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Persistent toxic substances in Mediterranean aquatic species

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HIGHLIGHTS

- PTS concentration levels in Mediterranean fish and fishery products were studied
- Data were collected on many contaminants in wild and farmed species of commercial interest
- Most samples were compliant with EU regulatory limits except for high predators.
- Mediterranean Sea areas with fish at background contamination levels were identified.
- The study outcome was evaluated in the light of the Marine Strategy Framework Directive.

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ABSTRACT

Fish and fishery products may represent one of the main sources of dietary exposure to persistent toxic substances (PTSs) such as polychlorinated dibenzodioxins, dibenzofurans, and biphenyls; polybromodiphenyl ethers; organochlorine pesticides; perfluorooctanoic acid and perfluorooctane sulfonate; and inorganic mercury and methyl mercury. In this study, PTS contamination of Mediterranean fish and crustaceans caught in Italian coastal waters was investigated in order to increase the representativeness of the occurrence database for wild species. The objectives were to verify the suitability of regulatory limits for PTSs, identify background concentrations values, if any, and examine the possible sources of variability when assessing the chemical body burdens of aquatic species. Twelve wild species of commercial interest and two farmed fish species were chosen. Excluding methyl mercury, chemical concentrations found in wild species fell generally towards the low ends of the concentration ranges found in Europe according to EFSA database and were quite lower than the tolerable maximum levels established in the European Union; farmed fish always showed contamination levels quite lower than those detected in wild species. The data obtained for wild species seemed to confirm the absence of local sources of contamination in the chosen sampling areas; however, species contamination could exceed regulatory levels even in the absence of specific local sources of contamination as a result of the position in the food web and natural variability in species' lifestyle. A species-specific approach to the management of contamination in aquatic organisms is therefore suggested as an alternative to a general approach based only on contaminant body burden. A chemical-specific analysis performed according to organism position in the food chain strengthened the need to develop this approach.

1. Introduction

Fish and fishery products may represent one of the main sources of dietary exposure to the following persistent toxic substances (PTSs): polychlorinated dibenzodioxins (PCDDs), dibenzofurans (PCDFs)

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(together also known as "dioxins"), and biphenyls (dioxin-like: DL-PCBs; non-dioxin-like: NDL-PCBs); polybromodiphenyl ethers (PBDEs); perfluorooctanoic acid and perfluorooctane sulfonate (PFOA, PFOS); organochlorine pesticides (OCPs) such as aldrin, *cis*- and *trans*-chlordane, 2,4′- and 4,4′-dichlorodiphenyldichloroethane (2,4′-DDD and 2,4′-DDD), 2,4′- and 4,4′-dichlorodiphenyldichloroethylene (2,4′-DDE and 4,4′-DDE), 2,4′- and 4,4′-dichlorodiphenyltrichloroethane (2,4′-DDT and 4,4′-DDT), dieldrin, endrin, heptachlor, *cis*- and *trans*-heptachlor epoxide, hexachlorobenzene (HCB), α-hexachorocyclohexane (α-HCH), β-HCH, and γ-HCH (lindane). The Mediterranean area is also considered to be a critical environment for coastal populations due to a potential overexposure to inorganic mercury (Hg) and methyl mercury (MeHg) when diets rich in fish are consumed (INRAN, 2012; UNEP, 2002).

Food safety and management measures in the European Union (EU) received much attention in recent years. On the basis of dioxin (2,3,7,8-T_4CDD) toxicity equivalency factors (TEFs) adopted by the World Health Organization (WHO) in 2005 (Van den Berg et al., 2006), Regulation (EU) 1259/2011 updated the maximum tolerable levels (MLs) in dioxin toxicity equivalents (TEQ units) for PCDDs + PCDFs and PCDDs + PCDFs + DL-PCBs (TEQ_{TOT}) in food, including fish and alike products. The Regulation also established *de novo* MLs for the sum of the six "indicator" NDL-PCBs 28, 52, 101, 138, 153, and 180 (Σ_6NDL-PCBs). Recommendation 2013/711/EU updated action levels (ALs) for PCDDs + PCDFs and DL-PCBs in food; in the case of fish and fishery products, an AL is available for the farmed produce. Regulation (EC) 1881/2006 provides MLs for Hg (expressed as total Hg, Hg_{TOT}) in fish and fishery products. No EU MLs concerning the same food are available for the remaining organohalogenated compounds of interest in this study (aldrin, chlordane, DDD, DDE, DDT, dieldrin, endrin, heptachlor, heptachlor epoxides, HCB, HCH, PBDEs, PFOA, and PFOS), although certain Member States have their own regulatory limits.

In early 2004, the Italian Ministry for Education, University, and Research financed a 5-year study (2004–2008) to characterize the chemical contamination and nutritional quality of fish and crustaceans caught in Italian coastal waters. The objectives of the study were (a) to increase the representativeness of chemical occurrence database for wild as well as farmed species collected in the same sampling areas so as to allow a statistically significant comparison of contaminant levels, (b) to compare these levels with regulatory limits and possibly provide suggestions for risk reduction strategies, (c) to explore the variability of experimental material in relation to possible influencing factors, and (d) to identify geo-referenced background values and their association with the Mediterranean fishing areas classified by the United Nations Food and Agriculture Organization (FAO). This last objective addressed the scope of the Marine Strategy Framework Directive (MSFD) (Directive 2008/56/EC), setting a framework within which EU Member States shall take the necessary measures to achieve or maintain good environmental status in the marine environment by the year 2020 at the latest.

