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ORIGINAL PAPER

Comparative absorption and tissue distribution of ^{14}C-benzo(a)pyrene and ^{14}C-phenanthrene in the polar cod (*Boreogadus saida*) following oral administration

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Abstract The Arctic is an important sink for organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) long-range transported from industrial regions. With the retreat of sea ice and increasing anthropogenic activities such as the oil and gas industries, local sources of PAHs are expected to increase both through operational and accidental discharges. There is a need to increase our knowledge concerning the uptake and distribution of organic pollutants, in particular PAHs, to evaluate the risk these toxic compounds may represent for Arctic species. The absorption and tissue distribution of ^{14}C-benzo(a)-pyrene (BaP) and ^{14}C-phenanthrene (Phen) were studied in the polar cod (*Boreogadus saida*), a key Arctic species. After a single oral dose of BaP (1.15 ± 0.36 mg/kg fish) or Phen (0.40 ± 0.12 mg/kg fish), corresponding to 0.12 ± 0.03 mCi/kg fish, the tissue distribution was followed through 30 days by means of whole-body autoradiography and liquid scintillation counting of liver and bile. For both compounds, radiolabeling was mainly present in the bile and the intestines throughout the study period. Phen-derived radioactivity, however, appeared to be more systemically distributed compared to BaP. Furthermore, a far higher amount of irreversibly bound BaP-derived radioactivity was present in the intestinal mucosa compared to Phen, indicating a more extensive formation of reactive intermediates from the former compared with the latter. Liquid scintillation counting confirmed that radioactivity was present in the liver at all time points for both groups although the levels were low in the BaP group. These results strongly indicated that both compounds and/or their metabolites undergo enterohepatic circulation.

Keywords Polar cod · Toxicokinetics · Benzo(a)pyrene · Phenanthrene · Enterohepatic circulation

Introduction

There are reasons to believe that the Arctic marine ecosystem is being increasingly loaded with organic pollutants, both from industrial discharges transported from temperate latitudes, and due to human activities in the Arctic itself (Friedman and Selin 2012). Polycyclic aromatic hydrocarbons (PAHs) are a large group of widely spread environmental contaminants, reaching the Arctic through long-range atmospheric transport (Laender et al. 2011; Friedman and Selin 2012) as well as through discharges from large Canadian and Russian river systems (Yunker and MacDonald 1995; AMAP 1998; Dahle et al. 2003). As the Arctic holds important oil reserves, local sources of hydrocarbons from oil seepage are also believed to contribute significantly to the release of PAHs in Arctic environments (Doré 1995; Blasco et al. 2010; Foster et al. 2015). The discovery of recoverable oil reserves in the Arctic will probably result in petroleum production in the near future, further increasing pollutant loading in Arctic ecosystems. Thus, there is an urgent need for increased research effort regarding the fate and effects of PAHs in Arctic wildlife.

This article belongs to the special issue on the “Ecology of Arctic Gadids”, coordinated by Franz Mueter, Jasmine Nahrgang, John Nelson, and Jørgen Berge.

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Polar cod (*Boreogadus saida*) is a cryo-pelagic key species in the Arctic shelf seas, also known from the Arctic Basin (Gradinger and Bluhm 2004). It is one of the most abundant fish species of the Arctic and constitutes a major link between lower and higher trophic levels (Hop and Gjøsæter 2013). A number of studies have investigated the biological effects of PAHs or other petroleum related products on this species (e.g. Nahrgang et al. 2009, 2010a, b; Dussauze et al. 2014; Geraudie et al. 2014); however, important gaps in knowledge remain concerning the toxicokinetics of PAHs in this species. In 2000, Ingebrigtsen and co-workers investigated the distribution and cellular binding of benzo(a)pyrene (BaP) in polar cod and showed an increased systemic availability of BaP through waterborne exposure compared with dietary exposure. More recently, Nahrgang et al. (2010b) suggested that polar cod exposed to dietary crude oil may show a potentially higher absorption of dietary PAHs compared to other fish species (James et al. 1996; van Veld et al. 1997; Couillard et al. 2009) due to slow gastric evacuation rates and high assimilation efficiency of lipids (Hop and Tonn 1998). Although of special importance for lipophilic contaminants, the dietary route of exposure has not been studied to the same extent as the waterborne exposure. Furthermore, studies have focused on a few model compounds, particularly BaP (James et al. 1991, 1996; Lemaire et al. 1992; Ingebrigtsen et al. 2000).

BaP, a five-ring PAH, is an ubiquitous contaminant that has been widely used as a model compound for pyrogenic-type PAH studies due to its carcinogenicity and mutagenicity, and risk of human and animal health (Phillips 1983; Stegeman and Lech 1991). On the other hand, the non-mutagenic tricyclic aromatic hydrocarbon phenanthrene (Phen) has received less attention, although it is highly abundant in crude oil comparatively to BaP. The aim of the present study was therefore to investigate and compare the tissue distribution of the model compound BaP with Phen in polar cod following oral administration.

Materials and methods

Chemicals

Uniformly labelled ^{14}C-benzo(a)pyrene (BaP) dissolved in toluene (1 mg BaP/ml, specific activity 20–50 mCi/mmol, purity >97 %) and uniformly labelled ^{14}C-phenanthrene (Phen) dissolved in ethanol (0.34 mg Phen/ml, specific activity 50–60 mCi/mmol, purity >97 %) were purchased from American Radiolabeled Chemicals, Inc.

