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 hr 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.

Keywords: *Calanus finmarchicus*, bioaccumulation, phenanthrene, benzo[a]pyrene

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. More ecotoxicology investigations 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, including alkanes, naphthenes, aromatic hydrocarbons (including polycyclic aromatic hydrocarbons (PAHs), and also non-hydrocarbon compounds (Wauquier, 1995). Among these, 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 7 for the heavier compounds (e.g. indeno(*1,2,3,cd)*pyrene) (Mackay, 2006; Neff and Burns, 1996). Many authors have found a linear relationship between log K_{ow} and log bioconcentration factor (BCF) (Hawker and Connell, 1986; Mackay, 1982; Veith et al., 1979) 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 (Baussant et al., 2001b; Southworth et al., 1980; Spacie et al., 1983; van Hattum et al., 1998).

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. 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 (Berrojalbiz et al., 2009; Cailleaud et al., 2009a; Cailleaud et al., 2007; Carls et al., 2006; Duesterloh et al., 2002; Harris et al., 1977) 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* (Soreide 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.

Materials and methods

The accumulation and depuration of phenanthrene and B[a]P was examined in stage V copepodites (CV) of *Calanus 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 %, 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 %, GE healthcare UK, Buckinghamshire, UK) were prepared daily by mixing filtered sea water with labeled and unlabeled chemicals from prepared stock solutions. Our target exposure concentration was 2 µg l $^{-1}$. 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

($69^{o}46'$N; $19^{o}08'$E) 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 beakers. These were kept at 2 °C in dimmed light. The exposure phase lasted for 8 days (192 h) followed by a 4 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, 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 hours. After a short mixing the vials were stored at room temperature (20 °C). Within 1 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 2 % 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_{lipid}^{-1}) (Livingstone, 1998). In the present study, lipid weights were analyzed by Unilab AS, using three replicates of non-exposed copepods sorted out along with experimental specimens i.e. prior to each experiment. These were then used to report the phenanthrene and B[a]P concentrations obtained in the present study on a lipid normalized basis.

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);

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

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

$\mathbf{Slope_{uptake\ curve}} = k_u C_w k_u = \frac{\mathbf{Slope_{uptake\ curve}}}{C_w}$Depuration was followed for 96 h and the depuration rate (k_d) was determined from

$$\ln C_a = \ln C_a^0 - k_e t \ln C_a = \ln C_a^0 - k_d t$$

Equation (2)

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).

Bioaccumulation Factors (BCF) were calculated based on the model derived k_u and either k_e (phenanthrene) or k_d (B[a]P) values, e.g. $BCF_{ku/ke(kd)} = k_u / k_e$ (k_d), which is equivalent to the BCF at steady state. In addition, the BCF at 192 h, i.e. when accumulation was terminated,

was calculated as $BCF_{192h} = C_{a(192h)}/C_w$. The 95 % confidence intervals for BCFs of phenanthrene and B[a]P was determined by a method developed by Bailer et al. (2000).

Results

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.

Toxicokinetics of phenanthrene

For phenanthrene the conditional uptake clearance coefficient (k_u) was 324 ml g^{-1} h^{-1} and the conditional elimination clearance coefficient (k_e) was 0.06 h^{-1} (Table 1). Steady state was reached within the exposure time (192 h) and the concentration in the copepods was 10.0 µg g_{lipid}^{-1}. There was no significant difference between tissue levels of phenanthrene at times 48, 96 and 192 (one way ANOVA, $p>0.05$) (Fig 1). The depuration rate (k_d) was 0.0089, but this was not significantly different from 0 (t-test, $p=0.12$). Figure 1 shows accumulation and depuration data fitted in the toxicokinetic model for phenanthrene in *Calanus finmarchicus* using elimination rate k_e and depuration rate k_d, respectively.

Toxicokinetics of B[a]P

The accumulation of B[a]P differed from the accumulation of phenanthrene as the steady state was not reached within the 192 hr accumulation phase (one way ANOVA $p<0.05$) (see Figure 2). The estimated uptake rate for B[a]P (201.5 ml g^{-1} h^{-1}) was a little lower than estimated for phenanthrene but the toxicokinetic model derived elimination rate (k_e) was much lower ($2.1*10^{-11}$, Table 1). Thus, the model also support that steady state was not reached during the exposure time. The depuration rate (k_d) was much higher than the estimated k_e and differed significantly from 0 (t-test, $p<0.0001$).

