

Di(2-ethylhexyl) adipate (DEHA) detection in Antarctic krill (*Euphasia superba* Dana)

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ABSTRACT

In this reported study, a novel high-performance thin-layer chromatography (HPTLC) method was developed for the detection and quantification of the toxic substance di(2-ethylhexyl) adipate (DEHA) in Antarctic krill. This procedure was based on the extraction of DEHA by ultrasonic solvent extraction with anhydrous ethanol, silica-gel column chromatographic separation, HPTLC detection and quantification using petroleum ether/ethyl acetate/acetone/glacial acetic acid (29:1:0.5:2d*, v/v/v/v) as the developing solvent and bromine thymol blue solution as the chromogenic agent. The content of DEHA in freeze-dried Antarctic krill was found to be ca. 0.63 ± 0.05 mg/g. The structure of DEHA in the Antarctic krill was subsequently determined by gas chromatography–mass spectrometry (GC-MS) and infrared chromatography, which verified the presence of this compound in the krill. The HPTLC method exhibited excellent accuracy, with a recovery of 97.1–101.6% and good precision with a relative standard deviation of 2.47–4.90%. The DEHA in Antarctic krill oil was extracted by n-hexane and detected using the same method described above, which verified that DEHA was also present in krill oil at a concentration of ca. 2.16 ± 0.08 mg/g. The presence of DEHA in krill oil is very concerning because of its demonstrated harmful ecotoxicity, and since Antarctic krill is the key link in the food chain in the Antarctic coastal marine ecosystem. The adverse effects of DEHA on Antarctic krill and the source of DEHA will be explored in future research.

KEYWORDS

toxicity; high-performance thin-layer chromatography (HPTLC); ecotoxins; plastic particles; marine pollution; krill oil

ABBREVIATIONS

2d: two drops; AU: absorbance unit; DEHA: di(2-ethylhexyl) adipate; FTIR: Fourier-transform infrared spectroscopy; GC-MS: gas chromatography-mass spectrometry; HPLC: high-performance liquid chromatography; HPTLC: high performance thin-layer chromatography; IR: infrared chromatography; RSD: relative standard deviation; SD: standard deviation; TLC: thin-layer chromatography

Introduction

DEHA is a suitable substitute for di(2-ethylhexyl) phthalate in some applications (Felder et al. 1986). This compound has been widely used in the plastics industry and other fields on account of its high boiling point, good electrical properties, good weathering resistance and good thermal and light stability (Abdul Rahman et al. 2009). It is primarily used as a plasticizer in food wraps, vinyl blood bags and haemodialysis bags (Yavan 1984). It is also added into plastic toys and other toys for children (Rahman & Brazel 2004). DEHA has been a component of cosmetic bases and solvents and it also has been widely used in the aerospace and automotive industries, as well as under extreme working conditions, such as high-temperature environments, on account of its high thermal-oxidation stability, large specific heat capacity, good stickiness index and excellent thermal conductivity (Yavan 1984; Qian et al. 2016). DEHA has been shown to migrate when packaging material is in direct contact with high fat content foods, which can lead to high DEHA concentration and contamination of the food (Badeka et al. 1999). DEHA can

be released into the environment during its synthesis and distribution and in consumer use of finished plastic products (Felder et al. 1986).

DEHA seriously inhibits the survival and growth of algae and the planktonic crustacean *Daphnia magna* at very low concentration (Felder et al. 1986; Bi et al. 2016). DEHA can induce remarkable histopathological changes in gill tissue and can produce hepatocellular changes in various species of fishes (Üçüncü et al. 2010; Üreten & Üçüncü 2013). It has been reported that DEHA seriously damaged the liver of rats and mice by affecting lipid metabolism, causing liver tumours and inducing DNA damage, among other negative effects (Bell 1983; Takagi et al. 1990; Lake et al. 1997; Dalgaard et al. 2003). Studies have found that DEHA can produce reproductive and developmental toxicity by interfering with the estrous cycle and increasing ovarian follicle atresia; it was found to be harmful to both the mother and foetus (Miyata et al. 2006). DEHA has caused adrenal weight decrease in adult male rats (Miyata et al. 2006). All of these results strongly suggest that the toxicity of DEHA, particularly its ecotoxicity, should

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receive great attention. Unfortunately, there is little available scientific data on the concentration of DEHA in Antarctic krill.

