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Urinary phthalate metabolite concentrations in relation to history of infertility and use of assisted reproductive technology (ART)

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Abstract

Objective—To examine urinary phthalate metabolite concentrations in pregnant women with planned pregnancies in relation to history of infertility and use of assisted reproductive technologies (ART).

Design—Phthalate metabolite concentrations were measured in first trimester urine samples collected from women participating in a prospective pregnancy cohort study.

Setting—Prenatal clinics affiliated with four U.S. medical centers.

Patients—750 women, of whom 86 had a history of infertility. Forty-one women used ART to conceive.

Intervention(s)—None.

Main Outcome Measures—Primary outcomes were concentrations of four metabolites of diethylhexyl phthalate (DEHP) and their molar sum (∑DEHP). Multivariable analyses compared phthalate metabolite levels in: (1) women reporting a history of infertility versus those who did not

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(comparison group); and (2) those who used ART to conceive the index pregnancy to women with a history of infertility who did not use ART.

Results—Among women with a history of infertility, ∑DEHP was significantly lower in women who conceived following ART compared to those who did not (geometric mean ratio: 0.83; 95% CI=0.71, 0.98). Similar significant associations were observed for all of the individual DEHP metabolites. There were no differences in DEHP metabolite concentrations between women with a history of infertility and the comparison group.

Conclusion—Women who used ART to conceive had lower first trimester phthalate metabolite concentrations than women with a history of infertility who did not use ART. Further research is needed to explore whether those pursuing fertility treatments take precautions to avoid exposure to environmental toxins in order to improve treatment outcomes.

Keywords

Phthalate; infertility; ART; endocrine disrupting chemicals (EDC)

Introduction

In 2000, 50 to 80 million people worldwide experienced some form of infertility, defined as failure to achieve clinical pregnancy after 12 consecutive months of unprotected intercourse. (1) In the United States, this number is expected to increase from 4 million in the early 1980s to 5.4-7.7 million by the year 2025, highlighting the importance of understanding factors contributing to this rise. (2)

Paralleling this increase in infertility, there has been a sharp rise in the production of synthetic chemicals, many of which may have endocrine-disrupting properties. (3) As a result, there has been concern as to whether endocrine disrupting chemicals (EDCs) may impact human fertility. (4, 5) Numerous EDCs, including DDT (dichlorodiphenyltrichloroethane), BPA (bisphenol A) and TCDD (2, 3, 7, 8-Tetrachlorodibenzodioxin), have now been linked to changes in ovarian function, longer time to pregnancy and increased risk of early pregnancy loss. (6-10) Although the mechanisms remain uncertain, EDCs may impact fertility by disrupting the hypothalamic-pituitary axis or by altering hormone synthesis and transport. (11-14) In 2013 the American College of Obstetrics and Gynecology (ACOG) and the American Society for Reproductive Medicine (ASRM) issued a joint committee opinion on the health impact of EDCs, underscoring the clinical relevance of better understanding these exposures. The statement emphasized how toxic environmental agents can have a profound effect on reproductive health and encouraged clinicians to educate patients on EDCs and how they might reduce their exposure. (15)

Among the chemicals highlighted in the report are phthalates, a class of EDCs widely used in the manufacture of industrial goods, pharmaceuticals, personal care products and foodstuffs. (16) In the 2011-2012 cycle of the National Health and Nutrition Examination Survey (NHANES), 9 of the 13 urinary phthalate metabolites measured were detectable in over 99% of individuals. (17) Phthalates have been linked to numerous adverse health

outcomes in human and animal models. (18-23) Of the phthalates studied thus far, di-2-ethylhexyl phthalate (DEHP) and its metabolites have attracted the most concern due to their anti-androgenic properties. In female animal models DEHP exposure is associated with decreased concentrations of key reproductive hormones, such as estradiol. (24, 25) Prenatal exposure to DEHP (and selected other phthalates) is linked to a spectrum of genital abnormalities in male rodents (termed the “phthalate syndrome”), including cryptorchidism, altered anogenital distance, and decreased sperm counts. (26) In humans, prenatal phthalate exposure is similarly linked to altered male reproductive development and changes in sex-specific childhood behavior. (27, 28) Phthalate exposure in adulthood has been associated with reduced semen quality and sex hormone concentrations. (18, 29) Despite intense interest in phthalate exposure and reproductive health in men, little is known about phthalates in relation to women's fertility. Given that testosterone and estradiol play integral roles in female reproductive capacity, and concentrations of both hormones appear to be altered by phthalate exposure, this question merits further attention.

