

OBJECTIVES

Method Development

- This study will develop and validate a protocol to detect phthalate metabolites in bottlenose dolphin blubber, a tissue more easily obtainable (via remote biopsy) by researchers than urine
- Method development will utilize tissue samples from a dolphin stranded near Charleston, SC

Address Knowledge Gaps

- Previous studies have reported concentrations of phthalate metabolites in blubber of marine fauna, but these studies had limited sample sizes and did not have well-defined methods

INTRODUCTION

What are phthalates?

- Phthalates are a family of plasticizing compounds added to a variety of consumer products
- Production is estimated at 5 million MT/year
- Detected in air, drinking water, natural water bodies, and soil
- Easily released from consumer products and are bioavailable to humans and wildlife

Do phthalates cause problems?

- Endocrine disruptors: impacts on development, reproduction, and metabolism
- May cause cardiovascular and neurological issues

How does phthalate exposure happen?

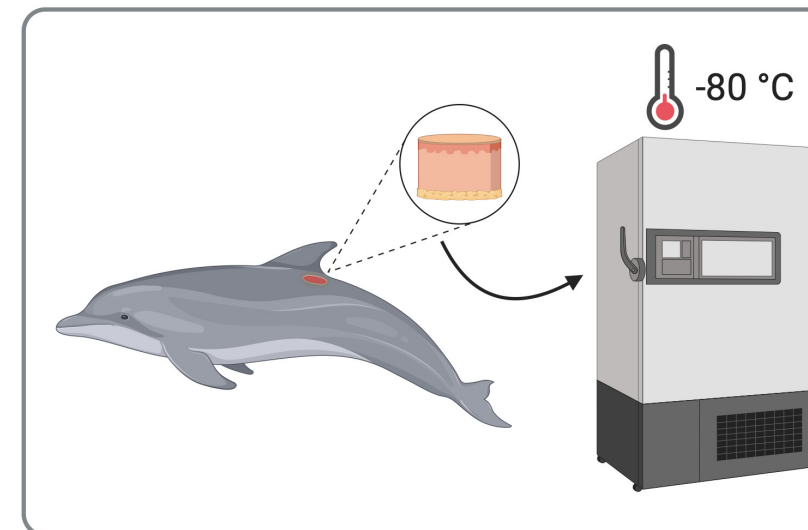
- Exposure in humans: ingestion, intravenous, inhalation, or dermal absorption
- Prevalent phthalate exposure has been observed in Sarasota Bay bottlenose dolphins
 - ~75% exposed to at least one type
- Dolphins likely exposed via similar mechanisms as humans, but the sources and types differ:
 - Exposure to a compound commonly added to plastic was higher in dolphins
 - Exposure to a compound commonly added to personal care products was higher in humans

How is phthalate exposure measured?

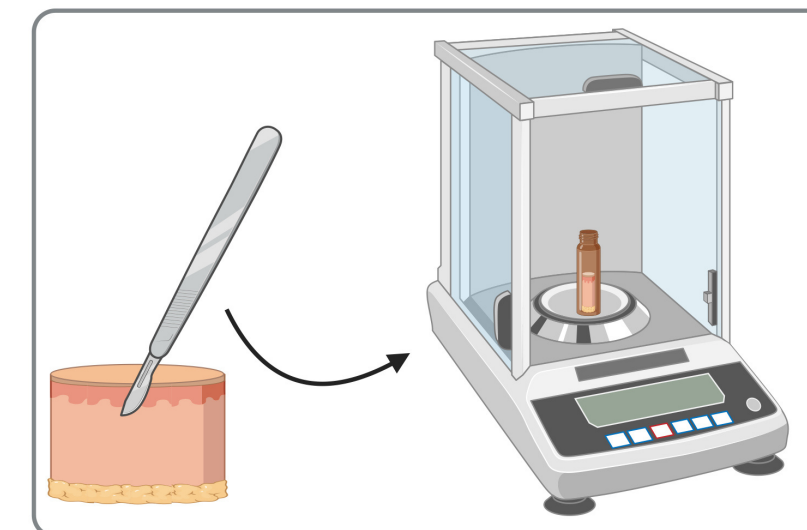
- Phthalate exposure surveillance has relied on urine samples for both humans and dolphins
- For dolphins, urine sample collection can be logistically complicated and requires that animals be physically handled

