Expression of common biomarkers in Antarctic krill (*Euphausia superba*) exposed to an organochlorine pesticide

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Abstract

Persistent organic pollutant (POP) contamination of Polar Regions continues to present a major ecological challenge and an environmental stressor to local biota. Antarctic krill *(Euphausia superba)* are a keystone species of the Antarctic sea-ice ecosystem. Krill have repeatedly been found to accumulate a diverse array of POPs and thereby serve as vectors of these to the remainder of the Antarctic food-web. p,p'-Dichlorodiphenyldichloroethylene (p,p'-DDE) is a dominant POP compound accumulating in Antarctic krill and higher trophic level predators. Recently, p,p'-DDE uptake dynamics, associated behavioural and developmental toxicity were evaluated in this species. The present study investigated the response of enzymes with known roles in exogenous compound metabolism (glutathione *S*-transferase, GST and cytochrome P450 2B, CYP2B), neurotoxicity (acetylcholinesterase, AChE) and oxidative stress (glutathione peroxidase GPx) in Antarctic krill exposed to p,p'-DDE. CYP2B was not detectable in Antarctic krill. No strong concentration-responses resulted from the exposure to p,p'-DDE. These findings do not provide evidence for an activated detoxification response to this compound via the tested biochemical pathways in Antarctic krill. This is the first time that GST, AChE and GPx have been characterised in this species following pollutant exposure. Further research with additional pollutants and compound mixtures is necessary to assess the practical role of these enzymes as biomarkers of pollutant exposure in Antarctic krill. These first exploratory findings present a valuable contribution to a critical knowledge gap in polar ecotoxicology, namely the comparative sensitivity of polar organisms relative to temperate and tropical counterparts.

Keywords

Crustacean; Antarctic krill; Antioxidant Enzymes; Biomarker; Detoxification; Persistent Organic Pollutant;

Introduction

Despite the apparent pristine nature of Antarctica and it's remoteness from industry, persistent organic pollutants (POPs) have been detected in Antarctic biota since the 1960s (George and Frear 1966; Sladen et al. 1966). The presence of POPs so far from their sources can largely be attributed to long range environmental transport pathways and more recently *in situ* human activities (Wild et al. 2015). Once deposited at high latitudes, the volatility of POPs are markedly reduced (Wania and MacKay 1996), with the Earth's poles proposed as major "environmental sinks" for most of the world's remaining POPs.

Antarctic krill, *Euphausia superba*, a swarming Euphausiid crustacean, are a commercially valuable Southern Ocean keystone species. Krill are a sympagic species that grazes on phytoplankton, with their abundance and distribution closely linked to the Antarctic sea ice extent (Flores et al. 2012). Despite being one of the most abundant species on the planet, there are concerns for the long term survival of krill stocks in the face of climate change and over-fishing. Several studies have documented decreased krill abundance and density linked with reduced sea ice extent, whilst ocean acidification is projected to have a dramatic effect on

recruitment and species survival if carbon emissions are left unmitigated (Kawaguchi et al. 2011). The pivotal ecological role of krill means that a biomass decline would have far reaching repercussions for the reliant ecosystem (Atkinson et al. 2004; Nicol et al. 2008).