Several studies have examined phthalate exposure in relation to female reproductive outcomes; however few have explicitly focused on fertility. (30) In female mice, exposure to DEHP and its metabolite Mono-2-ethylhexyl phthalate (MEHP) are associated with lower rates of live birth. (31) One possible mechanism is that phthalates may impair ovarian and follicular function, which consequently can interfere with conception. Supporting this possibility, in female rats, DEHP exposure is associated with lower estradiol concentrations and lower rates of ovulation. (24, 32) Similarly, MEHP induces ovarian toxicity in rodent ovarian follicles through suppression of follicular development. (33)

Few epidemiological studies have specifically examined women's fecundity in relation to phthalate exposure. In one study, occupational phthalate exposure was associated with impaired female fecundity; however, information on phthalate levels was inferred based on occupation rather than directly quantified. (34) A prospective pregnancy cohort study found that urinary levels of mono-methyl phthalate (MMP), mono-butyl phthalate (MBP) and mono-benzyl phthalate (MBzP) were inversely associated with male, but not female, fecundity. However, phthalate concentrations were lower than those reported elsewhere (including in NHANES), and by excluding anyone with diagnosed infertility, they may have removed the population of greatest concern. (35) A third pregnancy cohort study found non-significant associations between first trimester phthalate exposure and shorter time to pregnancy, but also excluded infertile women. (36)

Distinguishing between couples who conceive with assisted reproductive technologies (ART) versus those who do not is relevant because: a) couples requiring ART may represent a population with more severely impacted fertility; and b) couples who undergo ART receive more medical procedures, which presents additional opportunities for phthalate exposure through medical supplies and pharmaceuticals.

In this study we examined whether phthalate concentrations were associated with either women's history of infertility or use of ART. We used data from a large multicenter pregnancy cohort study to examine concentrations in a) women with a history of infertility compared to women with no history of infertility; and b) women who used ART to conceive

the index pregnancy compared to infertile women who conceived without ART. Because of the well-documented differences between planned and unplanned pregnancies, we limited the current analyses to planned pregnancies.(37-39) This work adds to the limited literature on phthalates in relation to women's infertility and is the first to examine phthalates in relation to infertility and use of ART.

Materials and methods

Overview of Recruitment

The Infant Development and Environment Study (TIDES), is a multi-center cohort study designed to examine the association between maternal phthalate exposures and infant health and development. Between 2010 and 2012, pregnant women ages 18 and over were recruited through prenatal care clinics at four academic medical centers across the United States: University of Minnesota, University of Rochester, Seattle Children's Hospital or University of Washington School of Medicine and University of California at San Francisco. Eligibility criteria included: less than 13 weeks pregnant, English-speaking (or Spanish-speaking at the CA center), planning to deliver at a study hospital, and no serious medical conditions (particularly psychiatric conditions that would make them poor candidates for longitudinal follow-up) or threats to the pregnancy (whereby a first trimester loss appeared probable). The institutional review boards at all participating institutions approved TIDES prior to study implementation and all subjects signed informed consent. Subjects provided spot urine samples and completed questionnaires in each trimester of pregnancy. A more detailed description of study procedures has been published elsewhere. (40)

Questionnaires

In each trimester, upon providing a urine sample, subjects completed questionnaires asking about maternal demographics, including age, race, education, income, smoking, and height and weight. Body mass index (BMI) was calculated based on first trimester values as weight/height^2 (in kg/m^2). The current analysis is based on first and second trimester questionnaire data and first trimester urine samples. We classified race as white/non-white and tobacco use as any/none. Highest level of educational attainment was categorized as less than college/college graduate or beyond. Household income was reported in seven categories ranging from less than $15,000 per year up to more than $75,000 per year. Subjects also reported on their current pregnancies as well as their reproductive histories. Women were asked whether the current pregnancy was planned and only women who answered affirmatively were included in the current analyses. To assess history of infertility, participants were asked: (1) if they had ever tried for 12 or more months without success to get pregnant; (2) whether they or their partner had ever been evaluated for infertility; and (3) whether they had received any fertility treatment including ART to conceive the current pregnancy. Women were considered to have a history of infertility if they answered yes to the first question. The comparison group was women who answered no to this question.

