Sentinel Indicators of Marine Microplastic and Phthalate Exposure

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I. Abstract

Phthalate esters are additives in common consumer products such as plastics, personal care products, and cleaning products. They are considered endocrine disrupting compounds, and pervasive use and the propensity to leach from these products has led to ubiquitous contamination throughout the terrestrial and marine environments. Recently, exposure has been reported in common bottlenose dolphins (*Tursiops truncatus*) sampled from Sarasota Bay, Florida. Previous research has sought to quantify and characterize exposure using urine samples with the understanding that quantifiable exposure may be linked to plastic pollution; however, it is not understood the extent of plastic exposure in these apex marine mammals. One potential route is via trophic transfer, where plastic particles consumed by lower-level prey fish are ingested by the dolphins when they feed. The objectives of this study are to quantify microplastic particles and phthalate metabolite concentrations in both dolphins and prey fish species to investigate whether plastic pollution may contribute to phthalate exposure and risk to seafood safety.

II. Introduction

Phthalates are chemical additives used to enhance desirable properties in common consumer products. Most commonly, they are added to plastics[1] and personal care products[2], but they can also be used in cleaning solutions[2], insect repellants[2], vinyl[3], and medical equipment[1,4]. Phthalates are not chemically bound to the products they modify[5,6], so pervasive anthropogenic use has led to contamination in every environmental compartment (e.g., air[7], freshwater[8], seawater[9], soil[10]) providing abundant exposure opportunities for humans and wildlife. Exposure is concerning due to evidence from human epidemiological and laboratory rodent studies of phthalate-induced endocrine disruption, resulting in reproductive and developmental abnormalities[11–14]. Following exposure, phthalates are rapidly biotransformed and excreted through urine and feces as metabolites[15,16]. As such, detectable phthalate metabolite concentrations can signal contact with parent compounds. Much of the current research on environmental phthalate pollution has focused on human populations; however, evidence of exposure in wildlife is diverse across all trophic levels, including benthic species (e.g., blue mussels; *Mytilus edulis*[17]), intermediate consumers (e.g., white-spotted greenlings; *Hexogrammos stelleri*[17]), and higher-level predators (e.g., common bottlenose dolphins; *Tursiops truncatus*[18–20]). In fact, recent studies of dolphins from Sarasota Bay, Florida, have not only found evidence of prevalent exposure (i.e., 70% of sampled individuals had detectable concentrations of at least one phthalate metabolite[19]), but detectable concentrations of mono(2-ethylhexyl) (MEHP), the first metabolite of di(2-ethylhexyl) phthalate (DEHP), were significantly higher in dolphins than a human reference population[21].

While specific sources and routes of phthalate exposure for dolphins are currently unknown, phthalates can be released into the marine environment through sewage or wastewater discharge[1,23], urban or agricultural runoff[1,2], or plastic waste[9]. Plastic debris as a source of marine phthalate pollution particularly concerning as plastics are found ubiquitously in the environment. It has been estimated that 5 trillion plastic pieces are floating in the ocean[24] in varying sizes (i.e., micro-are < 5.00 mm; meso- and macro-are ≥ 5.00 mm[25]). As plastics degrade, phthalates can be directly released into the marine environment[9], and ingested microplastic particles have been related to relevant MEHP concentrations in large filter feeders[26]. Phthalate bioaccumulation is expected to be outpaced by biotransformation in apex mammalian predators, so

it is unlikely that phthalate-contaminated prey fish would contribute to dolphin exposure; however, prey-based microplastic transfer and accumulation has been demonstrated in higher order marine mammals[27]. Further, MEHP has been found to be the most frequently detected phthalate metabolite in Sarasota Bay dolphins[19] likely indicating a plastic origin since the majority of DEHP use is as a plasticizer. It therefore seems plausible that plastic-contaminated prey could contribute to dolphin phthalate exposure. Given the overlap between consumed fish species, prey-based plastic and phthalate exposure raises concerns not only for dolphin health, but human health as well.

III. Goals

The overall goal of this research project is to understand whether microplastic trophic transfer may be a contributor to phthalate exposure. Resident Sarasota Bay dolphins are considered selective feeders, so this study will investigate the connections between microplastics detected in common prey fish (e.g., Gulf toadfish; *Opsanus beta*; spot; *Leiostomus xanthurus;* spotted seatrout; *Cynoscion nebulosus*; pinfish; *Lagodon rhomboides;* sheepshead; *Archosargus probatocephalus*; and mullet; *Mugil cephalus*[28,29]) and dolphins to determine whether consumed fish are a likely exposure route. Sarasota Bay dolphins were previously evaluated for spatial influences in overall phthalate metabolite detection[30]. Dolphins with detectable MEHP concentrations were found in areas of Sarasota Bay which may be sensitive to plastic pollution[30] (e.g., areas where freshwater and saltwater meet[31]). Based on these findings, and the ubiquity of marine plastic[32], we hypothesize that consumed plastic material is an important contributor to overall phthalate exposure.

