ABSTRACT

Title of Thesis: **INVESTIGATING THE ASSOCIATION OF PHTHALATE EXPOSURES AND ADVERSE REPRODUCTIVE HEALTH OUTCOMES IN A REPRESENTATIVE SAMPLE OF U.S. WOMEN**

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Phthalates are endocrine disrupting chemicals present in a large variety of consumer goods. There is supporting evidence from animal studies that exposure to phthalates affect the female reproductive system by disrupting the epigenome and folliculogenesis/oogenesis. Although women of reproductive age experience higher phthalate exposures versus males due to frequent use of cosmetics and personal care products (PCP), studies investigating reproductive health effects of these chemicals are scarce. In this study, a nationally representative sample was used to investigate the association between exposure to phthalates (primarily in PCPs and cosmetics) and subfertility in women aged 18-44. We observed significantly higher phthalate levels among minority women and positive associations between DBP exposure and subfertility in regressions when adjusted for important covariates This is the first study to use such a sample of women to study the effects of phthalates on subfertility. More epidemiological studies are needed to investigate phthalate levels among minorities.

INVESTIGATING THE ASSOCIATION OF PHTHALATE EXPOSURES AND ADVERSE REPRODUCTIVE HEALTH OUTCOMES IN A REPRESENTATIVE SAMPLE OF U.S. WOMEN

by

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Preface

This thesis was written to investigate the association between exposure to phthalates and reproductive health outcomes among women of reproductive age. My passion for this topic comes from the desire to investigate health disparities, especially due to environmental contaminants with regards to maternal and child health. Within the realm of environmental health, I am most interested in toxicology and genetics. My interest in this field stemmed from my experiences teaching at an inner-city high school and witnessing the disparities in health and cognitive abilities of my students. Following this experience, I worked at a clinical genetics lab analyzing genetic assays (microarrays) for copy number variants, designing real time polymerase chain reaction (qPCR) primers, performing clinical experiments via qPCR, and interpreting data within reports to determine copy number variants in specific genes. As I have moved through my applied environmental health MPH program at the University of Maryland, I have become even more interested in epigenetics specifically due to this genome's sensitivity to environmental contaminants. This has led me to my thesis topic of investigating the effects of exposure to phthalates and reproductive toxicology. It is my hope that the findings from this thesis shed light on the possible linkage between phthalates and subfertility in females of reproductive age and the lack of data surrounding this topic.

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List of Abbreviations

ANOVA	Analysis of Variance
ATSDR	Agency for Toxic Substances and Disease Registry
BBP	Butylbenzyl phthalate
CDC	U.S. Centers for Disease Control and Prevention
ΣDEHP	cumulative DEHP metabolites
D(n)BP	Di-n-butyl phthalate
DEHP	Di(2-ethylhexyl) phthalate
DEP	Diethylphthalate
DF	Detection Frequency
D(i)NP	Di-isononyl phthalate
DMP	Dimethyl phthalate
D(n)OP	Di-n-octyl phthalate
EDC	Endocrine Disrupting Chemical
EPA	U.S. Environmental Protection Agency
EWG	Environmental Working Group
FDA	U.S. Food and Drug Administration
HPLC	High Performance Liquid Chromatography
LLOD	Lower Level of Detection
LOD	Level of Detection
MBP	Mono-n-butyl phthalate

MCOP	Mono(carboxyisooctyl) phthalate
MCP	Mono-cyclohexyl phthalate
MEC	Mobile Examination Center
MEP	Mono-ethyl phthalate
MECPP	Mono(2-ethyl-5-carboxypentyl) phthalate
MEHHP	Mono(2-ethyl-5-hydroxyhexyl) phthalate
MEHP	Mono-(2-ethyl)-hexyl phthalate
MEOP	Mono(2-ethyl-5-oxohexyl) phthalate
MIAEH	Maryland Institute for Applied Environmental Health
MBP	Mono-benzyl phthalate
MMP	Mono-methyl phthalate
M(n)BP	Mono-n-butyl phthalate
M(i)NP	Mono-isononyl phthalate
M(n)OP	Mono-n-octyl phthalate
MS	Mass Spectrometry
NCHS	National Center for Health Statistics
NHANES	National Health and Nutrition Examination Survey
NHSA	National Health Survey Act
NIH	National Institutes of Health

Chapter 1: Background

Introduction

Historical context

Phthalates are a class of synthetic compounds that have been used as additives since the 1920s, originally in polyvinyl chloride (PVC) and insect repellants(Oehlmann et al., 2009). They are added to many consumer products such as plastic containers and medical tubing to make them softer and more flexible; hence why they are also referred to as "plasticizers" (NIH, 2017). They are also used as fragrance fixatives in products such as perfume and household cleaning agents(CDC, 2016d; NIH, 2017). Phthalates are also found in many other consumer products including cosmetics and personal care products (shampoos, lotions, nail polish, hair sprays, soap), toys and other infant products, solvents and wood varnishes, other household items (mini blinds, shower curtains) and medications (coating) (CDC, 2016d; NIH, 2017).

Currently, there are nearly ten different parent compounds of phthalates whose biotransformed metabolites can be measured in urine and are widely used as biomarkers of exposure in biomonitoring, exposure, and epidemiologic studies (Table 1). Phthalates are commonly classified as high (252.22-322.25 g/mol) or low molecular weight (180.16-222.24 b/mol) (MW). High molecular weight phthalates

such as DEHP, for example are commonly found in plastics, while low molecular weight phthalates are commonly found in personal care products.

Table 1 Parent phthalates and metabolites.

Parent Phthalate	Parent Phthalate Abbreviation	Urinary Metabolite(s)	Molecular Weights (MW) (g/mol)
Di(2-ethylhexyl) phthalate	DEHP	MEHP, MEHHP, MEOHP, MECPP	278.34-308.33 High
Butylbenzyl phthalate	BBP	Mono-benzyl phthalate (MBP)	256.25 High
Di-isononyl phthalate	DiNP	MCOP + MiNP	322.35 High
Di-n-octyl phthalate	D(n)OP	MCPP + MOP	252.22-252.24 High
Dimethyl phthalate	DMP	Monomethyl phthalate (MMP)	180.16 Low
Diethylphthalate	DEP	Mono-ethyl phthalate (MEP)	194.18 Low
Di-n-butyl phthalate Diisobutyl phthalate	D(n)BP DiBP	MnBP + MiBP	222.23-222.24 Low

(See Figure 1 in Appendix for structures and Table A for more uses of phthalates)

Phthalates are effective in making products more flexible because they lack covalent bonds between the phthalate and raw material, thereby, reducing the inter-molecule chemical affinity when embedded into the raw goods (Oehlmann et al., 2009). Thus, phthalates are not stable, allowing them to easily leach out of the products they have been added to, resulting in exposure in humans. Although they are not persistent and do not bioaccumulate, exposure from multiple pathways is an emerging health concern (CDC, 2016d). In addition, these chemicals are lipid soluble, meaning they enter human cells and easily cross placental barriers (Scowen, 1996).

Potential Adverse Reproductive Health Effects of Phthalates.

