Analysis and quantification urinary phthalate metabolite concentrations were based on a protocol established by the Centers for Disease Control and Prevention (CDC) as detailed in Hart et al. (2018). This broader temporal study includes bottlenose dolphin urinary metabolite concentrations reported for sample years 2016-2017 from Hart et al. (2018), as well as results from analyses conducted on samples collected during 2010-2015 and 2018-2019; for a total of 51 samples. Briefly, each urine sample (collected 2010-2015; 2018-2019) was screened for eight phthalate metabolites, including monomethyl phthalate (MMP), monoethyl phthalate (MEP), mono(2-ethylhexyl) phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), monobenzyl phthalate (MBzP), monoisobutyl phthalate (MiBP), and monobutyl phthalate (MBP). Male samples that had excess sperm were centrifuged (1,000 rpm for 10 minutes) prior to extraction to separate the urine and prevent the solid phase extraction (SPE) cartridge from becoming clogged. Urine samples (1 mL) were spiked with a suite of the following isotopically labeled internal standards: MBP, MiBP, MBzP, MEP, MEHHP, MEHP, MEOHP, MMP. After which, they underwent a deglucuronidation step allowing the monoesters to be released from their conjugated forms for extraction (Blount et al., 2000). After deglucuronidation, samples were extracted via SPE (Agilent Bond Elute Nexus), then separated and quantified using high-performance liquid chromatography (HPLC; Agilent 1100; Waters XBridge BEH C18, 2.5 µm, 2.1 x 50 mm analytical column) coupled to a triple quadrupole mass spectrometer (MS; Applied Biosystems Sciex API 4000) with electrospray ionization (ESI negative) interface. Sample integrations were performed using Analyst software (ver 1.5). Prior to the acquisition of sample data, the instrument was calibrated (standard reference material (SRM) 3060: monoester phthalates in acetonitrile); coefficients of determination ($r^2$) for all metabolites were $\geq 0.995$. Quality assurance/quality control (QA/QC) samples (reagent blanks, reagent spikes, matrix spikes, SRM 3672 Organic Contaminants in Smokers' Urine, and field blanks) were processed alongside the urine samples. Reagent blank values were subtracted from the determined concentration value to account for any metabolite contamination resulting from laboratory processes. Available field blank metabolite concentrations were not found to be statistically different from each other by year or by metabolite. Field blank concentrations were averaged for each metabolite and subtracted from urine samples for any contamination due to sample collection materials (e.g., catheters). Acceptable QA/QC criteria for spike (reagent and matrix) and SRM recoveries were 80-120%. The limit of detection (LOD) was determined for each metabolite and is based on the lowest point of the calibration curve that could be detected on the instrument divided by the volume of sample extracted. In addition, continuing calibration verification standards were run with each batch (n=10) of urine samples to ensure the integrity of the calibration curve.

References

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