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Biomonitoring of emerging DINCH metabolites in pregnant women in charleston, SC: 2011–2014

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HIGHLIGHTS

- We detect three urinary DINCH metabolites in low concentrations in pregnant women.
- OH-MINCH was the most prevalent DINCH metabolite detected.
- DINCH metabolites were higher in African American women than in Caucasian women.
- DINCH metabolite concentrations did not increase from 2011 to 2014.
- DINCH metabolites did not induce estrogenic or progestogenic activity.

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ABSTRACT

Due to the mounting evidence that phthalates, specifically di-2-ethylhexyl phthalate and dibutyl phthalate, produce adverse endocrine effects in humans and wildlife, the use of other chemicals as replacements has increased. One of the most commonly encountered phthalate replacements is di(isononyl)cyclohexane-1,2-dicarboxylate (DINCH). Currently, little is known about the prevalence of human exposure, bioactivity, and endocrine disrupting potential of DINCH. We sampled urine from 100 pregnant women during the second trimester of pregnancy living in Charleston, SC between 2011 and 2014 and measured the following DINCH metabolites by LC-MS/MS: cyclohexane-1,2-dicarboxylic acid-mono(hydroxy-isononyl) ester (OH-MINCH), cyclohexane-1,2-dicarboxylic acid-mono(oxo-isononyl) ester (oxo-MINCH), and cyclohexane-1,2-dicarboxylic acid-monocarboxy isooctyl ester (cx-MINCH). These metabolites were also tested on human estrogen receptor alpha and progesterone receptor beta transactivation assays *in vitro*. OH-MINCH was detected in 98% of urine samples. The specific gravity-adjusted median (interquartile range) OH-MINCH concentration was 0.20 (0.25) ng/mL, and concentrations were significantly higher in African American women compared to Caucasian women ($p = 0.01$). DINCH metabolite concentrations were consistent between years, and they did not exhibit estrogenic or progestogenic activity *in vitro*. Human exposure to these emerging compounds should continue to be monitored, especially in vulnerable populations, to ensure the replacement of phthalates by DINCH is not a case of regrettable substitution.

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Abbreviations: cx-MINCH, cyclohexane-1,2-dicarboxylic acid-monocarboxy isooctyl ester; DEHP, diethylhexyl phthalate; DF, detection frequency; DINCH, di(isononyl) cyclohexane-1,2-dicarboxylate; DINP, diisononyl phthalate; ESR1, estrogen receptor alpha; MEHP, mono-(2-ethylhexyl) phthalate; MINCH, cyclohexane-1,2-dicarboxylic acid mono isononyl ester; MUSC, Medical University of South Carolina; OH-MINCH, cyclohexane-1,2-dicarboxylic acid-mono(hydroxy-isononyl) ester; oxo-MINCH, cyclohexane-1,2-dicarboxylic acid-mono(oxo-isononyl) ester; PGRB, progesterone receptor beta; PVC, polyvinyl chloride; QC, quality control; SG, specific gravity.

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1. Introduction

With increasing evidence of phthalate-associated endocrine disruption from di-2-ethylhexyl phthalate and dibutyl phthalate over past decades comes a growing wariness of these compounds by both the general public and governmental regulatory bodies. As a result, some companies are seeking alternatives to phthalates in products in order to satisfy consumers and comply with stricter governmental regulations (CPSC, 2014). One of the most common phthalate replacements is di(isononyl) cyclohexane-1,2-dicarboxylate (DINCH). DINCH is manufactured by catalytic hydrogenation of the aromatic ring of diisononyl phthalate (DINP) and has been commercialized as Hexamoll® DINCH® (BASF, Ludwigshafen, Germany) since 2002. DINCH was introduced to replace high molecular weight phthalates, specifically diethylhexyl phthalate (DEHP) and DINP, in products leading to high human exposures such as toys, medical devices, and food packaging (Schütze et al., 2012). DINCH will likely have similar routes of exposure to humans as low molecular weight phthalates, which include inhalation and dermal absorption, and higher molecular weight phthalates, which includes ingestion (Giovanoulis et al., 2016).

In the human body, the metabolic degradation of DINCH begins with the partial cleavage of the diester into the simple hydrolytic monoester cyclohexane-1,2-dicarboxylic acid mono isononyl ester (MINCH). The alkyl chain of MINCH is then oxidized to produce secondary metabolites, which have been used as urinary biomarkers of DINCH exposure (Koch et al., 2013; Silva et al., 2013). Of the secondary metabolites, cyclohexane-1,2-dicarboxylic acid-mono(hydroxy-isononyl) ester (OH-MINCH) is most abundant, followed by cyclohexane-1,2-dicarboxylic acid-mono(oxo-isononyl) ester (oxo-MINCH), and cyclohexane-1,2-dicarboxylic acid-monocarboxy isooctyl ester (cx-MINCH). These three DINCH specific metabolites are excreted in urine within 24 h and represent approximately 13% of a single oral DINCH dose (Koch et al., 2013).