Diverse profiles and notable concentrations of POPs, including organochlorine compounds, have repeatedly been detected in Antarctic krill (Bengtson Nash et al. 2008; Corsolini et al. 2006; Corsolini et al. 2002a; Corsolini et al. 2002b). p,p'-Dichlorodiphenyldichloroethylene (p,p'-DDE) is one of the dominant compounds accumulating in Antarctic krill and higher trophic level Antarctic predators (Bengtson Nash et al. 2008; Bengtson Nash et al. 2013; Waugh et al. 2014). p,p'-DDE occurs in the environment as the most stable metabolite of the organochlorine compound dichlorodiphenyltrichloroethane, which is more commonly known as the pesticide DDT. Despite the basal position of krill in the Antarctic food web, investigations of the toxicological sensitivity of the species remain limited. Recently, the development of a series of Antarctic krill-based toxicological exposure assays permitted the first evaluation of p,p'-DDE toxicity in both larval and adult krill stages (Poulsen et al. 2012b; Poulsen et al. 2011; Poulsen et al. 2013). Measurement of p,p'-DDE in the bodies of Antarctic krill, which had been exposed to p,p'-DDE spiked into either seawater media or food in separate experiments, demonstrated efficient and linear uptake of p,p'-DDE over time via both exposure routes (Insert refs again..). Antarctic krill exposed to p,p'-DDE exhibited behavioural responses and altered larval development (Poulsen et al. 2012a; Poulsen et al. 2012b; Poulsen et al. 2011; Poulsen et al. 2013). Nothing is currently known about the underlying biochemical mechanisms of p,p'-DDE toxicity in Antarctic krill and their capacity for detoxification. It has often been proposed that the evolutionary isolation and underdeveloped detoxification systems of Antarctic species leave them vulnerable to the toxicity of anthropogenic contaminants (Chapman and Riddle 2005; Corsolini 2009). Further, polar organisms are characterized by gigantism and slow metabolism, making conventional toxicity evaluation time frames unsuitable and limiting comparison to temperate or tropical counterparts (King and Riddle 2001).

In crustaceans, the metabolism of foreign contaminants, or xenobiotics, predominantly occurs in the digestive gland and it is thought to proceed in a similar manner as in mammals. Metabolism is often described in three functional stages: Phase I, where xenobiotics are identified and transformed into more soluble metabolites; Phase II where metabolite solubility is further enhanced; and Phase III metabolism, which ensures effective excretion of by-products (Livingstone 1991; Rewitz et al. 2006). Phase I is primarily governed by a superfamily of specialised enzymes referred to as cytochrome P450, or CYPs for short. Phase II metabolism is performed by a variety of non-specific metabolising enzymes such as the glutathione *S*-transferases (GST). Up-regulation of GST activity has previously been observed in association with insecticide resistance (Enayati et al. 2005) and exposure to POPs (Gaume et al. 2014; Hoarau et al. 2001). Metabolism can lead to a state referred to as oxidative stress, which can be reduced by enzymes including, but not limited to, glutathione peroxidase (GPx), to prevent associated harmful effects such as lipid peroxidation and DNA damage (Livingstone 1991).

DDT is a neurotoxin, which primary mechanism affects crustacean sodium channels, subsequently leading to hyperactivity and death (Sánchez-Bayo 2012). A typically used marker

for neurotoxicity is acetylcholinesterase (AChE) inhibition, which impairs the transmission of signals between neurons, and which is the common mode of toxicity for organophosphate and carbamate pesticides (Hassall 1990). Previous research has also reported on AChE inhibition in response to organochlorine compounds, including DDT (Bhavan and Geraldine 2001; Galindo-Reyes et al. 2000; Martinez-Tabche et al. 1999). AChE inhibition may thus provide an easily measurable marker for exposure to DDT and its metabolites in krill.

Enzymes have been widely used as biomarkers of chemical exposure, with CYPs, GST, GPx and AChE some of the most frequently used. Biochemical screening is often more cost-effective than chemical analysis, permitting broad scale application. Further, molecular change signals a biological response to chemical exposure and serves as an early warning of possible toxic effects (Bengtson Nash et al. 2006). Successful implementation of the biomarker approach requires that a direct relationship is established between chemical exposure and expression of the target biomarker (Jemec et al. 2010). Nothing is currently known about the response of sub-cellular biomarkers to chemical exposure in Antarctic krill. The purpose of this exploratory study is to investigate the response of four commonly used biomarkers, GST, CYP2B, GPx and AChE, to sublethal p,p'-DDE exposure in Antarctic krill. The suitability of these enzymes as potential biomarkers of sub-cellular effects in Antarctic krill is discussed, thereby contributing new knowledge to the critical research gap surrounding the detoxification capabilities of endemic Polar species.

