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Urinary concentrations of cyclohexane-1,2-dicarboxylic acid monohydroxy isononyl ester, a metabolite of the non-phthalate plasticizer di(isononyl)cyclohexane-1,2-dicarboxylate (DINCH), and markers of ovarian response among women attending a fertility center

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Abstract

Di(isononyl)cyclohexane-1,2-dicarboxylate (DINCH), a non-phthalate plasticizer, was introduced commercially in 2002 as an alternative to ortho-phthalate esters because of its favorable toxicological profile. However, the potential health effects from DINCH exposure remain largely unknown. We explored the associations between urinary concentrations of metabolites of DINCH on markers of ovarian response among women undergoing in vitro fertilization (IVF) treatments.

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Author's Contribution

R.H. was involved in study concept and design, and critical revision for important intellectual content of the manuscript. P.L.W. was involved in study concept and design, and critical revision for important intellectual content of the manuscript and provided statistical expertise. L.M.A. analyzed data, drafted the manuscript and had a primary responsibility for final content; L.M.A., I.S., P.L.W. and R.H. interpreted the data; Y-H.C. reviewed the statistical analysis; I.S., J.B.F., X.Y. and A.M.C. were involved in acquisition of the data. All authors were involved in the critical revision of the manuscript and approved the final manuscript.

Conflict Of Interest

None of the authors has any conflicts of interest to declare. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC). Use of trade names is for identification only and does not imply endorsement by the CDC, the Public Health Service, or the US Department of Health and Human Services.

Between 2011 and 2015, 113 women enrolled a prospective cohort study at the Massachusetts General Hospital Fertility Center and provided up to two urine samples prior to oocyte retrieval. The urinary concentrations of two DINCH metabolites, cyclohexane-1,2-dicarboxylic acid monohydroxy isononyl ester (MHiNCH) and cyclohexane-1,2-dicarboxylic acid monocarboxyisooctyl ester (MCOCH), were quantified by isotope dilution tandem mass spectrometry. We used generalized linear mixed models to evaluate the association between urinary metabolite concentrations and markers of ovarian response, accounting for multiple IVF cycles per woman via random intercepts.

On average, women with detectable urinary MHiNCH concentrations, as compared to those below LOD, had a lower estradiol levels (-325 pmol/l, $p=0.09$) and number of retrieved oocytes (-1.8 , $p=0.08$), with a stronger association among older women. However, urinary MHiNCH concentrations were unrelated to mature oocyte yield and endometrial wall thickness.

In conclusion, we found suggestive negative associations between urinary MHiNCH concentrations and peak estradiol levels and number of total oocyte yields. This is the first study evaluating the effect of DINCH exposure on human reproductive health and raises the need for further experimental and epidemiological studies to better understand the potential effects of this chemical on health.

Keywords

DINCH metabolites; epidemiology; markers of ovarian response; phthalates; reproductive health

Introduction

Phthalates are chemicals widely used in consumer goods and personal care products (CDC 2009). Because of their widespread use, their urinary metabolites are generally detected in the U.S., European, and Canadian general populations (Dewalque et al. 2014, Saravanabhavan et al. 2013, Schutze et al. 2014, Tefre de Renzy-Martin et al. 2014, Zota et al. 2014). Because of their potential adverse effects on human health (Braun et al. 2013, Dodge et al. 2015, Hauser et al. 2015, Messerlian et al. 2016, Miodovnik et al. 2014), the use of certain phthalates in diverse products have been banned (or banned from use in high amounts) in the European Union (EU 2005), Canada (Canada 2012), and in the U.S. by the Consumer Product Safety Commission (CPSC 2008). Di(isononyl)cyclohexane-1,2-dicarboxylate (DINCH), a non-phthalate plasticizer, was introduced commercially in 2002 as a safer alternative to ortho-phthalate esters because of a more favorable toxicological profile (EFSA 2006, SCENIHR 2015). The use of DINCH was approved by the European Food Safety Authority in 2006 (EFSA 2006).