Bioaccumulation factors

The $BCF_{ku/ke}$ of phenanthrene in *Calanus finmarchicus* was 5,326, approximately equal to $BCF_{192\ h}$ (5,252) (Table 2). For B[a]P, the $BCF_{ku/kd}$ was 43,219 compared to the $BCF_{192\ h}$ of 42741 (Table 2). The 95 % confidence interval determined for BCF of B[a]P was $14{,}400<BCF_{B[a]P}<207{,}500$ and for phenanthrene the 95 % confidence interval was $2{,}963<BCF_{phen}<9{,}615$.

Discussion

A two phase experiment (192 h exposure + 96 h depuration) was performed on non-feeding stage V copepods of *Calanus finmarchicus*, a keystone species of high latitude marine ecosystems. The experiment was designed to simulate overwintering adult *C. finmarchicus* populations exposed to hydrocarbon compounds. The study highlights factors controlling the passive partitioning of selected PAHs between biota and their surrounding aquatic environment. The study further provides valuable quantitative data contributing to evaluations of the possible consequences of accident scenarios in Arctic ecosystems.

Uptake and depuration kinetics

Considerably faster uptake and depuration kinetics was observed for the lower molecular weight PAH (phenanthrene, molecular weight = 178.2) compared to the higher molecular weight PAH (B[a]P, molecular weight = 252.3). A first-order toxicokinetic model described the accumulation of phenanthrene relatively well and predicted the steady state bioconcentration factor to be over 5000. The depuration rate determined from the tissue concentrations of phenanthrene was not significant. In part, this may be explained by the large variation found in the measured concentrations of phenanthrene (Figure 1). On the contrary, steady state was not reached for B[a]P and the measured tissue concentrations fitted by the toxicokinetic model showed a continuous increase in the of concentration of B[a]P in *C. finmarchicus* over time. The depuration data, however, showed some excretion of B[a]P from the copepod tissues. Lipid normalized tissue concentration of B[a]P at the end of exposure phase was approximately 30 µg g $lipid^{-1}$. Our finding of relatively slow kinetics and thus the comparable long time to reach equilibrium for *Calanus finmarchicus* exposed to higher molecular weight compounds is in agreement with previous investigations of other aquatic organisms. For example, PAH exposure studies performed on oligochaetes resulted in slow uptake kinetics for compounds with log K_{ow} > 5.6 (Ingersoll et al., 2003; Leppanen and Kukkonen, 2000; Van Hoof et al., 2001). Lower metabolism and excretion rates for higher log

K_{ow} PAHs has been identified as two key factors leading to the observed difference in uptake kinetics between high and low molecular weight compounds. Cailleaud et al. (2009) also reported higher total uptake and lower depuration rates for higher log K_{ow} PAHs for the copepod *Eurytemora affinis*. However, it is not clear whether equilibrium was achieved in the study. We conclude that the kinetics governing the uptake and depuration of PAHs for *C. finmarchicus* is in accordance with the general understanding of these processes for marine organisms.

Bioaccumulation factors

The log bioaccumulation factors (log BCF_{192h} and log $BCF_{ku/ke}$) obtained for *Calanus finmarchicus* exposed to phenanthrene and B[a]P are about 20 % lower than their respective log K_{ow} values (Table 2). However, we would expect a linear 1:1 relationship between lipid based log BCF and log K_{ow} when uptake and elimination of chemical compounds is governed by passive partitioning alone (Hoekstra et al., 2002; Mackay, 1982). General explanations to account for the observed deviations from a 1:1 relationship are: insufficient time to achieve equilibrium, overestimated bioavailability of chemical compounds (Landrum, 1989; van Hattum et al., 1998), active uptake via food items (Magnusson et al., 2007; Magnusson and Tiselius, 2010) and metabolism and excretion of compounds (Barron, 1990).

