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Total mercury content in commercial swordfish (*Xiphias gladius*) from different FAO fishing areas

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HIGHLIGHTS

- Mercury analysis of muscular tissue of swordfish was performed.
- Samples from different FAO fishing areas were analysed and compared.
- Hg exposure risk should be considered for some groups of consumers like children.

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ABSTRACT

Mercury (Hg) is a global pollutant that affect human and ecosystem health. It is transferred through trophic level and bio magnification in the food chain. In this study, total Hg was measured in the muscular tissue of samples of swordfish (*Xiphias gladius*) from different FAO fishing areas and imported in Italy between 2014 and 2017. Total mercury concentrations of muscular tissues were determined using cold vapour atomic absorption spectrometry. In order to assess the health risk associated with human consumption of this fish, the Hg intake values were calculated and compared with those of provisional tolerable daily intake (PTDI) (0.57 μg/kg b.w.) as fixed by the Food and Agriculture Organization/World Health Organization (FAO/WHO). The estimated PTDI (provisional tolerable daily intake) were lower for adults (0.40 μg/kg b.w./day) but not for children (0.97 μg/kg b.w./day), and therefore is considered to pose an alert for children with the present fish consumption volume.

1. Introduction

The interest about the effects of urban and industrial pollution of marine ecosystems is increasingly high. Inorganic compounds from natural and anthropogenic sources continuously enter the aquatic environment where they pose a serious threat because of their toxicity, long time persistence, bioaccumulation and biomagnification along the food chain (Jarup, 2003; Gray, 2002). Trace elements, indeed, can reach high concentrations in marine organisms, similarly to the persistent organic pollutants (POPs) (Maisano et al., 2016). In particular, aquatic species take up trace metals through different organs and they can accumulate, whether essential or not, with the potential to cause toxic effects. The accumulation depends up on the intake and elimination from their body (Karadede et al., 2004) therefore tissue concentrations of trace elements show huge variability between metals and different species (Rainbow, 2007). Accumulated metals can be bio-available or stored in detoxified form and may or may not be excreted depending on the accumulation pattern. Some studies have revealed that metal concentrations in muscle are normally lower than levels in liver (Gašparík et al., 2017), even if muscle tissue of fish can be considered one of the most indicative biomarker for the estimation of trace metals pollution in water systems (Barak and Mason, 1990; Rashed, 2001) and, at the same time, it is of great importance for evaluation of risk for fish consumers.

Mercury (Hg) is a global environmental pollutant that has been in the past the cause of some outbreaks; its toxic effects have been well described in the literature (Morais et al., 2012; Hailemariam and Bolger, 2014; Okpala et al., 2017).

From both natural (erosion of the sediments) and anthropogenic sources (urban discharges, agricultural materials, mining and

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combustion and industrial discharges), mercury is released in to the atmosphere and the sea where aquatic organisms live and feed (Jackson, 1997; Pacyna et al., 2010). It is transferred through trophic level and can bio-magnificate in the food chain. So mercury is present in some fish and is of considerable interest because of its potential hazard to the health of people who consume them: its toxicity increases as a consequence of metals accumulated in aquatic organisms (Castro-Gonzalez and Méndez-Armenta, 2008).

Large predators, such as swordfish (*Xiphias gladius*) and tuna (*Thunnus spp.*), are at the top of aquatic food chains, and have very high metabolic rates (Storelli et al., 2005). Consequently their rate of food intake is elevated, hence they can accumulate large amounts of pollutants, such as mercury (Branco et al., 2004).

Over the past 40 years, several studies have evaluated the mercury concentrations in swordfish and modeled the change in mercury status as the fish matures. Most have studied swordfish from the Atlantic or Indian Oceans and determined significant positive relationships between mercury and weight and length of fish (Cladis et al., 2015). Data from the literature emphasise the importance of biometric parameters (species, sex, age, weight, size, metabolism) in studies on the bioaccumulation of mercury: its concentration in fish, in fact, increase with body size (Storelli et al., 2005). Consequently, consumption of larger fish leads to an increase in the exposure level for consumers. This is the reason why, between the edible fish, swordfish (*Xiphias gladius*) was already considered as a model for discussed processes relate to Hg exposure, including immunological aspects and risk assessment, vulnerability, toxico-kinetics, and toxico-dynamics (Okpala et al., 2017).

A consistent source of fish is essential for the nutritional and financial health of a large segment of the world's population (Tidwel and Allan, 2001). Fishery products are of great importance for Italian diet: they are man's most important single source of high-quality protein, providing ~15% of the animal protein consumed by the world's population, according to the Food and Agriculture Organization (FAO) of the United Nations (2012).