Biomonitoring efforts in the U.S. and worldwide have demonstrated that many humans are exposed to detectable amounts of DINCH, and exposure has been steadily increasing since its introduction in 2002. Between 2002 and 2012, the percentage of urine samples with detectable concentrations of DINCH metabolites increased from 0% to 21% in the U.S. and to 98% in Germany (Schütze et al., 2014; Silva et al., 2013). An important item to note is the limits of quantification for the two studies were an order of magnitude different. In the U.S. study the limit of quantification was 0.4 ng/mL and in the German study the limit of quantification was 0.05 ng/mL. This difference may account for some of the differences seen the detection of DINCH metabolites between the two studies. A second, more recent DINCH biomonitoring campaign from Germany found an increase in detectable DINCH metabolites to 100% starting in 2013 (Kasper-Sonnenberg et al., 2019). A Swedish study found that urinary concentrations of oxo-MINCH increased 20% each year, from 0.2 ng/mL to 0.7 ng/mL, between 2009 and 2014 (Gyllenhammar et al., 2017). DINCH metabolites have also been detected in fingernails, dust, and infant mattress covers (Boor et al., 2015; Fromme et al., 2016; Giovanoulis et al., 2016; Nagorka et al., 2011).

Due to the exposure potential of a fetus during gestation, pregnant women are a high-risk population for exposures to both phthalates and DINCH. However, unlike phthalates, the endocrine-disrupting potential of DINCH and its metabolites remain largely understudied. The non-aromatic structure of DINCH suggests it is less toxic to fertility and reproductive development than DEHP. *In vivo* toxicological studies have reported that DINCH exposure can exert negative effects on thyroid, liver, and kidney functions at high concentrations in rats, but these studies have shown no evidence of developmental or reproductive toxicity or endocrine disruption (EFSA, 2006). Data i*n vitro* investigating DINCH and its metabolites have shown effects at the molecular level (weak estrogenic effects), but were observed at concentrations more than three orders of magnitude higher than found in urine (Engel et al., 2018).

The aims of this study are to 1) determine urinary DINCH metabolite concentrations among pregnant women in Charleston, S·C., and 2) assess the potential estrogen or progesterone-like activity of DINCH metabolites. The estrogen receptor 1 (ESR1) and progesterone receptor B (PGRB) were tested as potential targets of DINCH metabolite activity due to their importance to fetal development and because the activity of these receptors is frequently modified by endocrine disrupting chemicals (Shanle and Xu, 2011; Viswanath et al., 2008). Based on the current literature and known exposure levels, we hypothesized that urinary DINCH metabolites would be present in our population at low concentrations. Because DINCH metabolites are non-aromatic, and because they are similar in structure to phthalate metabolites which are known more for their antiandrogenic effects, we also hypothesized that DINCH metabolites would exhibit no estrogenic or progesterone-like activity.

2. Materials and methods

2.1. Study population

Details of the study population have previously been described (Wenzel et al., 2018) Briefly, pregnant women living in the Charleston area and planning to deliver at the Medical University of South Carolina (MUSC) between 2011 and 2014 were recruited to participate in a larger study examining associations between maternal phthalate concentration and genital measurements of offspring. Women at least 18 years of age with uncomplicated singleton pregnancies dated by first trimester ultrasound were eligible. Exclusion criteria included women with fetal genetic anomalies, women who used steroids during pregnancy, and women diagnosed with any endocrine disorder.

At one point during gestational weeks 18 and 22, participants filled out a study questionnaire, provided a urine sample, and were physically evaluated. From this overall population (n = 378), urine samples from 100 women were selected for DINCH measurements. Of this subset of 100 study participants, 50 women were African American and 50 were Caucasian (very similar to the overall population of 49.2% African American and 50.8% Caucasian). We selected women that were representative of high, medium, and low phthalate exposures based on the summation of values determined in a previous study (then dividing these values into tertiles) (Wenzel et al., 2018) and ensured an even sampling distribution across years 2011–2014. Additionally, eight urine samples collected at delivery from participants in this subsample were selected at random for DINCH analysis. More information on the samples collected at delivery is provided in the SI. The institutional review board of MUSC approved this study protocol and informed consent was obtained from all participants prior to enrollment.

