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Bioaccumulation of phenanthrene and benzo[a]pyrene in *Calanus finmarchicus*

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ABSTRACT

With petroleum exploration and development expanding in the Arctic (AMAP, 2007) there is a need to obtain additional information on the ecotoxicology of Arctic organisms. Here we perform 192 h laboratory exposure experiments on the keystone Arctic zooplankton species, *Calanus finmarchicus*. We trace the accumulation and depuration of two polycyclic aromatic hydrocarbons (PAHs): phenanthrene and benzo[a]pyrene (B[a]P) using ^{14}C labeled PAH compounds. Copepods were not fed during the experiment, limiting uptake to diffusion processes alone. The lighter PAH compound, phenanthrene, accumulated rapidly in *C. finmarchicus*, reaching steady state within 96 h. The heavier PAH compound, B[a]P, accumulated more slowly and steady state was not reached within the 192 h exposure period. As expected, the bioconcentration factor (BCF) for B[a]P was higher than for phenanthrene in accordance with a higher octanol/water partition coefficient for B[a]P (log K_{ow}=6.04) compared to phenanthrene (log K_{ow}=4.53). However, for both compounds, log BCF was lower than log K_{ow} that may indicate active biotransformation and excretion of the selected PAH compounds. These findings on the bio-uptake kinetics for petroleum hydrocarbons are essential for evaluating the potential consequences of an oil spill in the Arctic.

1. Introduction

The petroleum industry is expanding exploration and development activities northward into the European Arctic from the north Atlantic into Greenland, northern Norway and northwest Russia (AMAP, 2007). As development moves northward, the associated increase in operational and transport activities will lead to a higher risk of accidental releases of oil to the marine environment. In order to evaluate the potential environmental consequences of increased activities, there is a need for more information on responses of individual organisms exposed to petroleum hydrocarbon compounds. Such data is sparse for Arctic dwelling organisms (Chapman and Riddle, 2005; Olsen et al., 2007) and it remains unclear whether Arctic and temperate dwelling organisms accumulate and respond differently to petroleum compounds. Further ecotoxicological studies with cold-water dwelling organisms are required as a basis for the development of appropriate environmental protection guidelines for both routine operations and emergency response procedures.

Crude oil is a complex mixture of chemical compounds, such as alkanes, naphthenes, aromatic hydrocarbons (including polycyclic aromatic hydrocarbons (PAHs) and also non-hydrocarbon compounds (Wauquier, 1995)). PAHs are considered the most toxic (Hylland, 2006). PAHs are hydrophobic exhibiting log octanol/water partition coefficients (log K_{ow}) ranging from 3.4 (e.g. naphthalene) to around seven for the heavier compounds (e.g. indeno(*1,2,3,cd*)pyrene) (Neff and Burns, 1996; Mackay, 2006). Many authors have found a linear relationship between log K_{ow} and log bioconcentration factor (BCF) (Veith et al., 1979; Mackay, 1982; Hawker and Connell, 1986) indicating that bioaccumulation is linked to hydrophobicity for a given PAH compound. However, several studies also show that lower bioavailability and higher metabolism of the heavier compounds may modify this linear relationship and the accumulation of the heavier compounds may be lower than expected (Southworth et al., 1980; Spacie et al., 1983; van Hattum et al., 1998; Baussant et al., 2001b).

Hence the resulting effect on an organism exposed to crude oil may vary depending on the combination of an organism's ability to bioaccumulate, metabolize and excrete these compounds.

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Vertebrates such as fish are generally able to metabolize and excrete PAHs (Spacie et al., 1983), while metabolism are known to vary considerably among invertebrate species (Livingstone, 1998). Several copepod species have been shown to accumulate PAHs (Harris et al., 1977; Duesterloh et al., 2002; Carls et al., 2006; Cailleaud et al., 2007; Berrojalbiz et al., 2009; Cailleaud et al., 2009a) but little is known on the uptake processes at low temperatures.