METHODOLOGY

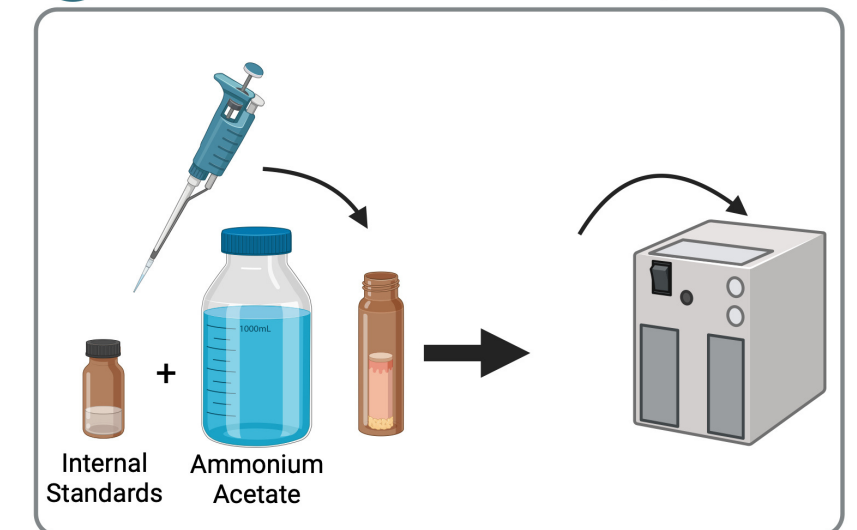
1 Sample collection and storage



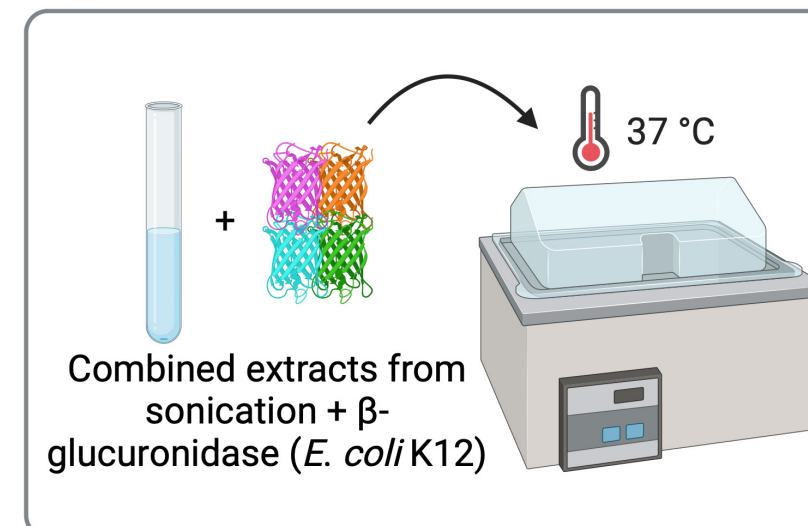
2 Subsample into 200 mg aliquots for analysis and store in glass