2.2. Urinary DINCH metabolite analysis

Urine was analyzed for three DINCH metabolites: OH-MINCH, oxo-MINCH, and cx-MINCH. All urine samples were collected in sterile glass jars at MUSC and transported to the Hollings Marine Laboratory (Charleston, South Carolina) for processing and analysis. The specific gravity (SG) of each urine sample was measured at room temperature with a handheld refractometer (Atago U.S.A., Inc., Bellevue, WA, USA), then the urine was aliquoted (1 mL) and stored at - 20 °C until analysis.

Methods for deconjugation, extraction, cleanup, and analysis were based on previously described methods from the U.S. Centers for Disease Control and Prevention and the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-University Bochum (IPA) (Koch et al., 2013; Silva et al., 2012). To summarize, DINCH metabolites were enzymatically deconjugated from their glucuronidated form by the addition of β-glucuronidase (*Escherichia coli*-K12), which was purchased from Roche Biomedical (Mannheim, Germany) and added to the urine sample. Extraction and cleanup of the urine samples was accomplished on automated solid-phase extraction workstations (RapidTrace, Biotage, Uppsala, Sweden) using polymeric sorbent-filled cartridges (60 mg, 3 mL; Bond Elut NEXUS, Agilent Technologies, Santa Clara, CA, USA). Compounds of interest were eluted with acetonitrile and ethyl acetate, which were then evaporated to dryness and reconstituted in water (200 μL) for analysis.

2.3. Instrumental analysis of DINCH metabolites

An Agilent 1200 Series liquid chromatography system with a 3 μm, 150 mm × 2.1 mm Betasil phenyl column (Thermo Fisher Scientific, Waltham, MA, USA) was used to separate urinary DINCH metabolites, which were then detected by tandem mass spectrometry on an AB Sciex API4000 QTRAP hybrid triple quadrupole linear ion trap mass spectrometer with electrospray ionization (Framingham, MA, USA). Isotopically labeled internal standards, generously provided by Dr. Holger Koch (IPA, Bochum, Germany), were used during DINCH analysis along with conjugated internal standards. Quality control samples and reagent blanks were analyzed alongside samples. Oxo-MINCH was determined semi-quantitatively by using the external calibration curve of Oxo-MINCH and d_4-OH-MINCH as the internal standard. Additional method description is provided in the Supplementary Material.

2.4. Statistical analyses

Statistical analyses were limited to metabolites with a detection frequency (DF) of greater than or equal to 50%. Urinary concentrations of metabolites that were less than the limit of detection (LOD) were given a value of half the LOD for statistical analysis. Concentrations of DINCH metabolites were adjusted for SG to account for urine dilution, using the formula: $P_c = P((SG_M - 1)/(SG - 1))$, where P_c is the concentration corrected for SG (ng/mL), P is the uncorrected concentration (ng/mL), SG_M is the mean SG for all study samples, and SG is the specific gravity of each individual urine sample (Boeniger et al., 1993).

Graphs were visually inspected, and all metabolite concentrations failed the Shapiro-Wilk test of normality, therefore we utilized nonparametric tests in subsequent analyses. We assessed bivariate associations between race, year, sampling time point, and DINCH concentrations by Mann-Whitney *U* test, Kruskal-Wallis test, and Wilcoxon signed-rank test, respectively. Correlations between OH-MINCH and values of oxo-MINCH above the LOD ($n = 44$) were determined by Spearman's rank-order correlation. Statistics were analyzed using IBM SPSS Statistics, version 22 (IBM Corp., Armonk, NY, USA) with significance set at $\alpha = 0.05$.

2.5. Transactivation and reporter gene assays

Transactivation assays were conducted according to previous publications (Katsu et al., 2006; Williams et al., 2017). For human ESR1 and PGRB transactivation assays, HEK293 T/17 cells (human embryonic kidney; ATCC CRL-11268) were maintained in phenol red-free DMEM (Sigma-Aldrich, St. Louis, MO, USA) containing 10% charcoal/dextran-treated fetal bovine serum (GE Healthcare, Little Chalfont, United Kingdom) and Penicillin-Streptomycin (Thermo Fisher Scientific). Cells were seeded in 96-well plates at a density of 10,000 cells per well. Twenty-four hours after seeding, the cells were transfected with plasmids DNAs (0.02 μg/well of human *ESR1* or *PGRB* in pcDNA3.1, 0.04 μg/well of *ERE* [estrogen response element] for *ESR1* or *MMTV* [mouse mammary tumor virus promoter] in pGL3 for PGRB), and 0.01 μg/well of pRL-tk (reference luciferase) using FuGENE® HD Transfection Reagent according to the manufacturer's protocol (Promega, Madison, WI, USA).