Sampling and acclimation

Sexually mature fish of both sexes were collected in the waters off the Svalbard Archipelago (latitude 78°N) with a Campelen bottom trawl onboard R/V *Helmer Hanssen* in October 2012. The sampled specimens were transported to the biological station of UiT The Arctic University of Norway (latitude 70°N) and maintained in 300-l holding tanks with running seawater at a temperature of about three degrees Celsius (3 °C) and constant dimmed light until experimental start in November 2012. During acclimation, polar cod were fed until satiation three times weekly with one of its natural prey items, i.e. calanoïd copepods *Calanus* spp. (purchased frozen from Calanus AS, Tromsø). The characteristics of the included specimens are listed in Table 1.

Test substances and experimental design

The test substances (BaP and Phen) were homogenously mixed in the *Calanus* spp. food at a concentration of 4.16 µCi/g wet weight food (solvent concentration of 42 µl/g food). One week prior to the exposure, polar cod were transferred to two experimental tanks and feeding was stopped. On the exposure day, polar cod (total $n = 24$, mean (±SD) total wet weight 31.8 ± 9.3 g and mean body length 16.8 ± 9.6 cm) were individually weighed, and 1-ml tuberculin syringes (BD Plastipak^{TM}) were filled with food mixture corresponding to 2.8 ± 0.8 % body weight (see Table 1 for details). Polar cod were exposed to a single oral dose, through force-feeding, to one of the mixtures at a final dose of 0.12 ± 0.03 mCi/kg fish, equivalent to a concentration of 1.15 ± 0.36 mg BaP/kg fish and 0.40 ± 0.12 mg Phen/kg fish. The fish were then observed during 3 min to control for potential regurgitation of the food, and only one specimen regurgitated during the course of the experiment (Table 1). Polar cod were then transferred to two 300-l experimental tanks (BaP and Phen-exposed fish, respectively) under running seawater of temperature of 3 °C and constant dimmed light. During the experiment, polar cod were not fed. Three specimens from each group were sampled 2, 6, 12, and 30 days after administration. Briefly, polar cod were euthanised with Finquel MS 222 (tricaine methanesulfonate) and immediately frozen at −80 °C.

Autoradiography

The frozen fish were individually prepared for tape-section autoradiography according to the method of Ullberg (Ullberg 1954, 1977). The fish were embedded in a precooled (1 °C) aqueous gel of carboxymethylcellulose (1 %) and

Table 1 Description of experimental specimens: sex (F: females, M: male), body weight (g) and total body length (cm) at sampling point, amount of food administered (g) by forced feeding and equivalent doses of radiolabeled PAHs µCi/g fish

	Sex	Body weight (g)	Body length (cm)	Administered food (g)	Dose µCi/g fish
BaP					
2 days	M	26.5	18.0	1.14	0.18
2 days	F	25.0	15.0	0.59	0.10
2 days	F	22.5	15.5	0.64	0.12
6 days	M	49.5	18.5	0.60	0.05
6 days	F	39.5	17.5	0.75	0.08
6 days	M	24.0	14.0	0.90	0.16[a]
12 days	F	52.0	22.0	1.31	0.11
12 days	M	30.0	16.0	0.94	0.13
12 days	M	29.5	16.0	0.88	0.12
30 days	M	21.5	16.0	0.74	0.14
30 days	F	36.5	18.5	0.68	0.08
30 days	F	35.0	17.5	0.99	0.12
Phen					
2 days	M	24.5	14.5	0.73	0.12
2 days	F	37.0	18.0	0.76	0.09
2 days	M	31.5	16.0	1.26	0.17
6 days	F	24.5	15.0	0.90	0.15
6 days	F	40.0	18.5	0.65	0.07
6 days	F	38.0	17.5	0.60	0.07
12 days	F	47.5	19.0	1.00	0.09
12 days	M	17.5	14.0	0.56	0.13
12 days	M	27.5	16.0	0.84	0.13
30 days	M	21.0	16.0	0.79	0.16
30 days	M	32.5	17.0	0.99	0.13
30 days	F	30.5	17.0	0.90	0.12

The feed contained 4.16 µCi/g wet weight of either ^{14}C-BaP or ^{14}C-Phen. No significant differences in length and weight were found among specimens ($p > 0.5$, Wilcoxon rank sum test)

[a] Polar cod observed to regurgitate some feed following force-feeding

frozen on dry ice. From each fish, sagittal whole-body sections (20 µm) were obtained on tape (no. 821, 3 M, St. Paul, Minn.) in a cryo-microtome (PMV 450 MP, Palmstierna Mekaniska, Stockholm) at −20 °C. All sections were freeze-dried at −20 °C for 24 h. In order to avoid artefacts due to melting and diffusion of fat in the dehydrated sections, all handling and exposure took place below −20 °C. Selected freeze-dried sections were extracted successively with polar and non-polar solvents (5 % trichloroacetic acid for 1 min, 50 % methanol for 30 s, 100 % methanol for 30 s, heptane for 15 s, 100 % methanol for 30 s, 50 % methanol for 30 s and tap water for 5 min). Both native and extracted sections were exposed to X-ray film (Structurix D7 DW ETE, Agfa, Belgium) for 14 days (−20 °C). Autoradiograms from the freeze-dried sections were considered to represent unchanged mother compound (^{14}C-BaP or ^{14}C-Phen) plus its soluble metabolites, while autoradiograms from the freeze-dried solvent-extracted sections were considered to represent metabolites firmly bound to tissue macromolecules (Bergman 1979; Brandt and Brittebo 1989; Ingebrigtsen et al. 2000).