2. Materials and methods

2.1. Sample collection and preparation

In order to characterize the contamination of Mediterranean aquatic species caught in relevant fishery zones presumably unexposed to direct contamination sources, the following areas were selected (Fig. 1): Southern Adriatic Sea (longitude 17° 20′ 42 E, latitude 41° 00′ 52 N, 10–40 miles off the coastline before Monopoli port); Ionian Sea (longitude 14° 54′ 625 E, latitude 36° 23′ 135 N, 15 miles off Portopalo port); and southern Tyrrhenian Sea (longitude 15° 46′ 16 E, Latitude 38° 17′ 54 N, 15 miles off the coastline, before Bagnara Calabra). These areas are all part of FAO Zone 37 (www.fao.org) and correspond to 18, 19, and 10 geographical sub-areas, respectively. The selection of sampling areas was critical as one of the objectives of the study was to collect aquatic species in the most relevant fishing areas in terms of catches (over 50% of the 17 kton of fish caught each year are from southern Italy) (IREPA, 2012). These fishing areas are less affected by anthropogenic pressures such as densely populated coastal towns and industrial settlements than those in the northern Adriatic and Tyrrhenian basins.

The sampling design covered many of the fish and crustacean species available on the Italian market (ISMEA, 2007). Twelve wild aquatic species, including two crustacean species and two farmed fish species, were chosen for this study (Table 1). The samples from Adriatic, Ionian, and Tyrrhenian seas are identified with the acronyms MO, PP, and BC, respectively (cf. Supplementary Material (SM), Table SM-1). In general, the same species were collected from the aforesaid areas with only few differences. Farmed gilthead sea bream and sea bass specimens were provided by COOP Italia consumer cooperative from offshore farming plants relatively close to the areas where wild species were caught. Specimens were obtained during 2007–2008. Wild fish and crustaceans were caught using bottom trawls, bottom trammel nets, and seine nets placed on the surface and at mid-water.

Sampled fish and crustaceans were stored in tanks with sea water ice; they were transported to the laboratory, where specimens were measured and their weight recorded. Fish specimens heavier than around 100 g, were skinned, gutted, beheaded, and filleted: fillet amounts of equal weight from individuals of the same species (and from the same site) were pooled together to obtain a composite sample. For smaller fish species (e.g., anchovy) and crustaceans, composite samples were made by using the edible portions of individuals of the same species after removal of the head, tail, and visceral package. Each composite sample was homogenized in a stainless steel jacketed cutter/mixer (Stephan UMC5) and aliquots were stored at −20 °C until analysis.

2.2. Chemical analysis

The quantification of PCDDs, PCDFs, and non-*ortho* DL-PCBs (congeners 77, 81, 126, and 169) was performed by adapting US EPA Methods 1613-B (1994) and 1668-B (2008), using high-resolution gas chromatography coupled with high-resolution mass spectrometry (HRGC-HRMS). High-resolution gas chromatography coupled with low-resolution mass spectrometry (HRGC-LRMS) was used for the following chemicals: mono-*ortho* DL-PCBs (congeners 105, 114, 118, 123, 156, 157, 167, and 189); 30 NDL-PCBs (congeners 18, 28, 31, 33, 49, 52, 66, 70, 74, 91, 95, 99, 101, 110, 128, 138, 141, 146, 149, 151, 153, 170, 174, 177, 180, 183, 187, 194, 196, and 203; cumulative analytical concentration, Σ_{30}NDL-PCBs); 13 PBDEs (congeners 28, 47, 49, 71, 85, 99, 100, 153, 154, 183, 197, 206, and 209; cumulative analytical concentration, Σ_{13}PBDEs). The analytical procedure described by Pacini et al. (2013) for the determination of PCDDs, PCDFs, and PCBs in fish was suitably modified for PBDEs. In line with European regulatory requirements, the cumulative concentrations of PCDDs, PCDFs, and DL-PCBs are expressed as upper bound (UB) dioxin (2,3,7,8-T_4CDD) toxicity equivalents (TEQ units). In addition, the parameter Σ_7PBDEs is reported providing the sum of the seven priority compounds identified by the European Food Safety Authority (EFSA, 2011): congeners 28, 47, 99, 100, 153, 154, and 183. Limits of quantification (LOQs) were normally better than 0.1 pg/g fw for PCDD, PCDF, and non-*ortho* DL-PCB congeners, and 0.01 ng/g fw for mono-*ortho* DL-PCB, NDL-PCB, and PBDE congeners.

The aforementioned eighteen OCPs were determined by an in-house validated HRGC-MS/MS method operating in the selective reaction monitoring (SRM) mode. LOQs were generally better than 0.5 ng/g fw for each OCP.

PFOA and PFOS were analyzed by liquid chromatography (LC) coupled *via* an electrospray interface (ESI) operating in the negative ion mode with a triple quadrupole mass spectrometer (MS) as described by Paiano et al. (2012). The limit of detection (LOD) was *ca.*