Bioavailability of compounds

Lower bioavailability has been identified as a factor leading to lower log BCF values compared to log K_{ow} values for high K_{ow} compounds (Landrum, 1989; van Hattum et al., 1998). Hydrophobic compounds such as B[a]P adsorb to surfaces such as the walls of experimental containers. Adsorption may lead to a relative reduction in the bioavailable fraction of B[a]P compared to phenanthrene. In the present experiment, initial concentrations of both phenanthrene and B[a]P were similar in the experimental containers. And there was no difference in the amount of chemical loss within the initial 24 h incubations for the two compounds (1-way ANOVA, $p > 0.05$). Bioavailability of high K_{ow} PAHs relative to low K_{ow} PAHs may also be lowered by the presence of particles in seawater as high K_{ow} PAHs readily bind to particles (Means et al., 1980). To control for this factor, we used the same seawater in both exposure experiments. Furthermore, seawater used in both exposure experiments was filtered through 1 µm filters. Our finding of higher bioaccumulation in *Calanus finmarchicus* for B[a]P relative to phenanthrene over the 192 h exposure period is therefore not attributed to a relative difference in bioavailability for these two compounds. However, we cannot rule out

231 that the observed lower BCF values relative to K_{ow} values for each compound is the result of
232 sorption to the surfaces of the experimental containers.
233
234 **Active uptake via food items**
235 Previous studies have shown higher bioaccumulation of chemical compounds in fed versus
236 unfed organisms but this may apply only for compounds which are not readily metabolized.
237 Copepods are found to metabolize PAHs but not PCBs (Cailleaud et al., 2009b) and this may
238 influence the active uptake via food. For example, Magnusson et al. (2007) examined the
239 uptake of PCB in *Calanus finmarchicus,* obtaining log bioaccumulation factors (BAFs)
240 exceeding their corresponding log K_{ow} values. In contrast, the BAF determined for active but
241 unfed copepods was lower than their corresponding log K_{ow} (Magnusson et al., 2007). In a
242 similar study of various PCB congeners, Magnusson and Tiselius (2010) observed higher
243 bioaccumulation in fed organisms relative to passive partitioning in *Acartia clausi*. However,
244 Berrojalbiz et al. (2009) observed no significant differences in BAF values between fed and
245 unfed copepods exposed to various PAHs. These were short term exposure experiments
246 lasting only 48 hours and the authors did not report whether equilibrium was achieved within
247 the exposure period but fed and unfed copepods were exposed to the same concentrations of
248 PAHs. An accumulation study showed that feeding reduced the accumulation of the readily
249 metabolized B[a]P but increased accumulation of hexachlorobiphenyl (HCB) which *Mysis*
250 *relicta* are not able to metabolize (Landrum et al., 1992a). Copepods may not respond like
251 mysids but these studies highlight the importance of dietary exposure as a pathway for
252 bioaccumulation in copepods. Feeding history should therefore be considered when
253 comparing BCF values from different studies. As we did not include fed copepods in the
254 experimental design, the BCF values obtained are a result of passive partitioning and
255 represent levels expected in overwintering specimens. The corresponding accumulation in fed
256 animals needs to be examined to establish if feeding status of exposed copepods explain the
257 deviation from the 1:1 relationship between log BCF and log K_{ow}.
258
259 **Metabolism and excretion of contaminants**
260 Metabolism and excretion may lead to the observed deviations between log BCF and log K_{ow}
261 based on the hydrophobicity model (Barron, 1990). PAH metabolism in copepods has been
262 investigated in several PAH exposure studies carried out on different marine species. In a
263 study of the copepod *Paracartia grani* Berrojalbiz et al. (2009) present evidence for PAH
264 metabolism based on a PAH mass balance analysis in their experimental treatments.