DINCH is used in many polyvinyl chloride (PVC) products particularly in food packaging and in the manufacturing of toys, building materials and medical devices (EFSA 2006). Before 2002, cyclohexane-1,2-dicarboxylic acid monohydroxy isononyl ester (MHiNCH) and cyclohexane-1,2-dicarboxylic acid monocarboxyisooctyl ester (MCOCH), two oxidative DINCH metabolites, were not detected in urine collected from convenience samples of German and US adults (Schutze et al. 2014, Silva et al. 2013). However, MHiNCH was

detected in 19.3% of urine samples from National Health and Nutrition Examination Survey (NHANES) 2011–2012 participants (CDC 2015) and in the urine of 98% of German business students during 2012 (Schutze et al. 2014). Moreover, two recent publications showed that MHINCH was also detected in pooled urine samples collected from a convenience group of Australians (Gomez Ramos et al. 2016) and a group of 27–80 month old children in Germany (Fromme et al. 2016). Therefore, taken together, these findings show that there is ongoing exposure to the non-phthalate plasticizer DINCH; due to its increased use in various products, higher exposure is expected in the future.

DINCH was not considered an endocrine disruptor or reproductive toxicant (EFSA 2006). No evidence was found of developmental or reproductive toxicity in rats subchronically gavaged for 13 weeks up to the highest dose of 1 g/kg bw/day; similarly, thyroid hyperplasia and renal toxicity occurred only at high doses (1 g/kg bw/day in female rats and 300 mg/kg bw/day in male rats) (EFSA 2006). Similarly, a recent in vitro experimental study by Boisvert and colleagues has shown no consistent effect of DINCH on Leydig and spermatogonial mouse testicular cell lines (Boisvert et al. 2016). In addition, based on another recent in vitro study using rat preadipocytes, Campioli and coworkers also found no effect of DINCH itself, but they suggested that cyclohexane-1,2-dicarboxylic acid monoisononyl ester (MINCH), a minor metabolite of DINCH (Koch et al. 2013), is a potent peroxisome proliferator-activated receptor (PPAR)- α agonist and a metabolic disruptor capable of inducing stromal vascular fraction preadipocyte differentiation, which may interfere with the endocrine system (Campioli et al. 2015). Whether DINCH behaves as an endocrine disruptor and has toxicological effects on reproduction is unclear due to the scarce data on experimental animals (Boisvert et al. 2016, Campioli et al. 2015, Campioli and Papadopoulos 2016, Otter 2016). Moreover, there are no data on the potential human effects of DINCH on reproductive health.

We recently published that urinary concentrations of the metabolites of di-(2-ethylhexyl) phthalate (DEHP), the most common PVC phthalate plasticizer (Hauser and Calafat 2005) were negatively associated with oocyte yield, clinical pregnancy and live birth among women following in vitro fertilization (IVF) treatments in an academic fertility clinic in Boston, MA (Hauser et al. 2015). In the current study, we explored the associations between urinary concentrations of DINCH metabolites on IVF outcomes in a subsample of women from the same study cohort with urinary concentrations of DINCH measured.

Methods

Study population

Study participants were women enrolled in the Environment and Reproductive Health (EARTH) Study, an ongoing prospective cohort established to evaluate environmental and dietary determinants of fertility (Hauser et al. 2006). Women between 18 and 45 years old were eligible to participate and approximately 60% of those contacted by the research nurses enrolled. Although DINCH was introduced commercially in 2002 and participants were recruited into this study beginning in 2004, analytical methods for measurement of urine DINCH metabolites were developed several years later (CDC 2015) and therefore, the measurement of DINCH metabolites in urine did not begin until June 2011, when these

chemicals were added to the study protocol. The current analysis includes 113 women who completed at least one IVF cycle between June 2011 and June 2015 (n=151 cycles) at the Massachusetts General Hospital (MGH) Fertility Center, and had provided at least one urine sample for the measurement of DINCH metabolites per IVF cycle. We excluded from this analysis IVF cycles for which women used an egg donor (n=1) and cryo-thaw cycles (n=29). The study was approved by the Human Studies Institutional Review Boards of the MGH, Harvard T.H. Chan School of Public Health, and the Centers for Disease Control and Prevention (CDC). Participants signed an informed consent after the study procedures were explained by a trained research study staff and all questions were answered.

