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Phthalate esters (plasticizers) in dead southern right whales (Eubalaena australis) at Península Valdes, Argentina.

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Abstract

Southern right whales (SRW, *Eubalaena australis*) use the waters off Peninsula Valdés mainly as a winter breeding area, yet feeding events occur in the spring and are increasingly recorded. Although the ingestion of plastic debris has been documented in this population, the transfer and accumulation of plastic additives has not been studied to date. In this study, three phthalate esters, dimethyl phthalate (DMP), dibuthyl phthalate (DBP), and diethylhexyl phthalate (DEHP), were quantified in the blubber of seven whales (one juvenile and six adults, one of these a male) stranded dead in 2021 at Península Valdés, Argentina. Our results indicate that DMP was detected in six whales (four adult females, one adult male and one juvenile; 57% at quantifiable levels and an additional 29% at trace levels) with an average concentration of 26.1 ± 31.7 ng/g (mean±SD for individuals with detectable levels). DBP was detected in three whales (two adult females and one juvenile; 29% at quantifiable levels and an additional 14% at trace levels), with an average concentration of 153.1 ± 37.9 ng/g. DEHP was detected in five whales (three female

adults, one male adult and one juvenile; all at quantifiable levels), with an average concentration of 58.4 ± 31.5 ng/g. These findings reveal exposure and potential for accumulation of plastic additives in southern right whales, providing relevant context for understanding sources of plastic pollution and potential health effects from exposure. More samples are available for the continuity of this work, which allow us to evaluate the transfer of phthalates between mothers and calves, assess differential exposure between age and sex categories, identify trends over time and compare our findings with other mysticetes worldwide. This is the first report of plasticizers in blubber of southern right whales.

Introduction

There is growing evidence of the threats that plastics pose to marine wildlife (Prokić et al. 2019, Puskic et al. 2020, Roman et al. 2021). To date, >1400 species are recorded to interact with marine litter through entanglement and ingestion (Claro et al. 2019). The impact of marine plastic pollution on cetaceans is a priority concern for the International Whaling Commission (IWC, Resolution 2022-1). Overall, 78% of cetacean species have been reported to be in some way affected by marine debris pollution (IWC Marine Debris Report 2019, Pieratonio et al. 2018).

Plastic debris can be physically harmful to an animal via entanglement or ingestion, causing gut obstruction or perforation or external wounds, all of which may lead to death (Bucci et al. 2020). Besides direct lethal effects, the presence of plastic debris may affect individual animal health if items persist in the gastrointestinal tract (GIT), for example by reducing the space for food and, subsequently, reducing their fitness and nutritional condition (Senko et al. 2020). Additionally, plastics can be chemically harmful due to the complex suite of chemicals with which they are made. This "chemical cocktail" consists of the residual monomers that make up the plastic polymer, the additives that are added during manufacturing, and the contaminants that adsorb from the surrounding environment (Teuten et al. 2009, Rochman 2015). Therefore, the ingestion of plastic debris may cause toxicological effects due to the exposure to both chemicals adsorbed by plastics and in plastic components.

Among plastic additives, phthalate esters (PAEs) are widely used as plasticizers by various industries (Hahladakis et al. 2018). Phthalates can be taken up by marine animals from water, through the food chain, and directly by plastic ingestion (Bakir et al. 2016, Baini et al. 2017, García-Garin et al. 2022). However, information on phthalate concentrations in cetaceans, and especially in the tissues of mysticetes, is scarce. Recently, scientific articles have reported on this topic in blubber of stranded specimens or skin biopsies mainly from fin whale (*Balaenoptera physalus*) (Baini et al., 2017; Fossi et al., 2012, 2014, 2016; Routti et al., 2021, García-Garin 2023, Galli et al. 2023), blue whale (*Balaenoptera musculus*) and bowhead whale (*Balaena mysticetus*) (Routti et al., 2021) in the northern Hemisphere. However, in most of these studies, the relationship between phthalate concentrations and key biological variables of the individuals (sex, age) as well as their trends over time are not assessed, probably due to reduced sample size and/or the difficulties in accessing biological information, particularly from free-living individuals (e.g. Baini et al. 2017, Fossi et al. 2012, 2014, 2016, Routti et al. 2021). Furthermore, because di (2-ethylhexyl) phthalate (DEHP) is the most widely produced and used, as well as the most persistent phthalate found in seawater (Chaler et al. 2004, Bergé et al. 2013), most studies are focused solely on this chemical (Fossi et al. 2012, 2014, 2016) although recent studies have included other toxics (Baini et al. 2017, Routti et al. 2021, Galli et al. 2023, García-Garin et al. 2023)

