HHS Public Access

Author manuscript

Environ Int. Author manuscript; available in PMC 2019 February 01.

Published in final edited form as:

Environ Int. 2018 February ; 111: 23–31. doi:10.1016/j.envint.2017.11.011.

Urinary concentrations of biomarkers of phthalates and phthalate alternatives and IVF outcomes

Ronit Machtinger, M.D.[1], **Audrey Gaskins, Sc.D.**[2,3], **Catherine Racowsky, Ph.D**[4], **Abdallah Mansour, M.Sc.**[1], **Michal Adir, B.Sc.**[1], **Andrea A. Baccarelli, M.D., Ph.D.**[5], **Antonia M. Calafat, Ph.D.**[6], and **Russ Hauser, M.D., Sc.D.**[7,8]

[1]Sheba Medical Center, Ramat-Gan and Sackler School of Medicine Tel- Aviv University, Tel Aviv, Israel

[2]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA

[3]Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

[4]Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

[5]Environmental Precision Biosciences Laboratory, Columbia University, Mailman School of Public Health, New York, NY, USA

[6]National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA, USA

[7]Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA, USA

[8]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA

Abstract

Phthalates are a class of chemicals found in a large variety of consumer products. Available experimental and limited human data show adverse effects of some phthalates on ovarian function, which has raised concerns regarding potential effects on fertility. The aim of the current study was to determine whether urinary concentrations of metabolites of phthalates and phthalate alternatives are associated with intermediate and clinical in vitro fertilization (IVF) outcomes. We enrolled 136 women undergoing IVF in a Tertiary University Affiliated Hospital. Participants provided one to two urine samples per cycle during ovarian stimulation and before oocyte retrieval. IVF outcomes

Address for Correspondence: Ronit Machtinger M.D, Infertility and IVF Unit, Department of Obstetrics and Gynecology, Chaim Sheba Medical Center, Tel Hashomer 52621 Israel. Tel: 972-3-5302882, Ronit.Machtinger@sheba.health.gov.il.

Conflicts of Interest: none

were abstracted from medical records. Concentrations of 17 phthalate metabolites and two metabolites of the phthalate alternative di(isononyl) cyclohexane-1,2-dicarboxylate (DINCH) were measured. Multivariable Poisson regression models with log link were used to analyze associations between tertiles of specific gravity adjusted phthalate or DINCH metabolites and number of total oocytes, mature oocytes, fertilized oocytes, and top quality embryos. Multivariable logistic regression models were applied to evaluate the association between tertiles of specific gravity adjusted phthalate or DINCH metabolites and probability of live birth. Urinary concentrations of the sum of di-2-ethylhexyl phthalate metabolites (ΣDEHP) and the individual metabolites mono-2-ethyl-5-hydroxyhexyl phthalate, mono-2-ethyl-5-oxohexyl phthalate, and mono-2-ethyl-5-carboxypentyl phthalate were negatively associated with the number of total oocytes, mature oocytes, fertilized oocytes, and top quality embryos. Of the low molecular weight phthalates, higher monoethyl phthalate and mono-n-butyl phthalate concentrations were associated with significantly fewer total, mature, and fertilized oocytes. None of the urinary phthalate metabolite concentrations were associated with a reduced probability implantation, clinical pregnancy or live birth. Metabolites of DINCH were not associated with intermediate or clinical IVF outcomes. Our results suggest that DEHP may impair early IVF outcomes, specifically oocyte parameters. Additional research is needed to elucidate the potential effect of DEHP on female fertility in the general population.

Keywords

IVF; phthalates; phthalate alternatives; oocytes; implantation; live birth

1. Introduction

Diester ortho-phthalates are synthetic chemicals widely used in personal care, consumer, and industrial products. Exposure to phthalates is ubiquitous and may occur through dermal absorption, inhalation, or ingestion (Lyche et al. 2009). After exposure, phthalates are metabolized to monoesters and oxidative products, some of which are biologically active and exert anti-estrogenic, anti-androgenic or anti-thyroid activity (Caserta et al. 2008; Lyche et al. 2009).

Phthalates can be classified into two groups based on their molecular weight. Low molecular weight phthalates include diethyl phthalate (DEP), di-n-butyl phthalate (DnBP), and di-iso-butyl phthalate (DiBP) and are used in personal-care products (e.g., cosmetics, hair spray, shampoos, deodorants, perfumes, nail polish, body lotions) but also in medication coatings (Hauser and Calafat 2005; Just et al. 2010; Meekeret al. 2009). High molecular weight phthalates include di(2-ethylhexyl) phthalate (DEHP), benzylbutyl phthalate (BzBP), and di(isononyl) phthalate (DiNP) and are mainly used in the manufacturing of flooring, carpet backings, adhesives, wallpaper and polyvinyl chloride (Hauser and Calafat 2005; Just et al. 2010; Meeker et al. 2009). Because phthalates are not covalently bound to the products in which they are incorporated, they can therefore leach into foods or into the environment during use (Koch et al. 2006).

Animal data indicate that exposure to some phthalates and their metabolites can alter folliculogenesis, steroidogenesis, oocyte maturation, and even impair embryo development (Grossman et al. 2012; Hannon et al. 2015). Although the data are limited (Minguez-Alarcon and Gaskins 2017), several epidemiology studies suggest that background low level exposure to some phthalates are associated with lower ovarian reserve parameters and increased pregnancy loss (Messerlian, C. et al., 2016a; Messerlian C., et al. 2016b).

For over a decade, some phthalates including DiNP, and non-phthalate plasticizers such as di(isononyl) cyclohexane-1,2-dicarboxylate (DINCH) have been used in specific countries (e.g., the United States, Germany, Switzerland, Austria) as alternatives to DEHP (Bui et al. 2016; Lessmann et al. 2016; Silva et al 2017; Silva et al. 2013) because they were expected to have lower toxicity than DEHP. However, any potential adverse effects from these phthalates and phthalates alternatives on human reproduction including on IVF outcomes, remain largely unexplored.

Given the animal data on the toxic effects of phthalates on the female reproductive system we conducted the present investigation to expand on the limited studies in humans. Specifically, we undertook a prospective cohort study of women that were followed for a single IVF cycle to explore the associations between a panel of 17 metabolites of phthalates, including DEHP, di(2-ethylhexyl) terephthalate (DEHTP), and DiNP, and two metabolites of DINCH and in-vitro fertilization (IVF) outcomes.

2. Methods

2.1 Institutional Review Board Approval

The study was approved by the Sheba Medical Center IRB and all patients signed informed consents. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subject research.

2.2 Study Population

From January 2014 through August 2016, 136 women undergoing a fresh IVF cycle at Sheba Medical Center, a tertiary university affiliated hospital in the center of Israel, and one of the largest infertility centers in Israel (1100 fresh cycles a year, of them 30% PGD), were enrolled into our prospective cohort study. Approximately 95% of those contacted by the research staff agreed to participate in the study. Participants were enrolled during ovarian stimulation and followed through one fresh IVF cycle. To avoid potential confounding by infertility diagnosis, we approached only women undergoing treatment because of male factor or unexplained infertility, who were oocyte donors, or couples undergoing preimplantation genetic diagnosis (PGD) of autosomal recessive diseases. Exclusion criteria for recruitment were age >38 yrs., BMI>30 kg/m^2, a diagnosis of polycystic ovary syndrome, endometriosis, social oocyte cryopreservation, poor responders according to Bologna criteria (Ferraretti et al. 2011) (which might affect oocyte quality) and frozen IVF cycles. To avoid potential confounding by the stimulation regimen on IVF outcomes (Orvieto and Patrizio 2013), only women using GnRH antagonist (first line protocol used in

our division) were included. For the analysis of clinical IVF outcomes, we excluded 15 women that were not supposed to undergo fresh embryo transfer, i.e. “freeze all” cycles.