Antarctic krill (*Euphausia superba*), a marine planktonic crustacean that lives in schools, is the key component of the food webs in the Antarctic coastal marine ecosystem (Kokubun et al. 2015). This abundant species comprises an estimated biomass of 400–1550 million metric tonnes, which may be the largest biomass of any multicellular species on the planet (Gigliotti et al. 2011). Antarctic krill contains high-quality lipids, and krill oil contains high levels of omega-3 polyunsaturated fatty acids (n-3 PUFA) such as eicosapentaenoic (EPA, 20:5n3) and docosahexaenoic (DHA, 22:6n3), which are easily absorbed by humans (Gigliotti et al. 2011; Ali-Nehari et al. 2012). The content of antioxidant astaxanthin in Antarctic krill is extremely high; astaxanthin can effectively scavenge oxygen free radicals in the body (Ali-Nehari et al. 2012). The lipids in Antarctic krill are beneficial to health in the areas of obesity, coronary heart disease, inflammation and brain function, among others (Maki et al. 2009; Fosshaug et al. 2011; Wibrand et al. 2013). These features, together with others, make Antarctic krill an outstanding food source and medical resource.

Methods for detecting DEHA have been presented in numerous reports. For instance, Di Bella et al. (2014) determined DEHA residues in moka pots, coffee pods and coffee capsules made from polypropylene and in polypropylene with a micro-perforated film of low-density polyethylene lids by GC-MS. Lo Turco et al. (2015) studied plasticizer residues in tea by solid phase extraction-GC-MS. Fromme et al. (2013) used a GC-MS method to study the daily intake of DEHA by infants through their standard diet by sampling for detection of DEHA in urine and proved that GC-MS was an excellent analytical method with good accuracy and high sensitivity. Wang detected DEHA in mixed plasticizer by HPLC (Wang 1996).

Although there is a wide choice of chromatographic methods for plasticizer analysis (GC, HPLC), the technology of HPTLC has gained greater acceptance as an analytical procedure because of its low operation cost and high sample throughput in recent years (Rezić et al. 2005; He et al. 2013; Li et al. 2016). As there has been no detailed HPTLC detection method for DEHA for krill and similar animals, the aim of the present study was to detect the presence of DEHA in Antarctic krill using an HPTLC detection method, determine its quantity and discuss its potential harmful effects.

Materials and methods

Sample, reagents and instrumentation

Frozen whole Antarctic krill were purchased from the Liaoning Fishery Group (Dalian, Liaoning, China). The

batch of Antarctic krill used in this study (35–55 mm in body length) was caught in the first quarter of 2015 from the waters surrounding the Chinese Great Wall Antarctic Station (48.1–48.3 zone in the Antarctic). A DEHA standard ($\geq 99\%$) was obtained from Aladdin Chemical (Shanghai, China). All the other reagents used in this study were analytically pure. An FUD-1200 freeze-dryer was obtained from Tokyo Rikakikai Co., Ltd (Tokyo, Japan). The ultrasonic cleaning device used in this study was purchased from Kun Shan Ultrasonic Instruments Co., Ltd (Jiangsu, China). A rotavapor-3 rotating evaporator was obtained from Buchi (Flawil, Switzerland). A silica gel chromatography column (3×60 cm) was obtained from Shandong Research Institute of Chemical Industries (Shandong, China). Pre-coated silica gel GF₂₅₄ high-performance TLC plates (10×10 cm²) and silica gel (200–300 mesh) were purchased from Haiyang Chemical (Qingdao, Shandong, China). A chromatography development chamber ($10 \times 12 \times 5$ cm³, consisting of a twin trough glass chamber) was obtained from Shanghai Xinyi Instrument Co., Ltd (Shanghai, China). A TLC Scanner 3 equipped with the Wincats 1.4.1 software was purchased from CAMAG (Muttentz, Switzerland). The Fourier transform infrared spectrometer used for this study (Bruker Tensor II) was purchased from Bruker Instruments (Karlsruhe, Germany). None of the experimental instruments and materials was composed of plastic to avoid any hint of contamination.