Four hours after transfection, cells were dosed with 10^{-14}, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, and 10^{-8} M E_2 for ESR1, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} M P_4 for PGRB, or vehicle alone (0.1% dimethyl sulfoxide). MINCH, OH-MINCH, cx-MINCH, and oxo-MINCH were tested at 10^{-9} M, 10^{-8} M, 10^{-7} M, 10^{-6} M, and 10^{-5} M. Additionally, we tested mono-(2-ethylhexyl) phthalate (MEHP) at 10^{-9} M, 10^{-8} M, and 10^{-7} M to compare results to a potent phthalate metabolite, as well as 10^{-7} M of a DINCH metabolite mixture (containing MINCH, OH-MINCH, cx-MINCH, and oxo-MINCH) to assess for potential mixture effects.

The cells were lysed after 44 h of exposure to compounds and firefly and Renilla luciferase signals were quantified using the Dual-Luciferase Reporter Assay System (Promega) and a Microbeta Workstation (PerkinElmer, Walktham, MA, USA) according to the manufacturer's protocols. Each experiment was repeated three times with triplicates at each repeat. Human ESR1 and PGRB and reporter constructs were kindly provided by Dr. Katsu (Hokkaido University, Japan), and Dr. Gellersen (Endokrinologikum, Hamburg, Germany). All data analyses were performed using GraphPad Prism software (GraphPad Software Inc., La Jolla, CA, USA).

3. Results

3.1. DINCH prevalence

The LODs for OH-MINCH, oxo-MINCH, and cx-MINCH were 0.04 ng/mL, 0.10 ng/mL, and 1.0 ng/mL, respectively (Table 1). Due in part to the relatively high LOD for cx-MINCH, we measured detectable concentrations in only one sample. We detected OH-MINCH in 98% and oxo-MINCH in 44% of the samples ($n = 100$). As expected, among those participants with OH-MINCH and oxo-MINCH levels above the LOD for both compounds, concentrations were positively correlated, $r_s(44) = 0.481$, $p < 0.01$ (Fig. 1). OH-MINCH, oxo-MINCH, and cx-MINCH were detectable in 100%, 50%, and 0% of the delivery urine samples ($n = 8$), respectively (data not shown).

The median (interquartile range) second trimester concentration of OH-MINCH was 0.18 (0.21) ng/mL for the overall population, 0.20 (0.24) ng/mL for African American women, and 0.12 (0.23) ng/mL for Caucasian women (Table 1). After adjusting for SG, median (interquartile range) OH-MINCH concentrations were 0.20 (0.25) ng/mL, 0.23 (0.27) ng/mL, and 0.13 (0.26) ng/mL for the overall population, African American women, and Caucasian women, respectively (Table 1). The maximum SG-adjusted concentration of OH-MINCH was 2.79 ng/mL. A Mann-Whitney *U* test determined that both unadjusted and SG-adjusted OH-MINCH concentrations were significantly higher in African American compared to Caucasian women ($p = 0.003$ for unadjusted; $p = 0.01$ for SG-adjusted; Table 1). We conducted a sensitivity analysis in which we dropped the three most extreme values (all African Americans), and found that the association between OH-MINCH and race remained significant ($p = 0.03$ for SG-adjusted concentrations).

3.2. Temporal trends in OH-MINCH exposure

Urinary concentrations of OH-MINCH in pregnant women did

Table 1
Limits of detection, detection frequency, range, and median concentrations of urinary DINCH metabolites (ng/mL) overall and by race during the second trimester of pregnancy.

DINCH Metabolite	LOD (ng/mL)	DF (%)	Range [a]	Median [a]		
				Overall (n = 100)	African American (n = 50)	Caucasian (n = 50)
OH-MINCH	0.04	98	<LOD – 1.58	0.18	0.20 *	0.12
			<LOD – 2.79	*0.20*	*0.23* **	*0.13*
oxo-MINCH	0.10	44	<LOD – 1.01	–	–	–
			<LOD – 1.06			
cx-MINCH	1.0	1	<LOD – 1.30	–	–	–
			<LOD – 1.04			

[a] Specific gravity-adjusted values are in italics beneath unadjusted values; *p = 0.003 using Mann-Whitney *U* Test for differences by race; **p = 0.01 using Mann-Whitney *U* Test for differences by race; LOD, limit of detection; DF, detection frequency.