An important link in the energy transfer from the lower to the higher trophic levels in the northern seas is the predominantly herbivorous copepods of the genus *Calanus* (Søreide et al., 2008). *Calanus* copepods accumulate lipids during the short Arctic productivity season, surviving the winter by diapausing in deeper waters (Falk-Petersen et al., 2009). The *Calanus* species complex consists of three species (*C. finmarchicus*, *C. glacialis* and *C. hyperboreus*) in the northern Atlantic. The further north and deeper the *Calanus* species are found, the larger size, lipid reserves and longer life span they exhibit (Falk-Petersen et al., 2009). The species in focus here is *C. finmarchicus,* the smallest of the northern residing *Calanus* species.

In this paper we report the uptake kinetics of two PAH compounds, i.e. phenanthrene and benzo[a]pyrene (B[a]P), in *C. finmarchicus*. Phenanthrene is a three ringed PAH with a log K_{ow} of 4.53 and B[a]P is a five ringed PAH with a log K_{ow} of 6.04 (Meador et al., 1995). Based on the distinct chemical characteristics of the two studied PAH compounds, we hypothesize that the more hydrophobic compound B[a]P will bioaccumulate to a greater degree than phenanthrene.

2. Materials and methods

The accumulation and depuration of phenanthrene and B[a]P was examined in stage V copepodites (CV) of *C. finmarchicus* using ^{14}C labeled compounds during March 2009. Solutions of 9-^{14}C phenanthrene (specific activity; 1.92 GBq mmol^{-1}, purity 99.7 percent, Moravek Biochemicals, Brea, CA, USA) and 7,10-^{14}C Benzo[a]-pyrene (B[a]P) (specific activity; 2.33 GBq mmol^{-1}, purity 99.2 percent, GE healthcare UK, Buckinghamshire, UK) were prepared daily by mixing filtered sea water with labeled and unlabeled chemicals from prepared stock solutions. Stock solution of phenanthrene was prepared in methanol (final solvent concentration in exposure was 15 μl l^{-1}), while B[a]P was solved in toluene (final solvent concentration in exposure was 15 μl l^{-1}). These solvent concentrations are below effect concentrations for aquatic invertebrates (Calleja and Persoone, 1993). Our target PAH exposure concentration was 2 μg l^{-1}. This is a realistic concentration that may be experienced near oil spills or as produced water from petroleum installations (Reddy and Quinn, 1999; Neff et al., 2006). For phenanthrene, the ratio labeled:unlabeled was 1:6.3, while for B[a]P the ratio was 1:3.7 labeled:unlabeled. These ratios, labeled:unlabeled, were used to calculate exposure concentrations in seawater and copepods over time. Copepod specimens were collected near Tromsø, Northern Norway (69°46′N; 19°08′E), by vertical hauls (180–0 m) using a WP-3 net mounted with a closed cod-end. After collection, specimens were maintained in 25 l polypropylene containers with filtered sea water. Prior to the start of the experiments, containers with copepods were stored in a temperature controlled room at 2 °C in dimmed light.

Experiments were run as semi-static exposures with copepods stored in 400 ml glass beakers. These were kept at 2 °C in dimmed light. The exposure phase lasted for eight day (192 h) followed by a four day (96 h) depuration phase. During the exposure phase, water solutions (filtered seawater+chemical compound) were exchanged daily. Similarly, during the depuration phase, filtered seawater was exchanged daily. The same beakers were used throughout each experiment to minimize chemical exchange to beaker walls, but changed on transition to depuration phase. In the phenanthrene experiment, the number of copepods in each beaker was 18–28 (n=5): in the B[a]P experiment, the number of copepods was 9–14 (n=4). Water samples were collected daily throughout the experiment. During the exposure phase, copepod samples were removed at the following time points: 0, 6, 12, 24, 96 and 192 h. During the depuration phase, copepod samples were taken at 198, 204, 216 and 288 h from the start of the exposure experiment.

The copepod samples were taken by sieving the animals onto a metal sieve, followed by rinsing in ammonium formate (CH_5NO_2, 24 g l^{-1}) to remove salts and adhered compounds. The copepod samples were then transferred to glass vials with 600 (phenanthrene) or 300 (B[a]P) μl tissue solubilizer (Soluene 350, Packard Instruments). Ten ml of scintillation cocktail (Ultima Gold, Packard Instruments) was added to each vial after 24 h. After a short mixing the vials were stored at room temperature (20 °C). Within one month, the vials were placed in a liquid scintillation counter with quench correction (Tri-Carb 2900 TR, Perkin Elmer) and counted for 20 min or until two percent sigma was achieved.