Urine sample collection and DINCH metabolite measurements

Women provided up to two spot urine samples per IVF cycle. The first specimen (not necessarily a fasting sample) was collected between Day 3 and Day 9 of the stimulation phase; the second, always a fasting sample, was collected on the day of oocyte retrieval, prior to the procedure or administration of IV fluids. Urine was collected in a sterile, clean polypropylene specimen cup at the MGH Fertility Center. Specific gravity (SG) was used to correct DINCH metabolites concentrations for urine dilution. SG was measured at room temperature and within several hours (typically within one hour) of the urine being produced using a handheld refractometer (National Instrument Company, Inc., Baltimore, MD, USA) calibrated with deionized water before each measurement. The urine was then divided into aliquots, frozen at -20°C , and then stored long-term at -80°C . Samples were shipped on dry ice overnight to the CDC where they were stored at or below -40°C until analysis. The urinary concentrations of MHiNCH and MCOCH were measured using online solid-phase extraction (SPE) coupled with isotope dilution- high-performance liquid chromatography (HPLC)- tandem mass spectrometry (MS/MS) as described before (Silva et al. 2013). Briefly, urine (0.1 mL) was spiked with an internal standard solution of stable-isotope labeled standards of the target metabolites and a buffered β -glucuronidase solution (*E. coli*-K12; 25 μL , pH 6.5/1 M), and incubated for a minimum of 120 min. After injection (450 μL), the target analytes in the spiked urine were extracted using online SPE (Chromolith high resolution RP-18e guard column, 4.6 mm \times 5 mm, Merck KGaA, Germany), chromatographically resolved by HPLC (Betasil phenyl HPLC column, 3 μm , 150 mm \times 2.1 mm, ThermoHypersil-Keystone, Bellefonte, PA, USA) using a nonlinear solvent gradient from 100% mobile phase A (0.1% water) to 100% mobile phase B (0.1% acetic acid in acetonitrile) at a flow rate of 0.35 mL/minute, and detected using negative ion electrospray-ionization MS/MS (Thermo Scientific TSQ Vantage Triple Quadrupole Mass Spectrometer, ThermoFinnigan, Bellefonte, PA, USA). The limit of detection (LOD) were 0.4 $\mu\text{g/L}$ (MHiNCH) and 0.5 $\mu\text{g/L}$ (MCOCH). In addition to study samples, each analytical run included low-concentration and high-concentration quality control urine pools and reagent blanks to assure the accuracy and reliability of the data (Silva et al. 2013). DINCH metabolite concentrations were adjusted for dilution using the following formula: $P_c = P[(1.015 - 1)/SG - 1]$, where P_c is the SG-corrected DINCH metabolite concentration ($\mu\text{g/L}$), P is the measured DINCH metabolite concentration ($\mu\text{g/L}$) of the urine sample, and 1.015 is the mean SG concentration in the study population (Smith et al. 2012). The geometric mean of the SG-adjusted DINCH metabolite concentrations from two spot urine samples collected during each IVF cycle was used as a measure of cycle-specific urinary

DINCH metabolites concentration. For cycles with only one urine sample (~19%), the DINCH metabolite concentration for that single urine sample was used as the cycle-specific urinary concentration.