Methods

Exposure design

The experimental design used for exposure of Antarctic krill to p,p'-DDE is further described in (Poulsen et al. 2012a). Adult Antarctic krill were collected in the austral summer of 2006 from the eastern Antarctic sector (66° S 80° E). Animals were housed at the Australian Antarctic Division krill culturing facilities, Tasmania, Australia until p,p'-DDE exposure in 2007. The weight of exposed krill ranged from 364 – 927 mg wet weight. Prior to exposure krill were acclimatised for 24 hours under experimental conditions without food. Five krill were housed in 5-L glass beakers. Exposure seawater was collected from Bruny Island, Tasmania, and pre-filtered to 0.2 µm. Exposure solutions were spiked with p,p'-DDE using acetone as a solvent carrier (in a final concentration of 0.03 mL/L). Antarctic krill were exposed for 96 h using five p,p'-DDE exposure treatments (1, 5, 10, 15 and 20 µg L^{-1}), plus an additional seawater-only and a seawater-acetone (0.03 mL L^{-1}) control. Exposure media was renewed every 24 h. The setup included three replicate five litre test beakers for each treatment and control to ensure statistical power. The krill were not fed for the duration of the experiment. Upon the end of the experiment all animals were euthanised by placing in liquid nitrogen and samples were stored at -80°C until the time of analysis. The exposure experiment was duplicated within one month, with krill from the first experiment used for GST, GPx and AChE analysis, and krill from the second experiment used for CYP2B analysis.

Exposure Concentrations

The actual concentrations of seawater and those accumulated by krill were not measured in the present experiment. We confirmed in a supporting experiment conducted under identical conditions, however, that actual seawater concentrations were in accordance with nominal concentrations (Poulsen et al., 2012a). We also confirmed in the supporting experiment that krill uptake of p,p'-DDE took place throughout 96 h of exposure by measuring the concentrations in krill bodies (Poulsen et al., 2012a). The uptake was concentration-dependent and linear over 96 h. The method used to measure p,p'-DDE in krill bodies is destructive and would have rendered enzyme analysis impossible. The concentrations of p,p'-DDE in exposure media and internal body residues at 96 h as measured in the supporting experiment are given in Table 1. As the concentrations were not measured in the present experiment, the exposure concentrations will continue to be referred to by their nominal concentrations from here on (1, 5, 10, 15 and 20 µg L^{-1}).

Sample preparation of sub-cellular fractions

Cytosolic fraction

Crustacean digestive glands are highly autolytic. In order to minimise the chances of enzyme degradation during sample preparation, whole body extracts were used for enzyme analysis. Two krill from each replicate of the first experiment were pooled for GST and GPx biochemical assays. The krill were decapitated; heads were kept for AChE analysis. The bodies were homogenised in approximately 10 mL phosphate buffer (100 mM potassium phosphate pH 7.4, 0.1 mM phenylmethylsulfonyl fluoride, 100 mM potassium chloride and 1 mM ethylenediaminetetraacetic acid, modified from Koenig et al. (2013)) with a hand held electric homogeniser. The homogenate was centrifuged at 12000×g for 90 minutes at 4°C, and the supernatant re-centrifuged at 100000×g for 90 minutes at 4°C. The resulting supernatant (cytosol) was kept on ice until use.

Post mitochondrial fraction

Krill heads were pooled to determine AChE activity (approximately 0.24 g wet weight). Homogenisation was carried out according to Minutoli et al. (2002). Samples were homogenised in a 0.1 M Tris-HCl pH 8, 0.1% triton buffer with 1 mL for every 0.06 g tissue. The homogenate was centrifuged for 12000×g for 10 minutes at 4°C and the resulting supernatant (post-mitochondrial fraction) was stored on ice until needed.

