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Bioaccumulation of nonylphenols and bisphenol A in the Greenland shark *Somniosus microcephalus* from the Greenland seawaters

Ademollo N^{a,*}, Patrolecco L^a, Rauseo J^a, Nielsen J^b, Corsolini S^c

^a Water Research Institute, National Research Council, Via Salaria km 29.300, 00015 Monterotondo Scalo, RM, Italy

^b Marine Biological Section, University of Copenhagen, Strandpromenaden 5, 3000 Helsingør, Denmark

^c Department of Physics, Earth and Environmental Sciences, University of Siena, via P.A. Mattioli 4, I-53100 Siena, Italy

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ABSTRACT

Recent climate changes associated with anthropogenic emissions of pollutants are triggering shifts in global biogeochemical cycles and polar marine ecosystem. The decrease of sea ice and the mechanism of ice formation/melting, may considerably have an impact on the mobility of contaminants and on the loss of biodiversity. In this work, we report the occurrence and bioaccumulation of selected endocrine-disrupting compounds (EDCs) in muscle and liver of the Greenland shark *Somniosus microcephalus*, an arctic species of interest for biogeography, migration, physiology, long- and short-term contaminant storage. The EDCs selected for this study were 4-nonylphenol (4-NP), its mono- (NP1EO) and di-ethoxylate (NP2EO) precursors and bisphenol A (BPA). There are currently very few scientific papers on the distribution and transport of these EDCs in the arctic marine food web and no such studies have been performed on the Greenland shark. Totally, muscles and liver samples were analyzed from 23 Greenland sharks (TL range 149–442 cm) sampled in W, SW, SE, and NE Greenland. Extraction of analytes from biological matrices were performed by ASE (Accelerated Solvent Extraction), followed by HPLC-Fluorescence (FLD) detection. Results showed higher contamination levels in muscle than in liver in the sharks from SE and NE Greenland, while in specimens from W and SW Greenland the liver was the tissue more contaminated. In fact, the 4-NP, NP1-2EO and BPA mean content in liver of SW Greenland specimens was 43.5 ng/g, 288.5 ng/g and 8.2 ng/g wet wt respectively, while in muscle mean concentrations was 20.3 ng/g for 4-NP, 171.1 ng/g wet wt for NP1-2EO and 7.9 ng/g for BPA. Results confirm the presence of selected EDCs in this species, suggesting the transfer of contamination in the Euro-Arctic marine trophic web.

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1. Introduction

Historically, the northern hemisphere and the Arctic Ocean ecosystems have experienced substantial contamination due to the presence of persistent xenobiotics; the lipophilic compounds are able to accumulate in marine organisms and circumpolar populations and they can elicit toxic effects [1].

The Arctic Ocean encompasses about 6% of the Earth' surface and is largely covered by sea ice throughout the whole year. The harsh climate has made the Arctic waters mostly inaccessible for sea transport and protected the region from oil spills in the past, condition that is evolving due to the climate change. Moreover, the list of chemicals found in arctic ecosystems is continuously growing with increasing temporal trends for some current-use chemicals [2]. The fate of contaminants in water and their bioaccumulation potential are closely interconnected and depend on environmental conditions influenced, in turn, by climate change. In this view, the ecotoxicological baseline data on marine

wildlife in remote regions of the Arctic, like Greenland, are particularly important due to the prospective oil, gas and mining activities in the fjords and on the shelf [3].

To monitor the spread and bioaccumulation of a variety of contaminants in the Arctic marine environments, a good sentinel species is the long-lived Greenland shark (*Somniosus microcephalus*) which recently has been found as the longest living vertebrate animals with a maximum lifespan exceeding centuries [4].

The presence of nonylphenols (NPs) and bisphenol A (BPA) in coastal and marine ecosystems is still not as well-documented as it is in freshwater environments [5], especially in the Arctic marine ecosystem [6]. Nevertheless, these compounds should be monitored also in marine ecosystems as required by the Marine Strategy Framework Directive (MSFD) [7].