Biospecimens

First trimester urine was collected in sterile and phthalate-free specimen cups during initial recruitment visits, transferred to cryovials, and stored in freezers at <−80° C. At the time of

urine collection specific gravity was measured using a handheld refractometer which was calibrated with deionized water before each measurement. (41) Phthalate metabolite concentrations were analyzed at two different laboratories due to funding issues: 313 subject samples were analyzed at the Environmental Health Laboratory at the University of Washington (UW) and 375 samples were analyzed by the Division of Laboratory Sciences, Center for Disease Control and Prevention (CDC). Ten urine samples were selected at random and analyzed at both laboratories for comparison. Per a modified version of the CDC method 6306.03, glucuronidated phthalate monoesters underwent enzymatic deconjugation, followed by online-solid phase extraction (SPE) coupled with reversed high performance liquid chromatography-electrospray ionization-tandem mass spectrometry to quantify the simple monoesters in urine. (42) Process, laboratory and instrument blanks as well as field blanks were run in each lab for quality assurance. The limit of detection (LOD) of metabolites was between 0.2 and 2.0 ng/mL for the UW samples and 0.2 and 0.6 ng/mL for the CDC samples.

Statistical Analysis

Primary analyses focused on the four metabolites of DEHP: MEHP, mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECCP), and their molar sum (∑DEHP). To calculate ∑DEHP, MEHP, MEHHP, MEOHP and MECPP were divided by their molecular weights and added (∑DEHP = (MEHP/278) + (MEHHP/294) + (MEOHP/292) + (MECPP/308)) * 1000).(41) Secondarily, we considered the following additional phthalate metabolites: MBZP, Monoethyl phthalate (MEP), Monoisobutyl phthalate (MIBP), Mono-3-carboxypropyl phthalate (MCPP) and MBP (Table 2). For concentrations below the LOD, a value equal to the LOD divided by the square root of 2 was used. (43) SpG adjusted phthalate metabolite concentrations were log-transformed for normality. Following methods widely used in the literature, specific gravity (SpG) was used to adjust for urine dilution using the formula: Pc = P [(1.014-1)/SpG-1)] where Pc is the SpG-adjusted phthalate concentration (ng/ml), P is the measured phthalate concentration of the individual urine sample (ng/ml), 1.014 is the mean SpG for all samples, and SpG is the specific gravity of the individual urine sample. (44-47) Concentrations of the DEHP metabolites were slightly lower in samples run at UW as compared to CDC; to compare across the two labs, we calculated Pearson's correlations on the log-transformed values of each metabolite.

Subjects with planned pregnancies were divided into three populations for the current analysis: women who used ART to conceive the current pregnancy, women with a history of infertility who did not use ART to conceive the current pregnancy, and women who reported no history of infertility (comparison group). Descriptive statistics were used to summarize all variables of interest including age, first trimester BMI, highest level of education, tobacco use, reproductive history, income, phthalate metabolite concentrations, and study center, for each population. These covariates were chosen *a priori* as factors that might be associated with fertility and/or use of ART. We compared our populations using Chi-square or Fisher exact tests (for categorical variables) and two-sample t-test or ANOVA (for continuous variables). No ART users or infertile non-ART users reported tobacco use therefore this was dropped from all subsequent analyses.

We then fit multi-variable models using Analysis of Covariance (ANCOVA) to examine the association between phthalate metabolite concentrations and fertility history, after adjusting for BMI, age, study center, lab at which samples were analyzed and household income. (Because income and education were highly correlated, education was not included in final models). We fit two main sets of ANCOVA models. The first set compared phthalate metabolite concentrations in women with a history of infertility versus those with no history of infertility. The second set compared phthalate metabolite concentrations in women who used ART to conceive the index pregnancy versus women with a history of infertility who did not. In sensitivity analyses, we considered education (rather than income) as a covariate in all models. In addition, because there is currently no consensus as to the best method for adjusting for urine dilution, we examined the robustness of our results by re-running all models using SG as a covariate rather than transforming the phthalate metabolite levels by SG using the equation specified above. All statistical tests were two-sided and the significance level was 0.05. Analyses were performed with SAS v.9.3 (Cary, NC).