Phthalates are known endocrine disrupting chemicals (EDCs), meaning they interfere with the endocrine system producing anti-androgenic or pro-estrogenic effects on hormones by mimicking natural hormones (CDC, 2016d; EPA, 2012). *The State of the Science of Endocrine Disrupting Chemicals –2012* report by the World Health Organization (WHO) and United Nations Environmental Program (UNEP), found that exposure to phthalates is linked to a range of adverse health outcomes including—adult male and female reproductive issues (e.g., decreased pregnancy rates and high miscarriage rates), pregnancy complications (e.g., anemia, preeclampsia, toxemia), decreased birth weight, endocrine-related cancers, obesity, asthma, diabetes, infections and learning disorders (Birnbaum, 2013; Hannon & Flaws, 2015). At this time, the potential long term health effects of phthalates at low, biologically relevant doses (i.e., doses at which the general population is exposed to) are unknown (CDC, 2016d).

In males, exposure has been linked to "phthalate syndrome", a term coined to describe a combination of effects— infertility, decreased sperm count, undescended testes, and other reproductive organ malformations (Swann, 2008). Female reproductive adverse health effects have not been studied as extensively, but are hypothesized to be more detrimental than in males, especially during childbearing years due to the higher exposure levels experienced by women which is hypothesized to result, in large part, from the use of cosmetics and personal care products (e.g., shampoos, cosmetics and other personal care products) (CDC, 2016d).

Exposure to Phthalates in the United States.

The ubiquitous presence of phthalates in many consumer products has led to widespread chronic exposure in the general U.S. population. In fact, it is estimated that 75-100% of the general population of the United States is exposed to phthalates on a daily basis (Hannon & Flaws, 2015). Exposure to phthalates primarily occurs by consuming foods and/or beverages that have been in contact with containers that contain phthalates, followed by exposure via cosmetics and toys (EPA, 2012). Research has demonstrated higher levels of phthalates among women versus men; and this is thought to be due, in part, to the fact that low molecular weight phthalates (e.g. DBP and DEP) are used heavily in cosmetics and personal care products (including lotions and nail polish), therefore, women have more frequent exposures versus men (CDC, 2016d; Huang et al., 2015). It is estimated that "more than a quarter of all women and one of every 100 men use at least 15 products daily" (EWG, 2013, 2017). Women who reported using more personal care products have also been found to have even higher levels of phthalates than those who use less of these products (Braun et al., 2014). Exposure to phthalates may also occur, to a lesser extent, via inhalation of ambient air that has been contaminated by phthalate particles and placental transfer is also possible (CDC, 2016d; National Research Council (US) Committee on the Health Risks of Phthalates, 2008).

Once a person is exposed to phthalates, excretion (clearance) from the body generally occurs via urine and feces within 24 hours (ATSDR, 2002). Because these chemicals are considered non-persistent and typically excreted within a few hours, human exposure is normally assessed via urine (CDC, 2016d; EPA, 2012). It is

important to also note that although half-lives have been recorded around 5 hours for phthalates, they can take up to 64 hours to break down in amniotic fluid(Genuis, Beesoon, Lobo, & Birkholz, 2012).

Preexisting Studies on Phthalates and Reproductive Health Outcomes

Mode of Action

It is well accepted that phthalates disrupt normal endocrine processes and it is further hypothesized that these EDCs do this within the female reproductive system by disrupting ovarian function, primarily folliculogenesis and ovarian steroidogenesis[1]. Female ovaries are paramount because their function moderates both reproductive and non-reproductive health in the mammalian body and are especially critical in terms of processes necessary for successful conception(Hannon & Flaws, 2015). Studies have found that EDCs as a whole, disrupt the epigenome by altering DNA methylation of particular genes, affecting histones and disrupting RNA function(Bernal & Jirtle, 2010). Particular points on chromosomes which are involved in these alterations were identified in the Agouti mouse model as imprinted genes[2] and metastable epialleles[3] (Bernal & Jirtle, 2010) (See Appendix, Figure 2). Culturing zygotes from these mice also led to findings which coincided with (inherited) human imprinting disorders(Bernal & Jirtle, 2010). The pattern of epigenetic changes necessary for proper development in utero is particularly vulnerable to environmental exposures. This has been applied to Barker's theory on "fetal origins of disease" which has long been accepted as a mechanism by which developmental exposures

[1] the biological process describing how to ovary produces sex steroid hormones
[2] inherited gene(s) from one parent which are necessary to be epigenetically silenced
[3] allele (particular form of gene) variably expressed in identical individuals due to environmental exposure

lead to adult (and possible transgenerational) adverse health outcomes, i.e. reproductive function.

In an *in vitro* study utilizing a yeast-based estrogen receptor gene transcription assay and methylation specific real time polymerase chain reaction (qPCR), it was found that estrogen receptor genes (ERα) were demethylated at promoter regions, thereby promoting the expression of these genes, by DBP and BBP (concentration 10^{-5} M) in human breast cancer cells(Kang & Lee, 2005). Although this study did not report reproductive ovarian effects, it illustrated a plausible mode of action for other, more recent reproductive studies on phthalates (Carnevali et al., 2010; Hannon, Brannick, Wang, Gupta, & Flaws, 2015; Kang & Lee, 2005; Sen, Liu, & Craig, 2015).

Animal studies

Studies in adult CD-1 mice have found that DEHP phthalates inhibit antral follicle[4] growth (Hannon et al., 2015). When antral follicles from these specimens were cultured in vitro and exposed to 1, 10 or 100 ug/ml of DEHP, it was found that mRNA regulators of the cell cycle, apoptosis and the enzymes responsible for estradiol generation were altered (Hannon et al., 2015). This alteration led to an increase in atresia, (the degeneration of ovarian follicles); this was also associated with the increase of mRNA expression in genes programmed for apoptosis. Hannon et al. (2015) also found that progesterone, androstenedione and testosterone were decreased, along with a number of enzymes, leading to their conclusion that DEHP

[4] functional units of the ovary which must grow properly to produce final, antral follicles for ovulation and hormone production

directly altered antral follicle functionality and proper ovulation (Hannon et al., 2015).

In another study on CD-1 mice by Niermann et al. (2015), DEHP was found to have reproductive effects on female pups. Authors reported an increase in preantral follicle numbers increase post-natally at day 21 and that over 20% of the mice who were treated with 20 ug/kg/day took over 5 days to get pregnant (at 3 months age). Findings were compared to a control group at different treatment ranges (20 and 200 µg/kg/day, 200, 500 and 750 mg/kg/day) due to monotonic dose-response relationship reported previously with DEHP (Flaws, Ph.D., 2015; Niermann, Rattan, Brehm, & Flaws, 2015).

