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Identification of potential biomarkers of exposure to diundecyl phthalate

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ABSTRACT

Diundecyl phthalate (DUP) is a high production volume chemical used as a plasticizer in polyvinyl chloride and other plastics. Specific biomarkers of DUP would be useful for human exposure assessment. To identify such biomarkers, we investigated the *in vitro* metabolism of DUP with human liver microsomes using online solid phase extraction coupled to HPLC-mass spectrometry. Using high resolution mass spectrometry, we conclusively confirmed the structures of four DUP specific metabolites: mono-undecyl phthalate (MUP), mono-hydroxyundecyl phthalate (MHUP), mono-oxoundecyl phthalate (MOUP), and mono-carboxydecyl phthalate (MCDP). We also used high resolution mass spectrometry to isolate MCDP and MHUP from co-eluting isobaric metabolites of diisononyl phthalate (*i.e.*, mono-carboxyisononyl phthalate) and diisododecyl phthalate (*i.e.*, monohydroxyisododecyl phthalate), respectively, that could not be separated with low resolution tandem mass spectrometry. To evaluate the potential usefulness of the newly identified DUP metabolites as exposure biomarkers, we analyzed 36 human urine samples by high resolution mass spectrometry. We detected MHUP and MCDP in $>83\%$ of the samples; median concentrations were 0.21 ng/mL and 0.36 ng/mL, respectively. MOUP was detected only in 14% of the samples analyzed, and MUP was not detected. All three metabolites eluted as peak clusters likely because of the presence of multiple oxidation sites and multiple isomers in DUP technical mixtures. Taken together, these findings suggest that with the appropriate mass spectrometry quantification techniques, MHUP and MCDP may serve as suitable biomarkers for assessing background exposure to DUP.

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1. Introduction

Diundecyl phthalate (DUP, Palatinol®111P-1, Jayflex™ L11P-E), a high molecular weight phthalate comprised of a pair of 11-carbon esters linked to a benzene-1,2-dicarboxylic moiety, is manufactured as a mixture of primarily branched chain isomers. DUP is a high production volume chemical (OECD, 2015) mainly used as a polyvinyl chloride (PVC) plasticizer in applications requiring low fog and low temperature flexibility such as wiring, cable jacketing and insulation, furniture and automobile upholstery, and flooring and wall covering (NICNAS, 2008; US Consumer Product Safety Commission, 2010). DUP can also be used in non-PVC polymers; in flame retardant nylon, rubbers, paints, and adhesives (NICNAS, 2008; US Consumer Product Safety Commission, 2010). US production of DUP and its proportion in the total phthalate production market have been slowly increasing since the implementation of chemical tracking in 1982 (US Consumer Product Safety Commission, 2010).

Toxicity data for DUP are relatively limited (Barber et al., 2000; David et al., 2001; Kwack et al., 2009, 2010; Saillenfait et al., 2013). Exposure to C7-11 phthalate monoester mixture did not show mutagenic potential in the L5178Y mouse lymphoma mammalian cell mutation assay, or genotoxicity in the Balb/3T3 cell transformation assay (Barber et al., 2000). In another *in vitro* study, cell-to-cell communication was inhibited by the C7-C11 phthalate monoester mixture with rat and mouse hepatocytes, but not with hamster, cynomolgus monkey, or human hepatocytes or in a human liver cell line; the same C7-11 monoester mixture did not markedly change peroxisomal beta-oxidation in hepatocytes from any species (Kamendulis et al., 2002). Exposure to DUP (500 mg/kg body weight/day) for 4 weeks in Sprague-Dawley male rats decreased sperm count and sperm mobility, although it did not significantly affect liver or testis weights (Kwack et al., 2009). In another study, male Sprague-Dawley rats administered 500 mg/kg body weight/day of DUP for two weeks