2.3 Exposure Assessment

In the majority of women (n=99/136; 73%), a spot urine was collected in a sterile polypropylene cup during ovarian stimulation (days 1–7 of gonadotropin injection) and on the day of oocyte retrieval, and the two specimens were pooled before further analysis. A minority of women contributed only one spot urine sample either during ovarian stimulation (n=1; 0.7%) or on the day of oocyte retrieval (n=36; 26%). After measuring specific gravity (SG) (Comber test strips, Roche, Switzerland), the urine was divided into aliquots and frozen at −80°C. Samples were shipped on dry ice to the CDC (Atlanta, GA, USA) for the quantification of concentrations of 17 phthalate metabolites and two metabolites of DINCH. The analytical approach, based on solid phase extraction coupled with high performance liquid chromatography-isotope dilution tandem mass spectrometry, followed standard quality assurance/quality control procedures as previously described (Silva et al. 2013; Silva et al. 2017a). We calculated the molar sum of DEHP metabolites (ΣDEHP) by dividing each DEHP metabolite concentration by its molecular weight and then summing: [(mono-2-ethylhexyl phthalate (MEHP)*(1/278.34)) + (mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP)*(1/294.34)) + (mono-2-ethyl-5-oxohexyl phthalate (MEOHP)*(1/292.33)) + (mono-2-ethyl-5-carboxypentyl phthalate (MECPP)*(1/308.33))].

2.4 Outcome Assessment

To reduce possible confounding by treatment protocol, all enrolled patients were treated with controlled ovarian stimulation using GnRH antagonist. For ovarian stimulation, a daily SC dose of recombinant FSH (Gonal-F, Merck-Serono or Puregon, Merck Sharp & Dohme) followed by hMG (Menopur; Ferring) was used starting on the third day of the menstrual cycle. The first dose administered was 150 IU, however the amount of the initial dose depended on the age, body mass index (BMI), and treatment history (Haas et al. 2014). Patients were monitored during gonadotropin stimulation for serum estradiol, follicle size measurements and counts, and endometrial thickness. Human chorionic gonadotropin (hCG) (Ovidrelle, Merck Serono) or GnRH agonist (Decapeptyl 0.2mg, Ferring) was administered approximately 36 hours before the scheduled oocyte retrieval to induce oocyte maturation. According to the policy of our department, the regimen of choice for ovulation induction was hCG. In cases that were scheduled for freezing of all the embryos (i.e., PGD in which embryos were frozen for further diagnosis or in cases with increased risk for ovarian hyper-stimulation syndrome), GnRH agonist was used to induce ovulation. Luteal phase support in cases of hCG were Crinone gel 8% (Merck-Serono) once a day or Crinone gel 8% bid and Progynova (Estradiol) 2mg (Zydus Cadila Healthcare) tid when GnRH agonist was used. Ovarian sensitivity index (OSI) was calculated was by dividing the total administered FSH dose (IU) by the number of oocytes retrieved (Biasoni et al. 2011). Women received conventional insemination or intracytoplasmic sperm injection (ICSI) as clinically indicated. Embryologists classified oocytes as germinal vesicle, metaphase I, metaphase II (MII), or degenerated. In ICSI, oocyte maturation was assessed during fertilization check. Oocyte maturity in conventional IVF was assessed as follows after removal of the cumulus/corona radiata cells at the fertilization check. The total number of mature oocytes in a conventional

IVF cycle was determined by summing the number of oocytes exhibiting one or more pronucleus combined with those without a pronucleus but exhibiting a polar body. Embryologists determined normal fertilization 16 to 18 hours after insemination or ICSI as the number of oocytes with two pronuclei. Embryos were further assessed on day 2 for cell number and on day 3 for cell number, symmetry, and fragmentation. Top quality embryos were classified as those with 7–8 cells on day 3 (or in cases of day 2 transfer, 4 cells) and <10% fragmentation. Patients were scheduled for day 3 transfer, unless day 3 occurred on a weekend (n=5). In these cases, embryos were transferred on day 2. In cases of PGD, embryos were biopsied on day 3 and transferred on day 4. Positive β-hCG (i.e., successful implantation) was defined as a serum β-hCG level >25 mIU/mL, typically measured 14 days after oocyte retrieval. Clinical pregnancy was defined as the presence of an intrauterine gestational sac and fetal heartbeat confirmed by ultrasound by 7 weeks of gestation, and live birth as the delivery of a live neonate on or after 24 weeks gestation. All clinical information was abstracted from medical records.

2.5 Covariate Assessment

Height and weight, measured at the start of the IVF cycle, were used to calculate body mass index (BMI) (kg/m^2). A woman's age was calculated based on date of birth. Smoking status, number of previous pregnancies and deliveries, duration of infertility, were self-reported and abstracted from patients' medical records. Total number of IVF attempts (defined as the total number of IVF attempts at our clinic or other clinics) was abstracted from patients' charts.

2.6 Statistical Analyses

We used instrumental reading values even for metabolite concentrations below the LOD. To adjust for urinary dilution, we used the following formula: $Pc = P[(1.014 - 1)/SG - 1]$, where Pc is the SG-corrected metabolite concentration (μg/L), P is the measured metabolite concentration (μg/L), and 1.014 is the mean SG level in our study population. We used SG-corrected metabolite concentrations in all analyses. To aid in the comparison of our cohort to those in other studies, descriptive statistics for each metabolite were calculated both before and after adjustment for specific gravity. Furthermore, to quantify the strength of the relationship between the various phthalate and phthalate alternative metabolites, we calculated Spearman correlation coefficients.

For metabolites where the percent of samples with detectable concentrations was >66%, women were placed into tertiles based on each of their metabolite concentrations. For the one metabolite (mono-isononyl phthalate (MiNP)) where the percent of samples with detectable concentrations was <66%, women with values below the LOD were placed in the first category and the women with detectable concentrations were placed in the remaining two levels based on the median metabolite concentration. Descriptive statistics were then calculated for demographic and reproductive characteristics in the entire cohort and by tertile of ΣDEHP concentration. For continuous and categorical variables, ANOVA and chi-square tests were used, respectively, to test for associations across tertiles of ΣDEHP concentration.

We fit multivariable Poisson regression models to evaluate the association between tertiles of metabolite concentrations and total oocyte, mature oocyte, fertilized oocyte, and top quality embryo yield (all count data). To evaluate the association between tertiles of metabolite concentrations and clinical outcomes, we used multivariable logistic regression models. For both intermediate and clinical outcomes adjusted marginal mean counts or proportions for each tertile were obtained. Tests for linear trend were conducted across tertiles using the median metabolite concentration in each tertile as a continuous variable in the regression models. We also performed a sensitivity analysis only among cycles with embryo transfer (n=116).

Potential confounders were identified using prior knowledge (with a focus on variables consistently related to ART outcomes and phthalate exposure) and descriptive statistics from our cohort. These variables were then explored using a directed acyclic graph to identify relevant confounders that were not on the causal pathway of analysis. Variables retained in the final multivariable models were maternal age, BMI, and current smoking status. We also ran sensitivity analyses where we further adjusted for the other specific phthalate metabolites that were associated with the outcome of interest. All analyses were conducted using SAS Software package 9.4 (Cary NC).

3. Results

Women were on average 30.9 years of age (range 19 to 38 years) with a mean duration of infertility of 1.2 years (Table 1). Most women were not current smokers (80%), were nulligravid (46%) and nulliparous (61%), and had no previous IVF attempts (66%). The mean + standard deviation BMI in our cohort was 23.5+4.7 kg/m^2. The women were distributed by primary infertility diagnosis as follows: 40% male factor, 43% PGD, 15% unexplained; 2% underwent IVF for social oocyte freezing or oocyte donation. The majority of cycles used hCG (90%) for ovulation induction while the rest used GnRH antagonist (10%). ICSI was primary method for fertilization (92%). There were no significant differences in the number of top quality embryos on day 3 and the number of embryo transferred among PGD and non-PGD cycles. Of the initial 136 cycles, 121 cycles were included in our analysis of clinical outcomes of ART and the majority of these cycles underwent embryo transfer (n=116). In 5 cases embryos were not transferred due to failed fertilization (n=1) or impaired quality (n=4). The percent of cycles undergoing embryo transfer that resulted in implantation, clinical pregnancy, and live birth were 39%, 35%, and 34%, respectively. There were few pregnancy losses in our cohort (5 cases of chemical pregnancies and 3 cases of miscarriages). There were no significant differences in demographic or reproductive characteristics across tertiles of SG-adjusted ΣDEHP concentrations although women with higher ΣDEHP concentrations were slightly younger (p-value=0.16) and were less likely to have had ≥2 previous pregnancies (p-value=0.11).

