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PAH metabolites in fish bile: From the Seine estuary to Iceland

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAH) are environmental contaminants that pose significant risk to health of fish. The International Workshop on Integrated Assessment of Contaminant Impacts on the North Sea (ICON) provided the framework to investigate biomarker responses as well as contaminant concentrations side by side in marine ecosystems. Concentrations of the main PAH metabolites 1-hydroxypyrene, 1-hydroxyphenanthren and 3-hydroxybenzo(a)pyrene were determined in bile by HPLC with fluorescence detection. Fish species under investigation were dab (*Limanda limanda*), flounder (*Platichthys flesus*) and haddock (*Melanogrammus aeglefinus*). A contamination gradient was demonstrated from the low contaminated waters of Iceland and off-shore regions of the North Sea towards higher concentrations in coastal areas. Concentrations of PAH metabolites differed primarily according to sampling region and secondarily to species.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants in the marine environment (Van der Oost et al., 2003). Because of some PAHs are known to be potent carcinogens, PAHs are generally regarded as priority contaminants for environmental monitoring. Whereas PAH concentrations in water or sediment samples can be used to describe the contamination, the quantification of parent PAHs in fish tissues may lead to an underestimation of the exposure level. This is due to the fast metabolic transformation of PAHs in fish as well as in many other vertebrates. During this enzymatic biotransformation PAHs and arising intermediate compounds such as epoxides may act as genotoxic carcinogens leading to e.g. DNA adducts (Aas et al., 2000) and neoplasia-related aberrations or tumors in fish liver (Myers et al., 2003; Vethaak et al., 2009). The resulting hydroxylated and/or conjugated PAH metabolites are mainly excreted via bile fluid. Therefore, the concentration of biliary PAH metabolites in fish can be used as a marker for assessing the recent PAH exposure. The main metabolite in fish bile is 1-hydroxypyrene which contributes up to 76% of the sum of PAH metabolites. Other metabolites, detected in considerably lower levels in fish bile are 1-hydroxyphenanthrene, 1-hydroxychrysene and three metabolites of benzo(a)pyrene (Ruddock et al., 2003).

Biological effect monitoring including determination of PAH metabolites is part of international monitoring programmes, among them “OSPAR Joint Assessment and Monitoring Programme” (JAMP; OSPAR Commission, 2008) with dab and flounder as target species. PAH metabolites measured in fish bile are recommended for monitoring the PAH contamination of the North Sea. The analytical procedure is well documented and has been described in detail (e.g. Ariese et al., 2005a) and reviewed by Beyer et al. (2010). In addition an European quality assurance trial has been published (Kammann et al., 2013) and further quality

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assurance is performed under the lead of the ICES working group of biological effects of contaminants (WGBEC). With this comprehensive background PAH metabolites have become popular parameters in environmental monitoring in several European countries and are consequently part of recent international environmental assessments as well as recommendations (OSPAR Commission, 2008; HELCOM, 2013). The increasing international popularity of the method is based on a fast, easy and reproducible analytical procedure. It should be noted that HPLC analysis and the alternative gas chromatography-mass spectrometry (GC–MS) gave comparable results of PAH metabolites in fish bile (Ariese et al., 2005b; Kammann et al., 2013), so that effect thresholds determined by GC–MS are valid for HPLC too (ICES, 2011).

In addition the matrix bile is attractive as a liquid which is free of lipids and can be subjected after a simple enzymatic deconjugation step to the HPLC device. Further on bile can be easily collected from almost every fish with a filled gall bladder. Just after feeding the bile is emptied into the intestine and the gall bladder starts to refill again. PAH metabolite data are available for many different marine fish species such as flounder (Vuorinen et al., 2006; Kammann, 2007), dab (Van Schanke et al., 2001; Devier et al., 2013), cod (Karl et al., 2016; Ruus et al., 2012; Aas et al., 2000), turbot (Le Dû-Lacoste et al., 2013) and eel (Ruddock et al., 2003; Kammann et al., 2014). The concentration of PAH metabolites in fish bile includes information of bioavailability of PAH as well as the metabolic capacity of the animal. Therefore, PAH metabolites can be counted to both chemical and biological effects parameter. In contrast to many organic contaminants PAH do not accumulate in the fish and the concentration of PAH metabolites in the bile reflects exposure in the near past.