Results

In total, 750 women completed a first trimester questionnaire and gave a first trimester urine sample in TIDES. Of those, 522 reported planning the index pregnancy and 97% of those (n=506) completed questionnaire items on infertility. Eighty-six women reported a history of infertility and 420 reported no history of infertility. Among the 420 women who reported no history of infertility, 8 had used ART to conceive the index pregnancy (presumably for non-fertility related reasons such as a same-sex partnership, although this was not specifically asked). Of the 86 women who reported a history of infertility, 63 answered a follow-up question about ART use; 37 women had used ART to conceive the index pregnancy while 26 had not. Fifteen women were missing data on income and/or BMI, resulting in a final sample of 402 women with no history of infertility, 41 ART users, and 25 women with a history of infertility who had not used ART to conceive the index pregnancy. Compared to women who were included in analyses, women who were not included were more likely to have reported tobacco use at the time of recruitment (p=0.02), be non-white (p=0.002), have less than a college education (p=0.02), and be from the UW study center (p=0.0001; not shown). There were no differences in age or BMI at recruitment.

On average, women with a history of infertility were older (p=0.001), had a higher household income (p=0.001), and were more likely to have a college education (p=0.05) than fertile women. BMI, race, and education did not differ significantly across groups. Women who used ART to conceive the index pregnancy and women who were infertile but did not use ART did not differ on any of the sociodemographic variables measured (Table 1).

Phthalate metabolites were detectable in 62-99% of urine samples, depending on the specific metabolite (Table 2). Of the metabolites measured, MEP was present in the highest concentrations in all three study groups. In general, the correlation between phthalate metabolite levels as measured in the two labs was very high. The correlation coefficients for our primary analytes of interest (DEHP metabolites and their molar sum) ranged from 0.78 (for MEHP) to 0.97 (for MECCP) and all were significant at p<0.001. The other phthalate

metabolites measured were also highly correlated (correlation coefficients ranging from 0.83 for MCPP to 0.98 for MEP), with the exception of MBZP (r=0.52).

After adjustment for covariates, MEP concentrations were higher (geometric mean ratio: 1.38; 95% CI=1.00, 1.92; Table 3) in women with a history of infertility compared to the comparison group. There were no significant differences between those two groups in the concentrations of any other phthalate metabolites, including those of DEHP and their molar sum (geometric mean ratio: 0.98; 95% CI=0.89, 1.07; Table 3).

In a second set of multivariable models (which compared women with a history of infertility who used ART to women with a history of infertility who did not use ART), ΣDEHP was significantly lower in ART users than in non-ART users (geometric mean ratio: 0.83; 95% CI=0.71, 0.98). Similar significant differences between those groups were observed across all individual DEHP metabolites studied (Table 4). The two groups did not differ in concentrations of any non-DEHP metabolites. Secondary analyses were conducted including SG as a covariate, rather than adjusting for SG using the equation presented earlier, in order to evaluate the robustness of our results. Results were noted to be similar with these analyses as well (Table 3 and 4). Across most multivariable models, young age and high BMI tended to be associated with higher phthalate metabolite concentrations whereas higher income and being from the UCSF study center were associated with lower concentrations of some, but not all, phthalates (not shown). The results were unchanged when education rather than income was included as a covariate. When SG was used as a covariate, the directions of the coefficients were similar with ART users having lower levels of DEHP metabolites than infertile non-ART users; however because sample sizes were small, results were no longer significant for MECCP and ΣDEHP (Table 4).

Discussion

In this large, multi-center pregnancy cohort, among pregnancy planners, after adjusting for potential confounders, first trimester ΣDEHP was significantly lower in women who had used ART in the index pregnancy compared to women with a history of infertility who had not. Similar associations were observed for the individual DEHP metabolites when comparing these two populations, but not for the metabolites of the other phthalate esters measured. Compared to women who reported no history of infertility, those with a history of infertility had higher MEP concentrations. For all other phthalates measured, including DEHP, there were no differences in concentrations between women with a history of infertility and the comparison group.