Preparation of sample solution and standard solution

Krill samples (ca. 200 g) were cut from the middle section of a chunk of frozen krill – to avoid sampling krill in contact with the plastic packaging – and were freeze-dried at -46°C to produce 40 g of dry Antarctic krill. This dry Antarctic krill sample was extracted three times with anhydrous ethanol (1:15, w/v) using ultrasound for 0.5 h, at 35°C , 350 w, 40 kHz. The extracts were combined, filtered (after flushing with anhydrous ethanol) and evaporated to dryness (50°C). The residue was dissolved in anhydrous ethanol and the volume of the mixture was accurately set to 10 ml in a volumetric flask. This original DEHA sample (Sample A) was used for analysis and refinement.

Standard solutions of DEHA were prepared by dissolving accurate quantities of DEHA ($\geq 99.5\%$) in methanol. The mass concentration of DEHA was 1.0 mg/ml.

Verifying the presence of DEHA and refining the original sample

The developing solvent petroleum ether/ethyl acetate/acetone/glacial acetic acid (29:1:0.5:2d, v/v/v/v) was added to the development chamber and incubated for 30 min in a fume hood. The 3 μL Sample A, DEHA

standard and blank solution (anhydrous ethanol solution, the DEHA extraction solution from Antarctic krill) were respectively spotted onto the same activated pre-coated silica gel HPTLC GF₂₅₄ plate (10 × 10 cm²). The plate was developed, air dried, dipped into a solution of bromine thymol blue for 15 s, removed and then dried at room temperature. A CAMAG TLC scanner 3 with Wincats 1.4.1 software was used to analyse the plate at the reflectance-absorbance wavelength of 400 nm using a tungsten lamp. The scanning speed was 20 mm/s and the data resolution was a 50 µm/step.

Activated silica gel (90 g, 200–300 meshes) was mixed with methylene chloride (1:2, w/v) and poured into a chromatography column (3.0 × 60 cm). 1 ml of the Sample A was applied to the column and eluted with methylene chloride /methanol (8:1, v/v). The 100 ml fractions of the eluent were collected and tentative identification was conducted using the HPTLC method described above. The fractions containing DEHA were pooled and dried by rotary evaporation. The residue was weighed and brought to a precise volume of 5 ml with methanol and then used for GC-MS and IR analysis and quantification (Sample B).

Quantifying DEHA in Antarctic krill

Select quantities (1 µl, 2 µl, 3 µl, 3.5 µl, 4 µl) of DEHA standard solutions were spotted on the same GF₂₅₄ HPTLC plate, which contained 1 µg, 2 µg, 3 µg, 3.5 µg and 4 µg of DEHA. Then 3 µl of Sample B was spotted on this same plate. This plate was then developed using the previously described HPTLC process, spots with peak areas were analysed using the Wincats 1.4.1 software to determine linearity of DEHA detection and to obtain the concentration of DEHA in Sample B. The DEHA concentration in the Antarctic krill was then calculated.

Validating the HPTLC method

Intraplate variation and interplate variation

To determine if there was any variation in the detection of DEHA, 2 µl of the DEHA standard solution was spotted on one GF₂₅₄ HPTLC plate in five repetitive spots; this plate was developed using the previously detailed mentioned to determine the relative intraplate variation in the DEHA detection. The peak area data were recorded and analysed using Wincats software. To evaluate the interplate variation, the same volume of DEHA standard solution (2 µL) was spotted on five individual GF₂₅₄ HPTLC plates. These plates were developed in the usual manner and the peak area data were recorded and analysed.

Stability

To determine the stability of the chromogen in the analysis, 2.5 µL of the DEHA standard solution was spotted on one GF₂₅₄ HPTLC plate in three replicate spots. After the plate was developed, it was scanned 1, 2 and 3 h after generating the chromogen and the peak areas were analysed using the scanning software.

Limit of detection and limit of quantification

The limit of detection and the limit of quantification were determined by serially diluting the DEHA standard solution. The limit of detection was defined as the amounts of DEHA that produced a 3:1 signal-to-noise ratio and the limit of quantification was defined as the amount of DEHA that produced a 10:1 signal-to-noise.

Extracting and detecting DEHA in Antarctic krill oil

An Antarctic krill oil sample was prepared using the method described above, but employing n-hexane as the extraction solvent. The purification and detection of DEHA in Antarctic krill oil were performed using the method and conditions described above.

