and 14% of polyunsaturated fatty acids among adults (AECOSAN, 2012). Similarly to other environmental contaminants, such as persistent organic pollutants or heavy metals (Domingo et al., 2012a,b; Perelló et al., 2014), diet could be the most important route of exposure to musks. Unfortunately, there is a complete lack of knowledge regarding the dietary intake of musks, in front of other potential exposure pathways.

The European Chemical Agency (ECHA) provided human risk assessment reports for HHCB (galaxolide), AHTN (tonalide), MX (musk xylene) and MK (musk ketone), where respective Oral Non Observed Adverse Effect Levels (NOAEL) of 150, 5, 7.5 and 2.5 mg kg^{-1} bw day^{-1} were established from studies with rats (ECHA 2008a,b). The toxicological information concerning cashmeran, celestolide, phantolide, traseolide or HHCB-Lactone is sparse and non-harmonised by panels of scientific experts. Therefore, threshold values for risk characterization have not been established yet.

The main goal of this study was to determine the occurrence of ten synthetic musks in tissues from ten widely consumed fish and shellfish species in Tarragona region (Spain). The content of the most used nitro and polycyclic musks, as well as one well-known transformation product, was determined. This information was used to assess the dietary exposure of the general population to these fragrances through seafood consumption and to characterize the human health risks.

2. Materials and methods

2.1. Reagents and standards

The six polycyclic musks studied, supplied by Promochem Iberia (Barcelona, Spain), were the following: 6,7-dihydro-1,1,2,3,3-pentamethyl-4(5H)-indanone (DPMI, cashmeran), 4-acetyl-1,1-dimethyl-6-tert-butyllindane (ADBI, celestolide), 6-acetyl-1,1,2,3,3,5-hexamethyllindane (AHMI, phantolide), 5-acetyl-1,1,2,6-tetramethyl-3-isopropylindane (ATII, traseolide), 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-[g]-2-benzopyran (HHCB, galaxolide), and 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphtalene (AHTN, tonalide). Two nitro musk fragrances, 2,4,6-trinitro-1,3-dimethyl-5-tert-butylbenzene (MX, musk xylene) and 1,1,3,3,5-pentamethyl-4,6-dinitroindane (MM, musk moskene), were purchased as 100 μg mL^{-1} individual solutions in acetonitrile from Sigma-Aldrich (Steinheim, Germany) and Riedel de Haën (Seelze, Germany), respectively. The musk fragance 4-aceto-3,5-dimethyl-2,6-dinitro-tertbutylbenzene (MK, musk ketone) was provided by Fluka (Buchs, Switzerland). International Flavors & Fragrances Inc. (Barcelona, Spain) supplied 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-[g]-2-benzopyran-1-one (HHCB-Lactone, galaxolidone), while the internal standard d15-musk xylene (d15-MX) came as a 100 μg mL^{-1} solution in acetone from Symta (Madrid, Spain).

Individual standard solutions of the synthetic musks were prepared in acetone at concentrations of 4000 μg mL^{-1}, for polycyclic musks, and 1000 μg mL^{-1}, for musk ketone and HHCB-Lactone. A working mixture solution of 100 μg mL^{-1} was prepared in methanol, containing all of the compounds except for MX, MM, and d15-MX, as well as HHCB-Lactone. An individual working solution of HHCB-Lactone was prepared in methanol at the same concentration (100 μg mL^{-1}). Acetone and methanol were GC grade with purity $>99.9\%$ from Prolabo (VWR, Llinars del Vallès, Barcelona, Spain) and from SDS (Peypin, France), respectively. Ultrapure water was obtained using an ultrapure water purification system from Veolia Water (Sant Cugat del Vallès, Barcelona, Spain). Helium gas with a purity of 99.999% was used for the chromatographic analysis (Carburos Metálicos, Tarragona, Spain).

2.2. Sampling and sample pre-treatment

Seafood species were selected among those most widely consumed by the Catalan population (AECOSAN, 2012). Samples of hake (*Merluccius merluccius*), tuna (*Thunnus Thynnus*), sole (*Solea solea*), sardine (*Sardina pilchardus*), codfish (*Gadus morhua*), salmon (*Salmo salar*), mackerel (*Scomber scombrus*), squid (*Loligo vulgaris*), shrimp (*Aristeus antennatus*) and mussel (*Mytilus galloprovincialis*) were purchased from three different commercial establishments (supermarket, local market and fish store) in Tarragona, Spain. This practice ensured a different origin of the samples. After collection, the samples were immediately preserved in a refrigerator box. Lateral fillets were then dissected from the fish, and the shells of mussels and shrimps were taken off. Subsequently, they were homogenized and stored in a freezer until analysis. Frozen homogenized samples were lyophilized using the freeze-drying system (Labconco, Kansas City, MO, USA) and crushed using a mortar and pestle. In addition, mussel samples were also sieved through a 125 μm screen to homogenize the diameter of the particles. Then, a composite sample for each species was obtained by mixing equal amounts from the three commercial establishments.

2.3. Analytical method

For sample analysis, synthetic musks were first extracted by QuEChERS (Quick, Easy, Effective, Rugged and Safe) extraction, and then determined by gas chromatography-ion trap-tandem mass spectrometry (GC–IT-MS/MS).

For QuEChERS extraction, 0.5 g (d.w.) of freeze-dried composite sample was weighed into 50 mL centrifuge tubes from Scharlab (Barcelona, Spain). Ten mL of ultrapure water was added to the tube, being shaken for 1 min. Then, 10 mL of acetonitrile (Prolabo) was added and followed by an extraction salt packet (Scharlab) according the Standard Method EN 15662 (Lehotay et al., 2010). This contained 4 g of magnesium sulfate, 1 g of sodium chloride, 0.5 g of sodium citrate dibasic sesquihydrate and 1 g of sodium citrate tribasic dihydrate. Then, the mixture was vortexed for 3 min and centrifuged for 5 min at 7000 rpm (Hettich Universal