Clinical management and assessment of outcomes

Clinical information was abstracted from the patient's electronic medical record by trained research staff. As part of the infertility work-up all patients had blood collected on the third day of the menstrual cycle. The serum concentrations of follicle stimulating hormone (FSH) and estradiol (E_2) were measured using an automated electrochemiluminescence immunoassay at the MGH Core Laboratory, as previously described (Mok-Lin et al. 2010). E_2 levels, defined as the highest level of E_2 prior to oocyte retrieval, was obtained on the day of trigger with human Chorionic Gonadotropin (hCG). Subsequent to an infertility evaluation, each patient was assigned an infertility diagnosis by a physician at the MGH Fertility Center according to the Society for Assisted Reproductive Technology (SART) definitions as previously described (Mok-Lin et al. 2010). The participant's date of birth was collected at entry, and weight and height were measured by trained study staff. Body mass index (BMI) was calculated as weight (in kilograms) per height (in meters) squared.

Women underwent one of three controlled ovarian stimulation IVF treatment protocols after completing a cycle of oral contraceptives: 1) luteal phase GnRH-agonist protocol, 2) follicular phase GnRH-agonist/Flare protocol, or 3) GnRH-antagonist protocol. FSH/hMG and GnRH-agonist or GnRH-antagonist was continued the night before of the day of trigger with hCG. Follicular response was monitored with serial ultrasounds and estradiol levels. The latter were determined at the MGH Core Laboratory using the Elecsys Estradiol II reagent kit (Roche Diagnostics). hCG was used to trigger oocyte maturation 36 hours before the scheduled oocyte retrieval and was administered when at least 3 follicles 16–18 mm in diameter were detected by transvaginal ultrasonography and when the peak E_2 level reached at least 800 pg/mL. Patients were monitored during gonadotropin stimulation for serum E_2 , follicle size measurements and counts, and endometrial thickness through 2 days before egg retrieval. Details of egg retrieval have been previously described (Mok-Lin et al. 2010). Embryologists determined the total number of oocytes retrieved per cycle and classified them as germinal vesicle, metaphase I, metaphase II (MII) or degenerated.

Statistical analysis

Demographic and baseline reproductive characteristics of the women were presented using median \pm interquartile ranges (IQRs) or percentages. Women's exposure to DINCH was categorized into 2 groups of urinary MHiNCH concentrations (below and above LOD). MCOCH was not considered for analysis because of the low percentage of detectable concentrations (9%). Associations of urinary MHiNCH concentrations with demographics and baseline reproductive characteristics were evaluated using Kruskal–Wallis tests for continuous variables and chi-squared tests for categorical variables (or Fisher's exact test where appropriate). An intraclass correlation coefficient (ICC) was calculated to explore variability of urinary MHiNCH concentrations within woman. Multivariable generalized linear mixed models were used to evaluate the association between urinary MHiNCH concentrations and markers of ovarian response, accounting for multiple IVF cycles per

woman using random intercepts. A Poisson distribution and log link function were specified for oocyte counts, and a normal distribution and identity link function were specified for endometrial wall thickness and E₂ trigger levels. To allow for better interpretation of the results, population marginal means (Searle et al. 1980) were presented adjusting for all the covariates in the model, at the mean levels of all covariates (including categorical measures).

Confounding was assessed using prior knowledge on biological relevance through descriptive statistics from our study population. The variables considered as potential confounders included factors previously related to ovarian outcomes in this and other studies, and factors associated with DINCH exposure and ovarian outcomes in this study, regardless of whether they had been previously described as predictors of ovarian outcomes (Table 1). Final models were adjusted for age (continuous), year of treatment (continuous), and infertility diagnosis (male factor, female factor, unexplained). Endometrial wall thickness, total and mature oocyte yields models were further adjusted by Day3 FSH levels (continuous). All tests were two-tailed and the level of statistical significance was set at 0.05. Statistical analyses were performed with SAS (version 9.4; SAS Institute Inc., Cary, NC, USA).