The distribution and limits of detection (LOD) for each of the phthalate and DINCH metabolites are shown in Table 2. The pairwise Spearman correlation coefficients between all of the metabolites are shown in Supplemental Table 1. The median concentrations of urinary metabolites in this population were similar to females participating in the 2011–2012 U.S. National Health and Nutrition Examination Survey (NHANES) with the exception of

monoethyl phthalate (MEP) (CDC 2017). Compared to NHANES females, the women in our cohort had almost five times higher MEP concentrations (150.5 vs 32.5 μg/L). All of the urinary phthalate metabolite concentrations were positively correlated with one another, with generally higher correlations, as expected, among metabolites of the same phthalate parent compound. The two metabolites of DINCH also strongly correlated with each other, but correlations between the concentrations of the DINCH metabolites and the phthalate metabolites were generally weak.

In multivariable models adjusted for age, BMI, and smoking status, there were statistically significant associations of higher urinary concentrations of ΣDEHP, MEHHP, MEOHP, and MECPP with reduced number of mature and total oocytes, number of fertilized oocytes, and number of top quality embryos (Table 3). Specifically, women in the highest tertile of urinary ΣDEHP concentrations (>0.22 μmol/L) had, on average, 2.9 fewer oocytes retrieved, 1.7 fewer mature oocytes, 1.2 fewer fertilized oocytes, and 1.1 fewer top quality embryos compared to women in the lowest tertile of ΣDEHP (<0.12 μmol/L). Of the low molecular weight phthalates, women in increasing tertiles of MEP and mono-n-butyl phthalate (MBP) had significantly fewer total, mature, and fertilized oocytes and women in increasing tertiles of MHiBP had significantly fewer top quality embryos (Table 4). When these phthalate metabolites were co-adjusted for one another in the same multivariable model, there was a −7.3% (95% CI −13.0, −1.2%), −10.3% (95% CI −15.4, −4.8%), and −5.7% (95% CI −10.8, −0.3%) decrease in total oocytes per 1 SD increase in log ΣDEHP, MEP, and MiNP urinary concentrations, respectively (Supplemental Table 2). For mature and fertilized oocyte yield, after co-adjustment, only urinary MEP concentrations were significantly associated with number of mature oocytes (−9.6% 95% CI −15.4, −3.4% per 1 SD increase in log MEP concentrations) and number of fertilized oocytes (−8.5% 95% CI −15.5, −0.8% per 1 SD increase in log MEP concentrations). Increasing concentrations of urinary cyclohexane-1,2-dicarboxylic acid- monohydroxy isononyl ester (MHiNCH) were significantly associated with higher fertilized oocytes (adjusted average fertilized oocyte count across tertiles of MHiNCH: 5.0, 4.7, 6.0; p-trend=0.008) yet the association was not entirely linear with the lowest counts observed for women in the second tertile (Table 3).

Despite inverse associations with some intermediate IVF endpoints, none of the urinary concentrations of metabolites of phthalates or phthalate alternatives were statistically significantly associated with probability of implantation, clinical pregnancy, or live birth (Table 5, Supplemental Table 3). These associations remained similar when analyses were restricted to the 116 cycles with embryo transfer (data not shown).

4. Discussion

We found significant associations between some of the urinary phthalate metabolite concentrations and intermediate IVF outcomes. Specifically, higher urinary concentrations of ΣDEHP metabolites, MEHHP, MEOHP, and MECPP were negatively associated with number of total and mature oocytes, fertilized oocytes, and top quality embryos. There was also a significant negative association between urinary concentrations of MiNP (but not with the other two DiNP biomarkers detected more frequently and at higher concentrations that MiNP) and total oocyte yield. Of the low molecular weight phthalates, women with higher

MEP and MBP concentrations had significantly fewer total, mature, and fertilized oocytes. None of the urinary phthalate concentrations were associated with implantation, clinical pregnancy, or live birth following IVF. Concentrations of MHiNCH, a metabolite of DINCH, a phthalate alternative, were not associated with intermediate or clinical IVF outcomes.

Animal studies to date have shown a possible association between chronic exposure to some phthalates and/or their metabolites and impaired female fertility (Ema et al. 2000; Heindel et al. 1989; Plummer et al. 2013; Shiota and Nishimura 1982; Zhang et al. 2013). In mice, DEHP and its metabolite MEHP inhibited follicular growth and reduced levels of E2 (Gupta et al. 2010), and exposure of mothers to DEHP caused depletion of the primordial follicle pool in their offspring (Zhang et al. 2013). *In vitro* studies have shown that exposure to DBP, the parent compound of MBP and mono-hydroxybutyl phthalate, suppressed antral follicular growth, altered cell cycle and increased apoptosis in mice (Craig et al. 2013), and impaired steroidogenesis in primary cultures of human granulosa cells (Adir et al. 2017). At high dose (>2000 times the estimated level of human intake), DEHP and DBP were embryo toxic and teratogenic in mice (Shiota and Nishimura 1982).

The findings from our study are supported by the previous work of the Environment and Reproductive Health (EARTH) study in the United States that found associations between phthalate metabolite concentrations and IVF outcomes. The median concentrations of ΣDEHP, MEHHP, MEOHP were similar in both populations while MECPP concentrations in the EARTH study were slightly higher compared with the concentrations of this metabolite in our cohort (26.3 μg/L compared with 19.7 μg/L). In that study of 256 women, urinary concentrations of ΣDEHP, MEHHP, MEOHP, and MECPP were inversely associated with number of oocytes retrieved and number of mature oocytes (Hauser et al. 2016). We found that urinary concentrations of ΣDEHP and the oxidative DEHP metabolites (MEHHP, MEOHP and MECPP) were also inversely associated with number of fertilized oocytes, while in the EARTH study, women in the second and third quartile (but not the fourth quartile) of ΣDEHP had lower number of fertilized oocytes compared to women in the lowest quartile. We also found that ΣDEHP and the DEHP oxidative metabolites (MEHHP, MEOHP and MECPP) were inversely related to the number of top quality embryos on day 3.

Recently, Wu et al., (Sperm Environmental Epigenetics and Development Study [SEEDS]) evaluated the association of urinary concentrations of 15 parental phthalate metabolites and two parental DINCH metabolites (MHiNCH and cyclohexane-1,2-dicarboxylic acid-mono(carboxyoctyl) ester (MCOCH)) with day 3 embryo quality in 50 couples (Wuet al. 2017). While paternal urinary MEP concentrations were positively associated with the number of high quality embryos on day 3, no associations were found in the female partner. The median concentrations of MEP among the female partner in the SEEDS study were much lower than the median MEP concentrations in our study (38.7 μg/L vs. 181.7 μg/L). The EARTH and SEEDS used the same CDC laboratory as the current study. In contrast to our study, the EARTH and the SEEDS studies, which both recruited patients undergoing IVF in Massachusetts, did not show an association between ΣDEHP and top embryo quality on day 3. Possible explanations for the difference between the associations we found between ΣDEHP and day 3 embryo quality in our cohort and EARTH and SEEDS studies can be attributed to the different patient characteristics (age, BMI, infertility diagnosis) as

well as different fertility clinic protocols, all of which may affect embryo quality. For instance, although we excluded patients with factors that might affect oocyte and embryo quality (older than 38 yrs., BMI>30 kg/m2, diagnosis of polycystic ovary syndrome, endometriosis and poor responders), 37% of the EARTH population were >37 years old. In the SEEDS study, 76% were between 30–40 years and 6% >40 yrs., however mean/median patient age was presented. In the SEEDS study, 36% were obese as compared to none in the current study. In the EARTH study, 30% had female factors, whereas 40% of the women in the SEEDS study had female factor and another 16% in the SEEDS study had both female and male factor. This is in contrast with only 1.5% of the participants in our study that underwent IVF due to female factor.

A recent cohort study of 112 infertile women undergoing fertility treatments in China explored associations between urinary concentrations of several phthalate metabolites (monomethyl phthalate, MEP, MBP, monobenzyl phthalate, MEHHP, MEOHP, MEHP, and mono-n-octyl phthalate) and intermediate IVF outcomes. The authors did not find associations between phthalate metabolite urinary concentrations and number of oocytes retrieved, number of mature oocytes, fertilized oocytes, number of top quality embryos or number of blastocyst formed (Du et al. 2016). The discordant results between this study and ours might be attributed to different patient age ranges, infertility diagnoses, stimulation protocols and/or fertilization methods.