This contrasts with findings from a large occupational study, in which phthalate exposure was associated with prolonged time to pregnancy, defined as taking longer than 6 months to conceive. The phthalate exposures differed between the studies, with the occupational study using estimated phthalate levels, rather than quantified values, resulting in the possibility of exposure misclassification. (34) In another study of sub-fertile couples, no significant association was found between phthalate metabolite levels and time to pregnancy, however infertile women were not included in that analysis. Notably, phthalate metabolite levels were much lower in that study than in NHANES. (35) While a third pregnancy cohort study also

did not find a significant association between phthalate metabolite levels and time to pregnancy, it suggested an association between phthalate exposure and shorter TTP. This study, however, also excluded women with infertility. (36)

Surprisingly, our study found that women who used ART to conceive the current pregnancy had significantly lower levels of all individual DEHP metabolites compared to women with a history of infertility who did not use ART to conceive. The reason for the difference is unclear. One possibility is that ART users pursue lifestyle choices that lower their DEHP exposure. A number of lifestyle factors have been identified which may lower exposure to phthalates and there has been interest in the medical community regarding the extent to which reducing such exposures can improve fecundity and protect fetal development. (15) It is estimated that 90% of DEHP exposure occurs through diet and fresher dietary choices with limited packaging have been demonstrated to decrease urinary DEHP metabolite levels even as soon as three days of implementation. (48, 49) Major dietary sources of DEHP may include beef, pork, certain seafood, cooking oils and cheese. (41, 50-53) Therefore, women who avoid unhealthy foods such as processed foods and foods high in animal fat could be decreasing their exposure to DEHP. Those avoiding exposure to polyvinyl chloride (PVC) containing cleaning materials, pharmaceuticals, or personal health care products could also be decreasing their exposure, as these are other well-known sources of phthalates and other endocrine disruptors. (54) Although we did assess some lifestyle factors in this population, our data specific to sources of phthalate exposure is insufficient to properly address these questions.

At present, little is known about the lifestyle habits pursued by women who conceive via ART. The International Fertility Decision-Making Study (IFDMS) found that women who reported feeling susceptible to infertility and being more knowledgeable about fertility also reported intending to optimize the chance of pregnancy through various lifestyle changes. (55) Receiving extensive pre-conception counseling, making large financial commitments to treatment, and having higher education levels are all other possible explanations as to why ART users may make healthier lifestyle choices. For example, a recent survey found that certain populations of infertile patients, such as those with diminished ovarian reserve, are more likely to consider it helpful to limit exposures to plastics in order to improve outcomes than are other IVF patients. (56) Further research is needed to evaluate whether infertile women adopt different lifestyle behaviors than fertile women, and if so, what motivates those changes. Dietary choices, use of personal care products and use of plastic containers are just some of the lifestyle measures that should be assessed in this population. Evaluating trends in their decision-making and the forces behind these decisions could help clinicians better serve this population and lead not only to higher treatment success rates but satisfaction rates as well.

Strengths of our study include a large, diverse study population and the study being nested within a prospective cohort study. Unlike previous studies, we included both infertile and fertile women, whereas most others have focused on one or the other, and have not specifically targeted ART users. Our main limitation was imprecision in timing of exposure and outcome. To determine the extent to which phthalates are associated with decreased fecundity, one would ideally measure exposure levels at the start of attempts to conceive,

rather than after a conception has occurred. Aside from issues of timing of sample collection, pregnancy may also alter phthalate metabolism, which further complicates inferences about earlier exposures during the peri-conception period. Other studies have suggested that it is reasonable to use urinary phthalate levels measured in spot samples to extrapolate exposure for several months, however because phthalates are rapidly metabolized, concentrations most reliably reflect the past day of exposure. (57) This issue is further compounded in the current analysis because our question on infertility was not specific to the index pregnancy. Instead, the question asked if women had ever tried for greater than 12 months to conceive; thus for a subject who had fertility problems years earlier, her phthalate metabolite concentrations are unlikely to reflect exposure levels during that time period. In addition, male fertility problems were not directly addressed within this analysis which could also have influenced results. We only included women who ultimately conceived, so potentially those most affected may have been excluded. Samples were also measured in two labs, however across the two labs the values measured were highly correlated with the exception of MBZP. The results for that metabolite should therefore be viewed cautiously. Lastly, the sample size of ART users was small.