Anyhow, evaluating the presence of this toxic metal is important for food safety, particularly in the case of mercury for which the European rule established maximum limits, on the basis of its TWI (tolerable weekly intake) of 1.6 μg/kg body weight for methylmercury and 4 μg/kg body weight for inorganic mercury (Reg. EC 1881/2006, EFSA, 2012a, b). While there are several studies on general seafood that examined associations with mercury levels focusing on regional context (Brambilla et al., 2013; Bodin et al., 2017; Galimberti et al., 2016; Gobert et al., 2006; Chen et al., 2007; Zaza et al., 2015), less is known about the impact of global differences of capture locations. Thus, further characterization of geographic variation is essential to better understand how fish origin can affect mercury exposition of consumers.

Swordfishes are distributed worldwide in the tropical and subtropical waters therefore, the goal of our study is to compare mercury content in fish caught in different FAO areas in order to provide an interesting case study in which to examine differences in mercury levels from different environments. This study could give a valid instrument to the consumers to assess health risk associated with human consumption of this fish.

2. Materials and methods

2.1. Sampling

Between January 2014 and September 2017, 220 fish samples were collected and sent to the Istituto Zooprofilattico Sperimentale del Mezzogiorno to determinate the presence of mercury. All samples were from different FAO fishing areas. The distribution of sampling location is shown in Fig. 1. They are all delivered from EU and extra EU countries, such as Spain, Portugal or Vietnam and Korea to be dispatched to the Italian market. Most of 220 samples were from Spain (104) and Portugal (66), only 10 from Greece. Fish products were collected by the Veterinary Health Authorities within monitoring plans, according to the performance criteria set in the Regulation 333/2007/EC. After collection, fish samples were immediately put into clean polyethylene bags and sent to the laboratory where they were stored at −20 °C until analysis.

2.2. Analytical procedure

2.2.1. Materials

High purity water was in house produced by a Milli-Q™ deionising system (Millipore), trace analysis boron hydride (pellets), nitric acid 70% w/v and hydrogen peroxide 30% v/v for heavy metal analysis were from Carlo Erba (Milan, Italy) and Romil (Cambridge, UK). Mercury standard solutions were prepared by diluting a standard stock solution at 1000 mg/L (Merck KGaA, Darmstadt, Germany). Glassware, before use, was washed with a solution of nitric acid (10% w/v), rinsed with high purity water and dried at 60 °C. For the cold vapour hydride technique (CV-AAS) a solution of boron hydride 0.2% w/v was freshly prepared by dissolving pellets of sodium boron hydride in NaOH 0.1 M.

2.2.2. Sample preparation for CV-AAS analysis

Fish was thawed, gutted and the edible part homogenized, than 0.75 ± 0.01 g of sample were weighted into a teflon tube with 5.0 mL of 70% nitric acid, 2.5 mL of 30% hydrogen peroxide and 2.5 mL of ultrapure water. The tube was sealed and placed into a microwave oven (Ethos E, Milestone) where the samples were digested under pressure for 10 min at 190 °C. After acid digestion, the samples were cooled at room temperature, filtered into Class A flask and brought to 50 (±0.06 at 20 °C) mL with ultrapure water.

2.2.3. CV-AAS analysis

Total mercury was determined by cold vapour atomic absorption (CV-AAS) using an atomic absorption spectrophotometer (3110, Perkin-Elmer) equipped with a quartz absorption cell, a hollow cathode mercury lamp (253.6 nm), a 50 mL hydride generation vessel were the mercury was evaporated using a reduction vaporizing equipment (MHS 10, Perkin-Elmer) by means of boron hydride 0.2% w/v. For the quantitative determination, linear regression calibration curves were calculated analysing standard solutions of mercury at different levels. Each sample was analysed in duplicate. All concentrations were expressed as mg/kg of wet weight (w.w.) tissue.

2.3. Method validation and quality assurance

Appropriate quality assurance procedures were implemented in order to ensure the reliability of the results in accordance with the UNI/EN/ISO/IEC 17025 Standard (2005). The method used was in house validated according to the requirements of the regulation 333/2007/EC and 582/2016/EC: quality control assessment was obtained by regular participation to proficiency tests (FAPAS-Proficiency testing from Fera) as well as laboratory procedures (blank reagent analysis, spiked samples during each working session, Shewart control charts). In particular, intra-laboratory reproducibility and accuracy studies were carried out through the analysis of ten replicates of the Certificate Reference Materials in three analytical sessions. Moreover, measurement uncertainty was evaluated, according to repeatability, calibration and recovery data, precision in pipetting, weighting and diluting. The limit of quantification (LOQ) (0.030 mg/kg) was finally assessed through the