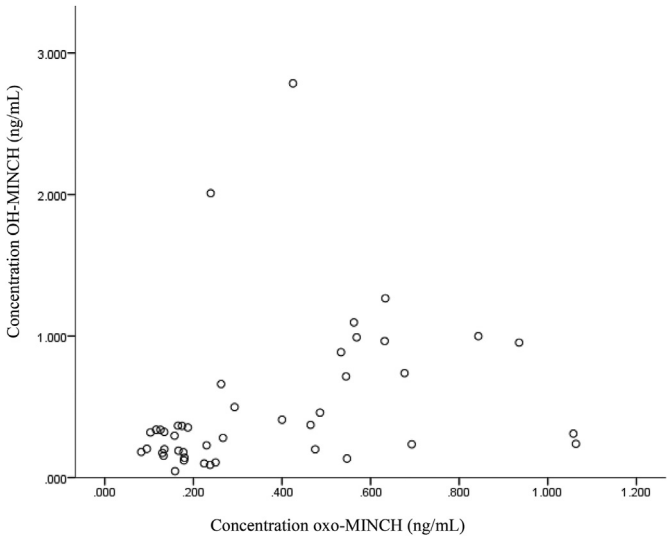

Fig. 1. Spearman's correlation analysis of SG-adjusted urinary concentrations of OH-MINCH and oxo-MINCH; $r_s(44) = 0.481$, $p < 0.01$.

not differ significantly between years 2011 and 2014 (Table 2, Fig. 2). The median SG-adjusted concentrations were 0.23 ng/mL in 2011, 0.22 ng/mL in 2012, 0.20 ng/mL in 2013, and 0.18 ng/mL in 2014. We also found no significant differences in OH-MINCH concentrations in second trimester urine samples (SG-adjusted median: 0.35 ng/mL) compared to paired delivery urine samples (SG-adjusted median = 0.13 ng/mL); however, OH-MINCH concentrations decreased in seven out of eight paired samples from the 2nd trimester to delivery (Fig. 3).

3.3. Transactivation assays

None of the DINCH metabolites tested demonstrated ESR1 or PGRB transactivation activity, even at high concentrations (Fig. 4). Similarly, neither MEHP nor the DINCH metabolite mixture elicited estrogenic or progestogenic responses. For the first of three experiments, the lowest concentration of MINCH tested (10^{-9} M) exhibited slight PGRB transactivation activity, but this was determined to be caused by contamination, as the response was not replicated in two additional experiments.

4. Discussion

At least one urinary DINCH metabolite in 98 of the 100 pregnant women participating in this cross-sectional study was detected. Concentrations of DINCH metabolites were low; the median concentration of most prevalent metabolite, OH-MINCH, was 0.18 ng/mL. African American women had higher urinary concentrations of OH-MINCH than Caucasian women, and OH-MINCH concentrations were similar between years 2011 and 2014. We found no significant differences in urinary DINCH metabolite concentrations between samples collected during the second trimester of pregnancy and at delivery. DINCH metabolites did not exhibit estrogenic or progestogenic activity when they were tested using an *in vitro* transactivation assay.

We detected OH-MINCH in 98% in samples, and oxo-MINCH and cx-MINCH were detected in 44% and 1% of samples, respectively. Our OH-MINCH and oxo-MINCH DFs were higher, but cx-MINCH DF

Table 2
Distribution of urinary OH-MINCH concentrations by year (ng/mL) [a].

Year	Mean (SD)	Median [b]	Range	N
2011	0.25 (0.31)	0.18	<LOD – 1.58	24
	0.33 (0.31)	*0.23*	*<LOD – 1.27*	
2012	0.30 (0.33)	0.19	<LOD – 1.44	24
	0.42 (0.48)	*0.22*	*<LOD – 2.01*	
2013	0.21 (0.18)	0.18	<LOD – 0.72	25
	0.26 (0.26)	*0.20*	*<LOD – 0.95*	
2014	0.30 (0.33)	0.17	<LOD – 1.39	27
	0.34 (0.53)	*0.18*	*<LOD – 2.79*	

[a] SG-adjusted values are in italics beneath unadjusted values.
[b] No differences according to the Kruskal-Wallis test (p = 0.86 for unadjusted; p = 0.67 for SG-adjusted); SD, standard deviation.

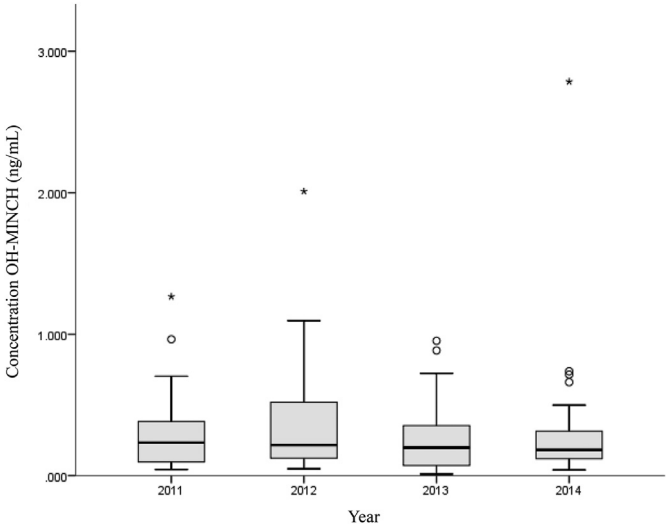

Fig. 2. Urinary OH-MINCH concentrations do not vary by year [a]. [a] p = 0.67 using Mann-Whitney *U* test for SG-adjusted differences by year; Box = 25th and 75th percentiles of SG-adjusted urinary OH-MINCH concentrations; bars = min and max values; circular dots = outliers; * = extreme outliers.