Eurytemora affinis eliminates PAHs at a higher rate than they eliminate PCB congeners with similar K_{ow}, suggesting that PAHs are actively metabolized (Cailleaud et al., 2009a) and earlier investigations indicate metabolism of naphthalene by *Calanus helgolandicus* (Corner et al., 1976; Harris et al., 1977). Metabolism and excretion of PAHs in invertebrates are presumably facilitated by the induction of cytochromes P450 enzymes (Rewitz et al., 2006). Induction of one P450 enzyme (CYP330A1) has been related to exposure to water soluble fraction of crude oil in *C. finmarchicus* (Hansen et al., 2009). However, further research is needed to reveal exact mechanisms involved in the metabolism and excretion of PAHs in copepods. In vertebrate species the comprehension of cytochromes P450 enzymes function is more complete. Studies on rainbow trout (*Oncorhynchus mykiss*) have shown that while B[a]P cause an induction of CYP1A enzyme activity, phenanthrene does not have that ability (Bols et al., 1999; Hawkins et al., 2002). However, when the CYP1A enzymes have been induced they do assist metabolism of phenanthrene in rainbow trout (Hawkins et al., 2002). The metabolism rate of B[a]P have been shown to be higher in the Brown Bullhead (*Ictalurus nebulosus*) compared to the metabolism rate of phenanthrene (Pangrekar et al., 1995). In the present study neither the metabolism of PAHs nor the presence of metabolites has been examined and whether copepod metabolism reassemble vertebrate metabolism still waits to be answered.

According to the hydrophobicity model, higher log K_{ow} compounds should produce higher log BCF values. While this continuous linear relationship is often seen in invertebrate species, e.g. *Daphnia pulex* (Southworth et al., 1980) and *Mytilus edulis* (Baussant et al., 2001a), fish species often show lower than expected BCF of compounds with log $K_{ow} > 6$ (Baussant et al., 2001a; Southworth et al., 1980; Spacie et al., 1983). This deviation may be caused by lower bioavailability of higher log K_{ow} compounds or a selected enhanced metabolism of the more hydrophobic substances. Hoekstra et al. (2002) examined bioaccumulation of an array of organochlorine (OCs) pollutants in the arctic *Calanus* species *C. glacialis* and *C. hyperboreus* by comparison of levels in the copepods and in water. While distribution of log BAFs of OCs with log $K_{ow} < 6$ vs their respective log K_{ow} values followed a linear regression, for the OCs with log $K_{ow} > 6$, a curvilinear model explained the distribution better. Hoekstra et al. (2002) explained this by overestimation of bioavailable OC concentrations as well as inaccurate octanol-water coefficients and insufficient time to reach equilibrium. In the present study of *C. finmarchicus*, the bioaccumulation of only two PAHs was assessed. Although this is not sufficient to evaluate if the bioaccumulation changes according to hydrophobicity within this

299 species, similar differences between log BCF and log K_{ow} for phenanthrene and B[a]P (20 %
300 difference, Table 2) indicate that this is not the case in our study.
301
302 *Relevance to Arctic O&G expansion*
303 Expansion into the Arctic by the petroleum industry calls for careful evaluation of the
304 environmental risks and potential impacts of development activities for Arctic organisms and
305 ecosystems. Arctic biota exhibit a number of unique features such as high and seasonally
306 varying lipid contents and longer life spans that are important variables influencing the
307 outcome of exposures to chemicals in their environment. Our study provides new information
308 on the baseline bioaccumulation of two PAHs by one of the most important zooplankton
309 species living south of the polar front in the Barents Sea: the lipid rich zooplankton species *C.*
310 *finmarchicus*. The derived data are relevant to winter conditions, where *C. finmarchicus* is
311 diapausing at deeper water depths, surviving with no food.
312
313 The temporal evolution of crude oil weathering is a key factor to be considered when
314 assessing the effect of an oil spill on impacted biological resources (National Research
315 Council, 2003). In this study, we have documented the corresponding temporal changes in the
316 accumulation of two important PAH compounds demonstrating that the bioaccumulation of
317 the heavier PAH compound increases more slowly over time for *C. finmarchicus* with steady
318 state achieved considerably later for this PAH compound.
319
320 When examined in context with other studies, the evidence obtained through the present study
321 suggests that *C. finmarchicus,* as well as other copepod species, are capable of metabolizing
322 PAHs, leading to a lowering of the total PAH bioaccumulation in these organisms. As
323 predicted from the difference in K_{ow} values of the two PAHs, the BCF of B[a]P is higher than
324 BCF for phenanthrene and we observe no preferential metabolism of B[a]P relative to
325 phenanthrene. The difference in uptake kinetics between the two PAHs and the time needed
326 to reach equilibrium with surrounding water masses may have implications for environmental
327 risk assessment (ERA). These findings provide important baseline information to support
328 analyses of the fate and behavior of crude oil in the event of an accidental release into the
329 Arctic.
330
331 The data obtained in the present study further helps to extend current risk assessment
332 procedures to include bioaccumulation and critical body residue (CBR) in assessments of