Other animal studies have also investigated the effects of phthalates at dosages that reflect those observed in the general population using animal models with similar genetic makeups, such as zebrafish. When investigated within zebrafish, DEHP was reported to significantly decrease fecundity by affecting the epigenome(Carnevali et al., 2010). Epigenetic signals for oocyte growth, maturation and ovulation were altered via nominal exposure to 0.02, 0.2, 2, 20 and 40 mg/l concentrations of DEHP over the course of 3 weeks; data was analyzed using qPCR, enzyme-linked immune sorbent assay (ELISA) and western blots. Genes necessary to be expressed for ovulation initiation were decreased for all dosages, leading to a reduction of embryos(Carnevali et al., 2010).

In a study performed by Sen, Liu and Craig (2015), 0.01, 0.1 and 1,000 mg/kg/day dosages of DBP were given to young CD-1 mice to investigate levels of exposure similar to humans, particularly female consumers who use beauty and other

medical products (supplements, medications)(Sen et al., 2015). Most notably, antral follicle numbers were decreased and mRNAs encoded for pro-apoptotic genes were increased(Sen et al., 2015). Steroidogenic enzymes were also increased in all dosage groups; it is said that all of these toxic effects on ovarian follicles "may result in blocked ovulation and estrogen deficiency, which in turn may lead to infertility"(Sen et al., 2015).

Lastly, a study tested a phthalate mixture consisting of 35% DEP, 21% DEHP, 15% DBP, 15% D(n)BP, 15% DiNP, 8% D(i)BP and 5% BBP on CD-1 mice at dosages of 20 and 200 μg/kg/day, 200 and 500 mg/kg/day; the mixture was based on phthalates in urine samples from pregnant women collected in Illinois(Zhou, Gao, & Flaws, 2017). Female mice from the F1 generation were tested for tissue alterations and fertility at various post natal periods. The mixture showed significant effects on body weights at onset of puberty in pups at 20 ug/kg/day and a decrease in days between weaning and vaginal opening (Zhou et al., 2017). Time to pregnancy was increased for all age groups at 200 mg/kg/day; the 3 month group was statistically significant(Zhou et al., 2017).

Human Studies

Few studies have evaluated phthalate exposures among women of reproductive age. In a cohort of pregnant women at a fertility clinic, women who reported using perfume had urinary concentrations of MEP 167% higher than those who did not use perfume. Those who used nail polish also had increased concentrations of both MEP and MBP (152% and 88%, respectively) versus non-

users (Braun et al., 2014). MBP concentrations of those who used lotions were also 28% higher than non-users. These preliminary findings further support that the major sources of phthalate exposure for women are cosmetics and personal care products.

To our knowledge, only one epidemiologic study to date has investigated the link between phthalate exposures and female reproductive health. Specifically, authors evaluated the effects of phthalate exposure on time to pregnancy and provided supporting evidence for the effects of phthalate exposures on the female reproductive system. Within this Danish cohort study, 229 women were enrolled in the early 1990s and urinary phthalate analyses was performed in 2009 (Thomsen et al., 2017). Morning spot urines were collected daily for 10 days and mean measurements from the 6 menstrual cycle collection period to test for MEP, MBP, MBzP and MEHP metabolites. Researchers reported a 21% decreased probability of conception (with each natural log unit increase in) as a result of exposure to MEP, illustrating an association between MEP and prolonged time to pregnancy (Thomsen et al., 2017).

Public Health Significance of Evaluating the Effects of Phthalates on Women's Reproductive Health and Current Research Gaps

As noted, the potential adverse health effects of phthalates on women's reproductive health have not been widely investigated although the biological plausibility of this link exists. Phthalate exposures can occur in utero and lay dormant for decades before creating adverse health effects or it could occur in adulthood and produce adverse health effects (Bernal & Jirtle, 2010). Even more concerning is the fact that vulnerable populations of pregnant women and their fetuses are at higher risk for phthalate toxicity due to an array of susceptibilities—

developing endocrine and reproductive systems, effects on folliculogenesis and oogenesis[5], and underdeveloped systems to detoxify these chemicals. Additionally, female reproductive systems are more complex than males and require specific timing of biochemical events, much unlike the male reproductive system, which does not fluctuate nearly as much (Tynes, 2016).

Phthalate levels have also been found to be higher in females of reproductive age versus any other gender or age group (Flaws, Ph.D., 2015). Interestingly, data from the general U.S. population also indicate that minority women, including Mexican Americans and non-Hispanic Blacks have elevated urinary levels for several phthalates commonly found in personal care products, suggesting that exposures could also lead to health disparities in these subgroups (Trasande, Attina, Sathyanarayana, Spanier, & Blustein, 2013; Varshavsky, Zota, & Woodruff, 2016). Women of Hispanic origin are said to be "the fastest-growing and highest-spending market segment for cosmetics" within the United States (EFE, 2016; The Nielson Company, 2015). Moreover, recent statistics have shown that overall infertility rates within the United Sates have declined in the past two decades, infertility rates among minority populations, including non-Hispanic black women have been increasing (Quinn & Fujimoto, 2016).

Lastly, although the Fair Packaging and Labeling Act (FPLA) requires ingredients to be included on cosmetic labels which are sold at the retail level, they do not require individual "fragrance" ingredients to be listed and phthalates may be included as one of the ingredients in "fragrance". To exacerbate the issue, most

[5] Figure 3 in Appendix

cosmetic products and ingredients are not subject to FDA approval, putting the potential health of females of reproductive age who use these products at risk. Although current regulations on phthalates exist to protect neonates and males, similar regulations have not been developed for other vulnerable and susceptible populations, e.g. women of reproductive age, due to gaps in research(McCormick, 2015).

Despite the lack of epidemiological data in females of reproductive age, the literature described herein illustrates the modes of action and biological plausibility by which phthalates could affect the female reproductive system not only animals, but humans as well. Thus, this thesis focuses on investigating the potential association of exposure to phthalates and female reproductive health outcomes (subfertility) in order to address this major research gap.

Research Question

Using data from the general U.S. population collected by the CDC, we sought to investigate if exposure to several phthalates (DEHP, DBP and DEP) previously linked to adverse reproductive health outcomes in animal studies, and commonly found in consumer products, is associated with subfertility among women of reproductive age. We hypothesized that exposure to phthalates (especially those found in personal care products and cosmetics) alters fertility in females of reproductive age. In alignment with the environmental health program, this thesis will use a number of environmental health competencies[6] to investigate this research question.

[6] Competencies can be found in Appendix, Tables B and C.

Chapter 2: Methods

NHANES

Data used for this analysis was obtained from the National Health and Nutrition Examination Survey (NHANES), which is a cross sectional survey conducted biannually by the Centers for Disease Control and Prevention's (CDC's) National Center for Health Statistics (NCHS) to provide data in order to assess civilian morbidity within the United States. Study activities include in home interviews and a standardized physical examination, which is conducted at a mobile examination center (MEC). The biannual survey randomly selects individuals across the nation and is both confidential and voluntary, per IRB (CDC, 2011, 2014). For our analyses, we focused on the NHANES data available for the 2013-2014 cycle year, including data on demographics, laboratory, and questionnaire datasets as described below.

Study Population

NHANES is designed to assess the health and nutritional status of both adults and children in the United States using interviews and physical examinations. NHANES has been gathering phthalate data since 1999. Through the use of questionnaires, NHANES has also collected reproductive health data on adults, ages 18 years and older. Specifically, in cycle 2013-2014, they began to include questions on women's history of infertility (CDC, 2014).