Scintillation counting

Liver and bile collected from all but one specimen (BaP, 2 days) were analysed through scintillation counting. Liver tissue was retrieved from the sectioned blocks and weighed (2.1–46.4 mg), 1 ml of Soluene was added, and the sample was allowed to digest overnight at 37 °C. Bile was collected from the native sections (20 µm thick) with a sharp, hollow probe (3 mm in diameter). From each fish, one to two bile samples were transferred to a vial and left to digest in 1 ml Soluene overnight at 37 °C. The following day, the samples were briefly whirl-mixed before 5 ml scintillation cocktail (Hionic fluor) was added each vial. A second round of whirl-mixing (30 s) was employed before the samples were left on the bench for at least 1 h to

equilibrate. The counting took place in a Tri-Carb liquid scintillation analyser (1900CA, Packard) with a built-in quencher standard. Quantified radioactivity was presented as disintegrations per minute (dpm)/mg for liver and dpm/µl for bile.

Results

Autoradiograms from native and extracted sections are presented in Fig. 1, and quantitative measure of the presence of radiolabeled compounds in liver and bile, through scintillation counting, is presented in Fig. 2.

Benzo(a)pyrene

Two days after administration, BaP-derived radioactivity was most prominent in the intestines and the gall bladder of the autoradiograms (Fig. 1). The same distribution pattern was observed 6 days after administration. Twelve days after administration, BaP-derived radiolabeling was very low in all tissues and all specimens, including bile and intestine, comparatively to the other time points. The quality of the autoradiograms was verified by the inclusion of standard microscales with known ^{14}C concentrations, and there were no indications of any technical failure during film exposure or development for any of the autoradiograms. Although not observed, part of these low levels may be attributed to regurgitation of a portion of the administered dose due to the presence of toluene in the BaP food mixture (see Table 1). Due to the uncertainty of the validity of these autoradiograms, this group is not included in Fig. 1. After 30 days, radioactivity was still observed in the bile and the intestines. The high levels of radioactivity in the content of intestine and bile on the autoradiograms made it impossible to evaluate radioactivity in the intestinal and gall bladder walls, as the films were saturated. However, sections extracted with polar and non-polar solvents revealed the presence of BaP-derived radioactivity in the walls of the gall bladder and the intestines proportionate to the levels found in the content at all time points. Radioactivity in the liver was not observable on the autoradiograms but was confirmed in all groups by liquid scintillation counting (Fig. 2a). The median values were 15.3, 3.6, 2.5, and 6.8 dpm/mg liver 2, 6, 12, and 30 days after exposure, respectively. One individual in the first group (2 days after exposure) contained higher levels in the liver (252.1 dpm/mg liver) than all of the other BaP