In our study, women with higher MEP and MBP concentrations had significantly fewer total, mature, and fertilized oocytes. In contrast with our results, no associations were found between these two biomarkers and IVF intermediate outcomes in the EARTH study (Hauser et al. 2016). Interestingly, in our cohort of Israeli women, we observed much higher MEP concentrations compared to U.S. women in the EARTH study and the Chinese women in Du et al. cohort (Du et al. 2016).

In the EARTH study, the proportion of cycles resulting in clinical pregnancy and live birth were significantly higher among women in the first quartile (lowest concentrations) as compared to women in the fourth quartile (highest concentrations) of ΣDEHP and the individual four DEHP metabolites, whereas we did not find significant associations in our study. One possible explanation for this difference is variation in the two populations. The EARTH study had a much higher proportion of women with female infertility, while those women were largely excluded from our cohort and instead we enrolled a high proportion of fertile women undergoing IVF for PGD, in order to generalize our results also to the fertile population. Also, in the EARTH study, ΣDEHP concentrations were positively associated with risk of pregnancy loss, while in contrast with the EARTH study our cohort included very few women with pregnancy losses (5 chemical pregnancies and 3 were missed abortions).

In recent years, some phthalates have been partially replaced in products by alternative substitute compounds such as DiNCH. The DEHP metabolite concentrations in our study were slightly below 2011–2012 NHANES concentrations (CDC 2015) but were very similar to those observed in EARTH study (Hauser et al. 2016). Interestingly, the detection frequency of the DINCH metabolites were much higher in our Israeli cohort (93%

[MHiNCH] and 68% [MCOCH] compared with the concentrations in the EARTH (29% and 9%, respectively) or the SEEDS (43% and 14%, respectively) U.S. cohorts (Minguez-Alarcon et al. 2016; Wu et al. 2017), and in a convenience sample of U.S. population adults in 2012 (16% and 17%, respectively) (Silva et al. 2013). Because the quantification of the metabolites of phthalates and phthalate alternatives in these three cohorts were performed by the same laboratory, the differences in detection frequencies and concentrations are likely related to real differences among the study populations.

In this study, no associations between the metabolites of DiNCH and intermediate or clinical IVF outcomes were observed. To the best of our knowledge, only one study to date has explored possible associations between urinary concentrations of MHiNCH, a DINCH metabolite, and markers of ovarian response (Minguez-Alarcon et al. 2016). In this study among a subset of participants in the EARTH cohort, women with detectable concentrations of MHiNCH had a lower number of oocytes retrieved (−1.8, p=0.08) compared with women with non-detectable concentrations of MHiNCH, and the association was stronger among older women. In line with our results, those authors reported no association between the number of mature oocytes and urinary MHiNCH concentrations (Minguez-Alarcon et al. 2016). We found that higher urinary concentrations of MHiNCH were associated with slightly higher number of fertilized oocytes although the association was not monotonic. However, similar to Wu et al., we found no association between urinary concentrations of MHiNCH and the number of top quality embryos on day 3 (Wu et al. 2017). As MHiNCH was significantly associated only with the number of fertilized oocytes and not with other intermediate IVF outcomes, it is possible that there are non-monotonic relationships between MHiNCH and intermediate IVF outcomes. However, larger studies are needed to confirm these results.

Our study has several limitations. First, our relatively small sample size limited our power to find and/or rule out clinically meaningful differences in clinical outcomes, particularly live birth, across tertiles of urinary concentrations of the biomarkers examined. It is possible that the intermediate IVF outcomes are more sensitive markers compared to live birth rates. If this is the case, larger studies may be needed to investigate potential influences of these chemicals on birth rates. We are also cognizant that the women in this study are likely exposed to a variety of environmental chemicals, not just DINCH and phthalates, and thus residual confounding may impact our results. Because of our strict inclusion/exclusion criteria, we were able to adjust for many clinical and treatment factors by design; however, the same criteria may limit the generalizability of our findings to all women undergoing IVF (particularly women with female factor infertility or poor responders who were largely under-represented in this cohort). Strength of our inclusion/exclusion criteria though was that we were able to enroll many fertile women undergoing IVF with PGD of autosomal recessive diseases, which may help to extrapolate our results to the general, fertile, population. Finally, we were able to quantify a wide range of metabolites of phthalates and phthalate alternatives (e.g., DINCH), and to evaluate the associations of these metabolites with IVF outcomes.

5. Conclusions

In conclusion, several phthalate biomarkers, specifically ΣDEHP, were inversely associated with total and mature oocyte yield, fertilization, and embryo quality. Interestingly, in this group of women widely exposed to DEHP alternatives, including DINCH, we found no associations between the concentrations of DINCH metabolites and IVF outcomes. To date, the best characterized predictors of IVF success are unmodifiable (such as patient age), hence identification of modifiable factors and alternatives to toxic phthalates is critical. Our understanding of the potential effects of phthalates or their alternatives on female fertility will benefit from further studies, particularly with large sample size cohorts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors gratefully acknowledge Xiaoyun Ye, Manori Silva, Ella Samandar, Jim Preau, and Tao Jia (CDC, Atlanta, GA) for measuring the urinary concentrations of the environmental biomarkers.

Study funding/competing interest(s): This work was supported by the National Institutes of Health (grant numbers P30ES00002, R21ES024236, and K99ES026648) by the Environment and Health Fund, Israel (grant award no. RPGA1301) and by the Israeli Science Foundation (grant award 1936/12).

References

Adir M, Combelles CM, Mansur A, Ophir L, Hourvitz A, Orvieto R, Dor J, Machtinger R. Dibutyl phthalate impairs steroidogenesis and a subset of LH-dependent genes in cultured human mural granulosa cell in vitro. Reproductive toxicology. 2017; 69:13–18. [PubMed: 28104404]

Biasoni V, Patriarca A, Dalmasso P, Bertagna A, Manieri C, Benedetto C, Revelli A. Ovarian sensitivity index is strongly related to circulating AMH and may be used to predict ovarian response to exogenous gonadotropins in IVF. Reproductive biology and endocrinology: RB&E. 2011; 9:112. [PubMed: 21824441]

Braun JM, Just AC, Williams PL, Smith KW, Calafat AM, Hauser R. Personal care product use and urinary phthalate metabolite and paraben concentrations during pregnancy among women from a fertility clinic. J Expo Sci Environ Epidemiol. 2014; 24:459–66. [PubMed: 24149971]

Bui TT, Giovanoulis G, Cousins AP, Magner J, Cousins IT, de Wit CA. Human exposure, hazard and risk of alternative plasticizers to phthalate esters. The Science of the total environment. 2016; 541:451–467. [PubMed: 26410720]

Caserta D, Maranghi L, Mantovani A, Marci R, Maranghi F, Moscarini M. Impact of endocrine disruptor chemicals in gynaecology. Human reproduction update. 2008; 14:59–72. [PubMed: 18070835]

CDC. Forth National Report on Exposure to Environmental Chemicals. 2015. https://www.cdc.gov/biomonitoring/pdf/fourthreport_updatedtables_feb2015.pdf

CDC. Forth National Report on Exposure to Environmental Chemicals Updated Tables. 2017. https://www.cdc.gov/exposurereport/index.html

Craig ZR, Hannon PR, Wang W, Ziv-Gal A, Flaws JA. Di-n-butyl phthalate disrupts the expression of genes involved in cell cycle and apoptotic pathways in mouse ovarian antral follicles. Biology of reproduction. 2013; 88:23. [PubMed: 23242528]

Du YY, Fang YL, Wang YX, Zeng Q, Guo N, Zhao H, Li YF. Follicular fluid and urinary concentrations of phthalate metabolites among infertile women and associations with in vitro fertilization parameters. Reproductive toxicology. 2016; 61:142–150. [PubMed: 27067915]

Ema M, Miyawaki E, Kawashima K. Effects of dibutyl phthalate on reproductive function in pregnant and pseudopregnant rats. Reproductive toxicology. 2000; 14:13–19. [PubMed: 10689199]

Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L, Definition, E.w.g.o.P.O.R. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. Human reproduction. 2011; 26:1616–1624. [PubMed: 21505041]