Because we conducted several sets of analyses, it is possible that some significant associations were due to chance. In particular, our findings that MEP levels were higher in infertile women may be spurious. Higher urinary MEP levels have been associated with DNA damage in sperm and have been linked to increased sperm aneuploidy in men. (58-60) To the extent that MEP levels are correlated in couples, it is possible MEP exposure in male partners could underlie fertility issues. Positive associations have been found between higher urinary MEP levels and BMI in female adults, although the correlation was not as strong as in adult males. (61) This association with obesity, which has independently been shown to negatively affect fertility, could partly explain the findings noted, however we did adjust for BMI. In general however, associations between MEP concentrations and female fecundity have been weak.

The high volume production of phthalates and their ubiquitous presence in our environment makes understanding their impact on our physiology of utmost importance. This can be especially relevant for the infertile population as they are particularly likely to seek information on how to improve their fecundity. (62) In addition, although several studies have examined potential lifestyle changes that could increase fertility, few have assessed whether infertility patients ultimately pursue different lifestyle behaviors in an effort to improve their treatment outcome. If we do find that the lifestyle behaviors pursued by women who use ART lower their phthalate exposure, this is important information that may then be used to develop recommendations for lowering levels in all women, not just the infertile population. Identifying behaviors associated with lower DEHP exposure may be important for improving prenatal health of both mother and fetus. (46) In conclusion, evaluating the effects of environmental toxins on the ability to conceive and understanding how lifestyle changes could mitigate these effects, is important for our improving our treatment of infertility.

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Table 1

TIDES subject demographics by fertility status and ART use.

	Women using ART (n=41)	Infertile not using ART (n=25)	Comparison Group[a] (n=402)
	Mean (SD)		
Age (years)	35.37 (4.78)	33.94 (4.46)	32.00 (4.63)
BMI (kg/m^2)	25.83 (4.74)	24.90 (6.42)	25.33 (5.21)
Gestational age (weeks)	12.12 (1.55)	11.12 (1.74)	11.26 (2.54)
	N (%)		
Tobacco use (any)	0 (0%)	0 (0%)	9 (2%)
Education			
Less than college	1 (2%)	1 (4%)	52 (13%)
College Graduate or more	40 (98%)	24 (96%)	350 (87%)
Income			
<$15,000	0 (0%)	1 (4%)	27 (7%)
$15,000-$25,000	0 (0%)	0 (0%)	24 (6%)
$25,001-$45,000	3 (7%)	1 (4%)	32 (8%)
$45,001-$55,000	0 (0%)	0 (0%)	27 (7%)
$55,001,$65,000	0 (0%)	2 (8%)	27 (7%)
$65,001-$75,000	1 (3%)	0 (0%)	24 (6%)
>$75,000	37 (90%)	21 (84%)	241 (60%)
Race			
Caucasian	32 (78%)	24 (96%)	307 (76%)
African- American	0 (0%)	1 (4%)	26 (6%)
Asian	5 (12%)	0 (0%)	34 (9%)
Other	4 (10%)	0 (0%)	35 (9%)
Unknown	0 (0%)	0 (0%)	0 (0%)
Study Center			
UCSF	17 (41%)	13 (52%)	114 (28%)
UMN	11 (27%)	4 (16%)	131 (33%)
URMC	8 (20%)	2 (8%)	64 (16%)
UW	5 (12%)	6 (24%)	93 (23%)

[a] Women who reported no history of infertility

Table 2

Phthalate Metabolite Concentrations (ng/ml) in comparison group[1], infertile women using ART, and infertile women who did not use ART in the TIDES study.