NPs are metabolites of nonylphenol ethoxylates (NPEOs), belonging to the group of non-ionic surfactants used as detergents, solubilizers, wetting agents, emulsifiers and dispersants. NPEOs are biodegraded during sewage treatment processes in wastewater treatment plants (WWTPs) and partially in the environment by the loss of the ethoxy groups, resulting in nonylphenols (NPs) and other mono- and di-

* Corresponding author.

E-mail address: ademollo@irsa.cnr.it (N. Ademollo).

ethoxylates precursors (NP1EO, NP2EO), which are more toxic and estrogenic than NPEOs [5]. In fact, NPs bind to oestrogen receptors and can block or alter endogenous endocrine functions in various reproductive and developmental stages, also affecting aromatase activity and the function of the aryl hydrocarbon receptor (AhR) [8,9].

Considering their endocrine disruptor (ED) potential and their high usage, NPs have been included in the European list of priority hazardous substances for surface waters in the Water Framework Directive [10], for which a drastic reduction policy is being implemented. In the USA, the Environmental Protection Agency (EPA) developed “chronic criteria recommendations” for NPs setting a limit of 1.7 µg/L in saltwater [11]. Many Asian and South American countries, however, still use NPEOs in large amounts without water quality control [6].

Bisphenol A (BPA) is an intermediated in the production of epoxy resins and polycarbonate plastics and is a suspected endocrine disruptor in the environment. From 1950 it was widely used in food cans and beverage [12,13]. During the past several years, BPA has attracted public and scientific attention due its potential adverse health effects on aquatic organisms and finally on humans [14,15]. BPA is subjected to atmospheric long range transport and a positive correlation was found with 1,3,5-triphenylbenzene, a tracer for plastic burning, indicating that open burning of plastics in domestic wastes should be a significant emission source of atmospheric BPA [16]. Although BPA was not included in water legislations, the Directive 2011/8/EU restricts its use in plastic infant feeding, showing its toxicity [17].

Unlike the BPA, there is no strong evidence for long-range transport of nonylphenols. A possible way for these compounds to reach the remote sites of the Earth, as the Arctic Ocean, is through the adsorption to plastic debris [18–20]. Coupled with extensive reports of ingestion of marine debris by fish and other organisms, the potential for marine plastic debris to be a transport mechanism and source of contamination of the food web is a concern [21]. The potential of endocrine disruption, even at low NPs concentrations, is likely to be enhanced by additive or synergistic effects due to the co-occurrence with other xenoestrogens, including plasticizers like BPA [5].

The degradation processes of NPs and BPA in polar seawaters are very slow, due the low temperatures and winter darkness allowing them to enter the trophic webs, bioaccumulate in the tissues of organisms and bio-magnify [22,23]. Evidence of reproductive adverse effects and behaviour changes of many persistent organic pollutants (POPs) have been already observed and reported for several predators, such seabirds, seals and whales [24,25]. To our knowledge, the information about the occurrence and the distribution of the NPs and BPA is very scarce in this environment.

The aim of this study was to evaluate: a) the occurrence of selected EDCs, such as nonylphenol, its ethoxylates and bisphenol A, in muscle and liver of the Greenland shark, caught in the Greenland seawaters; b) the bioaccumulation potential of NPs and BPA in this long-lived shark; and c) the possible relationship between the shark age/sex and the bioaccumulation of target EDCs.

2. Materials and methods

2.1. Sampling of Greenland shark

From April 2012 to September 2014, 23 specimens of Greenland shark were caught during Greenland Institute of Natural Resources (GINR) annual fish survey and during the international TUNU-Programme led by the UiT, the Arctic University of Norway. The sharks were caught at depths between 114 and 745 m from different regions in Greenland waters (Fig. 1). The general biology of this species mainly occupying cold and deep ocean waters is poorly known [26]. Greenland shark is considered a top predator in the marine food web with a diet composed by a variety of taxa but dominated by piscivore fishes and seals [4,27]. Such opportunistic feeding habits might affect the

contaminant input making this species interesting for ecotoxicology studies [23,28,29].