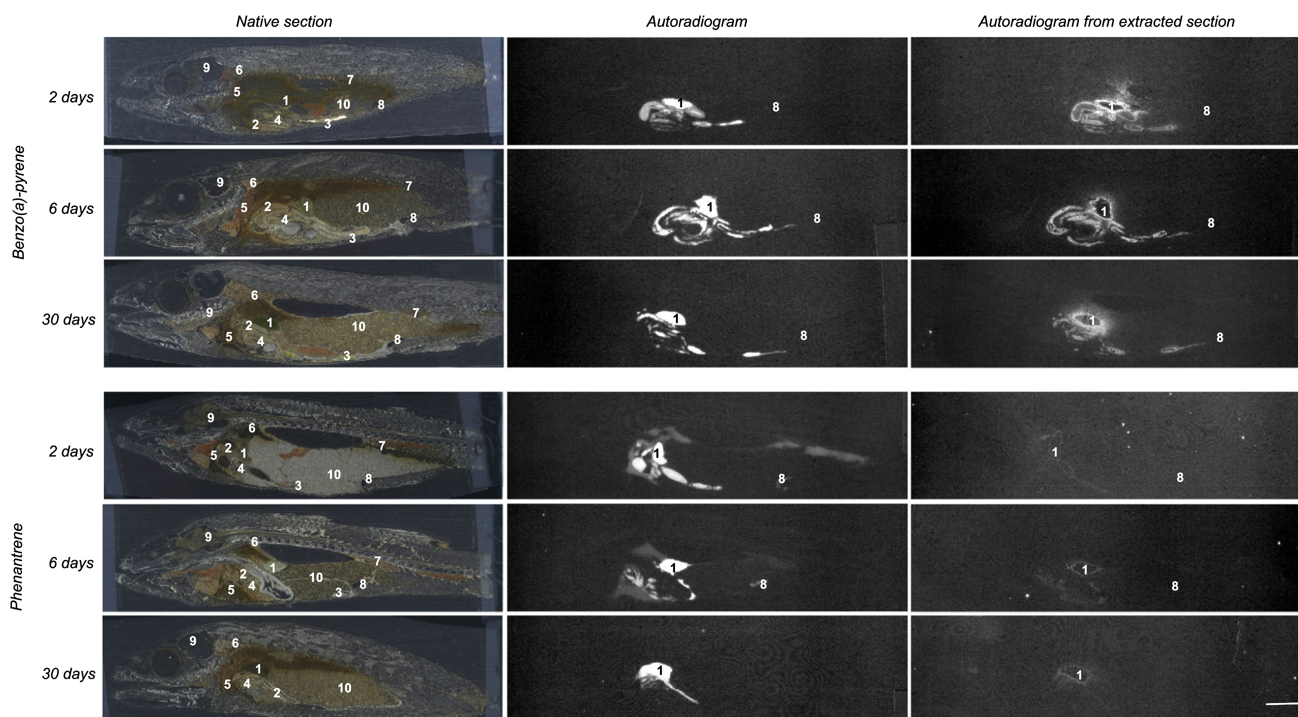

Fig. 1 Native tissue sections (20 µm, *first panel*) with corresponding autoradiograms (*second panel*) and autoradiograms from extracted sections (*third panel*) of polar cod (*Boreogadus saida*) 2, 6 and 30 days following intragastric administration of ^{14}C-benzo(a)pyrene (BaP) or ^{14}C-phenanthrene (Phen). Autoradiograms from native sections represent both mother compound and its soluble metabolites. Non-extractable PAH-derived radioactivity, regarded to represent reactive intermediates bound to tissue macromolecules, is presented in the autoradiograms from extracted sections. Numbering of tissues; *1* gall bladder, *2* mid-intestine, *3* posterior intestine, *4* pyloric caeca, *5* liver, *6* anterior kidney, *7* posterior kidney, *8* urinary bladder, *9* central nervous system, *10* gonad. Scale bar (*lower right*) is 1 cm

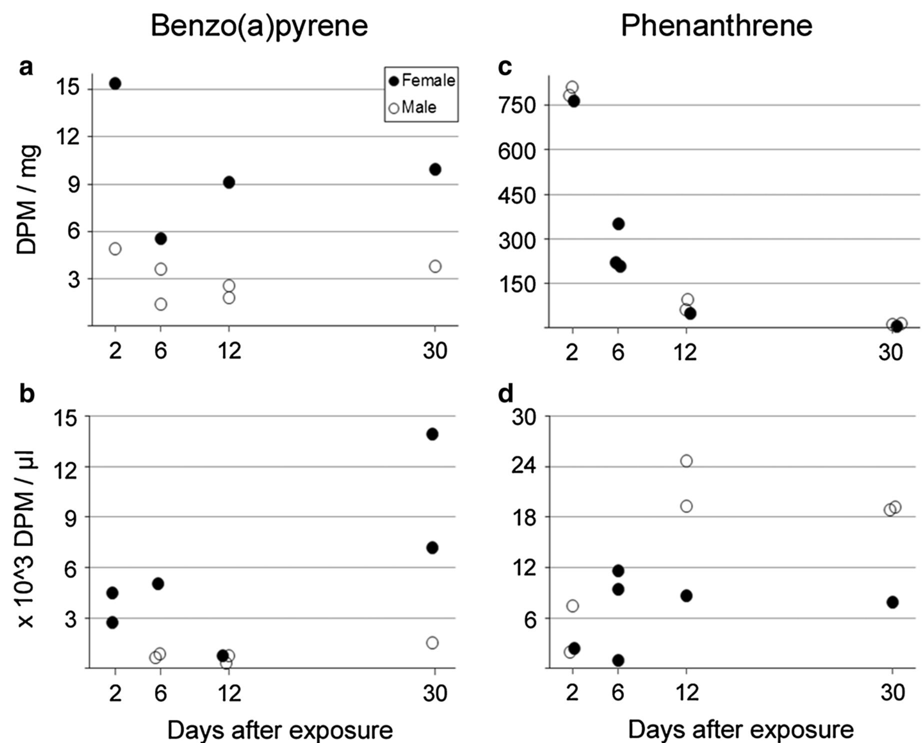

Fig. 2 Radioactivity measured by liquid scintillation counting in liver (**a**, **c**) sampled from the sectioned blocks and bile (**b**, **d**) sampled from native sections of polar cod (*Boreogadus saida*). The fish were administered an oral dose of either BaP (1.15 ± 0.36 mg/kg fish) or Phen (0.40 ± 0.12 mg/kg fish) and three fish from each group was collected 2, 6, 12 and 30 days after administration. *Black* and *open circles* represent individual females and males, respectively

exposed fish. Thus this value was outside the range of the figure but was included in the statistical analysis. Liquid scintillation counting showed that the bile content of radioactivity (Fig. 2b) dropped from 3.6×10^3 to 0.8×10^3 dpm/µl from 2 to 6 days after exposure (median values). Thirty days after exposure, the median value increased to 7.2×10^3 dpm/µl. Furthermore, male polar cod seemed to have lower levels of radioactivity compared with females both on autoradiograms (not shown) and after scintillation counting (Fig. 2a, b). There were no significant differences (Kruskal–Wallis, $p > 0.05$) among time points (males and females combined) in either liver or bile.

