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Behavioural sensitivity of a key Southern Ocean species (Antarctic krill, *Euphausia superba*) to p,p′-DDE exposure

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ABSTRACT

Persistent organic pollutants (POPs) have been frequently measured throughout the Southern Ocean food web for which little information is available to assess the potential risks of POP exposure. The current study evaluated the toxicological sensitivity of a key Southern Ocean species, Antarctic krill, to aqueous exposure of p,p′-dichlorodiphenyl dichloroethylene (p,p′-DDE). Behavioural endpoints were used as indicators of sublethal toxicity. Immediate behavioural responses (partial immobility and tail flicking) most likely reflect neurotoxicity, while the p,p′-DDE body residue causing a median level of sublethal toxicity in Antarctic krill following 96 h exposure ($IEC50_{sublethal\ toxicity} = 3.9 \pm 0.21$ mmol/kg lipid weight) is comparable to those known to cause sublethal narcosis in temperate aquatic species. Critical body residues (CBRs) were more reproducible across tests than effective seawater concentrations. These findings support the concept of the CBR approach, that effective tissue residues are comparable across species and geographical ranges despite differences in environmental factors.

1. Introduction

Antarctica is the world's most isolated continent and the Southern Ocean and Antarctic continent ecosystems are generally considered pristine and relatively free of pollution. Persistent organic pollutants (POPs) are however, ubiquitous in the environment and were first reported in Antarctic biota in the 1960s (George and Frear, 1966; Sladen et al., 1966; Tatton and Ruzicka, 1967). Semi-volatile, hydrophobic POPs reach remote regions far from their emission sources via long range atmospheric transport (Wania and Mackay, 1993; von Waldow et al., 2010). As compound volatility and degradation rates decrease markedly as the temperature drops with increasing latitude, polar regions represent important sinks for these xenobiotics (Aguilar et al., 2002). Due to their lipophilicity, POPs have high bioaccumulation potential in the food web, where they can cause toxic effects. A number of contemporary studies have reported on POP residues in Antarctic krill (*Euphausia superba*) (e.g., Corsolini et al., 2002; Chiuchiolo et al., 2004; Goerke et al., 2004), which are fundamental to the Southern Ocean ecosystem. Recently, a comprehensive profiling of organohalogen contaminant burdens in Antarctic krill corroborated the continued availability of particularly, organochlorine pesticides to this key prey species and hence, to the remainder of the Antarctic food chain (Bengtson Nash et al., 2008). p,p′-dichlorodiphenyl dichloroethylene (p,p′-DDE) was a dominant organohalogen contaminant in Antarctic krill with measured residues from below detection to 18.9 nmol/kg lipid weight (l.w.) (average = 8.2 nmol/kg l.w. or on mass basis, 2.6 μg/kg l.w.).

In addition to ecological significance of Antarctic krill, the species is of high commercial value and harvested for primarily aquacultural and pharmaceutical industries (Kawaguchi and Nicol, 2007). Recent compilation of long term monitoring data has indicated that krill densities around the productive Antarctic Peninsula may have decreased by as much as 80 percent since the 1970s (Atkinson et al., 2004). A continued decline in krill stocks could have devastating implications for the entire Antarctic ecosystem. Despite the pivotal role and vulnerability of Antarctic krill there is, to our knowledge, yet no ecotoxicological data available for assessing the potential risks posed by POP exposure to this or any other polar krill species.

The social behaviour of an obligatory schooling species such as Antarctic krill is critical for all aspects of its biology (Hamner and Hamner, 2000). Behavioural changes (e.g., swimming, feeding and predator avoidance) are valuable sublethal endpoints used in ecotoxicological testing (Garcia-de la Parra et al., 2006; Beggel

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et al., 2010) and strong behavioural responses have previously been observed in Antarctic krill exposed to mineral oil (Ogrodowczyk, 1981). Assessment of sublethal toxicity can be particularly challenging in non-model species for which standard test protocols are lacking (Werner et al., 2010). For polar species with adapted life strategies and detoxification systems, there is a conspicuous need for optimised methods (King and Riddle, 2001; Hatlen et al., 2009). Ecotoxicological approaches generally use external concentration (e.g., that of the exposure water) as a surrogate for exposure dose. Due to large heterogeneity in toxicant bioavailability, uptake route and absorption, high inter-species variability is often observed in organism sensitivity to the same external exposure concentration. The effective tissue concentration for a given toxic response is more comparable across species as the above external factors have been addressed. This is the premise of the critical body residue (CBR) – or tissue residue – approach, where external exposure concentration is substituted with internal exposure concentration (McCarty and Mackay, 1993). To date, little information is however available to assess the applicability of CBRs across temperate and polar species.

The current study implemented newly developed protocols for Antarctic krill in a controlled laboratory environment at the Australian Antarctic Division (AAD) in Kingston, Tasmania. Behavioural changes were used as indicators of sublethal toxicity, which was examined during 96 h of aqueous exposure. First, the observed toxicity was evaluated on a body residue/effect basis to assess the internal toxicological sensitivity of Antarctic krill to POP exposure. In a second experiment, a detailed behavioural analysis was conducted by monitoring two concurrent behavioural endpoints (immobility and tail flicking) at regular time points throughout the experimental period. This paper contributes the first toxicological threshold data for environmental risk assessment of POPs in Antarctic krill.