For our analyses, we used data from the NHANES 2013-2014 cycle, focusing on women of reproductive age for which subfertility data was available. Although the maximum reproductive age can exceed the age of 44 years, menopause may begin as early as 45, which is why we restricted the maximum age in our study population for our analyses (CDC, 2017). Since the minimum age for collection of this subfertility data was 18 years, we also restricted our minimum age for our target population our population, making our target age range 18-44 years rather than the CDC established range of 15-44 years (CDC, 2017). A total of 1,279 women were administered questionnaires, as described above, to assess subfertility and capture demographic information and data on smoking status. Due to missing data for phthalates or other demographic variables, the final sample size consisted of 425 women.

Exposure Assessment of Phthalates

Urine was collected from a subset of NHANES participants (age 6 and older) at MECs, each staffed with trained laboratory personnel(CDC, 2014, 2016c). A total of 15 phthalate metabolites were measures in participants' urine. For the analyses presented herein, we focused on (MEHHP, MEC(P), MEOHP, MEHP, MBP and MEP). Selection of parent phthalates and their respective urinary metabolite(s) (DEHP- MEHHP, MEC(P), MEOHP and MEHP; DBP- MBP; DEP- MEP) for this analysis was based on their potential for reproductive toxicity based on previous studies (Hannon & Flaws, 2015; Carnevali et al., 2010; Gray Jr, Laskey, & Ostby, 2006; Niermann, Rattan, Brehm, & Flaws, 2015; Sen et al., 2015; Zhou, Gao, & Flaws, 2017) and availability in NHANES data.

Target phthalates were quantified in urine samples using a validated laboratory method using High Performance Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry (HPLC-EDI-MS/MS) (CDC, 2016c). Samples were then processed utilizing enzymatic deconjugation of the glucuronidated metabolites and on-line solid phase extraction (SPE), which was coupled with reversed phase HPLC-ESI-MS/MS (CDC, 2016c). Limits of detection (LOD) for each analyte were: MEHHP 0.4, MEC(P) 0.4, MEOHP 0.2, MEHP 0.8, MBP 0.4, and MEP 1.2 ng/mL(CDC, 2016c). The detection frequencies were as follows: DEHP metabolites (MEHHP, MECPP, MEOHP, MEHP) 62.4% – 100%, DEP metabolite (MEP) 98.4% and lastly, DBP metabolite (MBP) 99.7% (Table 3). Urinary creatinine (milligrams per deciliter) concentrations were also collected and measured using an automated HPLC derived colorimetric method on a Roche/Hitachi Cobas 6000 analyzer (CDC, 2016b). Creatinine concentrations were used in our analyses to account for urine dilution.

Covariates

The following covariates were considered for inclusion in our multivariate logistic regression models: age, race/ethnicity, poverty income ratio (PIR), body mass index (BMI), smoking status and health insurance status. Age was categorized as 18-26, 27-35 and 36-44 years, and race/ethnicity was categorized as Mexican American/Other Hispanic as Hispanic, Non-Hispanic white, Non-Hispanic Black and Other (multi-racial). Federally based poverty threshold and household income was assessed by using the poverty income ratio and categorized as a binary variable, less

than 1 (below poverty level) and greater than or equal to 1 (above poverty level) as recommended previously by the CDC National Center for Health Statistics (CDC, 2013). BMI scores (in kilograms/meters^2) were categorized as normal (i.e., 18.5 to <25 or overweight/obese (i.e., 25 and over) per National Institute of Health (NIH) guidelines (NIH, n.d.). Smoking status was assessed by currently smoking everyday/some days versus non-smoker. Lastly, health insurance was categorized as a binary variable (yes/no) (Table 2). Anyone who refused to respond or did not know the answer to a question was coded as "missing".

Outcome Assessment

For the primary outcome variable subfertility, participants' fertility status was based on the response to two subfertility questions: "have you tried for a year to become pregnant" and "have you seen a doctor (DR) because unable to become pregnant". This question was asked only to women age 18 years and older. Each outcome was considered individually in separate models, but based on small sample sizes outcomes were combined into a binary outcome variable to increase statistical power in our models. We combined the response to these two questions into one binary outcome variable coded as yes if participants responded affirmatively to either or both questions and no if they responded negatively to either or both questions.

Statistical Procedures

First, we calculated descriptive statistics for phthalate concentrations in our population (e.g., GM, GSD, p25-p95, range). For the continuous analyte variables used in this thesis, those with a detection frequency (DF) over 90%, concentrations

below the LOD were imputed to (LOD/sqrt [2]). When the DF was below 90%, multiple imputation was used to replace the values below their respective LOD (CDC, 2016c). The molar sum of DEHP metabolites was calculated by dividing each metabolite by its respective molecular weight, e.g. {[MEHHP/294.34)] + [MECPP/308.33) + [MEOHP/292.33)] + [MEHP/278.34)]}. The molar sum of phthalates has been used as a measure of exposure in prior studies as it accounts for all possible metabolites more accurately than the non-molar sum of these metabolites (James-Todd et al., 2012; Russ Hauser et al., 2016; Varshavsky, et al., 2016).

Phthalate concentrations, along with creatinine, were then log10 transformed to approximate a normal distribution and reduce the potential influence of extreme outliers. Collinearity was also investigated among variables prior to running the regression models to limit overestimations. Bivariate analysis of these covariates and outcome were then used to inform covariates to be included in the regression based on a p-value<0.20. ANOVA comparisons were also conducted to investigate effect modification and determine if race should be stratified in the regression (Table 4).

Explanatory variables were evaluated in association to our subfertility outcome as follows: Model 1 represented the crude model, which adjusted for phthalate exposure and creatinine only and model 2 adjusted for all socio-demographic covariates (age, race/ethnicity, PIR and insurance) in addition to creatinine and phthalate exposure. The main regression model used for our multivariate logistic regression was as follows:

$$Logit\ [P(y=1)] = \beta_0 + \beta_1\ ((log)creatinine) + \beta_2((log)metabolite) + \beta_3(age) + \beta_4(race) + \beta_5(PIR) + \beta_6(insurance)$$

We evaluated smoking and BMI as part of our sensitivity analyses. Although smoking has been previously linked to fertility problems, we did not include it as a covariate in our main model because information on smoking was missing on many participants (73% of the women with phthalate measurements were missing information on smoking) (Sharma, Biedenharn, Fedor, & Agarwal, 2013). We also included BMI (and assessed interaction by BMI) in separate models instead of including it as a covariate in our main models because it is a potential mediator; that is, BMI could be in the causal pathway between our exposure to phthalates and subfertility (James-Todd et al., 2012; Russ Hauser et al., 2016; Yaghjyan, Sites, Ruan, & Chang, 2015). Sensitivity analysis was performed by way of the following models: Model 3a, which adjusted for all the same covariates but used only the subset of individuals who had smoking data (n=190), model 3b adjusted for all aforementioned covariates and smoking in the same subset of participants as model 3a; model 4a, which adjusted for all of the main covariates but used only the subset of individuals who had BMI data (n=380), model 4b adjusted for all aforementioned covariates and BMI, and lastly, model 5, adjusted for all of the previously mentioned covariates plus the interaction of BMI and phthalate exposure along with the interaction of race and phthalate exposure. Only one woman was underweight and was excluded from our analyses. Thus, BMI was categorized as 'normal' vs. 'overweight/obese'.