Filter-feeding whales are susceptible to high levels of microplastic ingestion and exposure to plastic- chemical components due to their feeding strategies and because of habitat overlap with oceanic microplastic hot spots (Germanov et al. 2018, Werth et al. 2024). Recently, Kahane-Rapport et al. (2022) reported that krill-feeding whales may be particularly susceptible to chemical harm associated to microplastic ingestion, as they have been described to filter and potentially ingest 10 million synthetic particles per day.

Southern right whales (SRW, *Eubalaena australis*) use the waters off Peninsula Valdés mainly as a winter breeding area, yet feeding events occur in the spring and have been increasingly recorded in the last decade (D'Agostino et al. 2023). The ingestion of plastic debris has been documented in one individual in this population (Alzugaray et al. 2020). However, the transfer and accumulation of plastic additives have not been studied to date. In this study, three phthalate esters, dimethyl phthalate (DMP), dibuthyl phthalate (DBP), and diethylhexyl phthalate (DEHP), were quantified in the blubber of whales stranded dead in 2021 at Península Valdés, Argentina.

Materials and methods

Samples of blubber (20x20 cm) were collected from seven whales (one juvenile, five adult females and one adult male) stranded dead in 2021 at Península Valdés, Argentina. Researchers from the Southern Right Whale Health Monitoring Program (SRWHMP) necropsied the animals following the SRWHMP necropsy protocol (Chirife et al. 2014). Samples were stored in ziploc bags at − 20 ◦C until analysis.

At the laboratory, subsamples of blubber (20gr mean weight from the core of the block) were collected, wrapped in heat treated aluminum foil (450°C/ six hours) and conserved frozen at −20 °C until analysis. To avoid contamination between individuals, reusable utensils (ie. blades, tweezers) were washed thoroughly with running water and detergent and then washed with solvents (3 times each): 1st methanol, 2nd dichloromethane, 3rd hexane (Uhart et al. 2020).

Blubber samples were lyophilized for 96 h, their dry weight (dw) and fresh weight were measured and water content (%) was calculated. About 1 g (dw) of sample was spiked with internal standard (DEPH/DMP/DBP-d4, 1000 µg/L) in a 10 ml glass centrifuge tube and vortexed for 10 seconds. Subsequently, 5ml of acetonitrile were added. The mixture was stirred during 30 min at 150 rpm (30 °C) and centrifuged for 5 min at 2500 rpm. Afterwards, 2 mL of supernatant were transferred to a glass tube with 40mg of octadecylsilane (C18) and freeze out was performed with liquid nitrogen (5min in LN Dewar). 3ul from this supernatant was used as the final extract subjected to analysis.

All analyses were performed using an Agilent 7890B GC coupled to an Agilent 7000C Triple Quadrupole GC-MS system (Agilent Technologies, France). A pre-connected LPGC (Low Pressure GC) column set consisting of a 5m, 0.18 mm ID uncoated guard/restrictor capillary at the inlet coupled to a 15 m 0.53 mm ID, 1 µm thickness fil Rtx-5MS analytical column with extra 1 m uncoated 0.53 mm ID integrated transfer lines capillary (Restek, Bellefonte, PA, USA). The carrier gas was high purity helium (99.999%) starting at 2.25 mL/min for 3 min which was lowered to1.5 mL/min until the end of the run. The injector operating conditions in Splitless mode were as follows: injection volume was 3 µL, injections were made at 280 °C and using a pressure pulse of 40 psi for 0.75 min after which the split vent was initiated. The oven temperature was set as follows: 80 ◦C for 1 min (hold 0.7min). The total run time was 7 min. The triple quadrupole mass spectrometer was operated in electron impact ionization (EI) with an ionizing energy of 70 eV. The temperatures of the transfer line, ion source and quadrupole 1 and 2 were 280 ◦C, 230 ◦C and 150 ◦C, respectively.