biological impacts. The current basis of risk calculations relies on external concentrations, e.g LC_{50} values and PEC/PNEC ratios (Singsaas et al., 2008; Smit et al., 2008). As these toxicity metrics are less relevant for bioaccumulative chemicals, using internal concentrations to derive bioaccumulation and critical body residue (CBR) data are considered more relevant for estimating risk (Tamis et al., 2009).

In the northern Atlantic, the primary production season is limited to late March to September (Wassmann et al., 1994), while in the Barents Sea, primary production extends from May to early September (Wassmann et al., 2006). Accumulation may be considerably higher during spring and summer, when *C. finmarchicus* is both actively feeding and developing large lipid reserves. Therefore, additional bioaccumulation studies are needed to assess the relationship between the timing of primary production on the diet of *C. finmarchicus* and its relationship to PAH bioaccumulation.

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Table 1: Toxicokinetic variables for phenantherene and BaP in *C. finmarchicus* resulting from fitting the measured tissue residues (C^{14} equivalents) into the first-order kinetic model (Equation 1): (a) the elimination rate (k_e) was estimated in the model; (b) the experimentally determined (k_d) value was fitted in the model as a fixed parameter. Also half-lives were calculated similarly by using k_e ($T_{1/2}$= *0.693/k_e)* and k_d ($T_{1/2}$= *0.693/k_d).*
The reported chemical half lives in the copepod tissues were calculated by using estimated k_e ($T_{1/2}$= 0.693/k_e) or experimentally defined k_d ($T_{1/2}$= 0.693/k_d). NA = not applicable for the specified variable.

	r^2 (model)	k_u (ml g^{-1} h^{-1}±S.E)	k_e or k_d (h^{-1}± S.E)	r^2 (k_d)	$T_{1/2}$ (h)*
(a) k_e estimated in the kinetic model			*k_e*		
Phenanthrene	0.69	323.9±61.3	0.0608±0.0135	NA	11.4
Benzo[a]pyrene	0.92	205.1±11.14	$4.1*10^{-13}$±$3.8*10^{-11}$	NA	$1.7*10^{\wedge-14}$
(b)Measured k_d fitted in the kinetic model			*k_d*		
Phenanthrene	0.18	79.2±21.4	0.0089 ±0.0047	0.10	77.9
Benzo[a]pyrene	0.79	354.4±104.2	0.0082 ±0.0051	0.68	84.5

Table 2: Bioconcentration factors t 192 h (BCF_{192}) and at steady state (BCF_{ss}) for *Calanus finmarchicus* exposed to phenanthrene and benzo[a]pyrene and the respective log values. For phenanthrene, the elimination rate (k_e) and the corresponding k_u value are used to calculate BCF at estimated steady state, while for B[a]P, the depuration rate (k_d) and the corresponding k_u value are used. The log octanol-water partitioning coefficient (log K_{ow}) for both PAHs is given as well as the relative proportion between log BCF and log K_{ow}.