Results

Most of the 113 women were Caucasian (80%), had a college degree (60%) and had never smoked (78%) (Table 1). Participants had a median (IQR) age and BMI of 35 years (31, 38) and 23.2 kg/m² (21.1, 26.3), respectively. Unexplained infertility (39%) was the primary SART diagnosis at enrollment and luteal phase GnRH-agonist was the most used initial treatment protocol (74%). Women were older across increasing urinary MHiNCH concentrations (p value=0.03), but no other covariates differed across urinary DINCH metabolite concentrations (Table 1).

The SG-adjusted GM and median MHiNCH urinary concentration for the 274 samples provided by the 113 women (contributing 151 IVF cycles) was <LOD (0.40 µg/L), with a 29% detection rate (Table 2). The SG-adjusted GM and median MCOCH urinary concentrations was also <LOD (0.50 µg/L), and 9% of them had detectable concentrations. Two urine samples were collected in 81% (123/151 for MHiNCH) of the IVF cycles. The intraclass correlation coefficient (ICC) (95% CI) was 0.22 (0.12, 0.38), which demonstrates high within woman variability.

In unadjusted and adjusted models, there were negative associations between SG-adjusted urinary MHiNCH concentrations and total oocyte yields and peak E₂ levels (Table 3). Although these relationships were of borderline statistical significance, in adjusted models, women with detectable urinary MHiNCH concentrations (> 0.40 µg/L) had a mean decrease of 1.8 total oocytes (p, trend= 0.08) and a mean decrease of 325 pmol/l E₂ levels (p, trend=0.09), respectively, compared with women in non-detectable urinary MHiNCH concentrations (<0.40 µg/L). However, SG-adjusted urinary MHiNCH concentrations were unrelated to other markers of ovarian reserve such as mature oocyte yields and endometrial wall thickness (Table 3).

We also explored whether age modified the associations between urinary concentrations and markers of ovarian reserve. The association between SG-adjusted urinary MHiNCH concentrations and total oocyte yields was stronger among women who were ≥ 37 years old as compared to younger women (Figure 1); however, the p-value for interaction was borderline (p-interactions=0.13). Women who were ≥ 37 years old and had SG-adjusted urinary MHiNCH concentrations above LOD, on average, had 2.9 fewer total oocytes (p, value=0.05) compared women who were <37 years old and had SG-adjusted urinary MHiNCH concentrations below LOD. This comparable difference in younger women was however, 0.4 fewer total oocytes (p, value= 0.80) (Figure 1).

Finally, because DINCH has been used as a substitute for DEHP in certain applications, we explored correlations between urinary DINCH and DEHP metabolites and also further adjusted our models for urinary concentrations of DEHP metabolites. We previously reported that urinary DEHP concentrations were negatively associated with oocyte yield (Hauser et al. 2015) and therefore, the negative association between urinary MHiNCH concentrations and total oocyte yield could be partially driven by DEHP exposure. As expected, urinary MHiNCH and DEHP metabolite concentrations were moderately correlated ($r=0.65$, $p=0.03$). However, in models additionally adjusted for sum of DEHP metabolites, results for the association of MHiNCH and oocyte yield did not change (data not shown).

Discussion

As far as we are aware, this is the first study exploring the association of DINCH metabolites with human reproductive health. We found suggestive associations of urinary MHiNCH concentrations with markers of ovarian response including the number of total oocyte and E_2 levels collected and measured at retrieval; however, MHiNCH was unrelated to other markers of ovarian response. The negative association between urinary MHiNCH concentrations and total oocyte yield was stronger in older women (≥ 37 years old) compared with younger women (<37 years old). This interesting finding could be explained by the important role of age in predicting infertility. Age has been considered the main predictor of infertility in women trying to become pregnant; the percentage of women experiencing infertility increases markedly with age, from 7%–9% among those aged 15–34 years to 25% among those aged 35–39 years and 30% among those aged 40–44 years (CDC 2014). As women age, the risk of infertility rises because of diminished oocyte quality and ovulatory function. The stronger association between higher urinary MHiNCH concentrations with lower total oocyte yield among older women may represent age as a marker of increased sensitivity to DINCH exposure. Interestingly, we also found that older women had higher urinary MHiNCH concentrations compared with younger women. It is unclear why they had higher urinary concentrations and this is worthy of further investigation in other cohorts.