Phthalate	Metabolite	Percent above LOD[2]	Comparison Group[1] (n=402)			Women using ART (n=41)			Infertile not using ART (n=25)		
			Percentiles			Percentiles			Percentiles		
			25th	50th	75th	25th	50th	75th	25th	50th	75th
Di-2-ethylhexyl phthalate (DEHP)	Mono-2-ethylhexyl phthalate (MEHP)	62.4	0.71	1.80	4.20	0.71	1.00	2.80	0.71	1.30	3.80
	Mono-2-ethyl-5-oxohexyl phthalate (MEOHP)	95.9	1.80	4.00	8.40	1.50	3.00	7.00	1.40	3.00	9.00
	Mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP)	97.3	2.40	5.70	12.20	2.00	4.40	9.60	1.90	4.60	15.00
	Mono-2-ethyl-5-carboxypentyl phthalate (MECCP)	97.9	2.90	8.20	16.70	2.60	5.70	15.40	4.80	7.20	12.70
	ΣDEHP[3]	--	26.31	66.22	139.68	22.95	47.38	116.76	29.41	54.01	136.88
Monobenzyl phthalate (MBZP)		83.7	0.80	2.70	7.00	0.71	2.00	5.90	0.71	1.60	7.00
Monoethyl phthalate (MEP)		98.9	7.90	22.05	57.00	13.00	22.00	73.00	8.70	23.60	95.00
Monoisobutyl phthalate (MIBP)		97.0	1.30	3.80	9.00	1.60	3.70	8.10	0.80	3.30	8.10
Mono-3-carboxypropyl phthalate (MCPP)		72.3	0.71	1.30	3.90	0.71	0.80	3.40	0.71	1.10	3.00
Mono-butyl phthalate (MBP)		91.7	2.00	6.00	14.50	2.00	6.00	10.40	3.00	5.00	11.00

[1] Women who reported no history of infertility

[2] LOD=Limit of Detection

[3] ΣDEHP = (MEHP/278) + (MEHHP/294) + (MEOHP/292) + (MECPP/308)) * 1000

Table 3

ANCOVA models comparing phthalate metabolite concentrations in women with a history of infertility (n=86) versus comparison group[1] (n= 402)[2]

	Model 1: Phthalate metabolite concentrations adjusted based on SG using formula[3]				Model 2: SG included as covariate			
	Geometric Mean Ratio	95% Confidence Limits		p-value	Geometric Mean Ratio	95% Confidence Limits		p-value
ΣDEHP	0.98	0.89	1.07	0.59	0.97	0.88	1.06	0.49
MEOHP	1.00	0.91	1.09	0.94	0.99	0.90	1.09	0.89
MEHHP	0.98	0.88	1.08	0.64	0.97	0.88	1.08	0.60
MEHP	0.96	0.87	1.06	0.40	0.96	0.87	1.06	0.40
MECCP	0.97	0.89	1.06	0.49	0.96	0.87	1.06	0.40
MBZP	0.99	0.78	1.25	0.93	0.97	0.77	1.22	0.77
MEP	1.38	1.00	1.92	0.05	1.36	0.98	1.89	0.07
MIBP	1.03	0.95	1.13	0.46	1.02	0.93	1.12	0.69
MCPP	0.83	0.62	1.11	0.21	0.82	0.61	1.10	0.19
MBP	0.97	0.89	1.06	0.55	0.96	0.88	1.05	0.40

[1] Women who reported no history of infertility

[2] Adjusted for BMI, age, center, lab and household income.

[3] Pc = P [(1.014-1)/SpG-1)]

Table 4

ANCOVA models comparing phthalate metabolite concentrations in infertile women who did (n=41) and did not use ART (n=25) to conceive.

	Model 1: Phthalate metabolite concentrations adjusted based on SG using formula[2]				Model 2: SG included as covariate			
	Geometric Mean Ratio	95% Confidence Limits		p-value	Geometric Mean Ratio	95% Confidence Limits		p-value
ΣDEHP	0.83	0.71	0.98	0.02	0.84	0.71	1.01	0.06
MEOHP	0.84	0.71	0.98	0.03	0.84	0.71	0.98	0.03
MEHHP	0.82	0.68	0.98	0.03	0.81	0.68	0.98	0.03
MEHP	0.83	0.69	1.00	0.05	0.83	0.69	1.00	0.05
MECCP	0.84	0.71	0.98	0.03	0.85	0.71	1.01	0.06
MBZP	0.87	0.53	1.43	0.58	0.90	0.52	1.54	0.69
MEP	1.00	0.44	2.27	1.00	1.04	0.45	2.42	0.92
MIBP	0.94	0.77	1.16	0.58	0.96	0.74	1.24	0.72
MCPP	0.96	0.50	1.83	0.91	1.00	0.52	1.93	1.00
MBP	0.90	0.73	1.10	0.30	0.91	0.72	1.16	0.44

[1] Adjusted for BMI, age, center, lab and household income.

[2] Pc = P [(1.014-1)/SpG-1)]