Grossman D, Kalo D, Gendelman M, Roth Z. Effect of di-(2-ethylhexyl) phthalate and mono-(2-ethylhexyl) phthalate on in vitro developmental competence of bovine oocytes. Cell biology and toxicology. 2012; 28:383–396. [PubMed: 22956148]

Gupta RK, Singh JM, Leslie TC, Meachum S, Flaws JA, Yao HH. Di-(2-ethylhexyl) phthalate and mono-(2-ethylhexyl) phthalate inhibit growth and reduce estradiol levels of antral follicles in vitro. Toxicology and applied pharmacology. 2010; 242:224–230. [PubMed: 19874833]

Hannon PR, Brannick KE, Wang W, Gupta RK, Flaws JA. Di(2-ethylhexyl) phthalate inhibits antral follicle growth, induces atresia, and inhibits steroid hormone production in cultured mouse antral follicles. Toxicology and applied pharmacology. 2015; 284:42–53. [PubMed: 25701202]

Haas J, Ophir L, Barzilay E, Yerushalmi GM, Yung Y, Kedem A, Maman E, Hourvitz A. GnRH agonist vs. hCG for triggering of ovulation–differential effects on gene expression in human granulosa cells. PloS one. 2014; 9:e90359. [PubMed: 24603682]

Hauser R, Calafat AM. Phthalates and human health. Occupational and environmental medicine. 2005; 62:806–818. [PubMed: 16234408]

Hauser R, Gaskins AJ, Souter I, Smith KW, Dodge LE, Ehrlich S, Meeker JD, Calafat AM, Williams PL, Team, E.S. Urinary Phthalate Metabolite Concentrations and Reproductive Outcomes among Women Undergoing in Vitro Fertilization: Results from the EARTH Study. Environmental health perspectives. 2016; 124:831–839. [PubMed: 26545148]

Heindel JJ, Gulati DK, Mounce RC, Russell SR, Lamb JCt. Reproductive toxicity of three phthalic acid esters in a continuous breeding protocol. Fundamental and applied toxicology: official journal of the Society of Toxicology. 1989; 12:508–518. [PubMed: 2731665]

Just AC, Adibi JJ, Rundle AG, Calafat AM, Camann DE, Hauser R, Silva MJ, Whyatt RM. Urinary and air phthalate concentrations and self-reported use of personal care products among minority pregnant women in New York city. Journal of exposure science & environmental epidemiology. 2010; 20:625–633. [PubMed: 20354564]

Koch HM, Preuss R, Angerer J. Di(2-ethylhexyl)phthalate (DEHP): human metabolism and internal exposure– an update and latest results. International journal of andrology. 2006; 29:155–165. discussion 181-155. [PubMed: 16466535]

Lessmann F, Schutze A, Weiss T, Langsch A, Otter R, Bruning T, Koch HM. Metabolism and urinary excretion kinetics of di(2-ethylhexyl) terephthalate (DEHTP) in three male volunteers after oral dosage. Archives of toxicology. 2016; 90:1659–1667. [PubMed: 27116293]

Lyche JL, Gutleb AC, Bergman A, Eriksen GS, Murk AJ, Ropstad E, Saunders M, Skaare JU. Reproductive and developmental toxicity of phthalates. Journal of toxicology and environmental health Part B, Critical reviews. 2009; 12:225–249.

Meeker JD, Sathyanarayana S, Swan SH. Phthalates and other additives in plastics: human exposure and associated health outcomes. Philosophical transactions of the Royal Society of London Series B, Biological sciences. 2009; 364:2097–2113. [PubMed: 19528058]

Messerlian C, Souter I, Gaskins AJ, Williams PL, Ford JB, Chiu YH, Calafat AM, Hauser R, Earth Study, T. Urinary phthalate metabolites and ovarian reserve among women seeking infertility care. Hum Reprod. 2016a; 31:75–83. [PubMed: 26573529]

Messerlian C, Wylie BJ, Minguez-Alarcon L, Williams PL, Ford JB, Souter IC, Calafat AM, Hauser R, Earth Study T. Urinary Concentrations of Phthalate Metabolites and Pregnancy Loss among Women Conceiving with Medically Assisted Reproduction. Epidemiology. 2016b; 27(6):879–88. [PubMed: 27299194]

Minguez-Alarcon L, Gaskins AJ. Female exposure to endocrine disrupting chemicals and fecundity: a review. Current opinion in obstetrics & gynecology. 2017; 29:202–211. [PubMed: 28557831]

Minguez-Alarcon L, Souter I, Chiu YH, Williams PL, Ford JB, Ye X, Calafat AM, Hauser R, Earth Study T. 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. Environmental research. 2016; 151:595–600. [PubMed: 27591839]

Orvieto R, Patrizio P. GnRH agonist versus GnRH antagonist in ovarian stimulation: an ongoing debate. Reproductive biomedicine online. 2013; 26:4–8. [PubMed: 23186555]

Plummer SM, Dan D, Quinney J, Hallmark N, Phillips RD, Millar M, Macpherson S, Elcombe CR. Identification of transcription factors and coactivators affected by dibutylphthalate interactions in fetal rat testes. Toxicological sciences: an official journal of the Society of Toxicology. 2013; 132:443–457. [PubMed: 23358192]

SE B. Hexamoll® DINCH Safe play with the non-phthalate plasticizer for toys and childcare articles. 2017

Shiota K, Nishimura H. Teratogenicity of di(2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DBP) in mice. Environmental health perspectives. 1982; 45:65–70. [PubMed: 7140698]

Silva MJ, Jia T, Samandar E, Preau JL Jr, Calafat AM. Environmental exposure to the plasticizer 1,2-cyclohexane dicarboxylic acid, diisononyl ester (DINCH) in U.S. adults (2000-2012). Environmental research. 2013; 126:159–163. [PubMed: 23777640]

Silva MJ, Wong LY, Samandar E, Preau JL, Calafat AM, Ye X. Exposure to di-2-ethylhexyl terephthalate in a convenience sample of U.S. adults from 2000 to 2016. Archives of toxicology. 2017 Mar 17. [Epub ahead of print]. doi: 10.1007/s00204-017-1956-3

Wu H, Ashcraft L, Whitcomb BW, Rahil T, Tougias E, Sites CK, Pilsner JR. Parental contributions to early embryo development: influences of urinary phthalate and phthalate alternatives among couples undergoing IVF treatment. Human reproduction. 2017; 32:65–75. [PubMed: 27927842]

Zhang XF, Zhang LJ, Li L, Feng YN, Chen B, Ma JM, Huynh E, Shi QH, De Felici M, Shen W. Diethylhexyl phthalate exposure impairs follicular development and affects oocyte maturation in the mouse. Environmental and molecular mutagenesis. 2013; 54:354–361. [PubMed: 23625783]

Highlights

1. We examined the associations between phthalates and phthalate alternative metabolites and IVF outcomes.
2. Exposure to ΣDEHP metabolites, MEP and MnBP was inversely associated with number of oocytes retrieved and fertilized.
3. We did not find associations between phthalate and phthalate alternative metabolites and IVF clinical outcomes.

Environ Int. Author manuscript; available in PMC 2019 February 01.

Table 1

Demographic and reproductive characteristics by tertiles of Σ**DEHP** concentrations.