It is difficult to interpret the potential pathways and mechanisms through which DINCH may impact oocyte yield given the dearth of in vitro studies and studies in experimental animals on this chemical and these outcomes. In a recent experimental study, Boisvert and coworkers showed differential in vitro effects of DINCH on Leydig and spermatogonial mouse testicular cell lines (Boisvert et al. 2016). While high concentrations of DINCH (10^{-4} M)

were found to decrease Leydig cell steroid production, lower DINCH concentrations (10^{-8} – 10^{-5} M) had a stimulatory effect on the production of these cells. Also, no consistent effects were found on Leydig cell viability, proliferation, and mitochondrial integrity, and spermatogonial cell viability and proliferation. Campioli and colleagues reported that DINCH itself had no toxicological effect; however, MINCH, a minor DINCH metabolite, promoted differentiation in a primary culture model of rat preadipocytes, which is mediated by PPAR- α in its first stage. Therefore, if MINCH acts as a potential PPAR- α agonist and is able to alter hormone signaling, it may be considered a metabolic disruptor interfering with the endocrine system (Campioli et al. 2015). However, they did not study MHiNCH and it is unknown if this metabolite has similar activities as MINCH. The woman in our study with the highest urinary MHiNCH and MCOCH concentrations (SG-adjusted=42.8 and 16.0 $\mu\text{g/L}$, respectively) also had a reduced number of total (4) and mature (3) retrieved oocytes. However, it is difficult to make conclusions based on a single participant.

The MHiNCH median in our study population was below the LOD of 0.4 $\mu\text{g/L}$, in agreement with data from NHANES 2011–2012 adult participants (CDC 2015), as well as other North American (Silva et al. 2013) and German (Schutze et al. 2014) populations. It is important to note that only in samples collected in 2012 among the Germans, the MHiNCH median was above the LOD (Schutze et al. 2014). Interestingly, frequency of detection of MHiNCH among convenience groups of adults changed from 0% to 19% in the U.S. (Silva et al. 2013) and from 0% to 98 % in Germany (Schutze et al. 2014) over a 12–13 year period (1999–2000 to 2012). The increased frequency of detection of MHiNCH in these studies is also consistent with a 21% increase observed in our study population between 2011 and 2015. Therefore, higher MHiNCH detection frequencies and urinary concentrations are expected in the future as DINCH becomes more widely used.

The present study has several limitations including small sample size. Due to its design, it may not be possible to generalize findings to couples conceiving without medical intervention. However, these findings may be applicable to other women seeking infertility treatment (Stephen and Chandra 2000). Also, misclassification of DINCH exposure based on urinary concentrations of MHiNCH from spot samples is possible because DINCH metabolites have relatively short elimination half-lives (Koch et al. 2013) and exposures to DINCH are likely to be episodic in nature. However, we collected multiple urine samples per participant and this will reduce exposure misclassification that would potentially lead to attenuated associations. Lastly, only 29% of urine samples had detectable MHiNCH concentrations suggesting limited exposure to DINCH in our population. Strengths of our study include its prospective design which minimizes the possibility of reverse causation and the comprehensive adjustment for possible confounding variables.

In conclusion, we found suggestive negative associations between urinary MHiNCH concentrations and peak E_2 levels and number of total oocyte yields, with stronger associations among older women. Because this is the first study evaluating the effect of DINCH exposure on human reproductive health, further experimental and epidemiological studies will be useful to better understand the effects of this chemical on health.

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Highlights

- Women with detectable urinary MHiNCH concentrations, as compared to those below LOD, had a lower estradiol levels and number of retrieved oocytes
- The negative association between urinary MHiNCH concentrations and total oocyte yield was stronger in older women (≥ 37 years old) compared with younger women (<37 years old).
- Urinary MHiNCH concentrations were unrelated to mature oocyte yield and endometrial wall thickness.

