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Maternal transfer of phenol derivatives in the Baltic grey seal *Halichoerus grypus grypus*☆

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ABSTRACT

Studies of circulating levels in difference sex and age classes, and maternal transfer of bisphenol A, 4-tert-octylphenol and 4- nonylphenol in the Baltic grey seal were performed from 2014-2017. Blood was collected from long-term captive adult males, pregnant females and pups. Milk was collected from nursing females. The aim of this study was not only to determine the concentrations of phenol derivatives, i.e. bisphenol A (BPA), 4-*tert*-octylphenol (OP) and 4-nonylphenol (NP), but also to try to evaluate the transfer of these compounds to the next generation in the final stage of foetal life and in the first few weeks of life in juvenile marine mammals. The measurements were carried out using high performance liquid chromatography. The obtained data show that all phenol derivatives are present in the blood of males, females and pups (range <0.07−101 ng·cm^{-3}) and in female milk (range <0.1 −406.3 ng·cm^{-3}). The main source of phenol derivatives in organisms is food exposure. Gender, age, or number of births were not observed to have a significant effect on changes in phenol derivative levels in seal blood within the breeding group. In the prenatal stage of life, a small amount of BPA and alkylphenols was passed on to the offspring through the placenta. In the blood of the offspring the concentration of these compounds exceeded the concentration in the mother's blood 1.5-fold. During nursing, females detoxified their systems. Level of phenol derivatives in the pups blood increased linearly with its increasing concentrations in the mother's milk. On the other hand, the seafood diet which started after the physiological fasting stage of the pup, stabilised the levels of phenol derivatives below 10 ng · cm^{-3}.

1. Introduction

Phenol derivatives such as bisphenol A (BPA), 4-*tert*-octylphenol (OP) and 4-nonylphenol (NP) are lipophilic compounds that have the capacity to bioaccumulate in the trophic chain (David et al., 2009) leading to homeostatic imbalance, and the development of many diseases (WHO, 2012; Snijder et al., 2013). Exposure to endocrine disrupting compounds (EDCs) is associated with a wide spectrum of adverse health effects, including impact on immunity, reproduction, and endocrine functions for example in rats, fish, humans, (Thibaut et al., 1999; Xiao et al., 2006; Chapin et al., 2008). Concentrations of EDCs do not need to be high in order to cause negative and harmful effects for organisms. Trace amounts can influence the activity of natural hormones (US EPA, 2010). An additional source of exposure is also the metabolism of these substances in organisms. Researchers indicate that some of the metabolites of both BPA and alkylphenols may be either as toxic as or more toxic than the starting substance (Thibaut et al., 1999; Pedersen and Hill, 2000; Inoue et al., 2001; Daidoji et al., 2006; Okuda et al., 2011; Li et al., 2011; Nachman et al., 2013).

NP and OP are considered by the European Parliament in the Framework Water Directive to be substances posing a particular threat to the water environment (EU, 2000). Despite this, BPA is permitted for use in food contact materials in the European Union (EU) under Regulation 10/2011/EU, relating to plastic materials and articles intended to come into contact with foodstuffs. However, in January 2011, the European Commission adopted Directive 2011/8/EU, prohibiting the use of BPA for the manufacture of polycarbonate

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infant feeding bottles. BPA is also permitted for food contact use in other countries such as the USA and Japan. Since 2003, a reduction policy has been implemented in the EU for NPs and their ethoxylates (EU, 2003), but for OP the limitations on usage are non-existent. In many countries outside Europe there are still no restrictions concerning the production and usage of phenol derivatives.

For many decades, man has been using NP for the production of non-ionic surfactants, cosmetics, as well as OP in the production of, among other things, car tyres, paints and varnishes. Bisphenol A has been widely used in the production of synthetic materials, mainly polycarbonate and epoxide resins (ECHA, 2008; HELCOM, 2010). The widespread use of phenol derivatives ensures their presence at municipal and industrial waste landfills, and the release of endocrine phenol derivatives from waste left on landfill sites has been reported by many researchers (Xu et al., 2011; Zhang et al., 2012; Morin et al., 2015). Phenol derivatives enter into the sea, where they occur at a measurable level, via rivers, sewage treatment plants and aeolian transport. A wide range of concentrations of BPA, OP and NP have been found in surface waters of the southern Baltic from <1.0 to 834.5 ng · dm^{-3}, sediments (<0.08 to 249.08 ng · g^{-1} dw.) and plankton (<0.8–769.2 ng · g^{-1} dw) and fish (<0.8–798.4 ng · g^{-1}dw), which are the food of seabirds, seals and humans (HELCOM, 2010; Koniecko et al., 2014; Staniszewska et al., 2014, 2015a; b; 2016a; b). Due to the ability of EDCs to bioaccumulate and biomagnify, organisms at the highest trophic levels, such as marine mammals like seals, are particularly exposed to the influence of a cocktail of EDCs in the sea by the alimentary route (Harban et al., 2011; Bełdowska; Falkowska, 2016; Routti et al., 2016). Their blubber contains other lipophilic compounds (dichlorodiphenyltrichloroethane, polychlorinated biphenyls, polybrominated diphenyl ethers) (Addison and Brodi, 1987; She et al., 2002; Hall et al., 2003; Larsson et al., 2004). During natural fasting periods, which take place e.g. when reproducing, post-weaning or moulting, fat tissue diminishes, and EDCs are released from the fat and transferred to the bloodstream, thereby exposing other tissues and organs to increased levels of xenobiotics (Debier et al., 2006).