Phenanthrene

Two days after administration, high levels of Phen-derived radioactivity were found in the intestines and gall bladder (Fig. 1). Comparatively, radioactivity was present to a visually lower degree in the liver, gonad, kidney and the urine. Furthermore, traces of radioactivity were observed in nervous tissue. Six days after administration, the levels of radioactivity were still prominent in the intestines and the bile and present in the liver, gonad, kidney and urine. After 12 days, radiolabeling as observed on autoradiograms was decreased in the liver, kidney and urine compared to the 6 days group, whereas levels in the bile and intestines seemed similar (not shown in Fig. 1). No traces of radioactivity were observable in the gonads at this time point. After 30 days, the levels of radioactivity were still prominent in the bile and the intestines, whereas the liver, kidney and urine did not contain observable amounts of radiolabeling. Liquid scintillation counting on liver showed a significant decline in Phen-derived radioactivity over time (Fig. 2c), confirming the trends observed on the autoradiograms. Furthermore, levels were remarkably higher for Phen-derived radioactivity compared with BaP-derived radioactivity (Fig. 2a). A drop in the median value from 782 to 217.2 dpm/mg tissue was observed in groups sampled two and six days after administration. The decline continued in groups sampled 12 and 30 days after administration to 58.5 and 11.2 dpm/mg tissue, respectively. Correspondingly, the levels in the bile (Fig. 2d) increased over time until 12 days after administration, from 2.3×10^3 via 9.4×10^3 to 19.3×10^3 dpm/µl bile (median values). The amount of radiolabeled compound 30 days after administration was almost identical to 12 days after administration (18.8×10^3 dpm/µl bile). Finally, sections extracted with polar and non-polar solvents contained traces of radioactivity in the walls of the gall bladder and the intestines at all time points. Apart from a significant difference between the liver content 2 and 30 days after exposure, there were no significant differ-

ences (Kruskal–Wallis, $p > 0.05$) among the time points (males and females combined) in either liver or bile.

Discussion

The high level of radioactivity in the bile clearly shows that absorption of both compounds had taken place within 2 days following administration. This is in accordance with previous studies of fish exposed intragastrically to BaP (James et al. 1991; McElroy et al. 1991; Lemaire et al. 1992) and Phen (Solbakken et al. 1979; Solbakken and Palmork 1980; Solbakken et al. 1982, 1983).

Furthermore, our results suggest that a larger fraction of Phen was subjected to systemic distribution than BaP. Indeed, the tissue distribution of BaP-derived radioactivity was confined to the intestinal tract and the biliary system. Liquid scintillation counting revealed the presence of radiolabeling in the liver at all time points, although levels of radioactivity were significantly reduced compared with those of Phen. The main load of Phen-derived radioactivity was present in the bile and intestines. However, unlike BaP, radioactivity derived from Phen was also observed on autoradiograms in the liver, kidney and urine through day 12, and traces were detected in nervous tissue and gonads. Several factors may influence differently the uptake and toxicokinetics of dietary-borne BaP and Phen, including molecular structure and size, the composition of the diet and transport across intestine (reviewed by Varanasi et al. 1989 and Ramesh et al. 2004). Dietary fats, for instance, have been shown to increase the bioavailability of lipophilic PAHs such as BaP in both mammals (Laurent et al. 2001) and fish (Vetter et al. 1985). The presence of bile has also been shown to play a crucial role in the uptake of large lipophilic PAHs such as BaP, but not for Phen (Rahman et al. 1986).

Hepatic and intestinal metabolism is an important factor in the elimination of PAHs. The low levels of radioactivity detected in the livers of specimens exposed to BaP compared to Phen could indicate a more efficient hepatic metabolism of BaP than Phen. Low levels of radioactivity in the liver have also been reported in sea bass (*Dicentrarchus labras*) force fed BaP (Lemaire et al. 1992). A more efficient metabolism of BaP compared to Phen is also supported by Niimi and Palazzo (1986) who showed that the half-life ($t_{1/2}$) of BaP was significantly shorter (<2 days) in rainbow trout (*Oncorhynchus mykiss*) intragastrically fed BaP in comparison to Phen ($t_{1/2} = 9$ days). Although Phen is metabolised by CYP450 isoenzymes, it has been shown not to induce CYP450 activity in several fish species (e.g. Goksøyr et al. 1986; Billiard et al. 2004), potentially leading to a reduced biotransformation efficiency of this compound compared to BaP. This was further supported by a lower rate of metabolism of Phen compared to BaP in liver microsomes of brown bullhead (*Ameriurus nebulosus*; Pangrekar et al. 1995). Although only based on a few specimens, females seemed to show higher tissue levels of BaP compared to males after both autoradiographical examination and scintillation counting. These gender differences are not yet understood but deserve further investigation. In the present study, polar cod was likely in an advanced gonadal developmental stage (Hop et al. 1995) at the time of the experiment (November), potentially showing gender differences in liver P450 content or activity and thus gender-specific bioaccumulation potential for lipophilic compounds. This is in accordance with data from channel catfish, *Ictalurus punctatus*, where general gender differences in P450 isoforms as well as gender differences in CYP1A induction after treatment with β-naphthoflavone have been described (Perkins and Schlenk 1998). Furthermore, there is a known cross-talk between the aryl hydrocarbon receptor and the estrogen receptor (Gräns et al. 2010), also potentially playing a role in this gender effect. Increased CYP1A induction in males would indicate a faster metabolism and thus lower retained amounts of the investigated substance. Since CYP1A is unlikely induced by Phen, also the lack of gender difference in the Phen-exposed group can be explained by this model.

Tissue-bound residues of radioactivity from both BaP and Phen were detected in the intestinal mucosa and most probably reflect site-specific CYP1A-catalysed metabolism and covalent binding of reactive intermediates. These observations are in accordance with previous studies with rainbow trout (Sandvik et al. 1998) and polar cod (Ingebrigtsen et al. 2000) where bound residues of BaP-derived radioactivity were detected in the intestinal mucosa. CYP1A activity has been reported in the intestinal tract of several fish species, localised in the epithelial cells of the mucosa as well as the vascular endothelium in the lamina propria (Stegeman et al. 1991; Smolowitz et al. 1991, 1992). In the present study, the tissue-bound BaP-derived radioactivity in the intestinal wall was far higher than the Phen-derived radioactivity. This observation strongly indicates that reactive intermediates of BaP are formed to a far greater extent compared with Phen in the intestinal mucosa of polar cod.