		Tertiles of SG-adjusted ΣDEHP (range, μmol/L)			
Number of Women	**Total Cohort 136**	**T1 (0.02–0.121) 45**	**T2 (0.122–0.220) 46**	**T3 (0.221–2.65) 45**	**p-value[1]**
Age[2], yrs	30.9 (3.7)	31.7 (3.4)	30.7 (4.1)	30.2 (3.6)	0.16
BMI, kg/m^2	23.5 (4.7)	23.8 (4.9)	22.9 (1.3)	23.8 (5.0)	0.60
Current Smoker, n (%)	27 (20.0)	10 (22.2)	8 (17.8)	9 (20.0)	0.68
Gravidity, n (%)					0.11
0	62 (45.6)	19 (42.2)	17 (37.0)	26 (57.8)	
1	39 (28.7)	10 (22.2)	17 (37.0)	12 (26.7)	
≥2	35 (25.7)	16 (35.6)	12 (26.1)	7 (15.6)	
Parity, n (%)					0.42
0	83 (61.0)	26 (57.8)	25 (54.4)	32 (71.1)	
1	39 (28.7)	13 (28.9)	15 (32.6)	11 (24.4)	
≥2	14 (10.3)	6 (13.3)	6 (13.3)	2 (4.4)	
Duration of Infertility, yrs	1.2 (1.5)	1.3 (1.7)	1.0 (1.3)	1.2 (1.4)	0.55
Previous IVF Attempts, n (%)					0.82
0	90 (66.2)	32 (71.1)	29 (63.0)	29 (64.4)	
1	26 (19.1)	6 (13.3)	10 (21.7)	10 (22.2)	
≥2	20 (14.7)	7 (15.6)	7 (15.6)	6 (13.3)	
Infertility Diagnosis, n (%)					0.51
Male	54 (39.7)	13 (28.9)	19 (41.2)	22 (48.9)	
Female	2 (1.5)	1 (2.2)	0 (0.0)	1 (2.2)	
Unexplained	21 (15.4)	9 (20.0)	6 (13.0)	6 (13.3)	
PGD	59 (43.4)	22 (48.9)	21 (45.7)	16 (35.6)	
Ovarian Sensitivity Index	284 (321)	221 (187)	270 (268)	360 (443)	0.11
Day 3 FSH[3]	7.0 (2.2)	7.1 (2.3)	6.7 (2.1)	7.1 (2.2)	0.66
Day 3 Estradiol[3]	191 (153)	204 (209)	194 (135)	176 (94)	0.68
Total FSH dose, IU	1771(889)	1756 (1098)	1740 (626)	1816 (903)	0.91
Mean daily FSH dose, IU	177 (66)	184 (82)	174 (56)	173 (57)	0.67

		Tertiles of SG-adjusted ΣDEHP (range, μmol/L)			
Number of Women	**Total Cohort 136**	**T1 (0.02–0.121) 45**	**T2 (0.122–0.220) 46**	**T3 (0.221–2.65) 45**	**p-value[1]**
Ovulation Induction, n (%)					0.88
hCG	122 (89.7)	40 (88.9)	42 (91.3)	40 (88.9)	
GnRH antagonist	14 (10.3)	5 (11.1)	4 (8.7)	5 (11.1)	
Fertilization Type, n (%)					0.86
Conventional Insemination	11 (8.1)	4 (8.9)	3 (6.5)	4 (8.9)	
ICSI	125 (91.9)	41 (91.1)	43 (93.5)	41 (91.1)	
Number of Embryos					
Transferred, n (%)					0.87
0		2 (4.9)	2 (4.6)	1 (2.8)	
1		17 (41.5)	17 (38.6)	14 (38.9)	
2		19 (46.3)	25 (56.8)	20 (55.6)	
3–4		3 (7.3)	0 (0.0)	1 (2.8)	

Abbreviations: BMI, body mass index; ΣDEHP, molar sum of di-2-ethylhexyl phthalate metabolites ; FSH, follicle stimulating hormone; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; PGD, pre-implantation genetic diagnosis; SG, specific gravity

[1]Differences across categories were tested using an ANOVA test for continuous variables and a Chi Square test (or Fisher's exact test when cell counts were <5) for categorical variables

[2]Data are presented as mean (standard deviation) or number of women (%) unless otherwise specified.

[3]There were 11 women missing data on FSH and 2 women missing data on estradiol.

Environ Int. Author manuscript; available in PMC 2019 February 01.

Table 2

Distribution of urinary metabolite or molar sum concentrations among 136 women undergoing assisted reproduction.[1]

Parent compound	Metabolite	Units	LOD	% Detect	SG-adjusted[2] Median (IQR)	Max	Unadjusted Median (IQR)	Max
DEHP	ΣDEHP	μmol/L	0.8	–	0.16 (0.11, 0.27)	2.7	0.17 (0.08, 0.28)	2.8
	MEHP	μg/L	0.4	91.2%	3.5 (2.2, 7.6)	71	3.8 (1.6, 8.4)	66
	MEHHP	μg/L	0.2	100%	13.2 (8.6, 22.2)	215	12.8 (7.2, 23.7)	230
	MEOHP	μg/L	0.4	100%	9.6 (6.4, 16.1)	145	9.9 (5.1, 16.9)	155
	MECPP	μg/L		100%	19.7 (13.3, 33.6)	371	20.1 (10.4, 35.1)	398
DEP	MEP	μg/L	1.2	100%	181.7 (71.1, 431.2)	9333	150.5 (61.7, 374.5)	10000
DBP or DnBP	MBP	μg/L	0.4	98.5%	18.8 (11.5, 32.0)	148	17.4 (8.9, 35.5)	212
	MHBP	μg/L	0.4	88.2%	1.3 (0.8, 2.3)	17	1.2 (0.6, 2.4)	18
DOP (DBP, minor)	MCPP	μg/L	0.4	90.4%	1.2 (0.7, 2.2)	130	1.2 (0.6, 2.2)	233
DiBP	MiBP	μg/L	0.8	99.3%	24.0 (15.9, 38.5)	188	24.9 (12.8, 41.7)	205 75
	MHiBP	μg/L	0.4	100%	6.6 (3.8, 13.2)	65	7.5 (4.8, 13.2)	
BBzP	MBzP	μg/L	0.3	77.2%	1.8 (1.0, 2.8)	382	1.9 (0.8, 3.2)	682
DiNP	MCOP	μg/L	0.3	100%	8.2 (5.3, 17.1)	1344	8.2 (5.0, 14.6)	2400
	MiNP	μg/L	0.9 0.4	51.5%	1.0 (<LOD, 1.6)	263	0.9 (<LOD, 1.8)	470
	MONP	μg/L		97.8%	3.5 (2.4, 6.0)	902	3.5 (1.8, 6.6)	1610
DiDP	MCNP	μg/L	0.2	98.5%	1.1 (0.7, 2.1)	46	1.1 (0.7, 2.1)	82
DINCH	MHiNCH	μg/L	0.4	92.6%	1.1 (0.7, 2.4)	75 27	1.2 (0.7, 2.1)	30
	MCOCH	μg/L	0.5	68.4%	0.6 (<LOD, 1.3)		0.6 (<LOD, 0.9)	10
DEHTP	MEHHTP	μg/L	0.4	90.4%	2.4 (1.2, 4.6)	386 2856	2.3 (1.0, 4.7)	140
	MECPTP	μg/L	0.2	100%	8.2 (4.3, 16.8)		7.7 (4.2, 15.7)	1020

Abbreviations: IQR, interquartile range; LOD, limit of detection; min, minimum; max, maximum; SG, specific gravity.

[1]Out of the 136 women, 99 (72.8%) women had 2 pooled urine samples from the day of stimulation and the day of retrieval, 36 (26.5%) women had one urine sample from the day of retrieval, and one (0.7%) woman had one urine sample from the day of stimulation.

[2]Instrumental reading values were used for metabolite concentrations below the LOD. To adjust for urinary dilution, we used the following formula: $P_C = P[(1.014 - 1)/SG - 1]$, where P_C is the SG-corrected metabolite concentration (μg/L), P is the measured metabolite concentration (μg/L), and 1.014 is the mean SG level in our study population.

Table 3

Associations between urinary concentration of DINCH metabolites, metabolites of high molecular weight phthalates, and ΣDEHP and intermediate outcomes of assisted reproduction (n=136 women/cycles).