To obtain the concentration of test solutions added daily to the experimental beakers, 5 ml of test solution was transferred to individual plastic vials and mixed with 5 ml scintillation cocktail (Insta-Gel Plus, Packard). These samples were counted concurrently with the copepod samples.

Concentrations of phenanthrene and B[a]P in solution (μg l^{-1}) and copepod (μg g^{-1}_{lipid}) samples were quantified from the count rates detected by the scintillation counter given as disintegration per minute (DPM) using the specific activity of the compounds.

It is assumed that the lipophilic PAH compounds accumulate in the lipid sacs of copepods. Therefore, chemical concentrations in biological specimens are reported on a lipid weight basis (μg g^{-1}_{lipid}) to facilitate the comparison to other experiments (Livingstone, 1998). In the present study, lipid weights were analyzed by Unilab AS by the gravimetric method developed by Folch et al. (1957). Three replicates from each experiment of non-exposed copepods were sorted out along with experimental specimens, i.e. prior to each experiment and analyzed for lipids. No difference was detected between the in total six replicates and the samples were pooled. These were then used to report the phenanthrene and B[a]P concentrations obtained in the present study on a lipid normalized basis.

2.1. Data analyses

Analyses of the data were performed using Sigmaplot 10.0 and SPSS 16.0. To evaluate differences in the uptake of phenanthrene and B[a]P in *C. finmarchicus,* the derived lipid based concentrations of each compound were fitted by a first order kinetic model (Landrum et al., 1992b):

$$C_a = \frac{k_u C_w}{k_e}(1-e^{-k_e t}) \quad (1)$$

where C_a is the concentration of chemical substance (i.e. phenantherene or B[a]P) in the copepods (μg g^{-1}_{lipid}), k_u is the conditional uptake clearance rate (ml g^{-1} h^{-1}), k_e is the conditional elimination rate constant (h^{-1}), C_w is the concentration of PAH in water (μg ml^{-1}) and t is the time in (h).

Depuration was followed for 96 h and the depuration rate (k_d (h^{-1})) was determined from

$$\ln C_a = \ln C_a^0 - k_d t \quad (2)$$

The model was run two times for each substance. In the first run both the conditional uptake clearance rate (k_u) and (k_e) are allowed to be estimated by the model based on the chemical concentrations in the different compartments (copepods and water) at the different time points. However, as we also measured the equivalent to the conditional elimination rate constant, i.e. the deputation rate (k_d), directly, a second model run included the experimentally measured constant as a fixed parameter. Based on k_e and k_d, the depuration half life of both PAHs was calculated as ln 0.5 divided by k_e or k_d.

To test if steady state was reached within the accumulation phase of the experiments, tissue accumulations at times 48, 96 and 192 was compared by a one way ANOVA (Honkanen and Kukkonen, 2006).

Kinetic bioconcentration factors were calculated based on the model derived k_u and either k_e (phenanthrene) or k_d (B[a]P) values, e.g. $BCF_{k_u/k_e(k_d)} = k_u/k_e(k_d)$. In addition, the BCF at 192 h, i.e. when accumulation was terminated, was calculated as $BCF_{192\ h} = C_{a(192\ h)}/C_w$. The 95 percent confidence intervals for BCFs of phenanthrene and B[a]P was determined by a method developed by Bailer et al. (2000).

3. Results

3.1. Water concentrations

In the phenanthrene experiment the measured average water concentration during the accumulation phase (day 1–8) was 1.9 ± 0.05 (average ± SD) μg l^{-1} while for B[a]P the average concentration was 0.7 ± 0.35 (average ± SD) μg l^{-1}. The measured concentrations were based on radiolabel equivalents. In the depuration phase (96 h) less than 0.1 μg l^{-1} was measured in both treatments. Because the beakers in each treatment were changed at the beginning of the depuration phase, the source of the PAHs (less than 0.1 μg l^{-1}) may be excretion from the copepods.