Detection DEHA Sample B by GC-MS

The DEHA in Sample B was detected using an Agilent-7890 GC-MS with an Agilent DB-1 ms (G3900-63017) chromatographic column (size: 30 m, 0.25 mm, 0.25 µm) employing the following conditions. The column temperature was initially set to 100°C and maintained for 4 min, then it was gradually increased to 120°C at 50°C/min and maintained for 10 min. The column temperature was then increased to 220°C at 50°C/min and maintained for 6 min. The injector and detector temperatures were set at 200°C and 250°C. The ionization mode had an electron impact ion source; the four stage rod temperature was 150°C and the ion power temperature was 230°C. The monitoring mode was full scanned (50–550 amu units) in the selective ion scanning mode. For GC-MS detection, an electron ionization system was used with an ionization energy of 70 eV, and the solvent delay was 2 min.

Detection DEHA Sample B by IR

IR spectroscopy was conducted on Sample B to determine the structure of the DEHA in the sample. For this work a Fourier transform infrared spectrometer (Bruker Tensor II) was used with a resolution ratio of 4 cm⁻¹. The background and the sample were each scanned 16 times and the range of determination was 4000–400 cm⁻¹.

Repetition and recovery

Eight 40-g samples of Antarctic krill were extracted, refined and quantified as described. The accuracy of the method was evaluated by a recovery test with three different DEHA concentrations. To accomplish this, three standard solutions of DEHA (1.25 mg, 2.5 mg, 3.75 mg) were individually added to three 1 ml aliquots of Sample A (DEHA concentration 2.5 mg/ml). After refining the solutions using silica gel column chromatography, the resulting combined eluents were spotted on a HPTLC plate in triplicate. Chromatograms were developed and scanned as previously described. The results of the analysis were used to calculate the total recovery (%) and the average recovery (%) of DEHA from the sample matrix.

Results

HPTLC and refinement results

The chromatographic spots of Sample A, Sample B, the DEHA standard solutions and blank solution, as well as the individual R_f, three-dimensional figure and the peak spectrodensitometric analysis curves are shown in Fig. 1.

Quantification results

The linear relationship between DEHA concentration and spot peaks areas for the standards can be expressed as:

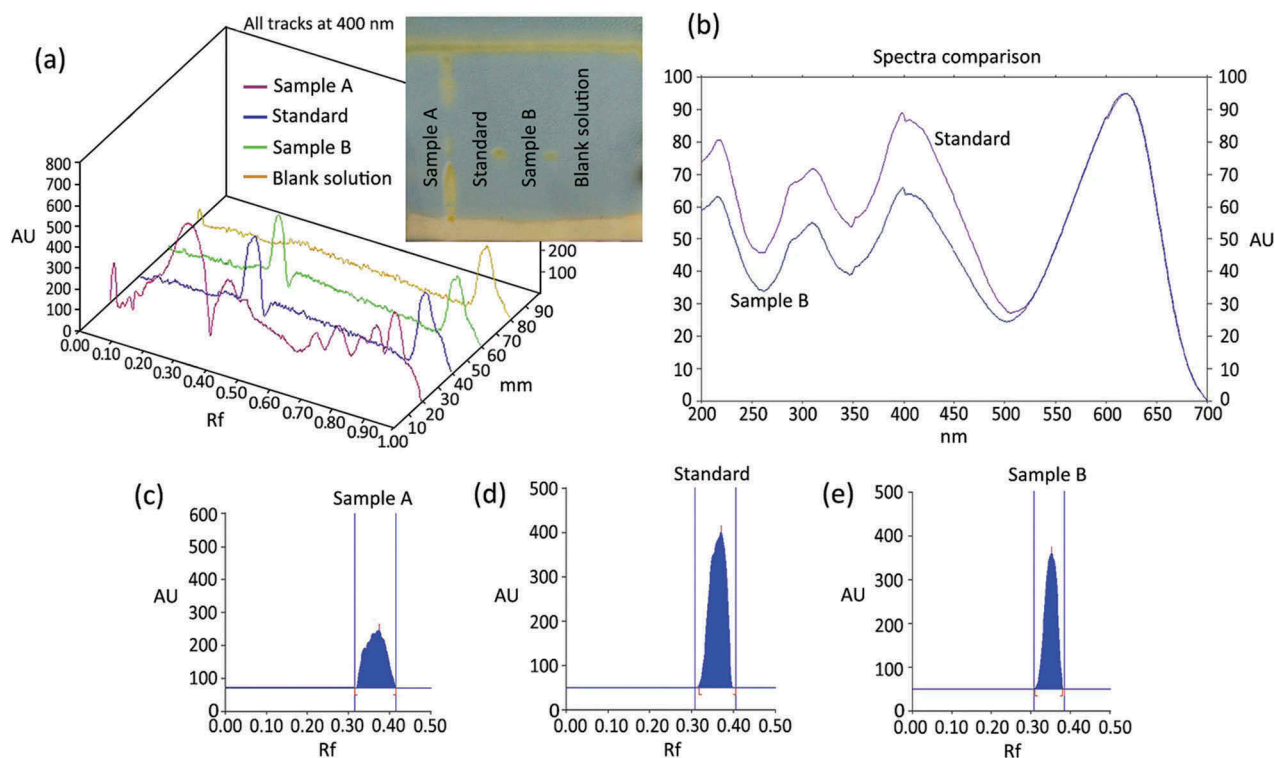


Figure 1. (a) The HPTLC plate after the staining process and the three-dimensional analysis of Sample A, the DEHA standard, Sample B and the blank solution. The spectral densitometric analysis of (b) Sample B and the DEHA standard and densitometric peaks for (c) Sample A, (d) the DEHA standard and (e) Sample B at an R_f of 0.36.

From these results it was found that the RSD value was 1.39%. The parameter Y was the peak area of DEHA and X was the quantity of DEHA in the sample. The linear range for the detection of DEHA using this method was between 1 µg and 4 µg (Table 1). The DEHA concentration of 3 µl of Sample B was 1.39 µg. The concentration of DEHA in the dry Antarctic krill was found to be 0.58 mg/g.

Validating the HPTLC method

Intraplate variation and interplate variation

The results for the intraplate and interplate variation tests are shown in Table 2. The RSD of the data for the intraplate variation was 1.03% and the interplate variation in the test was found to be 1.56%. These results demonstrated adequate accuracy and precision for the HPTLC analysis of DEHA.

Stability

The light absorption of the peak areas was similar 2 h after staining the plate, as demonstrated by the data in Table 3. Therefore, it was concluded that the chromogen was sufficiently stable during the time of analysis to ensure the accuracy of this technique.

Limit of detection and limit of quantification

The detection limit of DEHA as determined by HPTLC was 32 ng (at a signal-to-noise ratio of 3:1). The limit of quantification was determined to be 125 ng (at a signal-to-noise ratio of 10:1). These levels

Table 1. Average peak areas corresponding to different amounts of DEHA.

| Spot | Volume (μL) | DEHA (μg) | Average peak areas (AU) |
|------------|--------------------------|------------------------|-------------------------|
| Standard 1 | 1 | 1 | 2843.12 |
| Standard 2 | 2 | 2 | 4447.07 |
| Standard 3 | 3 | 3 | 6041.82 |
| Standard 4 | 3.5 | 3.5 | 6904.96 |
| Standard 5 | 4 | 4 | 7510.64 |
| Sample | 3 | 1.39 | 3487.10 |

Table 2. Intraplate variation and interplate variation of the HPTLC analysis.

| Spot | Intraplate variation | | Interplate variation | |
|------|----------------------|---------|----------------------|---------|
| | Peak area | RSD (%) | Peak area | RSD (%) |
| 1 | 4404.6 | 1.03 | 4474.2 | 1.56 |
| 2 | 4493.8 | | 4543.3 | |
| 3 | 4488.5 | | 4448.7 | |
| 4 | 4397.7 | | 4480.1 | |
| 5 | 4429.4 | | 4351.4 | |

Table 3. Stability of DEHA-chromogen based on peak area analysis after staining.

| Time after staining | Area of spot 1 (AU) | Area of spot 2 (AU) | Area of spot 3 (AU) |
|---------------------|---------------------|---------------------|---------------------|
| 0 h | 5646.5 | 5690.6 | 5731.8 |
| 1 h | 5615.8 | 5683.1 | 5708.0 |
| 2 h | 5571.6 | 5594.5 | 5627.3 |
| 3 h | 5094.1 | 5116.2 | 5014.8 |
| RSD (0–2 h)% | 0.67 | 0.95 | 0.96 |
| RSD (0–3 h)% | 4.75 | 4.95 | 6.16 |