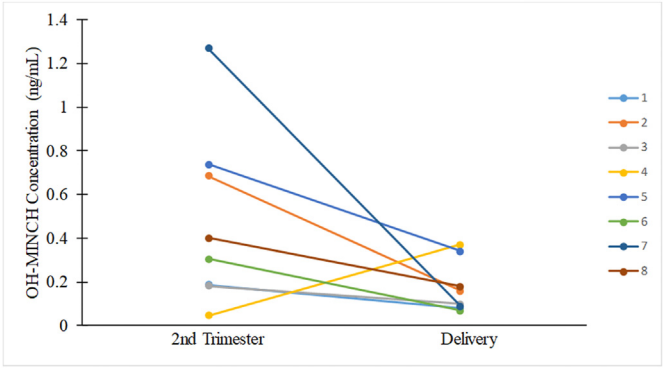

Fig. 3. Concentrations of OH-MINCH are lower at delivery than during the 2nd trimester in seven out of eight paired samples. Concentrations are higher at delivery than during the second trimester for Subject 4.

was lower than previously described for the U.S. population in 2012 (DF for OH-MINCH, oxo-MINCH, and cx-MINCH = 19%, 16%, 21%, respectively; Silva et al., 2013). The OH-MINCH DF reported here resembles those from abroad, but our oxo-MINCH and cx-MINCH DFs fall below those reported in Germany, Norway, and Sweden. Two studies in Germany reported 98% and 100% DF for OH-MINCH, 85% and 99% DF for oxo-MINCH, and 88% and 100% DF for cx-MINCH in 2011 and 2012 (Fromme et al., 2016; Schütze et al., 2014). A Norwegian study reported 85%–90% DF for OH-MINCH, depending on the time of day, and 85% DF for cx-MINCH (Giovanoulis et al., 2016). The Swedish study reported a DF of 90% for oxo-MINCH (Gyllenhammar et al., 2017). Taken together, these results suggest DINCH exposure is ubiquitous in our population and is more prevalent than in the general U.S. population, but similar to exposures detected in European populations. Since the LOD largely determines DFs of low-concentration compounds, variations in DFs between studies could also be the result of differences in measurement sensitivity. The LOD for OH-MINCH in this study was very similar to the LOD reported for the German studies and the Swedish study (0.04 ng/mL for this study compared to 0.05 ng/mL (German) and 0.08 ng/mL (Swedish)); however the LOD for OH-MINCH in this study was an order of magnitude lower compared to the previous U.S. study (Gyllenhammar et al., 2017; Schütze et al., 2014; Silva et al., 2013).

In addition to being more frequently detected, concentrations of OH-MINCH reported here (95th percentile = 0.99 ng/mL) are slightly higher than concentrations reported for U.S. females (95th percentile = 0.90 ng/mL) (CDC, 2017). Internationally, urinary concentrations of OH-MINCH metabolites measured here (median = 0.18 ng/mL) are lower than those reported in Portuguese children from 2014 to 2015 (median = 2.14 ng/mL) (Correia-Sá et al., 2017), German children from 2011 to 2012 (median = 1.7 ng/mL) (Fromme et al., 2016), Germans in 2012 (geometric mean = 0.4 ng/mL) (Schütze et al., 2014), and Australians from 2012 to 2013 (OH-MINCH range = 1.2–16.2 ng/mL) (Gomez Ramos et al., 2016). Additionally, urinary oxo-MINCH concentrations measured here (median = 0.24 ng/mL for values above the LOD) are slightly lower than those reported in Swedish first-time mothers from 2009 to 2014 (median = 0.37 ng/mL) (Gyllenhammar et al., 2017). The only European study in which DINCH metabolite concentrations were similar to concentrations reported here comes from a study of Norwegian adults between 2013 and 2014, in which their OH-MINCH creatinine-corrected