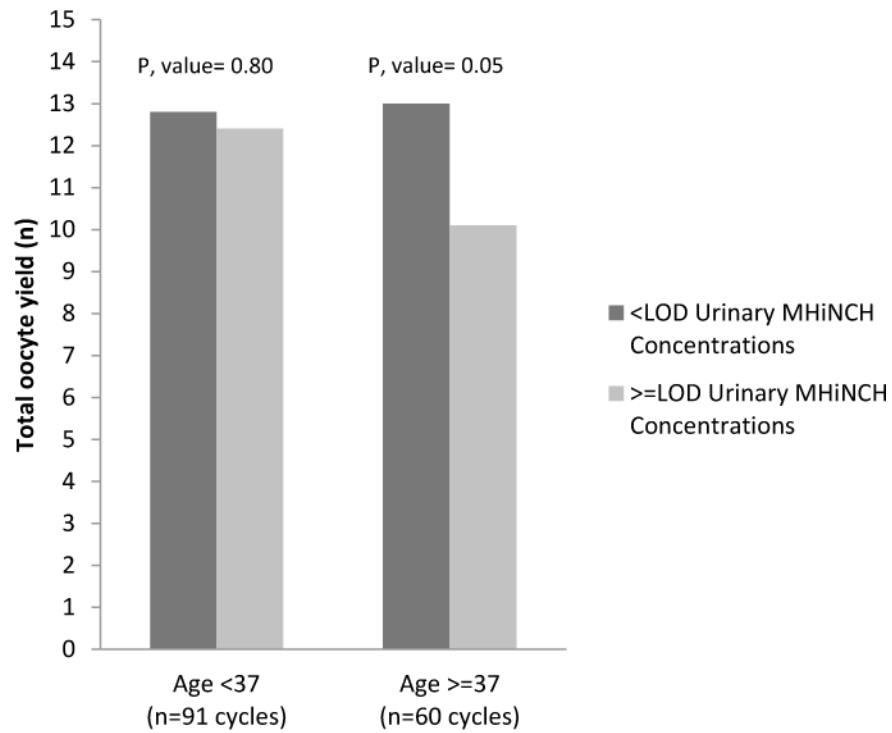


Fig 1. Age modification on the association between urinary MHiNCH concentrations and total oocyte yield among 113 women contributing to 151 fresh IVF cycles in the EARTH
Models are adjusted for age (continuous), year of treatment (continuous), Day3 FSH levels (continuous), and infertility diagnosis (male, female and unexplained). P, interaction=0.13. LOD for MHiNCH is 0.40 $\mu\text{g/L}$.

Table 1Baseline characteristics^a of 113 women in the EARTH by urinary MHiNCH concentrations (µg/L).