Quantification was based on 6-point calibration curves generated for each analyte, ranging from 1 to 100 µg /mL (1, 5, 10, 25, 50 and 100 µg/mL). The correlation coefficient (R^2) and relative standard deviation (RSD) of the calibration curve were higher than 0.987 and less than 15%, respectively. One spiked and repeated samples were analyzed in each batch. Surrogate and internal recovery standards were added to all samples and concentrations of PAEs were all corrected for recovery. The recoveries ranged from 86% to 108%. The quantification limits (LOQ) for individual PAEs were as follows: DBP= 1 ng/g, DMP= 10 ng/g, DEHP= 25 ng/g.

Results and Discussion

Our results indicate exposure and potential for accumulation of plasticizer chemicals in southern right whale blubber, the first report for this species. Specifically, DMP was detected in six whales (four adult females, one adult male and one juvenile; 57% at quantifiable levels and an additional 29% at trace levels) with an average concentration of 26.1 ± 31.7 ng/g (mean±SD, range=12-72 ng/g for individuals with concentrations above LOQ). DBP was detected in three whales (two adult females and one juvenile; 29% at quantifiable levels and an additional 14% at trace levels), with an average concentration of 153.1 ± 37.9 ng/g (range=126-180 ng/g, for individuals with concentrations above LOQ). DEHP was detected in five whales (three female adults, one male adult and one juvenile; all at quantifiable levels), with an average concentration of 58.4 ± 31.5 ng/g (range=30-98 ng/g). At least one phthalate compound was above the limit of quantification in 6/7 samples, and the simultaneous occurrence of all phthalates analyzed was observed in one adult female. These findings provide relevant context for understanding sources of marine plastic pollution and potential health effects on baleen whales from exposure. Unlike odontocetes which may become exposed to phthalates via prey species (eg. fish), mysticetes may ingest large quantities of microplastics directly while filtering (Kahane-Rapport et al. 2022). Notwithstanding, exposure through water and prey may be additional sources of toxic chemical uptake. Phthalate concentrations in our study are similar or lower to those previously reported in mysticetes from the northern hemisphere, which possibly reflects greater exposure to plastic additives in northern oceanic feeding grounds (Baini et al. 2017, Routti et al. 2021, Galli et al. 2023, García-Garin et al. 2023). Experimental studies on animals, supported by clinical human studies, indicate that phthalates can impair development, reproduction, metabolism and cause neurological issues in addition to being carcinogenic (NRC 2008, Kamrin 2009, Benjamin et al. 2017). Recently, in vitro studies on fin whales, showed potential for DEHP to modulate the transcriptional activity of thyroid hormone receptor beta (THRB) at considerably higher concentrations than reported baselines (Routti et al. 2021). The SRWHMP has been collecting tissue samples from southern right whale strandings in Península Valdés since 2003. The continuity of this work will allow us to evaluate the transfer of phthalates between mothers and calves, assess differential exposure between age and sex categories and identify trends over time. Future studies should aim to assess toxicological effects related to chronic exposure to plastic additives in wild animals. Moreover, many filter-feeding marine megafauna are charismatic and iconic species, with the potential to act as sentinels of ocean health, stimulating awareness of scientific and local communities and encouraging actions to tackle microplastic oceanic pollution (IWC Marine Debris Report 2019).