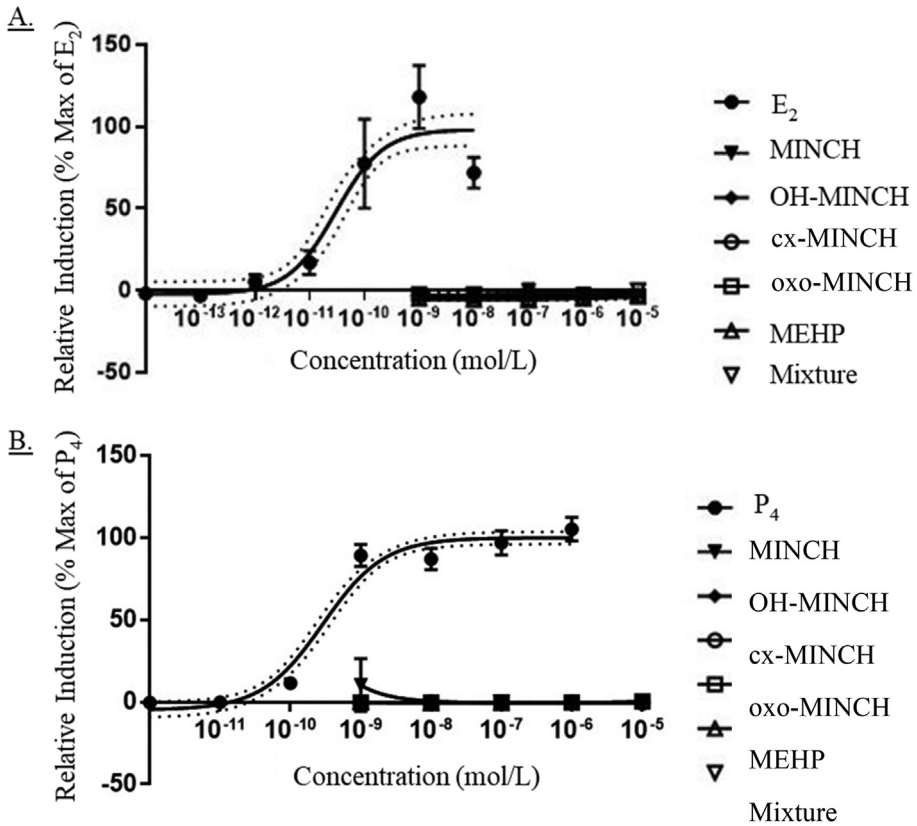

Fig. 4. ESR1 (ERα) and PGRB transactivation by DINCH metabolites and MEHP A) Test chemicals did not demonstrate estrogenic activity at any concentration (10^{-9} through 10^{-5} M). B) Test chemicals did not demonstrate progestogenic activity at any concentration (10^{-9} through 10^{-5} M).

geometric mean and our OH-MINCH SG-adjusted median were both 0.2 ng/mL (Giovanoulis et al., 2016). Laws banning the use of certain phthalates are more stringent in the European Union, thus we might expect the use of phthalate replacements such as DINCH to be more widespread abroad than in the U.S.

According to the National Health and Nutrition Examination Survey (NHANES; CDC, 2017), concentrations of OH-MINCH were highest in Mexican Americans, followed by non-Hispanic blacks, then non-Hispanic whites; however, when urine samples were creatinine-corrected, non-Hispanic whites had higher OH-MINCH concentrations than non-Hispanic blacks. Conversely, we found that with our study population both SG-adjusted and unadjusted urinary OH-MINCH concentrations were higher in African American women than in Caucasian women. Specific information on commercial phthalate replacement use and product formulations is not typically disclosed; therefore, we were unsure if DINCH is only used in certain phthalate-free products, or if DINCH implementation is widespread across the consumer market. Because both phthalate and DINCH metabolites were found at significantly higher concentrations in African Americans in our population, we speculate that DINCH is replacing phthalates in a wide variety of dietary and/or personal care products and may be indicative of a similar type source.

Increasing levels of urinary DINCH metabolites have been documented worldwide. We were surprised to find that urinary concentrations of DINCH metabolites did not increase from 2011 to 2014 in our study. In fact, median SG-adjusted OH-MINCH concentrations non-significantly decreased each year. These results suggest that while DINCH use may be increasing globally, local exposure to DINCH remained consistent from 2011 to 2014. It is possible that following an initial replacement of phthalates for DINCH to fulfill governmental requirements, little to no further substitution has occurred. Another possibility is that personal choices (exclusion of purchasing and using plastic materials, purchasing certain products) may play a role in the uniform levels which were seen.

Some studies have reported high exposure to phthalates during hospitalization in both preterm and full-term infants, suggesting that medical bags and tubing are acute sources of phthalate exposure (Frederiksen et al., 2014; Mallow and Fox, 2014; Su et al., 2012; Weuve et al., 2006). To determine if hospitalization was an acute source of DINCH exposure, we measured urinary DINCH metabolites in samples collected from eight study participants while hospitalized for delivery. We found no significant differences between urinary DINCH metabolite concentrations from the second trimester and at delivery in eight study participants. Therefore, medical devices and tubing used at MUSC between 2011 and 2014 were not significant sources of DINCH exposure. Current U.S. regulations encourage, but do not require, the replacement of DEHP-containing polyvinyl chloride (PVC) materials for medical use. As public awareness of adverse health effects associated with DEHP and other phthalates grows, it is likely that hospitals will voluntarily replace PVC materials with phthalate-free versions, and concentrations of urinary DINCH metabolites following hospitalization may increase.