IV. Methods

Phthalate Metabolites: Urine is considered an ideal matrix to study and quantify phthalate metabolites in mammals as analytical methods are sensitive enough to detect low concentrations, and it is less susceptible to contamination than other matrices (e.g., milk or serum[33,34]). In fish, detectable phthalate metabolite concentrations have been found in the liver, plasma, and bile[35]. Bottlenose dolphin urine (2-10 mL) will be opportunistically collected via catheterization during health assessments conducted by the Sarasota Dolphin Research Program (SDRP). All urine and fish tissue samples will be screened for five phthalate metabolites including: monoethyl phthalate (MEP), mono-(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and mono-isononyl phthalate (MINP). Fish will be caught during surveys conducted by SDRP, dissected, and the tissues of interest for phthalate analysis (i.e., bile, liver, muscle[35]) will be collected, weighed, homogenized. Methods for analyzing phthalate metabolite concentrations in bottlenose dolphin urine and fish tissue samples will be based on protocols established in Hart et al., (2018) and Valton et al., (2014) respectively. Briefly, urine samples will be spiked with 13C-labeled internal standards prior to an enzymatic de-glucuronidation step. Phthalate metabolites will be isolated by solid phase extraction (SPE) using an Agilent Bond Elute Nexus SPE. Analytical separation, detection, and quantification of phthalate metabolites in urine will rely on high performance liquid chromatography (Agilent 1100) with electrospray ionization tandem mass spectrometry (AB Sciex API400) and standard calibration curves. A series of laboratory spikes, field blanks, and matrix spikes will be used to ensure data quality[18,19].

Microplastics*:* Microplastic particle screening will occur in dolphin gastric and fecal samples, as well as fish tissue samples, including the esophagus, stomach, intestines, and muscle. Dolphin samples (2019, 2022-2023; n~20) will be emptied into a 250 mL glass beaker and weighed, and fish samples (2022-2023; n~150) will be dissected and placed in a 1000 mL glass beaker and weighed. Organic (non-plastic) material in the samples will be digested using potassium hydroxide (KOH[35]) and incubated at 60°C for 24 hours. Following digestion, samples will be vacuum filtered onto a GF/A 1.6 µm glass fiber filter that will be left to dry in a covered petri dish. Microplastic particles will be characterized using a dissection microscope (Leica EZ4, magnification 8-35x) according to physical attributes including shape (e.g., fiber, film, fragment, foam), texture, and color (e.g.,

transparent, blue, black[36]). Suspected plastic materials will be tested with a hot needle (250°C); plastic particles are confirmed if the needle melts or leaves a mark on the particle surface[37,38]. Microplastic particles ranging in size between 500µm and 5mm will be further characterized using Fourier Transform Infrared (FTIR) spectroscopy to determine polymer composition.

V. **Importance/Contribution**

Both phthalate and plastic pollution have previously been documented in apex marine mammals[19,20,39,40]. Plastic degradation can release phthalates into the surrounding environment[9]; however, the link between plastic exposure and subsequent phthalate exposure is not well studied. This project aims to fill in that gap in knowledge by evaluating paired samples indicating exposure to microplastics and phthalates to identify any links between exposures. **This study will be the first to characterize microplastic ingestion in live, free-ranging bottlenose dolphins relative to detectable phthalate metabolite concentrations**. Previous marine mammal studies of microplastic ingestion utilized scat samples from pinnipeds under managed care[27], and gastrointestinal tracts from stranded animals[26].

VI. **One health framework**

This project exemplifies a One Health framework by using bottlenose dolphins as sentinel of environmental microplastic and phthalate contamination. Dolphins from Sarasota Bay are long-lived apex predators who show a high degree of site fidelity, making them sensitive gauges for evaluating local environmental conditions. Further, given similarities in coastal water use and seafood selection between humans and dolphins, contaminant exposure routes in dolphins may be relevant to humans as well. As such, risks posed to dolphin health likely threaten human health as well, warranting further understanding of marine chemical contamination.

VII. **Use of funds**

Funds to analyze phthalate metabolite concentrations in dolphin urine have already been obtained, so requested funds will be used to purchase necessary laboratory supplies to analyze phthalate metabolite concentrations in fish samples, including a liquid chromatography column, a suite of native phthalate metabolite compounds, and SPE cartridges. Funds will also be requested to support student travel and lodging to participate in prey fish surveys in Sarasota Bay, Florida, as well as to support travel to present findings from this project at the Society for Marine Mammalogy's 25th biennial conference on the Biology of Marine Mammals in 2024.

LC column: XBridge Peptide BEH C18 XP Column, 300 Å, 2.5 µm, 2.1 mm X 50 mm, 1/pk (Waters)	Native phthalate metabolite compounds: MEP, MEHP, MEHHP, MEOHP, and MINP*	SPE cartridges (Agilent Bond Elute Nexus)	Travel to present at the Society for Marine Mammalogy biennial conference (2024)	Travel and lodging to participate in field work and prey fish surveys in Sarasota Bay	Total budget
$900	$2,500	$200	$1,000	$400	$5,000

*Estimated total cost for native phthalate metabolite compounds from Sigma Aldrich

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