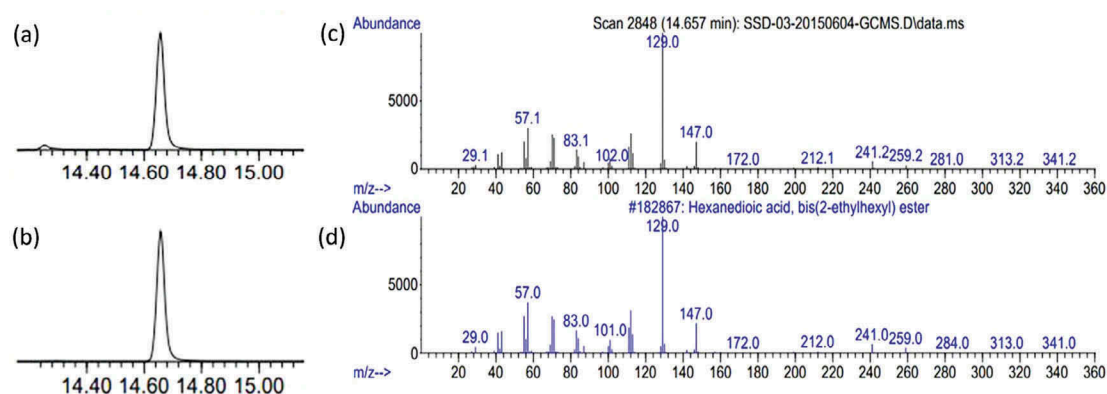
of sensitivity should allow for detecting and quantifying DEHA in many different types of matrices.

Extraction and detection results for DEHA in Antarctic krill oil

Employing the described methods, the concentration of DEHA in the Antarctic krill oil was calculated to be ca. 2.16 ± 0.08 mg/g ($n = 3$).

GC-MS analytical results

The results of the GC-MS analysis of Sample B and the DEHA standards are shown in Fig. 2. This result proved the presence of DEHA in Sample B.

**Figure 2.** Retention time (a) of Sample B and (b) the DEHA standard, and mass spectra of (c) Sample B and (d) the DEHA standard.

FTIR results for Sample B and the DEHA standard

The FTIR spectra for Sample B and the DEHA standard are shown in Fig. 3. The two spectra were very similar, indicating that Sample B contained DEHA.

Repetition and recovery results

The results for the test repetition are shown in Table 4, where the DEHA concentration of the dry Antarctic krill was calculated to be 0.63 ± 0.05 mg/g. The results for the recovery tests are shown in Table 5 and the average percentage recovery of DEHA at three different levels was 97.3% with an RSD of 2.47–4.90%.

Discussion

In this study, we extracted DEHA in Antarctic krill with anhydrous ethanol by an ultrasound method with the aim of reducing solvents and pollution. As a method to detect DEHA, GC-MS demands complicated operation and purification procedures. The HPLC method also needs complex pretreatment and consumes much solvents. Because of its low operation cost, simple pretreatment, lower solvent use, good accuracy and high sample throughput (Rezić et al. 2005), HPTLC is potentially an excellent method for detecting DEHA. This was demonstrated in our study, in which we detected and quantified DEHA in Antarctic krill using an HPTLC analysis. In this method, a new developing solvent and chromogenic agent were employed. Validation tests (according to International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines) confirmed the utility of this method as a way to detect and quantify DEHA, especially in Antarctic krill (Human Medicines Evaluation Unit 1994).

This is the first report concerning the presence of DEHA in Antarctic krill. The content of DEHA in the freeze-dried Antarctic krill that we tested was 0.63 ± 0.05 mg/g (630 ± 50 mg/kg). The content of DEHA in Antarctic krill oil using the described