Microsomal Fraction

Five krill from each replicate of the second experiment were pooled for CYP2B activity. Microsomes were prepared as per the cystolic fraction, and the resulting pellet (microsomes) from the 100000×g centrifuge was kept on ice until use.

Biomarker Assays

Biomarker assays were carried out in triplicate with positive and negative controls using a BioRad microplate spectrophotometer or a PolarStar Optima microplate reader. GST activity

was quantified by measuring the reaction of 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM reduced glutathione (Habig et al. 1974) with 150 µL of sample. The absorbance at 340 nm (25°C) was recorded for 5 minutes. GPx was determined using a commercial assay kit (Cayman Chemical). The decrease in absorbance was measured at 340 nm (25°C) for 5 minutes. AChE activity was determined by measuring the reaction of 0.425 mM 5,5′-dithiobis(2-nitrobenzoic acid), 1 mM acetylthiocholine iodide and 10 µL of sample at 405 nm (25°C) for 10 minutes (Ellman et al. 1961). Blank activity was measured in the absence of post-mitochondrial fraction. CYP2B activity was determined using the pentoxyresorufin *O*-dealkylase assay. The increase in fluorescence of 0.25 mM of NADPH, 6.2 µM pentoxyresorufin and 50 µL of sample was measured at 30°C for 10 minutes (λ excitation= 537 nm and λ emission= 583 nm). The reaction was calculated with a resorufin sodium salt standard curve with seven concentrations (0 nM – 80 nM) (Koenig et al. 2013). Protein content was determined using bovine serum albumen as a standard, ranging from 0–1000 mg mL^{-1}. The reaction was measured at 562 nm.

Statistical Analysis

All measurements are reported as mean ± standard error (S.E) unless otherwise stated. Activity is expressed as nmol min^{-1} mg $protein^{-1}$. Homogeneity of variance was assessed using a Levene Test and data was checked for normality using a Shapiro-Wilk test. AChE activity was analysed using a Kruskal Wallis test. GST activity was analysed using Welch's ANOVA with Dunnett's post hoc and GPx responses between exposure concentrations were analysed using a one way ANOVA with a Tukey post hoc test. Pearson's or Spearman's correlation was used to evaluate linear relationships between concentration and enzymes response. All statistical analyses were performed in SPSS, Inc., version 20.0 with p values of < 0.05 considered to be statistically significant. The acetone solvent control did not differ significantly from the seawater control; therefore both controls were pooled for the analysis (student's t-test, GST $t16 = -0.26$, $p = 0.800$, GPx $t16 = -1.18$, $p = 0.280$, AChE $t9.5 = -1.4$, $p = 0.193$).

Results

There was no mortality of any krill throughout the exposure period. Of the enzymes measured, none produced a p,p'-DDE concentration-dependent response at the range tested in Antarctic krill ($r = 0.190$, $n = 63$, $p = 0.135$; $r = 0.246$, $n = 63$, $p = 0.052$; $rs = -0.02$, $n = 57$, $p = 0.887$ for GST, GPx and AChE respectively). CYP2B was not detectable in these specimens. Glutathione *S*-transferase activity was elevated at all p,p'-DDE exposure concentrations compared to the control (48.63 ± 4.54) (**Error! Reference source not found.**), however, no treatment induced a significant increase in GST activity in Antarctic krill ($F(5,22.33) = 1.92$, $p = 0.130$). Interestingly the 1 and 20 µg L^{-1} concentrations expressed higher GST activity than the 5, 10 and 15 µg L^{-1}. Treatment activity ranged from 48.71 ± 2.34 for exposure to 15 µg L^{-1} to 63.24 ± 5.93 for 20 µg L^{-1}. Acetylcholinesterase activity was not correlated with p,p'-DDE exposure concentration. All exposure concentrations displayed lower activity than the control however no treatment was significantly inhibited by p,p'-DDE in Antarctic krill ($H(5) = 5.03$, $p = 0.412$, range 0.22 ± 0.12 – 0.27 ± 0.30). Glutathione peroxidase activity ranged from 14.04 ± 2.11 to 19.06 ± 0.98. There was no significant change in activity for any treatment ($F(5,57)$

= 1.97, p = 0.097). GPx and GST followed similar trends throughout the treatment responses, possibly suggesting there was an underlying factor, such as gender, which remained unaccounted for.