All models were run separately for each phthalate and the molar sum of DEHP metabolites (MBP, MEP, ∑DEHP). Unadjusted and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to investigate the

association between each phthalate and subfertility. NCHS created sample weights, strata and primary sampling units for this subgroup were used to take into account the complex survey design. We also conducted a secondary analysis for women who had missing covariate data in our study population, but had data on phthalate levels (Table 2). SAS Studio 9.04 was used to perform all data analyses. The threshold for significance of variables (p-value) in our regression models was set to 0.05, except for the interaction terms investigating BMI and phthalate exposure, which was set to 0.20.

Chapter 3: Results and Discussion

Results

Descriptive Statistics

Of the 425 women included in our analyses, 35% were non-Hispanic White, 24% were Hispanic (Mexican American and Other Hispanic), 23% were non-Hispanic Black, and 18% were categorized as Other, including multi-race. Missing data was found within poverty income ratio (PIR), body mass index (BMI) and smoking; most of the women (66%) in our analysis subsample were smokers to some extent. Both age and BMI and age and smoking were weakly correlated (r^2=0.17, p=<0.0001; r^2=0.10, p=<0.005, respectively). PIR was also weakly associated with race, age and smoking (r^2=0.16, p<0.0001, r^2=0.17, p=<0.0001 and r^2=0.25, p=<0.001). No other demographic variables were positively correlated with one another, including insurance and PIR[7]. Both age and BMI were kept in the models (in our sensitivity analyses) despite this based on other literature and *a priori* reasoning that both may be confounders in subfertility. BMI was not considered in our main models as it may also be a mediator in the relationship between phthalates and subfertility as phthalates have been shown to be linked to BMI in prior studies of adult women (James-Todd et al., 2012; Russ Hauser et al., 2016; Yaghjyan et al., 2015).

[7] see Table D and E in Appendix for correlation tables

There were no statistically significant differences in demographic characteristics between participants who were included in our analyses and those who were excluded from our analysis due to missing data (i.e., women missing phthalate data, data on subfertility, and/or data on the main covariates.) (Table 2).

Table 2 Study population characteristics of women 18-44 years of age (NHANES 2013-2014).

Variable		Study Population n=425 n(%)	Excluded[a] n=2740 n(%)	P value[b]
Age	18 - 26	164 (39)	1010 (37)	0.33
	27 - 35	114 (27)	833 (30)	
	36 - 44	147 (35)	897 (33)	
Race/ Ethnicity	Non-Hispanic White	149 (35)	1016 (37)	0.47
	Hispanic (Mexican American & Other Hispanic)	100 (24)	692 (25)	
	Non-Hispanic Black	98 (23)	551 (20)	
	Other (incl. multi-racial)	78 (18)	481 (18)	
Insurance	Yes	308 (72)	1916 (69)	0.31
	No	117 (28)	820 (30)	
Poverty Income Ratio (PIR)	Below Poverty (<1)	126 (32)	711 (28)	0.13
	Above Poverty (≥ 1)	269 (68)	1810 (72)	
	Missing	30	219	
Body Mass Index (BMI)	Healthy (18.5 to < 25)	149 (37)	912 (36)	0.75
	Overweight/obese (≥ 25)	259 (63)	1642 (64)	
	Missing	17	186	
Smoking	Yes	75 (66)	577 (65)	0.91
	No	39 (34)	307 (35)	
	Missing	311	1856	

[a] Those excluded from the study population had missing data for phthalates, subfertility and/or any of the covariates

[b] Chi-squared test of significance between populations; no significance found at p value 0.05

Descriptive statistics for phthalate metabolite concentrations in our population are presented in Table 3. We observed that the greatest concentrations of phthalate metabolites were found for MEP, followed by, MEC(P)P, MEHHP, MEOHP, MBP; MEP levels were nearly 200% higher than the lowest max of MEHP (152.3 ng/mL) (Table 3). Geometric means were in alignment with the trends in maximum levels with MEP being the highest at 177.5 ng/mL and the lowest being 8.8 ng/mL for MEOHP. We also observed that phthalate concentrations were generally highly correlated with one another and the most of these correlations were significant.[8]

Table 3 Descriptive statistics of phthalate metabolites in study population (N=425).

Parent Phthalate	Metabolite	Lower LOD (ng/mL)	DF (%)	GM (ng/mL)	GSD	min	p25	p50	p75	max
DEHP	MEHHP	0.4	99.3	14.4	46.5	<LOD	<LOD	7.7	13.7	1029.7
	MEC(P)P	0.4	100	21.0	59.6	0.28	6.2	11.8	20.6	1422
	MEOHP	0.2	99.5	8.8	25.8	<LOD	<LOD	5.0	9.0	585.2
	MEHP	0.8	62.4	2.97	7.45	<LOD	<LOD	1.50	3.00	152.3
DBP	MBP	0.4	98.4	17.9	30.2	<LOD	<LOD	11.2	20.6	489.6
DEP	MEP	1.2	99.7	177.5	799.5	<LOD	<LOD	37.0	102.9	12958

LOD: limit of detection, >LOD: below lower LOD, DF%: detection frequency percentage, GM: geometric mean (ng/mL), GSD: geometric standard deviation;

Pairwise Comparisons

We observed significant differences in phthalate concentrations between different racial/ethnic groups; i.e., Hispanic vs. Non-Hispanic Black, Non-Hispanic White vs. Non-Hispanic Black, and Non-Hispanic Black vs. Other (incl. multi-racial) were significantly different between one another. Hence, most groups versus Non-

[8] see Table D and E in Appendix for correlation tables

Hispanic Black had significantly lower differences in urinary metabolite phthalate levels. Geometric means of phthalate levels found among women in different racial/ethnic groups, along with p values, are presented in Table 4. In general, we observed that phthalate metabolite concentrations were highest among non-Hispanic Blacks compared to women in other racial/ethnic groups.