Maximum length is >5 m making Greenland shark the largest fish in the Arctic, yet specimens typically measures 2.5–3.5 m in length. Females outgrow males and reaches sexual maturation >4 m at an age well above 100 years [4]. Table 1 shows the samples identity (ID), the biometric measure (total length and body mass) and the sampling area of each specimen. In West Greenland (W) five females that measured between 228 and 410 cm and one male of 322 cm were sampled. Samples were also collected in South West Greenland (SW) from five females measuring between 149 and 442 and four males between 281 and 326 cm. In South East Greenland (SE) four females measuring between 264 and 355 cm and two males of 290 and 313 cm were sampled. Lastly, from North East Greenland (NE) samples from two females of 230 and 330 cm were available for analysis. All samples were kept frozen after sampling at –20 °C until further analysis.

In the laboratory, muscles and livers samples were lyophilized in a freeze-drier (CHRIST, Alpha 1-2/LD plus, Osterode, Germany) and then analyzed to evaluate the presence of bisphenol A (BPA), 4-nonylphenol (NP), nonylphenol monoethoxylate (NPE1O) and nonylphenol diethoxylates (NPE2O).

2.2. Chemicals

4-Nonylphenol (NP), nonylphenol monoethoxylate (NPE1O), nonylphenol diethoxylate (NPE2O) and bisphenol A (BPA) standards, purity >99%, were purchased from Fluka Analytical Sigma-Aldrich (Steinheim, Germany) at concentration of 50 µg/mL in acetone. Methanol, ammonium acetate, acetonitrile of HPLC-grade and hydrochloric acid (37%) were obtained from VWR (Radnor, PA, USA). Water (18 MΩ/cm quality) was prepared on a Milli-Q purification system (Millipore, Bedford, MA, USA). A PHM 240 Model pH-meter (Radiometer, Copenhagen, Denmark) with combined glass electrode was used for the pH adjustment of mobile phase. Sodium sulphate anhydrous (ACS-ISO for analysis) was obtained from Carlo Erba Reagents (Milan, Italy). For ASE extraction, diatomaceous earth (Thermo 062819) was purchased from Thermo Scientific (USA). Stock solutions of 4-NP and BPA compounds were prepared at 1000 mg/L in methanol and stored in dark flasks at –20 °C. Working standards (1 mg/L) were achieved by dilution of stock solutions with methanol, stored in the dark at 4 °C and analyzed as soon as possible. Stock solutions of NPEO1 and NPEO2 were prepared at 1 mg/L in methanol and stored at –20 °C.

2.3. Extraction procedure

Extraction of BPA, NPE1O, NPE2O and 4-NP from lyophilized muscles and livers of the Greenland shark samples was performed using the Thermo Scientific™ Dionex™ ASE™ 150 Accelerated Solvent Extractor system. About 2 g of lyophilized muscle/liver samples were mixed to the appropriate amount of a dispersant agent (diatomaceous earth, Thermo 062819) and carefully homogenized. Moreover, in order to reduce matrices interferences, an extra dispersant agent, the sodium sulphate anhydrous, was introduced simultaneously with diatomaceous earth. This mixture was then packed into 10-mL stainless steel cells topped up with diatomaceous earth. The operating pressurized liquid extraction conditions were optimized by modifying a previous method from literature [30]: solvent extraction methanol; pressure 1500 psi; temperature 50 °C; static time 10 min; number of cycles 3; flush volume 100%; purge time 60 s. The extraction was replicated two or three times for each lyophilized sample (muscle or liver). The ASE extracts (20–25 mL) were then purified with a clean-up step on C18 cartridges and reduced almost to dryness in a Rotavapor system followed by a gentle stream of nitrogen and then dissolved in 1 mL of acetonitrile before injection in HPLC.

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