		Adjusted Mean (95% CI)[1]			
SG-adjusted Biomarker Concentration (Range)	**N**	**Total Oocytes**	**Mature Oocytes**	**Fertilized Oocytes**	**Top Quality Embryos**
Σ DEHP (μmol/L)					
T1 (0.02–0.12)	45	11.4 (10.4, 12.4)	8.8 (8.0, 9.7)	5.9 (5.3, 6.7)	3.0 (2.5, 3.5)
T2 (0.12–0.22)	46	9.0 (8.1, 9.9) *	7.3 (6.6, 8.1) *	5.0 (4.4, 5.7)	2.2 (1.8, 2.7) *
T3 (0.22–2.65)	45	8.5 (7.7, 9.4) *	7.1 (6.4, 7.9) *	4.7 (4.1, 5.4) *	1.9 (1.5, 2.3) *
P-trend[2]		<0.001	0.02	0.02	0.002
MEHP (μg/L)					
T1 (0.00–2.71)	45	9.8 (8.9, 10.7)	7.3 (6.6, 8.2)	4.8 (4.2, 5.5)	2.5 (2.0, 3.0)
T2 (2.72–6.07)	46	10.1 (9.3, 11.1)	8.1 (7.3, 9.0)	5.6 (5.0, 6.4)	2.5 (2.1, 3.0)
T3 (6.08–70.6)	45	8.9 (8.0, 9.8) †	7.8 (7.0, 8.6)	5.2 (4.6, 5.9)	2.1 (1.7, 2.6)
P-trend		0.07	0.69	0.78	0.19
MEHHP (μg/L)					
T1 (2.10–9.73)	45	11.6 (10.7, 12.7)	9.0 (8.1, 9.9)	6.0 (5.3, 6.8)	2.9 (2.4, 3.4)
T2 (9.74–17.9)	46	9.3 (8.5, 10.3) *	7.5 (6.8, 8.3) *	5.1 (4.5, 5.8)	2.3 (1.9, 2.8)
T3 (18.0–215)	45	7.9 (7.1, 8.7) *†	6.7 (6.0, 7.5) *	4.6 (4.0, 5.2) *	1.9 (1.5, 2.4) *
P-trend		<0.001	<0.001	0.006	0.006
MEOHP (μg/L)					
T1 (1.40–7.49)	45	11.6 (10.6, 12.7)	9.0 (8.1, 9.9)	6.1 (5.4, 6.9)	3.0 (2.5, 3.6)
T2 (7.50–13.3)	46	9.2 (8.4, 10.2) *	7.5 (6.8, 8.3) *	5.1 (4.5, 5.8) *	2.2 (1.8, 2.7) *
T3 (13.4–145)	45	8.0 (7.2, 8.8) *†	6.7 (6.0, 7.5) *	4.5 (3.9, 5.1) *	1.9 (1.5, 2.3) *
P-trend		<0.001	<0.001	0.002	0.002
MECPP (μg/L)					
T1 (1.68–15.3)	45	11.0 (10.1, 12.0)	8.3 (7.5, 9.2)	5.5 (4.8, 6.2)	2.5 (2.1, 3.1)
T2 (15.4–26.3)	45	9.4 (8.5, 10.3) *	7.8 (7.0, 8.6)	5.5 (4.8, 6.2)	2.8 (2.4, 3.3)
T3 (26.4–371)	46	8.5 (7.7, 9.4) *	7.1 (6.4, 7.9) *	4.8 (4.2, 5.4)	1.8 (1.4, 2.2)
P-trend		<0.001	0.03	0.10	0.004

SG-adjusted Biomarker Concentration (Range)	N	Adjusted Mean (95% CI)[1]			
		Total Oocytes	Mature Oocytes	Fertilized Oocytes	Top Quality Embryos
MBzP (μg/L)					
T1 (0.00–1.23)	45	9.6 (8.7, 10.6)	7.6 (6.9, 8.5)	5.2 (4.6, 5.9)	2.5 (2.1, 3.0)
T2 (1.24–2.35)	46	9.5 (8.7, 10.4)	7.7 (7.0, 8.6)	5.0 (4.4, 5.7)	2.2 (1.8, 2.6)
T3 (2.35–382)	45	9.7 (8.8, 10.6)	7.8 (7.1, 8.7)	5.4 (4.8, 6.2)	2.5 (2.0, 3.0)
P-trend		0.86	0.74	0.57	0.93
MCPP (μg/L)					
T1 (0–0.91)	44	8.9 (8.1, 9.9)	7.2 (6.4, 8.0)	4.8 (4.2, 5.5)	2.4 (1.9, 2.9)
T2 (0.92–1.68)	46	10.1 (9.3,11.1)	8.3 (7.5, 9.2)	5.6 (5.0, 6.4)	2.6 (2.2, 3.1)
T3 (1.69–131)	46	9.7 (8.8, 10.6)	7.7 (6.9, 8.6)	5.2 (4.6, 5.9)	2.2 (1.8, 2.6)
P-trend		0.48	0.62	0.64	0.37
MCOP (μg/L)					
T1 (1.68–6.15)	45	9.2 (8.3, 10.1)	7.2 (6.4, 8.0)	5.0 (4.4, 5.7)	2.5 (2.0, 3.0)
T2 (6.16–11.14)	46	10.2 (9.3, 11.2) *	8.4 (7.6, 9.3) *	5.7 (5.0, 6.4)	2.5 (2.1, 3.0)
T3 (11.15–1344)	45	9.4 (8.5, 10.3)	7.6 (6.8, 8.4)	5.0 (4.4, 5.7)	2.1 (1.7, 2.6)
P-trend		0.75	0.91	0.51	0.20
MiNP (μg/L)					
T1 (<LOD)	66	9.9 (9.2, 10.7)	7.8 (7.2, 8.5)	5.2 (4.7, 5.8)	2.6 (2.2, 3.0)
T2 (0.50–1.40)	35	9.5 (8.5, 10.5)	7.6 (6.8, 8.6)	5.0 (4.4, 5.8)	2.1 (1.7, 2.7)
T3 (1.41–263)	35	9.2 (8.2, 10.2) *	7.6 (6.7, 8.6)	5.4 (4.7, 6.3)	2.1 (1.7, 2.7)
P-trend		0.28	0.74	0.63	0.18
MONP (μg/L)					
T1 (0.56–2.79)	45	9.8 (8.9, 10.7)	7.7 (6.9, 8.5)	5.3 (4.6, 6.0)	2.5 (2.0, 3.0)
T2 (2.80–4.83)	46	9.4 (8.5, 10.3)	7.6 (6.8, 8.4)	4.9 (4.3, 5.6)	2.4 (2.0, 2.9)
T3 (4.84–902)	45	9.7 (8.8, 10.6)	7.9 (7.2, 8.8)	5.5 (4.9, 6.3)	2.2 (1.8, 2.7)
P-trend		0.98	0.57	0.41	0.48
MCNP (μg/L)					
T1 (0.21–0.83)	47	10.1 (9.2, 11.1)	7.8 (7.1, 8.7)	5.3 (4.7, 6.0)	2.4 (2.0, 2.9)
T2 (0.84–1.67)	44	9.0 (8.2, 10.0)	7.2 (6.4, 8.1)	4.9 (4.2, 5.6)	2.0 (1.6, 2.5)
T3 (1.68–46.0)	45	9.6 (8.7, 10.6)	8.1 (7.3, 9.0)	5.5 (4.9, 6.2)	2.7 (2.2, 3.2)
P-trend		0.76	0.36	0.44	0.22

SG-adjusted Biomarker Concentration (Range)	N	Adjusted Mean (95% CI)[1]			
		Total Oocytes	Mature Oocytes	Fertilized Oocytes	Top Quality Embryos
MHiNCH (μg/L)					
T1 (0.19–0.91)	45	10.1 (9.2, 11.1)	8.0 (7.2, 8.9)	5.0 (4.4, 5.7)	2.0 (1.6, 2.4)
T2 (0.92–1.77)	45	8.3 (7.5, 9.2) *	6.6 (5.9, 7.4) *	4.7 (4.1, 5.3)	2.5 (2.0, 3.0)
T3 (1.78–74.8)	46	10.3 (9.4, 11.3)	8.6 (7.8, 9.5)	6.0 (5.3, 6.8) *†	2.6 (2.2, 3.2) *
P-trend		0.20	0.06	0.008	0.07
MCOCH (μg/L)					
T1 (0–0.45)	45	9.0 (8.1, 9.9)	7.4 (6.6, 8.2)	4.7 (4.1, 5.4)	2.3 (1.9, 2.8)
T2 (0.46–0.84)	45	10.4 (9.5, 11.4)	8.0 (7.2, 8.9)	5.4 (4.7, 6.1)	2.1 (1.7, 2.6)
T3 (0.85–26.6)	46	9.5 (8.6, 10.4)	7.8 (7.0, 8.6)	5.6 (4.9, 6.3)	2.6 (2.2, 3.2)
P-trend		0.97	0.70	0.15	0.18
MEHHTP (μg/L)					
T1 (0–1.62)	45	10.0 (9.1, 11.0)	7.9 (7.1, 8.8)	5.3 (4.6, 6.0)	2.3 (1.9, 2.8)
T2 (1.63–3.71)	46	9.6 (8.7, 10.5)	7.8 (7.0, 8.7)	5.3 (4.6, 6.0)	2.6 (2.1, 3.1)
T3 (3.72–386)	45	9.3 (8.4, 10.2)	7.5 (6.7, 8.3)	5.1 (4.5, 5.8)	2.2 (1.8, 2.7)
P-trend		0.32	0.47	0.75	0.54
MECPTP (μg/L)					
T1 (0.84–5.53)	45	8.8 (8.0, 9.7)	6.9 (6.1, 7.7)	4.6 (4.0, 5.2)	2.0 (1.6, 2.5)
T2 (5.54–13.25)	45	10.0 (9.2, 11.0)	8.3 (7.5, 9.2) *	5.6 (5.0, 6.3) *	2.6 (2.2, 3.1)
T3 (13.26–2856)	46	9.9 (9.1, 10.9)	8.0 (7.2, 8.8)	5.5 (4.8, 6.2) *	2.5 (2.0, 2.9)
P-trend		0.19	0.21	0.16	0.40

[1] Models were run using Poisson regression with log link. All data are presented as adjusted mean counts controlling for maternal age, body mass index, and current smoking status.