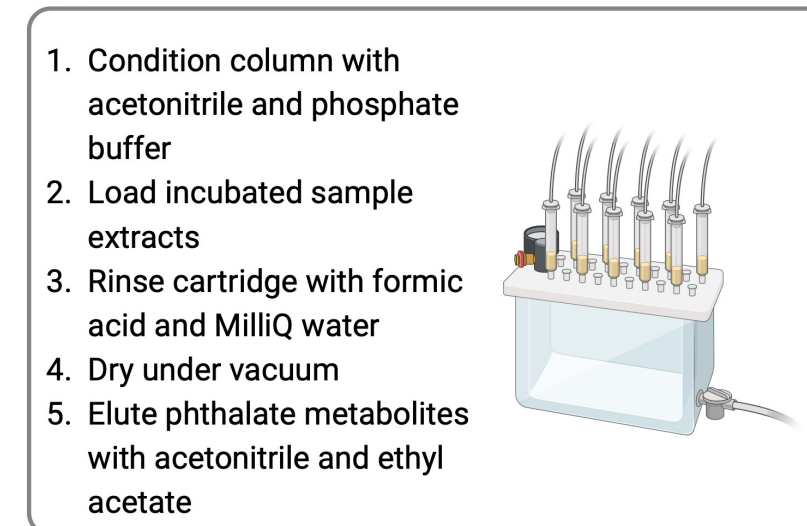
3 Samples spiked with internal standards and sonicated in ammonium acetate (2X)



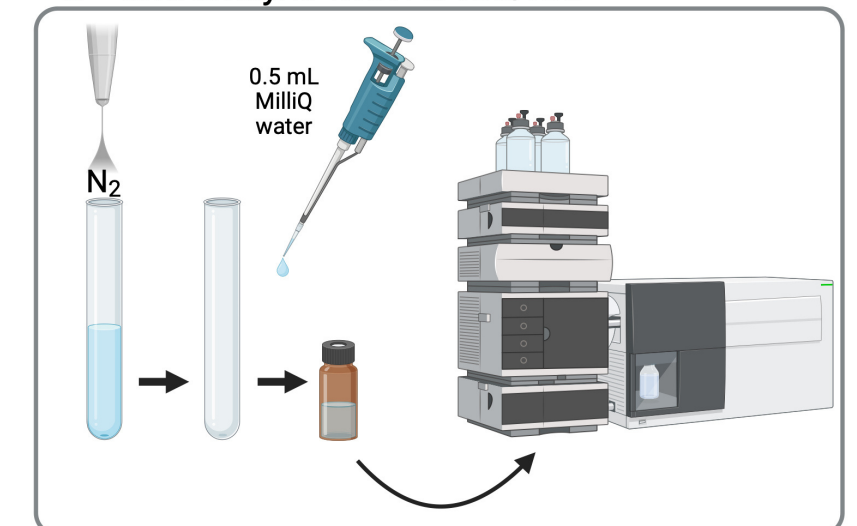
4 Deglucuronidation reaction (90 min)