Little is known about the bioactivity of DINCH metabolites in humans. According to results from initial rodent-based toxicological testing, high DINCH exposure can cause renal toxicity and thyroid hyperplasia, yet it was determined to be neither a reproductive toxicant nor an endocrine disruptor (EFSA, 2006). Upon testing DINCH metabolites individually and in a mixture on a transactivation assay, we found that none of the metabolites exhibited estrogenic or progestogenic activity at any doses tested. With the exception of the peroxisome proliferator-activated receptor (Lovekamp-Swan et al., 2003; Xu et al., 2010), phthalate metabolites do not typically exert their effects by targeting hormone receptors; therefore, we were unsure if DINCH metabolites would be bioactive at the hormone receptor level or not. Phthalate metabolites reduce the expression of enzymes and proteins involved in steroidogenesis, ultimately inhibiting Leydig cell production of testosterone (Martinez-Arguelles et al., 2013). Therefore, further experiments are required to test potential activities of DINCH metabolites on steroidogenic pathways.

Despite our null findings, others have recently discovered potential adverse health effects associated with DINCH and its metabolites, including hormonal (Boisvert et al., 2016), genetic (Nardelli et al., 2015), cytotoxic (Eljezi et al., 2017), and reproductive effects (Mínguez-Alarcón et al., 2016). Therefore, additional toxicological and epidemiological studies on this non-phthalate plasticizer and its metabolites are needed to determine its safety for human exposure.

These results should be interpreted with the consideration of several limitations. First, a single (spot) urine sample was used to estimate exposure, therefore, we were unable to control for the timing of sample collection or potential exposures before sampling. Previous studies have demonstrated that a single urine sample may represent exposure of semi-bioaccumulative compounds (like phthalates) to over several days (Hoppin et al., 2002) or months (Hauser et al., 2004), so the possibility exists that a spot urine sample is also adequate for DINCH measurement. Second, we assume that DINCH is metabolized and excreted similarly in all study participants. This is improbable, but we used specific gravity to correct for urine concentration in an attempt to reduce inter-individual pharmacokinetic variability. Third, the evaluation of exposure trends across years may not have enough statistical power since there were only approximately 25 samples for each of the four years. Strengths of this study include the diversity of the racial makeup of this population and robust sample size ($n = 100$). With a study population consisting of exactly half African American women and half Caucasian women, we were able to explore racial disparities in DINCH concentrations.

5. Conclusions

Here we report results from only the second biomonitoring assessment of urinary DINCH metabolite concentrations during pregnancy in the U.S., as well as the first results from a test of DINCH metabolites on ESR1 and PGRB transactivation assays. In summary, we found urinary DINCH metabolites in relatively low concentrations in this population of pregnant women. Similar to other biomonitoring studies, OH-MINCH was the most prevalent metabolite. African American women had significantly higher DINCH, and phthalate metabolite concentrations (Wenzel et al., 2018) than Caucasian women, suggesting the two plasticizers share common exposure pathways. We found that DINCH metabolite concentrations did not vary between 2011 and 2014, which is discordant from previous results showing increasing exposures. Urinary DINCH metabolite concentrations did not increase when sampled at delivery compared to the second trimester, suggesting that DINCH is not used in medical devices at MUSC. Finally, DINCH metabolites did not induce estrogenic or progestogenic activity. Body burdens of this phthalate replacement are likely to further increase. It is important to continue to monitor human exposure, especially in vulnerable populations, and to perform rigorous testing to ensure the replacement of DEHP for DINCH is not a case of regrettable substitution. From the “fetal origins of adult disease” standpoint, it is important to investigate effects of DINCH and its metabolites on both fetal health and health later in life.

Author contribution

Abby G. Wenzel, Conceptualization, Writing - original draft, preparation, sample preparation, Investigation; Jessica L. Reiner, Conceptualization, Methodology, writing, writing – reviewing and editing; Satomi Kohno, Methodology, Formal analysis, writing – reviewing and editing; Bethany J. Wolf, Conceptualization, Formal analysis, writing – reviewing and editing; John W. Brock, writing – reviewing and editing; Lori Cruze, Formal analysis, Resources, writing – reviewing and editing; Roger B. Newman, Conceptualization, Resources, writing – reviewing and editing; John R. Kucklick, Conceptualization, Supervision, writing – reviewing and editing

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.128369.

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