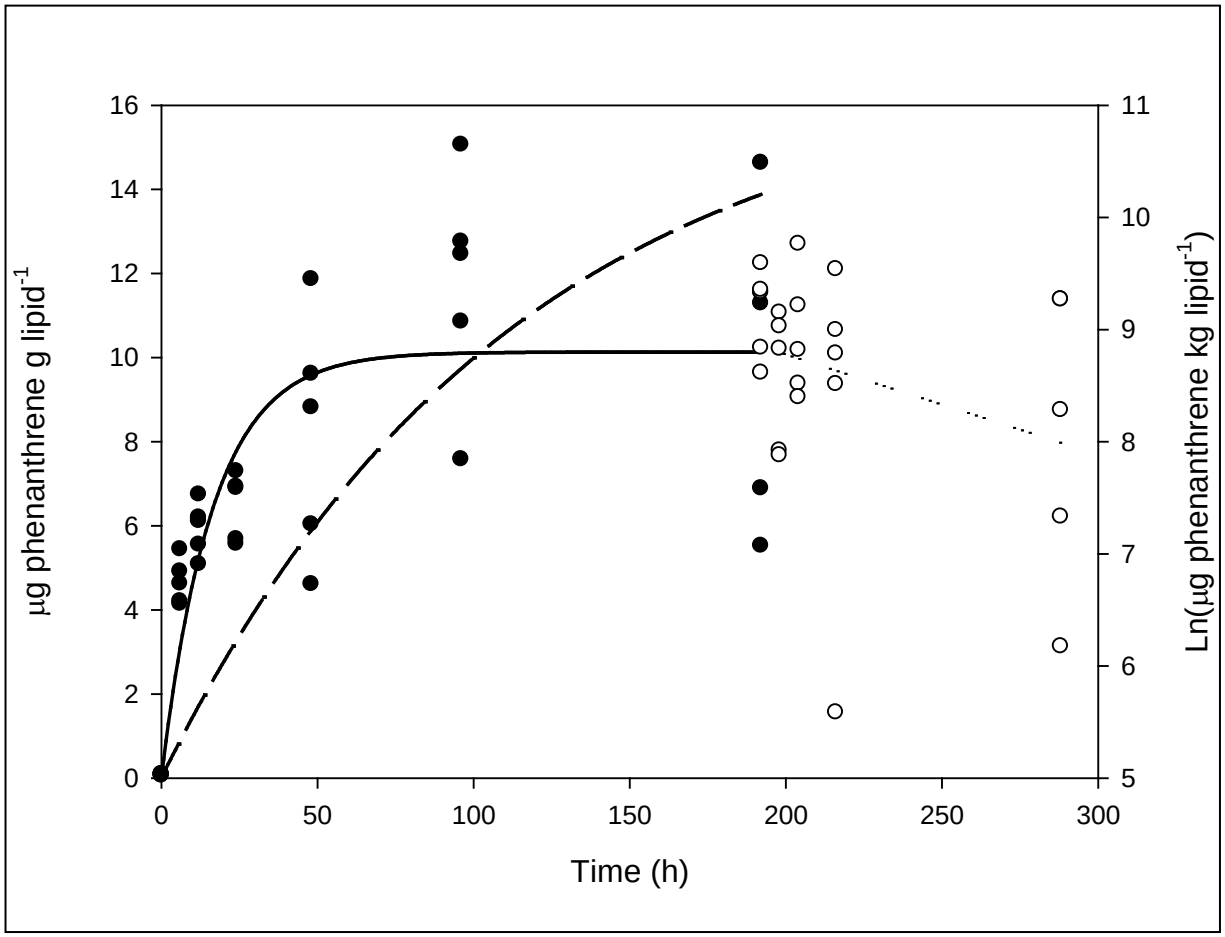
	log K_{ow}	BCF_{192}	logBCF_{192}	$BCF_{ku/ke}$/ $BCF_{ku/kd}$	log $BCF_{ku/ke}$/ $BCF_{ku/kd}$	log BCF/log K_{ow}
Phenanthrene	4.5	5,281	3.7	5,327	3.7	0.82
Benzo[a]pyrene	6.0	42,741	4.6	43,219	4.6	0.79

Figure legends

Figure 1: Lipid normalized accumulation of phenanthrene (^{14}C equivalents) in *Calanus finmarchicus.* Solid circles are the measured accumulation over a 192 h experiment (*n*=5) and solid line represents the toxicokinetics model run. Dashed line show the toxicokinetics model run based on k_d. Open circles refer to right y-axis and are concentration of phenanthrene in *C. finmarchicus* during depuration (ln(µg phenanthrene kg lipid^{-1})). Dashed & dot line is the linear regression (r^2=0.10) of the depuration data. Please note that the y-axis differ between Figure 1 and 2.