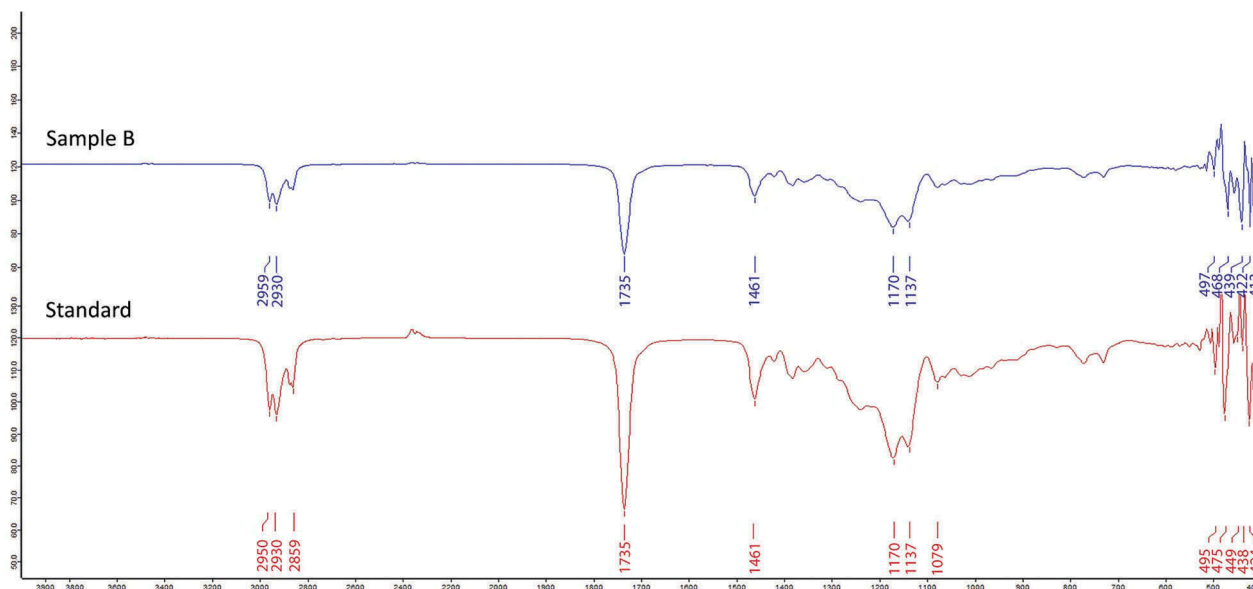


Figure 3. FTIR spectra of Sample B and the DEHA standard.

Table 4. DEHA concentration for repeated sampling of freeze-dried Antarctic krill.

| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Mean \pm SD |
|---|----------|----------|----------|----------|----------|----------|----------|----------|------------------|
| DEHA ($\mu\text{g}/\mu\text{L}$) in refined sample (Sample B) | 0.463 | 0.520 | 0.487 | 0.514 | 0.445 | 0.584 | 0.516 | 0.474 | 0.500 \pm 0.04 |
| DEHA (mg/g) in Antarctic krill | 0.58 | 0.65 | 0.61 | 0.64 | 0.56 | 0.73 | 0.65 | 0.59 | 0.63 \pm 0.05 |

Table 5. DEHA recovery determination.

| Compound | Amount of standard added (mg) | Amount of standard determined (mg) | Recovery (%) | Mean recovery (%) | RSD (%) |
|----------|-------------------------------|------------------------------------|--------------|-------------------|---------|
| DEHA | 1.25 | 1.27 \pm 0.060 | 101.6% | 97.3% | 4.72% |
| | 2.50 | 2.33 \pm 0.114 | 93.2% | | 4.90% |
| | 3.75 | 3.64 \pm 0.090 | 97.1% | | 2.47% |

method was found to be 2.16 ± 0.08 mg/g (2160 ± 80 mg/kg). The structure of the DEHA found in the Antarctic krill as determined by GC-MS and IR confirmed its presence. GC-MS further validated the feasibility of using HPTLC as a method for determining DEHA.

As shown in Table 6, DEHA can have negative effects on a wide variety of organisms. The highest and lowest concentrations that can affect organisms are 1000 mg/kg and 0.087 mg/L. The concentration of DEHA in dry Antarctic krill was found to be 630 ± 50 mg/kg (126 mg/kg in fresh Antarctic krill according to 80% water content), which is very high. Although the effects of DEHA on Antarctic krill have not been defined, based on the known effects of this contaminant on other species, it is reasonable to assume that DEHA may harm Antarctic krill at the high concentrations determined in this study. The accumulation of DEHA in krill could lead to the poisoning of other animals in this food chain, seriously affecting the Antarctic ecosystem. As people consume krill-based products, it could also seriously affect human health.

There are no detailed data on the sources and concentrations of DEHA in Antarctic biota. DEHA may be entering Antarctic krill through several pathways. The natural concentration of DEHA in water is 0.78 ± 0.16 mg/L (Felder et al. 1986), which is quite low, but there has been little research dealing with the concentration of DEHA in the Southern Ocean, so whether Antarctic krill absorb DEHA from seawater is not known. One study showed that algae can produce DEHA internally in high concentrations (Bi et al. 2016). Consequently, Antarctic krill may ingest DEHA directly by consuming these algae as food.