The grey seal *Halichoerus grypus grypus* is one of three protected seal species found in the Baltic Sea. Despite the fact that the grey seal population represents the most numerous population of predatory mammals, the levels of phenol derivatives in their bodies are not very well understood. Researchers consider seals to be indicators of environmental pollution (Bossart, 2006) and of the condition of the marine ecosystem (Reddy et al., 2001). In the literature there is also little information on the effects of chronic exposure to phenol derivatives, their distribution in the mother's body and transfer to the offspring. Other EDCs such as chloroorganic compounds (chlordanes, DDTs, HCBs, HCHs, PCBs, s-triazine derivatives) can be transferred from the mother to offsprings during the prenatal period and during lactation (Sørmo et al., 2003; Reindl et al., 2015). We have thus far published the results on phenol derivatives in grey seals placenta from the breeding group of the Marine Station in Hel (Nehring et al., 2017). In the literature researchers have indicated that young seals exposed to endocrine-active compounds, even at low concentrations, may have impaired thyroid function and vitamin A homeostasis (Simms and Ross, 2000; Hall et al., 2003; Sørmo et al., 2003). That's why blood biochemistry and haematological reference ranges are used to assess the general health and gross physiological status of individual animals, but they may also be useful for identifying diseases and malnutrition in populations (Dierauf; Gulland, 2001; Gulland and Hall, 2007; Hall et al., 2009) and long-term monitoring of endangered species (Reif et al., 2004).

By using a minimally invasive method of blood and milk collection, the authors of the present study determined the concentrations of bisphenol A, 4-*tert*-octylphenol and 4-nonylphenol. We investigated the maternal transfer of these compounds to the next generation in the prenatal stage and the first few weeks of life in juvenile grey seals. In addition, we wanted to check the relationship between the age and the number of deliveries of female seal and the concentrations of phenol derivatives in the blood. The authors' decision to conduct such studies was prompted by the fact that the mortality of young Baltic seals is relatively high (Harding et al., 2007). In the global literature there is no information about the concentration of BPA, OP, NP in the initial stages of the pups' life, including at the time of birth, or the effects of diet and physiological stages (fasting, pregnancy or lactation) on the development of young organisms. The blood carries contaminants throughout the body. Therefore, the determination of concentrations of BPA, OP and NP in the blood provides a proxy for the whole organism phenol-derivative burden.

On the other hand, the knowledge about the amount of phenol derivatives in breast milk makes it possible to assess the alimentary exposure of grey seal pups. The study included males as a comparison with females, which are able to offload some of their contaminant burden to their offspring.

2. Materials and methods

2.1. Sample collection

The research was carried out at the Professor Krzysztof Skóra Hel Marine Station (HMS) belonging to the Institute of Oceanography of the University of Gdansk (website: http://www.hel.ug.edu.pl/). The station is the national center for the research of marine mammals that live in the Polish part of the Baltic. The Marine Station has all the permits necessary to conduct research on grey seals. The station is permanently inhabited by a group of seals consisting of 4 females and 2 males. Up to 4 seal pups are born there every year. The females from which blood was collected, had given birth to 6 to 19 pups during their lifetime. All young seals were fed by their mothers and learnt to hunt for live fish (this period lasted about 3 months) and only after this period were they released into the wild. After birth, pups suckle exclusively on the mother's milk for about 3 weeks, followed by a period of natural fasting of ca. 2 weeks. In captivity about 5 weeks after the birth of a grey seal pup, it begins to feed on fish. Pups born at the Hel Marine Station are released into their natural environment and start their fish diet in ca. 2–3 weeks after weaning to shorten the time spent in captivity. If the pup shows no interest in food for a long time it is force-fed to start eating by itself and gain weight in order to be ready for release at the same time as other individuals. Pups are fed mainly with Baltic defrosted herring supplemented with vitamins. Occasionally they are given sprats and in the last 2 days before release they are also given few rainbow trout. It was therefore possible to carry out our research in terms of changes in concentrations of phenol derivatives in the bodies of young seals in the first weeks of their life. The cooperation with the Hel Marine Station made it possible to conduct unique studies on live seals. Grey seals are a protected and rare species in Poland, therefore it is not possible to conduct research on free-living subjects. Such studies had never been conducted before and were authorised by the Local Ethics Commission for Experimentation on Animals at the Gdansk Medical University (Act no. 2/2016 and supplemented by act no. 12/2016).

The female seals in the breeding group were born in the wild. The oldest female was found as a pup on the coast of Sweden. By the time she moved into HMS in 1999 she had already mothered two pups. The other three females come from Allirahu Island (Estonia) where, having been nursed for 3 weeks by their mothers, they were caught and transported to the Hel Marine Station in 1998.

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