Table 4 Tukey and Bonferroni pairwise comparison results of phthalate levels among women of different racial/ethnic groups (N=425)

Metabolite	Race/Hispanic Origin	P value	$GM_{phthalate}$ (ng/mL)
∑DEHP	Hispanic vs. Non-Hispanic White	0.80	0.14 vs. 0.14
	Hispanic vs. Non-Hispanic Black	0.04*	0.14 vs. 0.26
	Hispanic vs. Other (incl. multi-race)	0.14	0.14 vs. 0.28
	Non-Hispanic White vs. Non-Hispanic Black	0.04*	0.14 vs. 0.26
	Non-Hispanic White vs. Other (incl. multi-race)	0.17	0.14 vs. 0.28
	Non-Hispanic Black vs. Other (incl. multi-race)	0.63	0.26 vs. 0.28
	Non-Hispanic White vs. Hispanic/ Non-Hispanic Black	0.27	0.14 vs. 0.14/0.26
MBP	Hispanic vs. Non-Hispanic White	0.89	19.43 vs. 18.66
	Hispanic vs. Non-Hispanic Black	0.05*	19.43 vs. 20.12
	Hispanic vs. Other (incl. multi-race)	0.95	19.43 vs. 17.23
	Non-Hispanic White vs. Non-Hispanic Black	0.04*	18.66 vs. 20.12
	Non-Hispanic White vs. Other (incl. multi-race)	0.84	18.66 vs. 17.73
	Non-Hispanic Black vs. Other (incl. multi-race)	0.05	20.12 vs. 17. 73
	Non-Hispanic White vs. Hispanic/ Non-Hispanic Black	0.43	18.66 vs. 19.43/20.12
MEP	Hispanic vs. Non-Hispanic White	0.13	154.65 vs. 174.66
	Hispanic vs. Non-Hispanic Black	0.70	154.65 vs. 281.88
	Hispanic vs. Other (incl. multi-race)	0.29	154.65 vs. 95.58
	Non-Hispanic White vs. Non-Hispanic Black	0.04*	174.66 vs. 281.88
	Non-Hispanic White vs. Other (incl. multi-race)	0.78	174.66 vs. 95.58
	Non-Hispanic Black vs. Other (incl. multi-race)	0.15	281.88 vs. 95.58
	Non-Hispanic White vs. Hispanic/ Non-Hispanic Black	0.03*	174.56 vs. 154.65/281.88

*: significance <0.05

Regressions

Overall, no significant associations were observed in crude models for all phthalate metabolites and only odds ratios for MBP were above the null value of 1.0 (cOR=1.07, CI: 0.73-1.58). After including other covariates, including age, race, PIR and insurance (model 2) adjusted odds ratios (aOR) were as follows: ∑DEHP (aOR=0.72, CI: 0.45-1.16), MBP (aOR=1.12, CI: 0.74-1.70) and MEP (aOR=0.84, CI: 0.61-1.11). Although we observed a positive association between exposure to MBP and an increased odds of subfertility, this was not a statistically significant finding. To investigate the possible change in odds for individuals within our population who had smoking data (n=109), the previous full model was repeated but only including women with smoking data; effect estimates are presented in model 3a and differed for ∑DEHP and MBP only. The next model (model 3b) included all of the aforementioned covariates (age, race, PIR and smoking), along with (log-10)phthalate and (log-10)creatinine concentrations. The adjusted odds ratio for ∑DEHP and MEP were attenuated, but increased for MBP; the highest aOR in all of the regression models was observed for MBP in this model (aOR=2.43 CI: 0.98-5.99); however, results were not statistically significant.

As aforementioned, BMI was added in our main models as part of our sensitivity analyses as it may be in the causal pathway. The model was repeated with all of the same sociodemographic covariates as the full model, but performed only on those who had BMI data (n=380; model 4a). The aORs for this model were as follows: ∑DEHP (aOR=0.73, CI: 0.46-1.17), MBP (aOR=1.14, CI: 0.75-1.72) and MEP aOR=0.84, CI: 0.64-1.11). BMI was then added into the model (model 4b); odds

ratios did not differ between these two models. All odds ratios are presented in Table 5. In summary, we observed positive associations between MBP exposure and an increased odds of subfertility and an inverse association between exposure to ∑DEHP and MEP and subfertility; however, none of these findings were statistically significant.

Table 5 Odds ratio estimates from logistic regressions

Phthalate	Regression Model [c]	cOR (95% CI)	aOR (95% CI)
∑DEHP	Model 1	0.71 (0.46-1.09)	-
	Model 2	-	0.72 (0.45-1.16)
	Model 3a	-	0.96 (0.38-2.42)
	Model 3b	-	0.74 (0.27-2.03)
	Model 4a	-	0.73 (0.46-1.17)
	Model 4b	-	0.73 (0.46-1.17)
MBP	Model 1	1.07 (0.73-1.58)	-
	Model 2	-	1.12 (0.74-1.70)
	Model 3a	-	2.08 (0.92-4.66)
	Model 3b	-	2.43 (0.98-5.99)
	Model 4a	-	1.14 (0.75-1.72)
	Model 4b	-	1.14 (0.75-1.73)
MEP	Model 1	0.82 (0.64-1.06)	-
	Model 2	-	0.84 (0.64-1.11)
	Model 3a	-	0.84 (0.48-1.45)
	Model 3b	-	0.79 (0.42-1.50)
	Model 4a	-	0.84 (0.64-1.11)
	Model 4b	-	0.84 (0.64-1.10)

cOR: Crude Odds Ratio; aOR: Adjusted Odds Ratio
[c] All models included the following covariates:
Model 1: adjusted for (log)phthalate exposure and (log)creatinine only (n=425)
Model 2: adjusted for (log)creatinine, (log)respective phthalate and all socio-demographic covariates—age, race/ethnicity, PIR and insurance
Model 3a: adjusted for full model but with only the subset of those who had smoking data (n=109)
Model 3b: adjusted for full model + smoking
Model 4a: adjusted for full model but with only the subset of individuals who had BMI data (n=380)
Model 4b: adjusted for full model + BMI (n=380)
Model 5: adjusted for full model + interaction of BMI and (log)phthalate (n=380)

We only observed a significant interaction between BMI and MBP (p-interaction=0.06) and between BMI and MEP (p-interaction=0.11) (Table 6). Still, although we observed a positive association between phthalate metabolites and an increase risk of subfertility only among women who had a normal BMI, none of the stratified results were significant.

Table 6 Sensitivity analysis results stratified by BMI

	Overall model n=380 aOR[d] (95% CI)	Normal BMI n=141 aOR (95% CI)	Overweight/ obese BMI n=239 aOR (95% CI)	P-interaction
∑DEHP	1.05 (0.50-2.21)	1.39 (0.55-3.53)	0.72 (0.30-1.82)	0.22
MBP	0.75 (0.41-1.40)	1.39 (0.62-3.15)	0.72 (0.32-1.62)	0.06
MEP	0.64 (0.40-1.01)	1.17 (0.55-2.48)	0.76 (0.33-1.74)	0.11

[d] Adjusted for creatinine, respective phthalate, age, race PIR and insurance.

Discussion

It is estimated that 12.1% of women in the United States have impaired fecundity, e.g. the potential to reproduce(CDC, 2016a). In this analyses, we used several models to investigate the effects of phthalate exposure on subfertility, specifically taking over one year prolonged time to pregnancy (PTT). Positive associations between phthalate exposure and subfertility, i.e., increased risk of subfertility, were found for MBP. Increased risk was also found in two models of ∑DEHP—when run only on women in our population who had smoking data (n=109) and when adjusted for sociodemographic factors (age, race and PIR) and the interaction of BMI and phthalate exposure. Lastly, an increased odds of subfertility within all phthalates for an individual with a normal BMI when stratified for BMI. However, none of our findings were statistically significant. To our knowledge, this is the first study to use a nationally representative sample to evaluate the association between exposure to phthalates commonly found in personal care products and subfertility among women of reproductive age.