The present study suggested that the main excretory route for both compounds and their related metabolites was via the bile. The absence of BaP-related radioactivity in the urine and the low levels of urinary radioactivity related to Phen (through day 12) indicated that urinary excretion played a secondary role. Biliary excretion of PAHs is well documented in fish (e.g. Solbakken and Palmork 1981; James et al. 1991; Lemaire et al. 1992; Van Veld et al. 1997), and PAH metabolites in fish bile are widely used as

biomarker of PAH exposure (Beyer et al. 2010). PAHs taken up via the diet will primarily be transported to the liver via the hepatic portal vein and undergo first path metabolism, where their metabolites will be secreted to the bile (Varanasi et al. 1989; Kleinow et al. 2008). This pathway was supported for polar cod in the present study as radioactivity was detected in both liver and bile after administration of both compounds. Nevertheless, some renal elimination seemed to take place for Phen but not for BaP. This reflects important toxicokinetic differences between these two compounds and should be further investigated. Finally, polar cod as well as other polar fish species possess aglomerular kidneys (Christiansen et al. 1996), an adaptation to retain small antifreeze proteins (Eastman and DeVries 1986). As earlier suggested, this adaptation strengthens the hypothesis that elimination of PAHs and their related metabolites is primarily occurring via the bile in polar cod (Christiansen et al. 1996; Ingebrigtsen et al. 2000).

The high levels of radioactivity in the bile and intestinal tract throughout the study were most probably a result of enterohepatic circulation. Furthermore, the higher levels of Phen-derived radioactivity compared to BaP-derived hepatic radioactivity indicated a higher metabolic rate of the latter. Enterohepatic circulation implies that hepatic metabolites excreted in bile are reabsorbed from the intestine and transported to the liver via portal blood. Accordingly, the retention time of the metabolites may be prolonged depending on the extent of reabsorption. Furthermore, enterohepatic circulation implies that the liver will be continuously exposed to the mother compound and its metabolites during the entire elimination period. This mechanism is regarded to be important for PAH compounds undergoing metabolic bioactivation, such as BaP, because reactive intermediates readily bind to macromolecules, such as proteins and nucleic acids which may in turn result in cellular damage. To our knowledge, the significance of enterohepatic circulation for the kinetics of organic contaminants has not been as well studied in fishes as it has been in mammals (Dutczak et al. 1991; Ramesh et al. 2004; Jandacek and Tso 2007).

In conclusion, the present study showed a more important systemic distribution of Phen compared to BaP. This finding may be of significance for an Arctic species potentially at risk of exposures to PAHs of petrogenic origin. Further work is necessary to investigate the toxicokinetics of both single and mixtures of PAHs and the factors contributing to their bioavailability and transport across the intestines in dietarily exposed fish (Erickson et al. 2008). Ongoing studies will complement these results by evaluating the comparative toxicokinetics of Phen between water and dietary exposure. A comprehensive understanding of the mechanisms controlling uptake, distribution and elimination of this complex and vast group of organic contaminants is still largely lacking for fish species. This knowledge is important for both the evaluation of the risk these pollutants may represent to wild life species and for interpretation of their toxicity, especially for Arctic species where little data are yet available.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed were in accordance with the ethical standards of the Norwegian animal welfare authorities.

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References

AMAP (1998) AMAP assessment report: Arctic pollution issues. Arctic Monitoring and Assessment Programme (AMAP), Oslo

Bergman K (1979) Whole-body autoradiography and allied tracer techniques in distribution and elimination studies of some organic solvents. Scand J Work Environ Health 5:1–263

Beyer J, Jonsson G, Porte C, Krahn MM, Ariese F (2010) Analytical methods for determining metabolites of polycyclic aromatic hydrocarbon (PAH) pollutants in fish bile: a review. Environ Toxicol Pharmacol 30:224–244

Billiard SM, Bols NC, Hodson PV (2004) In vitro and in vivo comparisons of fish-specific CYP1A induction relative potency factors for selected polycyclic aromatic hydrocarbons. Ecotoxicol Environ Saf 59:292–299

Blasco KA, Blasco SM, Bennett R, MacLean B, Rainey WA, Davies EH (2010) Seabed geologic features and processes and their relationship with fluid seeps and the benthic environment in the Northwest Passage. Geological Survey of Canada, Open File 6438, Ottawa

Brandt I, Brittebo EB (1989) The use of autoradiography as a tool to study xenobiotic metabolism. In: Huston DH, Caldwell J, Paulson GD (eds) Intermediary xenobiotic metabolism in animals: methodology, mechanisms and significance. Taylor and Francis, London, pp 295–314

Christiansen JS, Dalmo RA, Ingebrigtsen K (1996) Xenobiotic excretion in fish with aglomerular kidneys. Mar Ecol Prog Ser 136:303–304

Couillard CM, Laplatte B, Pelletier E (2009) A fish bioassay to evaluate the toxicity associated with the ingestion of benzo[a]-pyrene-contaminated benthic prey. Environ Toxicol Chem 28:772–781

Dahle S, Savinov VM, Matishov GG, Evenset A, Næs K (2003) Polycyclic aromatic hydrocarbons (PAHs) in bottom sediments of the Kara Sea shelf, Gulf of Ob and Yenisei Bay. Sci Total Environ 306:57–71

Doré AG (1995) Barents sea geology, petroleum resources and commercial potential. Arctic 48:1–15