Baseline characteristics	Total cohort n=113	Urinary MHiNCH concentrations (µg/L)		P,value ^b
		<LOD (n=80)	>LOD (n=33)	
Age, years	35.0 (31.0, 38.0)	33.5 (31.0, 37.0)	37.0 (33.0, 39.0)	0.03
Race/Ethnic group, n (%)				0.44
White/Caucasian	90 (79.7)	62 (77.5)	28 (84.8)	
Black	3 (2.7)	3 (3.8)	0 (0)	
Asian	15 (13.3)	10 (12.5)	5 (15.2)	
Other	5 (4.3)	5 (6.2)	0 (0)	
Body Mass Index, kg/m ²	23.2 (21.1, 26.3)	23.1 (21.0, 25.8)	24.6 (21.4, 27.3)	0.24
Ever smoker, n (%)	25 (22.1)	17 (21.3)	8 (24.2)	0.80
Education, n ^c (%)				0.53
< College graduate	8 (7.1)	6 (8.6)	2 (21.4)	
College graduate	30 (30.6)	24 (34.3)	6 (21.4)	
Graduate degree	59 (60.2)	39 (55.7)	20 (71.4)	
Baseline Reproductive characteristics				
Initial infertility diagnosis, n (%)				0.96
Male factor	33 (29.2)	24 (30.0)	9 (27.3)	
Female factor	36 (31.9)	24 (30.0)	12 (36.4)	
Diminished Ovarian Reserve	4 (3.5)	3 (3.8)	1 (3.0)	
Endometriosis	8 (7.1)	5 (6.2)	3 (9.2)	
Ovulation Disorders	10 (8.9)	6 (7.5)	4 (12.1)	
Tubal	14 (12.4)	10 (12.5)	4 (12.1)	
Uterine	0 (0)	0 (0)	0 (0)	
Unexplained	44 (38.9)	32 (40.0)	12 (36.4)	
Initial treatment protocol, n (%)				0.14
Antagonist	17 (15.0)	10 (12.5)	7 (21.2)	
Flare ^d	13 (11.5)	7 (8.9)	6 (18.2)	
Luteal phase agonist ^e	83 (73.5)	63 (78.8)	20 (60.6)	

^aValues are presented as median (IQR) unless otherwise noted.^bFrom Kruskal-Wallis test for continuous variables and chi-squared tests (or Fisher's exact test where appropriate) for categorical variables.^cThis variable has missing data.^dFollicular phase GnRH-agonist/Flare protocol.^eLuteal phase GnRH-agonist protocol.

Distribution of cycle-specific urinary concentrations of DINCH metabolites among 113 women in the EARTH undergoing 151 IVF cycles.

Table 2

	Detection frequency	N Urines	Percentile				
			50th	75th	90th	95th	Max
MHINCH	29	274	<LOD	0.47	0.80	1.30	67.2
SG-adj			<LOD	0.44	0.68	1.12	42.8
MHINCH							
MCOCH ^a	9	167	<LOD	<LOD	<LOD	0.72	25.1
SG-adj			<LOD	<LOD	<LOD	0.98	16.0
MCOCH							

MHINCH LOD=0.40 µg/L and MCOCH LOD=0.50 µg/L. All concentrations below LOD were assigned a value equal to the LOD divided by 2.

^a69 women contributing to 99 IVF cycles with data for MCOCH since this metabolite was added to the protocol later than MHINCH.

Table 3

Specific gravity adjusted cycle-specific urinary MHiNCH concentrations in relation to markers of ovarian response^a among 113 women contributing to 151 fresh IVF cycles in the EARTH.

	Urinary MHiNCH concentrations (µg/L)		
	<LOD (n=110)	>LOD (n=50)	P,value
Total Oocyte Yield, n			
Unadjusted	12.9 (11.7, 14.2)	11.4 (9.9, 13.1)	0.13
Adjusted ^a	12.9 (11.7, 14.2)	11.1 (9.7, 12.8)	0.08
MII Oocyte Yield, n			
Unadjusted	10.3 (9.3, 11.3)	9.4 (8.1, 10.8)	0.28
Adjusted ^a	10.2 (9.2, 11.2)	9.2 (8.0, 10.7)	0.25
Endometrial wall thickness, mm			
Unadjusted	10.4 (9.9, 11.0)	10.6 (9.8, 11.3)	0.75
Adjusted ^a	10.5 (10.0, 11.0)	10.5 (9.7, 11.3)	0.99
Peak E2 levels, pmol/l			
Unadjusted	2344 (2109, 2580)	2038 (1709, 2367)	0.09
Adjusted ^a	2342 (2102, 2582)	2017 (1681, 2354)	0.09

^aData are presented as predicted marginal means (95% CI) adjusted for age (continuous), year of treatment (continuous), and infertility diagnosis (male, female and unexplained). Endometrial wall thickness, total and mature oocyte yields models were further adjusted by Day3 FSH levels (continuous). LOD for MHiNCH is 0.40 µg/L.