In a study on couple fecundity and phthalate exposure by Louis et al. (2014), researchers found negative associations with a number of phthalate metabolites

(including MEP and MEHP). Positive associations (aOR=1.02-1.06) were found among specific DEHP metabolites (MEHHP, MEOHP and MECPP) when adjusted for creatinine and age, BMI, cotinine and research site (Buck Louis et al., 2014). However, unparalleled demographics in this study compared to ours included majority white (79%), college educated women (95%) with health insurance (92%), versus our study population which was nearly 50% Hispanic or Non-Hispanic Black and 72% had insurance (Buck Louis et al., 2014). Furthermore, in a study by Thomsen et al. (2017) which investigated PTT and phthalates, specifically in women, a positive association was found among prolonged time to pregnancy and MEP, yet subfertile women were overrepresented in this sample. The unmatched demographics of these different studies make it difficult to compare results with our study.

Despite these varying results in comparison to other studies, our initial pairwise analyses are consistent with other studies that have demonstrated that minority women, specifically non-Hispanic Black women, have higher phthalate levels than non-Hispanic White women(Branch, Woodruff, Mitro, & Zota, 2015; Varshavsky, et al., 2016). In Varshavsky, Zota and Woodruff's cumulative exposure analysis of phthalate levels among racial groups (2016), higher levels of phthalates DBP, BBzP and DEHP were found among black women. DBP and BBP have also been found to be higher in low socioeconomic status (SES) communities, which are primarily composed of minorities(Varshavsky, et al., 2016; Wilson, 2009). This could be due their built environment consisting of numerous industries that release emissions comprised of phthalates, chemical exposures in food containers, and/or a

number of other variables related to lifestyle, including personal care practices, e.g. vaginal douching(Branch et al., 2015; Wilson, 2010).

Our current study has several limitations. First, our small sample size and stratification could have led to possible statistical power issues, limiting the scope of this investigation as only data from one NHANES cycle was available for our analyses. Model specification error, i.e. the lack of additional explanatory variables within the model may have also contributed to some level of analysis error. These additional covariates may have included alcohol intake and covariates for metabolism/diet (James-Todd et al., 2012; Russ Hauser et al., 2016). These additional covariates were not included in our main models because they were missing for a large portion of our population and would have further reduced our sample size. Lastly, a primary limitation of TTP is the fact that it is a "functional measure of couple fecundity" and, therefore, is difficult to determine if the subfertility is due to the female, male or is couple mediated (Buck Louis et al., 2014).

Despite these limitations, our study has several strengths. First, we used data from a nationally representative sample of women from the general US population, focusing on women of reproductive age. Table 5 shows the similarities and importantly, insignificant differences among our population and those who had phthalate data, but were excluded from our study, highlighting the ability of our findings to be applied to this general dataset of individuals with phthalate measurements. This strength counteracts the lack of outcome assessment for women aged under 18 or over 44 years. This is also the first study of its kind using the subfertility data provided by NHANES in terms of exposure to phthalates.

Chapter 4: Conclusion

Major findings

Our analyses suggest that there may be a positive association between exposure to MBP-related phthalates with an increased odds of subfertility (at 12 months prolonged time to pregnancy); however, results were not statistically significant. Several limitations could have contributed to our overall null findings as specified above. Additionally, evidence is available in animal models for the plausibility of prolonged time to pregnancy in humans (females specifically) due to exposure to other phthalates, primarily DEHP (Carnevali et al., 2010; Flaws, Ph.D., 2015; Gray Jr et al., 2006; Sen et al., 2015). To date, this is the first analysis of phthalates and subfertility reproductive health outcomes using a nationally representative sample (NHANES). Although only two other human studies has have investigated the effect of phthalates on fecundity, this research provides more justification for the need to investigate phthalate exposure among women in their child bearing years, especially women of minority backgrounds given they experience elevated exposures (Buck Louis et al., 2014; Thomsen et al., 2017).

Future Research

Although the FDA performed a survey of roughly 200 cosmetic and personal care products, this is just a small fraction of the consumer products that need to be investigated (Table F in Appendix) (FDA, 2016). Despite this limitation, DEP levels were found anywhere from 480-40,00 ppm in fragrance alone, confirming the presence of a wide range of phthalates across personal care products. As illustrated by

the data in this analyses, many more products specific to women of reproductive age should be investigated to determine why phthalate levels in this group of women are so much higher than others and to better inform these women of potentially harmful additives that could be present in their frequent beauty routines and increase their risk of adverse health effects.

Additionally, minorities, especially women of color, are reported to have higher levels of chemicals related to beauty products, e.g. skin lighteners, hair straighteners, and feminine hygiene products, versus white women (Zota & Shamasunder, 2017) Furthermore, while cosmetic sales have declined overall in past years, purchases made by Latinas have actually been increasing exponentially (-1.2 versus 7.4 dollar percentage change over previous year)(Gustafson, 2015; The Nielson Company, 2015). Facial cleansers and moisturizers, hand and body lotion sales have also more than doubled in Latinas due to endorsements by Latina celebrities(The Nielson Company, 2015). This information highlights the need to evaluate the potential health effects of these chemicals on women's health given their pervasive use, particularly among select minority groups.

Lastly, single phthalate exposures as used by NHANES are not a complete representation of overall exposure. As aforementioned, people are exposed to phthalates through numerous pathways (air, dust, food, water, soil and synthetic products) and multiple routes of exposure (inhalation, ingestion and dermal in the case of cosmetics and personal care products). Body burdens should also be investigated in a more holistic way to account for cumulative exposures and/or synergistic interactions, e.g. by an all-encompassing potency calculation, as described by Varshavsky et al. (2016).

In summary, this is the first work to evaluate the association between exposure to phthalates and subfertility using a nationally representative sample of women from the general population within the United States. Our sample size may have limited statistical power justifying the need for more studies to assess the potential adverse health effects of these chemicals on women of reproductive age given their widespread use and detection. The fact that phthalate exposures are observed to be higher among minority women is also of concern and further supports the need for more epidemiologic research.