Dussauze M, Camus L, Le Floch S, Pichavant-Rafini K, Geraudie P, Coquillé N, Amérand A, Lemaire P, Theron M (2014) Impact of dispersed fuel oil on cardiac mitochondrial function in polar cod *Boreogadus saida*. Environ Sci Pollut Res Int 21:13779–13788

Dutczak WJ, Clarkson TW, Ballatori N (1991) Biliary-hepatic recycling of a xenobiotic: gallbladder absorption of methyl mercury. Am J Physiol 260:G873–G880

Eastman JT, DeVries AL (1986) Renal glomerular evolution in Antarctic notothenioid fishes. J Fish Biol 29:649–662

Erickson RJ, Nichols JW, Cook PM, Ankley GT (2008) Bioavailability of chemical contaminants in aquatic systems. In: Di Giulio RT, Hinton DE (eds) The toxicology of fishes. CRC Press, Boca Raton, pp 9–54

Foster KL, Stern GA, Carrie J et al (2015) Spatial, temporal, and source variations of hydrocarbons in marine sediments from Baffin Bay, Eastern Canadian Arctic. Sci Total Environ 506–507:430–443

Friedman CL, Selin NE (2012) Long-range atmospheric transport of polycyclic aromatic hydrocarbons: a global 3-D model analysis including evaluation of Arctic sources. Environ Sci Technol 46:9501–9510

Geraudie P, Nahrgang J, Leray J, Minier C, Camus L (2014) Endocrine disrupting effects of produced water in polar cod (*Boreogadus saida*). J Toxicol Environ Health A 77:557–573

Goksøyr A, Solbakken JE, Klungsøyr J (1986) Regioselective metabolism of phenanthrene in Atlantic cod (*Gadus morhua*): studies on the effects of monooxygenase inducers and role of cytochromes P-450. Chem Biol Interact 60:247–263

Gradinger R, Bluhm B (2004) In-situ observations on the distribution and behavior of amphipods and Arctic cod (*Boreogadus saida*) under the sea ice of the High Arctic Canada Basin. Polar Biol 27:595–603

Gräns J, Wassmur B, Celander MC (2010) One-way inhibiting cross-talk between arylhydrocarbon receptor (AhR) and estrogen receptor (ER) signaling in primary cultures of rainbow trout hepatocytes. Aquat Toxicol 100:263–270. doi:10.1016/j.aquatox.2010.07.024

Hop H, Gjøsæter H (2013) Polar cod (*Boreogadus saida*) and capelin (*Mallotus villosus*) as key species in marine food webs of the Arctic and the Barents Sea. Mar Biol Res 9:878–894

Hop H, Tonn WM (1998) Gastric evacuation rates and daily rations of Arctic cod (*Boreogadus saida*) at low temperatures. Polar Biol 19:293–301

Hop H, Graham M, Trudeau VL (1995) Spawning energetics of Arctic cod (*Boreogadus saida*) in relation to seasonal development of the ovary and plasma sex steroid levels. Can J Fish Aquat Sci 52:541–550

Ingebrigtsen K, Christiansen JS, Lindhe Ö, Brandt I (2000) Disposition and cellular binding of 3H-benzo(a)pyrene at subzero temperatures: studies in an aglomerular arctic teleost fish—the polar cod (*Boreogadus saida*). Polar Biol 23:503–509

James MO, Schell JD, Boyle SM, Altman AH, Cromer EA (1991) Southern flounder hepatic and intestinal metabolism and DNA binding of benzo[a]pyrene (BaP) metabolites following dietary administration of low doses of BaP, BaP-7, 8-dihydrodiol or a BaP metabolite mixture. Chem Biol Interact 79:305–321

James MO, Kleinow KM, Tong Z, Venugopalan C (1996) Bioavailability and biotransformation of 3 H-benzo[a]pyrene metabolites in in situ intestinal preparations of uninduced and BNF-induced channel catfish. Mar Environ Res 42:309–315

Jandacek RJ, Tso P (2007) Enterohepatic circulation of organochlorine compounds: a site for nutritional intervention. J Nutr Biochem 18:163–167

Kleinow KM, Nichols JW, Hayton WL, McKim JM, Barron MG (2008) Toxicokinetics in fishes. In: Di Giulio RT, Hinton DE (eds) The toxicology of fishes. CRC Press, Boca Raton, pp 55–152

Laender FD, Hammer J, Hendriks AJ et al (2011) Combining monitoring data and modeling identifies PAHs as emerging contaminants in the arctic. Environ Sci Technol 45:9024–9029

Laurent C, Feidt C, Lichtfouse E, Grova N, Laurent F et al (2001) Milk-blood transfer of ^{14}C-tagged polycyclic aromatic hydrocarbons (PAHs) in pigs. J Agric Food Chem 49:2493–2496

Lemaire P, Lemaire-Gony S, Berhaut J, Lafaurie M (1992) The uptake, metabolism, and biological half-life of benzo[a]pyrene administered by force-feeding in sea bass (*Dicentrarchus labrax*). Ecotoxicol Environ Saf 23:244–251

McElroy AE, Cahill JM, Sisson JD, Kleinow KM (1991) Relative bioavailability and DNA adduct formation of benzo[a]pyrene and metabolites in the diet of the winter flounder. Comp Biochem Phys C 100:29–32

Nahrgang J, Camus L, Gonzalez P, Goksøyr A, Christiansen JS, Hop H (2009) PAH biomarker responses in polar cod (*Boreogadus saida*) exposed to benzo(a)pyrene. Aquat Toxicol 94:309–319