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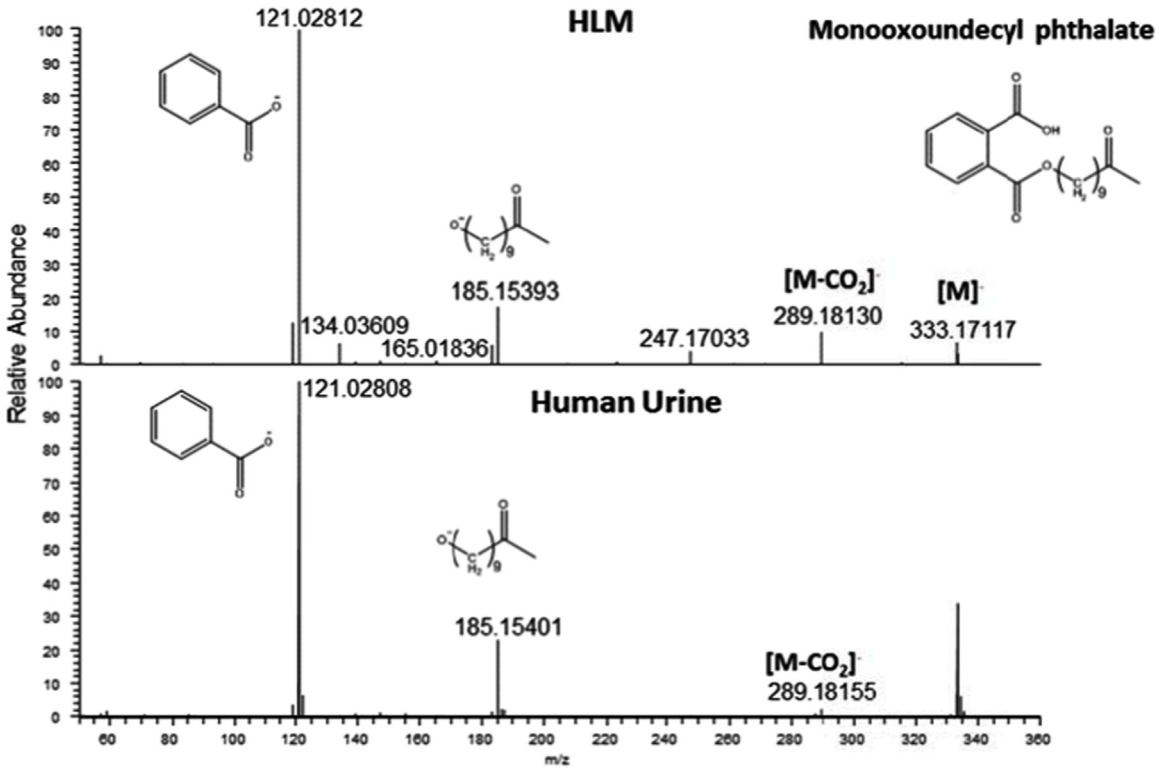


Fig. 1. High resolution mass spectrometric fragmentation of mono-oxoundecyl phthalate identified in human liver microsomes (HLM) after *in vitro* metabolism of DUP (top) and in human urine (bottom).

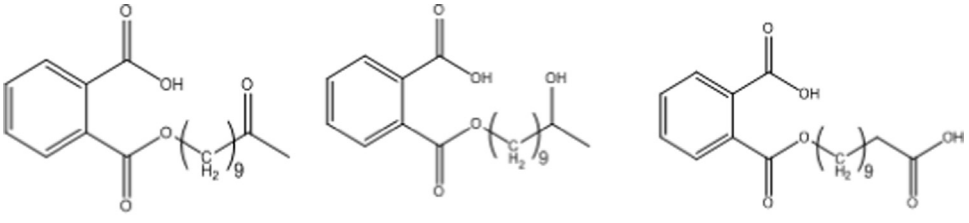
showed significant increases in aspartate aminotransferase and alkaline phosphatase (Kwack et al., 2010). In Sprague-Dawley rats dosed with 0.5 and 1 g DUP/kg body weight/day by gavage on gestation days 6–20, maternal body weight and food consumption were not affected, but, compared to controls, treated fetuses showed small decreases in anogenital distance (males) as well as a higher incidence of supernumerary lumbar ribs (Saillenfait et al., 2013). Although health effects of DUP in humans are largely unknown, identifying biomarkers of DUP would facilitate human exposure assessment, particularly because exposures to phthalates and phthalate alternatives may be changing as a result, at least in part, of legislative activity, industrial practices, public concerns and consumers' demands (Goen et al., 2011; Health Canada 2013; Schutze et al., 2014; Silva et al., 2013; Wittassek et al., 2007; Zota et al., 2014).

In vitro metabolism has been used to identify potential biomarkers of exposure to select environmental chemicals (Choi et al., 2013; Roberts et al., 2012; Silva et al., 2015). For the current study,

Table 1
QExactive high resolution mass spectrometric parameters for measuring the metabolites of diundecyl phthalate.

DUP metabolite	*m/z*		NCE*
	Precursor	Product	
Mono-oxoundecyl phthalate (MOUP)	333.1708	121.030	29
Mono-hydroxyundecyl phthalate (MHUP)	335.1864	187.170	25
Mono-carboxydecyl phthalate (MCDP)	349.1657	201.150	12

NCE*–normalized collision energy



The structure shown is for one isomer only.