Discussion

Glutathione *S*-transferase

The observation of a slight induction of GST activity may suggest that a generic detoxification response was triggered in the krill, however with limited capacity. Indeed a basic capacity for detoxification of chemical exposure may explain the declining potency of p,p'-DDE noted during the exposure period (Poulsen et al. 2012a). After initial exposure to p,p'-DDE, krill mobility was observed to decrease and as the experiment progressed the response lessened (Poulsen et al. 2012a). p,p'-DDE is the most stable metabolite of DDT and it is unknown if this product is further metabolised in Antarctic krill, as is the case for some freshwater invertebrates (Lotufo et al. 2000; Lydy et al. 2000). Based on the present assay results, it does not appear that GST plays a major role in p,p'-DDE detoxification in this species. These findings contrast the DDT detoxification pathways known in other species. For example GST causes insecticide resistance and is responsible for the detoxification of DDT to p,p'-DDE, in insects and freshwater invertebrates (Clark and Shamaan 1984; Livingstone 1991). Further, increased GST activity is exhibited by molluscs exposed to p,p'-DDE in similar exposure concentrations to those used in this study (Hoarau et al. 2001). Although, the latter study found that only one specific class of GSTs were induced by p,p'-DDE, which have not yet been described in Antarctic krill (Clark et al. 2011).

A drawback of the use of GST as a biomarker is the variability of baseline levels demonstrated in previous studies (e.g. Koenig and Solé 2012). Being a generalist family of enzymes, GST induction is triggered by many endogenous and exogenous stimuli such as fasting, gender, vertical migration, season, metabolic rate, *etc*. (Jemec et al. 2010; Jemec et al. 2012; Tremblay et al. 2010). As this variability can mask the response of GST to xenobiotic exposure, further characterisation is needed, but was beyond the scope of this study. The effects of environmental stimuli can be reduced by sufficient acclimation to stable laboratory conditions (Jemec et al. 2010). The present experiment included a 24 h acclimation period to test conditions. It cannot be ruled out that the variability exhibited in krill in this study resulted from environmental factors. More likely the observed variation was caused by biological factors such as age or gender, for which a baseline has not been established in Antarctic krill.

Acetylcholinesterase

Acetylcholinesterase activity between crustacean species seems to be inversely proportional to size. Previous studies have reported low basal activity of AChE in *Euphausia superba* compared to smaller krill species (Minutoli et al. 2002). AChE activity exhibited in this study was slightly lower compared to reports from similarly sized crustacean species (Bolton-Warberg et al. 2007; Key and Fulton 2002; Lavarias et al. 2011). In the present study, the individual responses measured most likely reflect baseline levels. AChE is responsible for the

breakdown of neurotransmitters allowing for normal neurological function. Although the inhibition of AChE is not typically associated with DDT and its metabolites, AChE inhibition has previously been observed in crustaceans following DDT exposure (Galindo-Reyes et al. 2000; Martinez-Tabche et al. 1999). There is a small body of research that brings into question the specificity of AChE inhibition to organophosphate and carbamate pesticides. Non-dose dependent inhibition of AChE was noted in response to polycyclic aromatic hydrocarbon mixtures in Antarctic scallops (Bonacci et al. 2009), and metals have also been shown to inhibit AChE activity (Frasco et al. 2005). It is therefore recommended that AChE activity in *E. superba* be examined further, particularly in light of the low basal activity exhibited in this study. It may be valuable for future work to test if other OCs, including the parent metabolite DDT, and known AChE inhibitors such as chlorpyrifos, which was recently detected in Antarctic air and seawater (Bigot et al. 2016), can inhibit this enzyme in Antarctic krill.