Nahrgang J, Camus L, Carls MG, Gonzalez P, Jönsson M, Taban IC, Bechmann RK, Christiansen JS, Hop H (2010a) Biomarker responses in polar cod (*Boreogadus saida*) exposed to the water soluble fraction of crude oil. Aquat Toxicol 97:234–242

Nahrgang J, Camus L, Gonzalez P, Jönsson M, Christiansen JS, Hop H (2010b) Biomarker responses in polar cod (*Boreogadus saida*) exposed to dietary crude oil. Aquat Toxicol 96:77–83

Niimi AJ, Palazzo V (1986) Biological half-lives of eight polycyclic aromatic hydrocarbons (PAHs) in rainbow trout (*Salmo gairdneri*). Water Res 20:503–507

Pangrekar J, Kandaswami C, Kole P, Kumar S, Sikka HC (1995) Comparative metabolism of benzo(a)pyrene, chrysene and phenanthrene by brown bullhead liver microsomes. Mar Environ Res 39:51–55

Perkins EJ, Schlenk D (1998) Immunochemical characterization of hepatic cytochrome P450 isozymes in the channel catfish: assessment of sexual, developmental and treatment-related effects. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 121:305–310

Phillips DH (1983) Fifty years of benzo (a) pyrene. Nature 303:468–472

Rahman A, Barrowman JA, Rahimtula A (1986) The influence of bile on the bioavailability of polynuclear aromatic hydrocarbons from the rat intestine. Can J Phys Pharmacol 64:1214–1218

Ramesh A, Walker S, Hood D, Guillén M, Schneider K et al (2004) Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons. Int J Toxicol 23:301–333

Sandvik M, Horsberg TE, Skaare JU, Ingebrigtsen K (1998) Comparison of dietary and waterborne exposure to benzo(a)pyrene: bioavailability, tissue disposition and CYP1A1 induction in rainbow trout (*Oncorhynchus mykiss*). Biomarkers 3:399–410

Smolowitz RM, Hahn ME, Stegeman JJ (1991) Immunohistochemical localization of cytochrome P-4501A1 induced by 3,3′,4,4′-tetrachlorobiphenyl and by 2,3,7,8-tetrachlorodibenzo-furan in liver and extrahepatic tissues of the teleost *Stenotomus chrysops* (scup). Drug Metab Dispos 19:113–123

Smolowitz RM, Schultzan ME, Stegeman JJ (1992) Cytochrome P4501A induction in tissues, including olfactory epithelium, of topminnows (*Poeciliopsis* spp.) by waterborne benzo(a)pyrene. Carcinogenesis 13:2395–2402

Solbakken JE, Palmork KH (1980) Distribution of radioactivity in the chondrichthyes *Squalus acanthias* and the osteichthyes *Salmo*

gairdneri following intragastric administration of (9-^{14}C) phenanthrene. Bull Environ Contam Toxicol 25:902–908

Solbakken JE, Palmork KH (1981) Metabolism of phenanthrene in various marine animals. Comp Biochem Phys C 70:21–26

Solbakken JE, Palmork KH, Neppelberg T, Scheline RR (1979) Distribution of radioactivity in coalfish (*Pollachius virens*) following intragastric administration of [9-^{14}C] phenanthrene. Bull Environ Contam Toxicol 23:100–103

Solbakken JE, Knap AH, Palmork KH (1982) Disposition of (9-^{14}C) phenanthrene in a subtropical marine teleost (*Haemulon sciurus*). Bull Environ Contam Toxicol 28:285–289

Solbakken JE, Solberg M, Palmork KH (1983) A comparative study on the disposition of three aromatic hydrocarbons in Flounder (*Platichthys flesus*). Fisk Dir Skr Ser HauUnders 17:473–481

Stegeman JJ, Lech JJ (1991) Cytochrome P-450 monooxygenase systems in aquatic species: carcinogen metabolism and biomarkers for carcinogen and pollutant exposure. Environ Health Perspect 90:101–109

Stegeman JJ, Smolowitz RM, Hahn ME (1991) Immunohistochemical localization of environmentally induced cytochrome P4501A1 in multiple organs of the marine teleost *Stenotomus chrysops* (scup). Toxicol Appl Pharm 110:1–17

Ullberg S (1954) Studies on the distribution and fate of ^{35}S-labelled benzylpenicillin in the body. Acta Radiol 118:1–110

Ullberg S (1977) The technique of whole-body autoradiography. Cryosectioning of large specimens. Sci Tools LKB Instrum J, Special issue:1–29

Van Veld PA, Vogelbein WK, Cochran MK, Goksøyr A, Stegeman JJ (1997) Route-specific cellular expression of cytochrome P4501A (CYP1A) in fish (*Fundulus heteroclitus*) following exposure to aqueous and dietary benzo[a]pyrene. Toxicol Appl Pharm 142:348–359

Varanasi U, Stein JE, Nishimoto M (1989) Biotransformation and disposition of PAH in fish. In: Varanasi U (ed) Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment. CRC Press, Boca Raton, pp 93–149

Vetter RD, Carey MC, Patton JS (1985) Coassimilation of dietary fat and benzo(a)pyrene in the small intestine: an absorption model using the killifish. J Lipid Res 26:428–434

Yunker MB, Macdonald RW (1995) Composition and origins of polycyclic aromatic hydrocarbons in the Mackenzie River and on the Beaufort Sea shelf. Arctic 48:118–129