The ICON project (Hylland et al., 2012, 2017a) is a demonstration program for integrated marine monitoring including biological effects in European marine waters. ICON provided the frame to investigate biomarker responses as well as contaminant concentrations side by side in marine samples. Several laboratories in charge for national monitoring joined this project to exchange knowledge and contribute to a joint assessment on European scale. This approach is in line with the goals of the EU Marine Strategy Framework Directive (MSFD, 2008). The MSFD requires that “Concentrations of contaminants are at levels not giving rise to pollution effects” in order for Good Environmental Status to be achieved. Monitoring and assessment strategies concerning MSFD are currently under international discussion. ICES and OSPAR provide a detailed framework and example for environmental assessment as demanded by MSFD (Davies and Vethaak, 2012). A central part of the recommended strategy is a categorization of results by assessment criteria acting as (1) thresholds between background and elevated but acceptable levels (background assessment concentration, BAC), and (2) between acceptable and unacceptable levels (environmental assessment concentration, EAC).

The aims of the present study are related to the integrated assessment addressed in ICON: (1) To compare levels of PAH metabolites in fish from NW European marine and estuarine waters; to (2) compare levels of PAH metabolites in different fish species and (3) to provide background concentrations needed for environmental assessment.

2. Material and methods

2.1. Fish collection and bile sampling

Fish were sampled during cruises of RV ″Walther Herwig III″ in 2008 by means of bottom trawling in the North Sea and western Baltic. The sampling stations are shown in Fig. 1. For this study adult female dab (*Limanda limanda*) with a body length between 20 and 26 cm as well as adult male or female flounder (*Platichthys flesus*) with a body length between 24 and 31 cm were used. Haddock (*Melanogrammus aeglefinus*) of both sexes with body length between 28 and 45 cm were also included in the study. In the Dutch Wadden Sea adult male or female flounder with a body length between 24 and 30 cm were caught in September 2008 using a small beam trawl. Dab and flounder from the Seine estuary were sampled during cruises of the French ship “Gwendrez” in September 2008 by means of bottom trawling. The body length was determined with 20–26.5 cm for dab and 24.5–31.5 cm for flounder (Table 1). All sampling campaigns took place in August or September and were in accordance with the OSPAR sampling strategy (OSPAR Commission, 2008). Fish were killed by a blow on the head and bile fluid was sampled using a 1 ml disposable syringe with a disposable hypodermic needle (0.6 × 30 mm). Bile samples of approximately 0.1–0.5 ml were immediately frozen and stored at −20 °C or lower until analysis.

2.2. Chemicals

The hydroxylated PAH standards 1-hydroxypyrene (1OHPyr) and 1-hydroxyphenanthrene (1OHPhen) were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany) as certified solutions. Acetonitrile (hypergrade for HPLC), methanol (gradient grade, for HPLC), ethanol (absolute), trifluoroacetic acid (spectroscopic quality) and β-glucuronidase/arylsulfatase (30/60 U/ml) were purchased by VWR International GmbH (Darmstadt, Germany). Ascorbic acid and 3-hydroxybenzoapyren (3OHBAP) as neat certified reference material (BCR 343) were purchased by Sigma–Aldrich (Taufkirchen, Germany) and water in HPLC gradient grade quality was purchased by J.T. Baker (Netherlands).

2.3. Treatment of bile samples

PAH metabolites in bile samples were determined as described by Kammann et al. (2014). Briefly, 25 μl bile was mixed with 95 μl water and 5 μl of an enzyme solution containing β-glucuronidase/arylsulfatase (30/60 U/ml). The resultant mixture was shaken for 2 h at 37 °C. The reaction was stopped by addition of 125 μl ethanol containing 5 mg/ml ascorbic acid. The final solution was centrifuged (5 min, 700 × g). The clear supernatant was transferred to HPLC vials, stored at 10 °C in the auto sampler and used for HPLC analysis at the same day.

2.4. HPLC analysis for PAH metabolites

The concentrations of the three metabolites were determined using a LaChrom HPLC system (Merck Hitachi) comprising a quaternary pump (L-7100), an auto sampler (L-7200) and a fluorescence detector (L-7480). Samples were chromatographed on a Nucleosil 100-3 C18 (3 × 125 mm) reverse phase column equipped with a 10 × 3 mm guard column filled with the same material. Standard solutions were diluted in acetonitrile containing 5 mg/ml ascorbic acid and stored in the dark. Calibrations consisting of five standard concentrations were repeated daily with every sample batch. The initial mobile phase was acetonitrile/0.1% trifluoroacetic acid 50/50 (v/v) pumped with 0.5 ml/min. After 10 min the solvent composition progressively changed to 60/40 within 4 min and afterwards to 100% acetonitrile over 2 min. The gradient remained on this level for additional 3 min and returned to the initial composition within 1 min. The excitation/emission wavelength pairs for 1OHPyr and 1OHPhen were 346/384 and 256/380 nm respectively. The wavelength pair used for 3OHBAP was 380/430 nm.