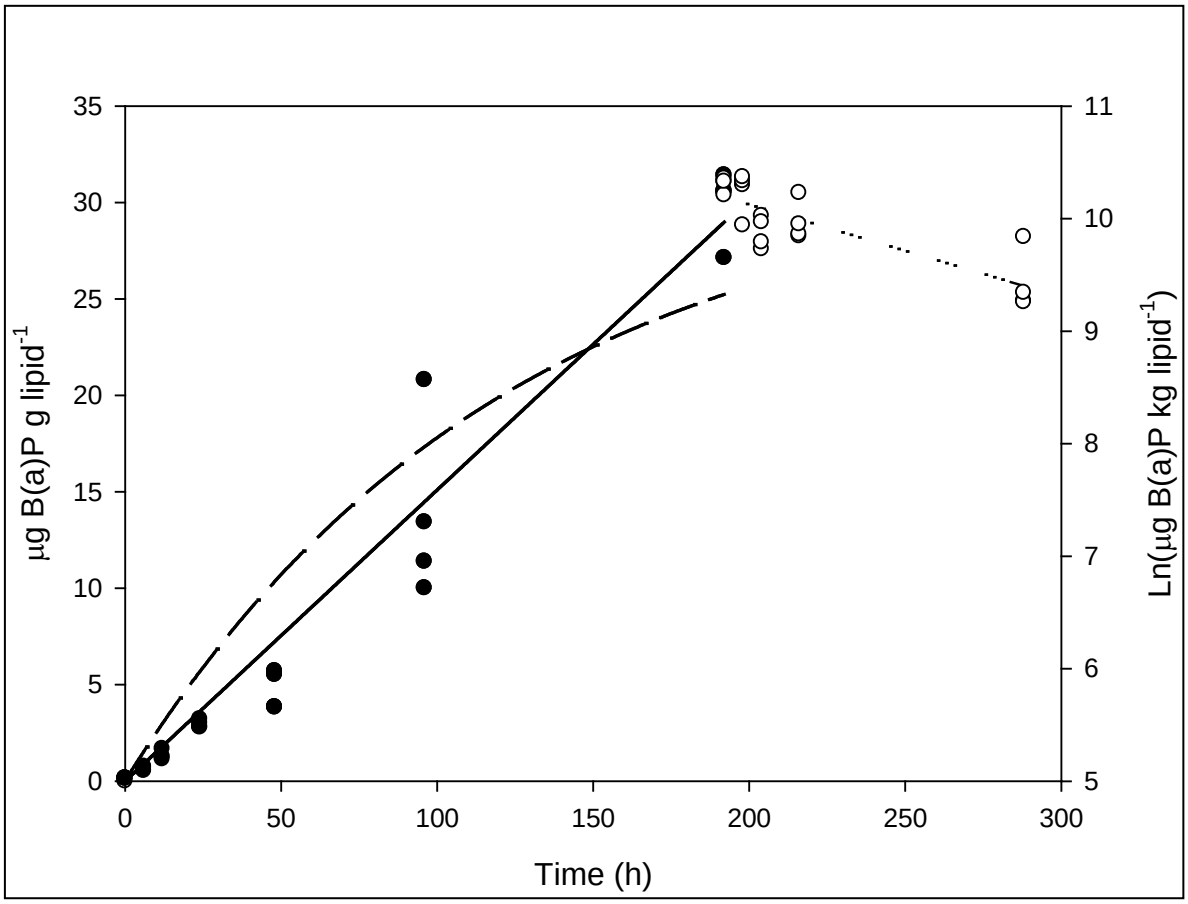
Figure 2: Lipid normalized accumulation of benzo[a]pyrene (^{14}C equivalents) in *Calanus finmarchicus.* Solid squares are the measured accumulation over a 192 h experiment (*n*=4) and solid line represents the toxicokinetics model run. Dashed line show the toxicokinetics model run based on k_d. Open circles refer to right y-axis and are concentration of B[a]P in *C. finmarchicus* during depuration (ln(µg B[a]P kg lipid^{-1})). Dashed & dot line is the linear regression (r^2=0.68) of the depuration data. Please note that the y-axis differ between Figure 1 and 2.

392 Figure 1:
393



394 Figure 2:

395



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