Table 1. Studies in mysticetes. Mean phthalate-compound concentrations expressed in dry (dw) or fresh weight (fw), concentration range (between brackets, when available), and frequency of occurrence (FO=samples at detectable levels/samples evaluated). n=sample size, na=not analyzed, nd=not detectable or concentration values below limit of detection (LOD).

Reference	Our study	Fossi et al. 2012, 2014	Fossi et al. 2016	Baini et al. 2017	Routti et al. 2021	Galli et al. 2023	García-Garin et al. 2023
Species	Southern Right Whale (n=7)	Fin whale (n=5)	Fin whale (n=40)	Fin whale (n=3)	Fin whale (n=6) Blue whale (n=7) Bowhead whale (n=5)	Fin whale (n=3)	Fin whale (n=31)
Sample	blubber (stranded)	blubber (stranded)	Skin biopsies (live) ie. epidermis, dermis and blubber	Skin biopsies (live) ie. epidermal, dermal and blubber tissue	Blubber biopsies (live) ie. The upper layer of whale blubber, approximately 1–5 cm from the skin	Skin biopies (live) ie. 1–2 g of epidermal, dermal, and blubber tissue	Muscle (hunted)
Site	South west Atlantic Ocean (7)	Mediterranean Sea (MS)	MS (n=30) Sea of Cortez (n=10)	MS	Svalbard Archipelago	Gulf of California	Western Iceland
DEHP - diethylhexyl phthalate	FO=5/7 58.4 ± 31.5 ng/g dw	nd	<LOD	7051ng/g dw	FO Fin= 6/7 42 ng/g fw (12-300). FO Blue= 4/7 20ng/g fw (<12-101) FO bowhead=2/5 (<12-398)	FO=3/3 1942.0±402.3 ng/g dw	FO=58% 0.010 ug/g (10ng/g) dw
MEHP - Mono(2-ethyl-1-hexyl) phthalate	na	FO=4/5 57.97 ng/g fw	54.8±27.7ng/g fw 40.0±23.2 ng/g fw	<LOD	nd	FO=3/3 736.3±100.1 ng/g dw	na
DBP - dibuthyl phthalate	FO=2/7 153.1±37.9 ng/g dw	na	na	na	na	na	FO= 81% 0.303 ug/g (303ng/g) dw
DMP - dimethyl phthalate	FO=5/7 26.1±31.7 ng/g dw	na	na	na	na	na	FO=100% 0.008 ug/g (8ng/g) dw
Other detectable phthalates analyzed*				BBzP DIOIP MBzP MBP	DiBP	BBzP DNHP MBzP MBP	DEP DCHP
Other non-detectable phthalates				DNHP (<LOD) DNDP (<LOD)	DEP BBzP DCHP		DMEP DPP DEEP

analyzed*					BMPP DNHP DNOP DnBP DiNP DnNP DiDP MMP MEP MEOHP MiBP MnBP MBzP MEHHP		BBzP BMPP DNHP DBEP DNOP

***Notes:**

diethylphthalate (DEP), bis(2-methoxyethyl) phthalate (DMEP), dipentylphthalate (DPP), bis(2-ethoxyethyl) phthalate (DEEP), phthalic acid dicyclohexyl ester (DCHP), benzyl butyl phthalate (BBzP), bis(4-methyl-2-pentyl) phthalate (BMPP), di-n-hexyl phthalate (DNHP), bis(2-n-butoxyethyl) phthalate (DBEP), di-n-octyl phthalate (DNOP), di-n-decyl phthalate (DNDP), di-Isooctyl iso phthalate (DIOIP), Di-isobutyl phthalate (DiBP), Di-n-butyl phthalate (DnBP), Diisononyl phthalate (DiNP), Di-n-nonyl phthalate (DnNP), Diisodecyl phthalate(DiDP), Mono-benzyl phthalate (MBzP), Mono-butyl phthalate (MBP), Monomethyl phthalate (MMP), Monoethyl phthalate (MEP), Mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), Monoisobutyl phthalate (MiBP), Mono-n-butyl phthalate (MnBP), Mono(2-ethyl-5-hydroxyhexyl) (MEHHP).

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