Glutathione peroxidase

The absence of concentration dependent response for GPx activity indicates the detection of baseline levels of GPx. Up-regulation of antioxidant enzymes would be expected if krill were experiencing oxidative stress relating to p,p'-DDE exposure, *i.e.*, oxidative stress would trigger increased production of antioxidant enzymes. In mammalian systems, p,p'-DDE is known to cause oxidative stress through the generation of reactive oxygen species (ROS) (Pérez-Maldonado et al. 2005). This response has also been noted in bivalves, for which the production of ROS was suggested to be the main mode of toxicity (Dowling et al. 2006). The absence of a significant increase in GPx activity suggests that either; a) GPx is unable to respond to the high level of p,p'-DDE-induced ROS in Antarctic krill, *i.e.*, an inefficiency of the system potentially capped by a limiting component such as glutathione co-factor (as noted in response to other organochlorines e.g. Numan et al. 1990) or, b) the krill are not experiencing oxidative stress induced by p,p'-DDE. As the assessment of oxidative stress indicators (such as lipid peroxidation or protein carbonyl content) were outside the scope of this study, it is difficult to conclude whether or not the krill were experiencing oxidative stress. Generally the antioxidant capacity of polar invertebrates is quite high (Abele and Puntarulo 2004; Regoli et al. 2000). Antarctic krill have been found to experience very little oxidative stress in response to hypoxia despite the absence of significant up-regulation of antioxidant enzymes, including GPx (Tremblay and Abele 2015). Previous studies have found that Antarctic krill exhibit high levels of low molecular weight antioxidants, reduced glutathione and vitamin E (Dunlap et al. 2002), which afforded adequate ROS elimination through scavenging. It is possible that Antarctic krill may be able to cope with a p,p'-DDE mediated increase in ROS under normal basal GPx expression due to the abundance of these other antioxidants.

Detoxification capabilities of *Euphausia superba*

The applied exposure concentrations, although in line with routine toxicity testing protocols, were appreciably higher than those measured in Antarctic seawater (Bigot et al. 2016). Interestingly, despite these artificially elevated concentrations, Phase II enzyme activity did not provide support for an induction of a detoxification response in the species. These findings correspond well with the observation of toxic effects expressed throughout the original

behavioural experiment (Poulsen et al. 2012a). Enzymes of the cytochrome P450 complex are known to be induced by DDT and p,p'-DDE in mammals (Nims et al. 1998), and may be better candidates for studying p,p'-DDE detoxification in krill. Furthermore, CYPs have been associated with organochlorine insecticide resistance in crustaceans (Brausch and Smith 2009). DDT resistant fairy shrimp treated with cytochrome P450 inhibitors became more sensitive to DDT induced toxicity. It is possible that CYPs played a role in the apparent declining toxicity of p,p'-DDE throughout the behavioural experiment, perhaps more so than the limited capacity expressed by GST. Despite the non-detectability of CYP2B, cytochrome P450 is a large and diverse group of enzymes and further study of other CYPs may provide insights into krill detoxification mechanisms.

An in-depth comparison of the detoxification capabilities between analogous species or contaminants is not possible due to an almost complete lack of published data in this field. Very little is known about polar crustacean detoxification capabilities. It has been suggested that polar species may be more susceptible to foreign chemical contaminants compared to tropical or temperate species (Chapman and Riddle 2005; Corsolini 2009; de Hoop et al. 2011), and that the detoxification capabilities of polar species are lower than temperate and tropical counterparts. This seems to be the case for fish (e.g. Strobel et al. 2015), but the limited number of studies on polar crustaceans present conflicting results. A recent study compared the GST detoxification capabilities of Arctic and sub-Arctic copepods exposed to marine diesel. Interestingly the Arctic species expressed an almost 25 times increase in GST following diesel exposure, whereas GST in the sub-Arctic species only expressed a twofold increase (Hansen et al. 2013). By contrast, a study on the same two species of copepod found the opposite trend when exposed to artificially weathered marine diesel (Hansen et al. 2011). Compared to temperate species, adaption to a polar climate has led to reduced metabolic rates (Peck 2002), which effectively delay the onset of toxicity (King and Riddle 2001; Payne et al. 2014). Reduced metabolism is thus also expected to delay detoxification, possibly explaining the absence of induced GST activity found in this study over a 96 h exposure timeframe. Wild krill are likely to be exposed over a long period of time. Chronic exposure studies may be needed to determine if the absence of significant up regulation of GST is a function of delayed detoxification.