5 Solid phase extraction



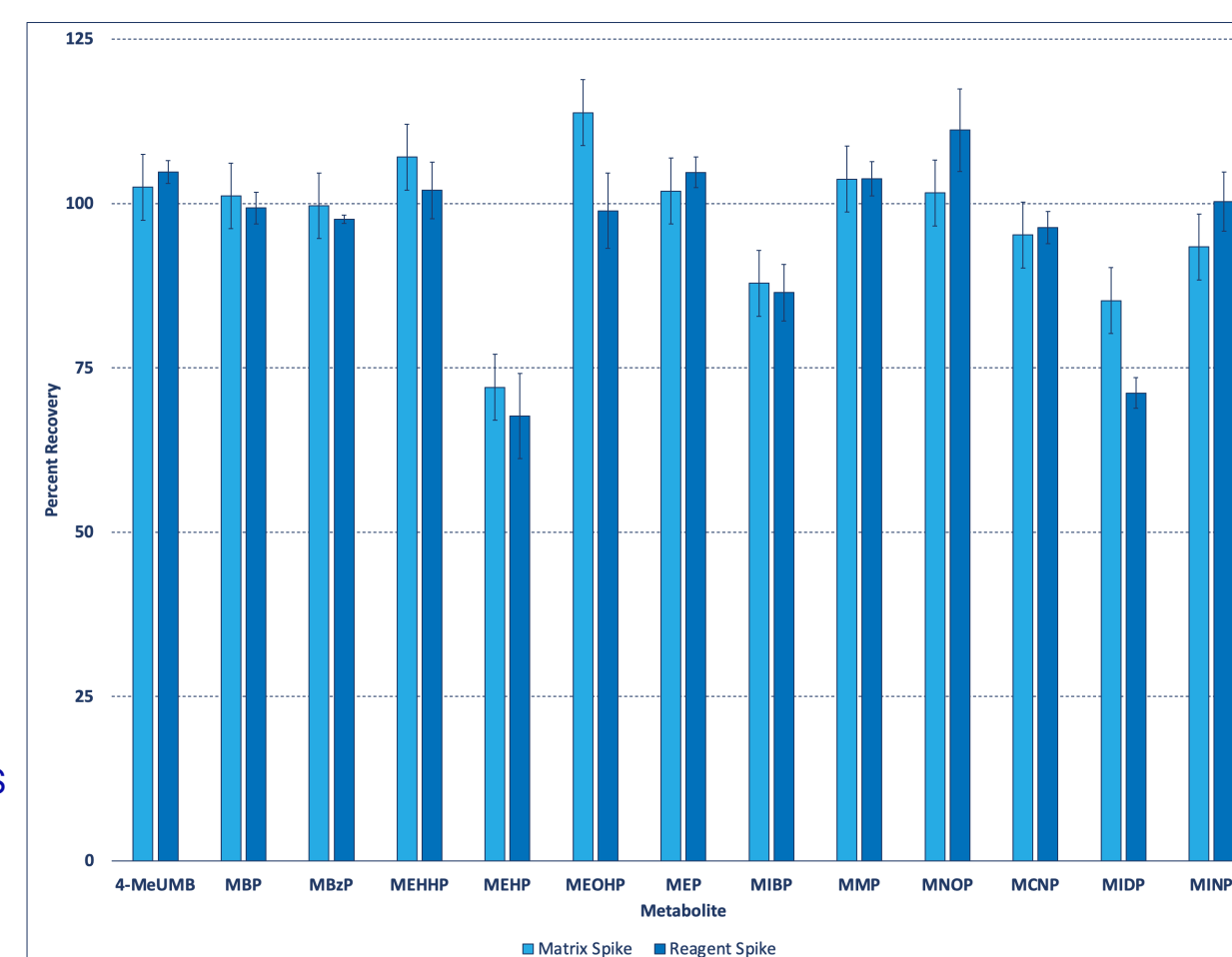
6 Evaporate under N₂, reconstitute in H₂O, and analyze with LC-MS/MS



Modified from Hart et al., 2018, GeoHealth, 2(10), 313-326 & Rian et al., 2020, Environment International, 137, 105525

FINDINGS

- A method was successfully developed to quantify phthalate metabolites in bottlenose dolphin blubber
- QA/QC samples fell within acceptable ranges for 13 metabolites
- 89% of matrix spikes and 82% of reagent spikes fell within acceptable recovery range (80-120%)



Average percent recoveries for matrix and reagent spikes. Bars represent mean recovery across five batches and error bars represent standard error.

- Preliminary analysis shows detectable metabolite concentrations in 35% of sampled dolphins (n=28)

FUTURE DIRECTIONS

- Measure phthalate metabolites in archived blubber samples obtained during catch-and-release health assessments in 2016, 2017, 2022, and 2023
- Examine the relationship between phthalate metabolite measurements in blubber and urine collected from individual Sarasota Bay dolphins during catch-and-release health assessments
- Validate and characterize phthalate metabolites spatiotemporally in blubber from Sarasota Bay dolphins using samples obtained in 2010-2024

ACKNOWLEDGEMENTS

Special thanks to my advisory committee (Dr. Leslie Hart, Dr. Ed Wirth, Dr. Randall Wells & Dr. Paul Sandifer) as well as Emily Pisarski and Miranda Dziobak. Funding support was provided, in part, by NIEHS grant #P01ES028942 to the University of South Carolina and a subward to the College of Charleston's School of Health Sciences. Laboratory resources and support were provided by the Ecotoxicology Group at NOAA's Hollings Marine Laboratory. Sampling efforts were supported by Wayne McFee as well as Sarasota Dolphin Research Program staff and volunteers.