The presence of DEHA in the Antarctic marine ecosystem may result primarily from plastic pollution. It has been shown that Antarctic waters are contaminated with plastic particles (Fendall & Sewell 2009), which marine organisms readily absorb. The main route by which krill are contaminated by DEHA may therefore be direct absorption of large quantities of plastic particles. The global production of plastics is around 300 million metric tonnes annually, much of which eventually finds its way to the sea (Brink et al. 2017). The plastic garbage directly discarded by humans in the Antarctic may also be a source of DEHA as human Antarctic activities continue to increase (Stark et al. 2006).

DEHA may move to the Antarctic by long-range atmospheric and hydrospheric transport. Migratory species (such as seabirds) may bio-transport DEHA

Table 6. Harmful effects of DEHA on organisms and their related concentrations.

| Species | Concentration | Harmful effects on organisms | Reference |
|--|---------------------------|--|------------------------|
| Algae | | | |
| <i>Heterosigma akashiwo</i> | 5.82 mg/L | Strongly inhibiting growth | Bi et al. (2016) |
| <i>Gymnodinium breve</i> | 2.74 mg/L | Strongly inhibiting growth | Bi et al. (2016) |
| Zooplankton | | | |
| <i>Daphnia magna</i> | 0.087 mg/L and 0.180 mg/L | Notable effect on survival; notable effect on growth | Felder et al. (1986) |
| Fishes | | | |
| <i>Sparus aurata</i> (sea bream) | 250 ppm | Notable changes of gill histopathological indices; liver hepatocellular changes (steatosis, necrosis and fibrosis) | Üreten & Üçüncü (2013) |
| <i>Labidochromis caeruleus</i> (yellow princess) | 0.75 ppm | Notable changes of gill histopathological indices (hypertrophy, severe hyperplasia, aneurysma, oedema, lifting epithelial and striking fusion) | Ücücü et al. (2010) |
| Mammals | | | |
| Female F344 rats | 0%–4% | Dose-dependent increase in liver weight; dose-dependent increase in hepatic peroxisome proliferation | Lake et al. (1997) |
| Female B6C3F1 mice | 0%–2.5% | Dose-dependent increase in liver weight; dose-dependent increase in hepatic peroxisome proliferation | Lake et al. (1997) |
| | 1.2% and 2.5% | Sustained stimulation of replicative DNA synthesis | |
| Wistar rats | 800 mg/kg | Prolonged gestation period | Dalgaard et al. (2003) |
| | 400 mg/kg | Dose-dependent increase in postnatal death | |
| | 800 mg/kg | Permanent decrease in offspring body weight | |
| F344 rats | 2.5% | Increase in 8-OH-Dg levels in liver oxidative DNA damage hepatocarcinogenesis | Takagi et al. (1990) |
| Male rats | 0.5% | Inhibition hepatic cholesterolgenesis and modification of phospholipid synthesis in liver | Bell (1983) |
| | 1% | Cholesterol-lowering effect | |
| Rats | 1000 mg/kg | Disturbed estrous cycle; increased ovarian follicle atresia; adrenal weight decrease | Miyata et al. (2006) |

into the Antarctic from food chains elsewhere. Determining the sources of DEHA in Antarctic requires further research.

Fresh Antarctic krill, Antarctic krill powder and Antarctic krill oil are the main krill products. These products are exported and widely consumed throughout the world. The tolerable daily intake of DEHA by humans is 0.3 mg/kg bw/day (Fromme et al. 2013). The concentration of DEHA that we found in freeze-dried Antarctic krill is significantly higher, and the content of DEHA in Antarctic krill oil was even higher. DEHA in krill is a serious concern in human food consumption.

DEHA may present a growing threat to the survival and reproduction of Antarctic krill, which is an important part of the food web in the Southern Ocean. The adverse effects of DEHA on Antarctic krill and the source of DEHA in the Antarctic should be explored in future research.

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Compliance with ethical standards

This study was approved by the Shandong Institute of Zoology and followed all applicable international, national and/or institutional guidelines for the care and use of animals.

Disclosure statement

No potential conflict of interest was reported by the authors.

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