Conclusions

As a result of their unique adaptations to a cold climate, many biomarker responses for tropical or temperate species may not be directly comparable to polar species. True polar bio-indicator species are rare. To the authors' knowledge, this is the first time that the detoxification response of any polar crustacean has been assessed in response to organochlorine compound exposure. The findings presented here provide an important baseline for future work to establish the mechanisms of organochlorine toxicity and further our understanding of Antarctic krill detoxification capabilities. The activity of glutathione *S*-transferase, glutathione peroxidase and acetylcholinesterase do not to respond to p,p'-DDE in a concentration-dependent manner in Antarctic krill.

Compliance with Ethical standards

The authors declare no conflict of interest. All procedures performed involving animals were in accordance with the ethical standards of the institution at which the studies were conducted

Acknowledgments

The authors would like to acknowledge Mr. Michael Arthur for assistance with statistical analysis. This work received partial funding from ARC Discovery Grant DP0666891. Amanda Dawson is supported by PHD scholarship from ARC Discovery DP140100018. We would like to thank the anonymous reviewers whose comments greatly improved this manuscript.

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Table 1 Concentration of p,p'-DDE in exposure seawater media and the accumulated internal body burden of Antarctic krill (*Euphausia superba*) measured in a supporting experiment (reproduced with some modification from Poulsen et al. 2012a). The supporting exposure experiment was carried out under identical conditions and in duplicate (Test A and B). Body residues are reported in wet weight (w.w) and lipid weight (l.w)

Nominal Exposure Concentration		Exposure Media (2 hour)[a]		Exposure Media (24 hour)[a]		Internal Body Residue (µmol kg^{-1} w.w.)[b]			Internal Body Residue (mmol kg^{-1} l.w.)[b]		
µg L^{-1}	nM	µg L^{-1}	nM	µg L^{-1}	nM	A	B	Average[c]	A	B	Average[c]
1	3.1	1.1 (0.083)	3.5 (0.26)	1.1 (0.51)	3.4 (1.6)	8.6 (0.99)	13.6 (0.51)	11.1 (3.5)	0.28 (0.03)	0.66 (0.02)	0.47 (0.27)
5	15.7	4.4 (0.80)	13.9(2.5)	3.9 (0.69)	12.2 (2.2)	31.0 (7.7)	64.5 (2.2)	47.8 (24)	1.0 (0.25)	3.2 (0.11)	2.1(1.6)
10	31.4	10.7 (1.5)	33.8 (4.7)	12.9 (5.7)	40.6 (17.9)	58.8 (8.8)	117 (3.5)	87.7 (41)	1.9 (0.28)	5.7 (0.17)	3.8 (2.7)
15	47.2	15.1 (1.5)	47.6 (4.7)	16.8 (3.6)	52.7 (11.3)	90.7 (8.3)	157 (8.4)	124 (47)	2.9 (0.27)	7.7 (0.41)	5.3 (3.4)
20	62.9	22.0 (5.4)	69.0 (17.0)	42.7 (21)	135 (66.0)	110 (7.2)	169 (24)	140 (42)	3.5 (0.23)	8.3 (1.2)	5.9 (3.4)

[a] Average (±S.D) seawater concentration measured in test A (n=4 days, three replicates each).

[b] Average (±S.D) krill body residues (n=3, five individual measurements each)

[c] Average (±S.D) between tests A and B.