* p-value for T3 or T2 vs. T1 < 0.05;

† p-value for T3 vs. T2 < 0.05

Table 4

Associations between urinary concentrations of metabolites of low molecular weight phthalates and intermediate outcomes of assisted reproduction (n=136 women/cycles).

		Adjusted Mean (95% CI)[1]			
SG-adjusted Phthalate Metabolite Concentration (Range)	**N**	**Total Oocytes**	**Mature Oocytes**	**Fertilized Oocytes**	**Top Quality Embryos**
MEP (μg/L)					
T1 (12.9–91.7)	45	10.8 (9.9, 11.9)	8.7 (7.8, 9.6)	5.8 (5.1, 6.6)	2.6 (2.1, 3.1)
T2 (91.8–286)	46	10.2 (9.3, 11.1)	8.2 (7.4, 9.0)	5.5 (4.9, 6.3)	2.3 (1.9, 2.8)
T3 (287–9333)	45	7.8 (7.0, 8.6) *†	6.4 (5.7, 7.2) *†	4.3 (3.7, 5.0) *†	2.2 (1.8, 2.7)
P-trend[2]		<0.001	<0.001	<0.001	0.38
MBP (μg/L)					
T1 (0.00–12.7)	45	10.9 (10.0, 11.9)	8.6 (7.7, 9.5)	5.8 (5.2, 6.6)	2.8 (2.4, 3.4)
T2 (12.8–26.6)	46	9.7 (8.8, 10.6)	7.9 (7.1, 8.7)	5.3 (4.7, 6.0)	2.1 (1.7, 2.6) *
T3 (26.7–148)	45	8.2 (7.4, 9.1) *†	6.8 (6.1, 7.6) *	4.5 (3.9, 5.2) *	2.2 (1.8, 2.6) *
P-trend		<0.001	0.002	0.006	0.07
MHBP (μg/L)					
T1 (0.00–0.93)	46	8.1 (8.0, 9.8)	7.2 (6.5, 8.0)	4.9 (4.3, 5.6)	2.3 (1.9, 2.8)
T2 (0.94–1.87)	45	11.3 (10.4, 12.3) *	8.9 (8.1, 9.8) *	6.1 (5.4, 6.9) *	2.8 (2.4, 3.4)
T3 (1.88–17.1)	45	8.7 (7.8, 9.6) †	7.1 (6.4, 7.9) †	4.7 (4.1, 5.3) †	1.9 (1.6, 2.4) †
P-trend		0.17	0.32	0.22	0.09
MiBP (μg/L)					
T1 (0.28–17.9)	45	10.6 (9.6, 11.6)	8.5 (7.7, 9.4)	5.8 (5.1, 6.6)	2.9 (2.4, 3.4)
T2 (18.0–31.1)	46	8.7 (7.9, 9.6) *	6.7 (6.0, 7.5) *	4.6 (4.0, 5.3)*	2.0 (1.7, 2.5) *
T3 (31.2–188)	45	9.5 (8.7, 10.5)	8.0 (7.2, 8.8) †	5.3 (4.6, 6.0)	2.2 (1.8, 2.7) *
P-trend		0.28	0.72	0.48	0.08
MHiBP (μg/L)					
T1 (0.67–5.6)	45	9.5 (8.6, 10.5)	7.7 (6.9, 8.5)	5.2 (4.6, 6.0)	2.7 (2.2, 3.2)
T2 (5.7–10.4)	46	10.1 (9.2, 11.0)	8.0 (7.3, 8.9)	5.5 (4.9, 6.3)	2.5 (2.2, 3.0)
T3 (10.5–75)	45	9.2 (8.3, 10.1)	7.5 (6.7, 8.3)	4.9 (4.3, 5.6)	2.0 (1.6, 2.4)
P-trend		0.46	0.61	0.32	0.03

[1] Models were run using Poisson regression with log link. All data are presented as adjusted mean counts controlling for maternal age, body mass index, and current smoking status.

[*] p-value for T3 or T2 vs. T1 < 0.05;

[†] p-value for T3 vs. T2 < 0.05

Table 5

Associations between tertiles of specific gravity adjusted urinary metabolites and ΣDEHP and live birth following assisted reproduction (n=121 women/initiated cycles).

	Adjusted Mean Proportions of Live Birth (95% CI)[1]			
	T1	T2	T3	p-trend
ΣDEHP	0.27 (0.16, 0.43)	0.36 (0.22, 0.51)	0.28 (0.16, 0.46)	0.96
MEHP	0.25 (0.14, 0.41)	0.31 (0.19, 0.47)	0.36 (0.22, 0.52)	0.36
MEHHP	0.35 (0.21, 0.51)	0.21 (0.11, 0.36)	0.37 (0.23, 0.55)	0.56
MEOHP	0.35 (0.22, 0.51)	0.26 (0.15, 0.41)	0.31 (0.18, 0.49)	0.90
MECPP	0.36 (0.23, 0.52)	0.26 (0.15, 0.42)	0.29 (0.17, 0.45)	0.60
MCPP	0.29 (0.17, 0.45)	0.33 (0.20, 0.50)	0.29 (0.17, 0.46)	0.95
MCOP	0.27 (0.15, 0.43)	0.40 (0.25, 0.56)	0.25 (0.13, 0.41)	0.48
MiNP	0.24 (0.14, 0.37)	0.35 (0.21, 0.52)	0.38 (0.22, 0.57)	0.25
MONP	0.32 (0.20, 0.49)	0.30 (0.18, 0.47)	0.29 (0.16, 0.45)	0.73
MCNP	0.23 (0.13, 0.38)	0.38 (0.24, 0.55)	0.32 (0.19, 0.48)	0.61
MHiNCH	0.33 (0.20, 0.49)	0.28 (0.16, 0.45)	0.30 (0.17, 0.46)	0.81
MCOCH	0.33 (0.20, 0.48)	0.26 (0.14, 0.43)	0.32 (0.19, 0.49)	0.88
MEHHTP	0.24 (0.13, 0.40)	0.37 (0.23, 0.53)	0.31 (0.18, 0.47)	0.77
MECPTP	0.16 (0.08, 0.31)	0.47 (0.31, 0.63)	0.30 (0.18, 0.46)	0.59
MEP	0.27 (0.15, 0.44)	0.38 (0.24, 0.54)	0.27 (0.15, 0.43)	0.73
MBP	0.22 (0.12, 0.37)	0.35 (0.21, 0.51)	0.36 (0.22, 0.53)	0.23
MHBP	0.31 (0.19, 0.47)	0.25 (0.14, 0.41)	0.36 (0.22, 0.54)	0.53
MiBP	0.23 (0.12, 0.38)	0.31 (0.18, 0.48)	0.39 (0.24, 0.55)	0.14
MHiBP	0.28 (0.16, 0.44)	0.25 (0.13, 0.41)	0.39 (0.24, 0.55)	0.28
MBzP	0.24 (0.13, 0.40)	0.28 (0.16, 0.43)	0.41 (0.26, 0.57)	0.12

Models were run using logistic regression. All data are presented as adjusted mean proportions controlling for maternal age, body mass index, and current smoking status.