2. Materials and methods

2.1. Chemicals

^{14}C-labelled p,p′-DDE (ring-UL-C14, ≥95 percent purity, 13.0 mCi/mmol) and unlabelled p,p′-DDE (1,1-dichloro-2,2-bis(4-chlorophenyl)ethene, 99 percent purity) were obtained from Bioscientific and Sigma Aldrich (both NSW, Australia). Nitric acid (69 percent), hydrogen peroxide (100 v/v) and high performance liquid chromatography (HPLC) grade acetone, methanol and chloroform were purchased from Merck (VIC, Australia). Scintillation cocktails (Aquassure and Ultima Gold XR) were from Perkin Elmer (VIC, Australia).

2.2. Test organisms

Antarctic krill were sampled from the Eastern Antarctic sector in 2006. As Antarctic krill are long-lived animals, they could be maintained at the AAD krill experimental aquarium (Kawaguchi et al., 2010) until tests were conducted in 2007 and 2009. Active adult krill used in tests were 2.4–4 cm in length (measured from rostrum to end of telson) and 90–400 mg wet weight. Test krill were pre-acclimated to experimental conditions for 24 h.

2.3. General experiment and exposure design

Two experiments were conducted, both in duplicate; (1) the CBR experiment (Tests 1A and B), where sublethal toxicity was evaluated on a body residue/effect basis in order to determine Antarctic krill internal sensitivity to p,p′-DDE exposure and (2) the behavioural experiment (Tests 2A and B), which focused on quantitative analysis of relevant behavioural endpoints without measurement of body residues.

All tests were carried out in a temperature and light controlled room at 0 °C (±0.5 °C). A 12:12 h light:dark cycle with continuous variation of light intensity (i.e., 0 lx at 6 AM increasing to 45 lx by midday then decreasing to 0 lx by 6 PM) was applied to simulate Southern Ocean spring/autumn conditions. Only solvent rinsed glassware and seawater grade stainless steel equipment were used in tests. Each test beaker contained five krill. During each 96 h exposure duration, krill were transferred to fresh exposure media every 24 h (static renewal). Exposure beakers were covered tightly with aluminium foil to keep dust out and minimise volatilisation of p,p′-DDE. Antarctic krill tolerate prolonged starvation (Nicol, 2006). To ensure that exposure took place via aqueous uptake only; no food was supplied during tests. On termination of the experiments, krill were snap frozen in liquid nitrogen. Each test incorporated the same exposure series with three replicates of each of the seawater control, the solvent control and the five p,p′-DDE treatments. Repeat tests were conducted within one month.

2.4. Exposure solutions

A nominal exposure concentration range of 1–20 μg/L (3.1–63 nM, molecular weight=318.03 g/mol) was applied to achieve effective tissue concentrations in 96 h. Aqueous solubility of p,p′,-DDE (40 μg/L (Bigger and Riggs, 1974)), corrected for salinity according to Setschenow relationship (Schwarzenbach et al., 2003) falls in the range of ca. 25–32 μg/L. At 0 °C, the applied concentration range is expected to approach the solubility limit. No precipitation of p,p′-DDE was however, observed in any treatment.

Seawater collected at Bruny Island, Tasmania, was filtered (0.2 μm) and equilibrated to 0 °C overnight prior to use. p,p′-DDE stock and working solutions were made up in acetone by weight. Exposure solutions were made up to the desired volume (4 L for the CBR experiment and 5 L for the behavioural experiment) in 5 L test beakers by simultaneous addition of chemical standard and seawater during mixing. The final concentration of acetone in treatments and solvent controls did not exceed 0.03 mL/L.

To facilitate liquid scintillation counting (LSC) of krill and seawater samples in the CBR experiment, ^{14}C-labelled p,p′-DDE standard was added to exposure solutions to yield 22.5 and 90 disintegrations per minute (dpm)/mL exposure water. Then unlabelled p,p′-DDE standard was added to achieve the desired exposure concentration. Correcting for isotopic dilution, total p,p′-DDE concentrations in exposure water and krill samples were determined by LSC analysis (Section 2.5.2). p,p′-DDE seawater exposure concentrations were determined in CBR test 1A, where 2 mL of exposure solution was sampled from each beaker twice daily; 2 h after renewal of exposure water to allow thorough mixing and again at the end of each 24 h static period. The measured seawater exposure concentrations are listed in Table 1. An apparent concentration increase in some treatments during static exposure is most likely an artefact due to formation and accidental sampling of particulate matter in the exposure water. The average initial (2 h) measurements of seawater concentrations were consistent throughout

Table 1
p,p′-DDE seawater concentrations and krill body residues measured in the CBR experiment.

Nominal conc.		Measured seawater conc.[a]				Average body residue (w.w. basis)[b]			Average body residue (l.w. basis)[b]			BCF[d]
		t=2 h		t=24 h		μmol/kg w.w.			mmol/kg l.w.			L/kg w.w.
μg/L	nM	μg/L	nM	μg/L	nM	Test 1A	Test 1B	Average[c]	Test 1A	Test 1B	Average[c]	Average
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 (0.032)	0.66 (0.025)	0.47 (0.27)	3171
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)	3439
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)	2595
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)	2605
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)	2029

[a] Average (±SD) seawater concentration measured in test 1A (n=4 (days), three replicates each).
[b] Average (±SD) krill body residues (n=3, five individual measurements each) on wet weight (w.w.) and lipid weight (l.w.) basis.
[c] Average (±SD) between tests 1A and 1B.
[d] BCF is the bioconcentration factor (average body residue/average measured seawater concentration).