Appendix

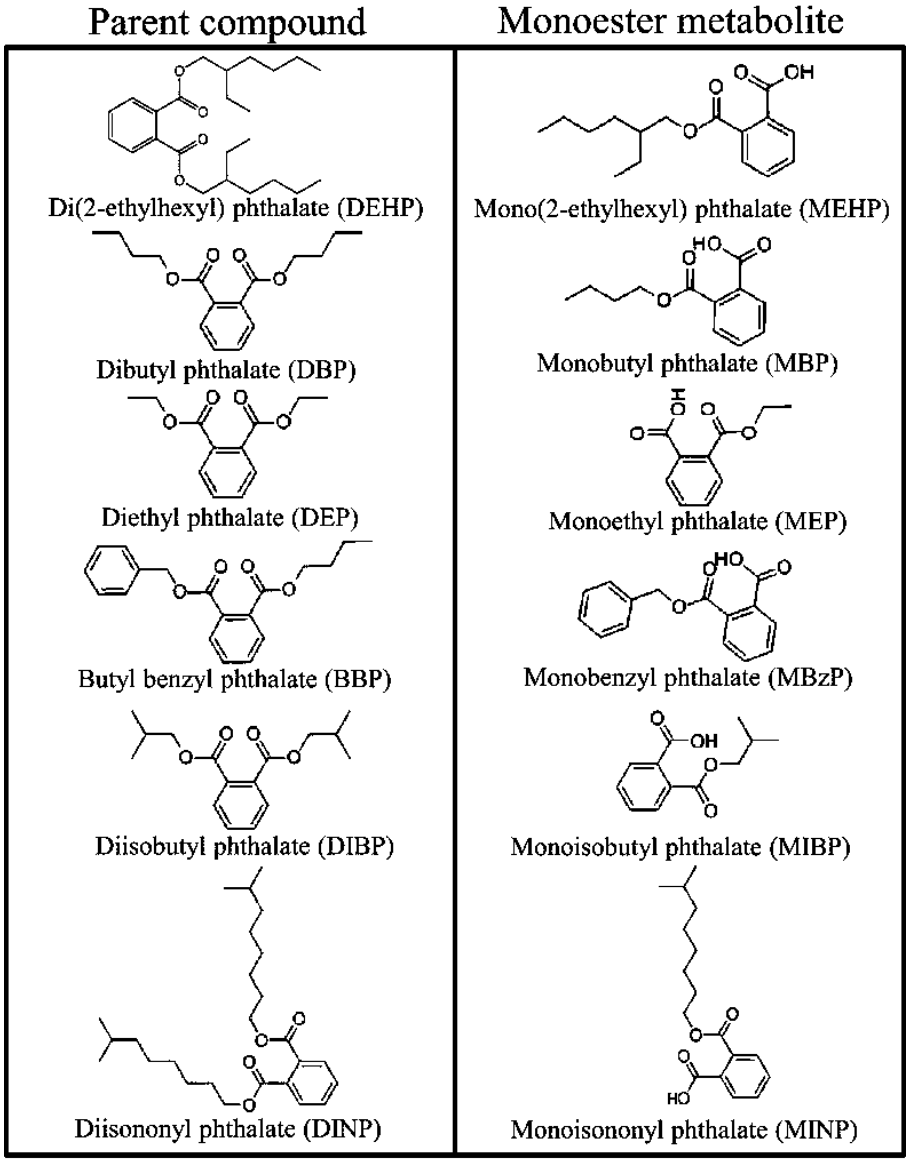


Figure 1 More parent phthalates and respective metabolites. Adapted from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4313599/

Table A Phthalates uses.

Parent Phthalate	MW	Source
DEHP BBP	High	Polyvinyl chloride (PVC), added to other plastics to soften (food containers, medical tubing)
DiNP	High	Mixture used as plasticizer, also in insecticides, herbicides, dyes
D(n)OP	High	Plasticizer in PVC production and other polymers (rubber, dye)
DMP	Low	Insect repellant, bottles, cosmetics, personal care products (hairspray)
DEP	Low	Solvents, fixative in fragrances/lotions, medications
D(n)BP, DBP	Low	Plastics, paints (incl. nail polish), inks, cosmetics, food packaging

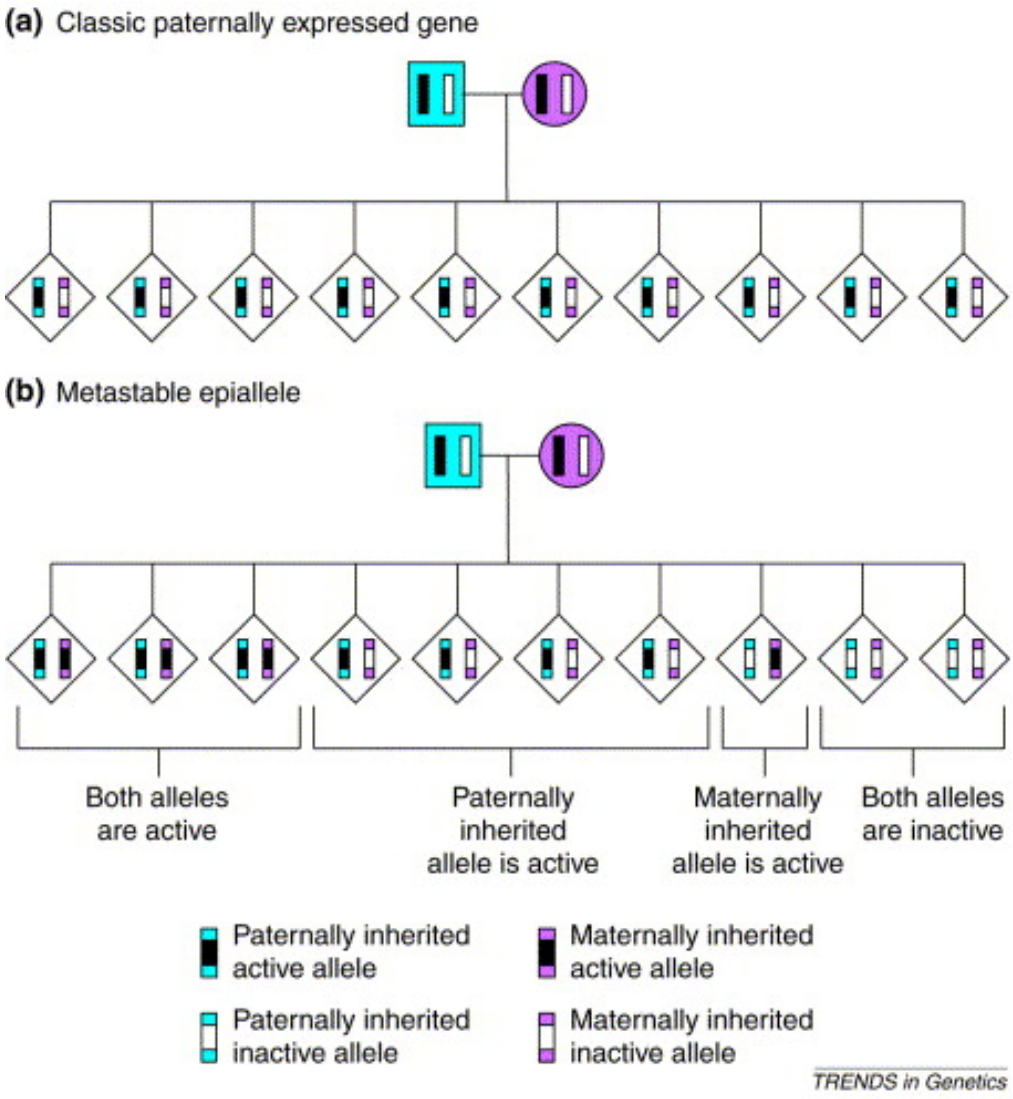
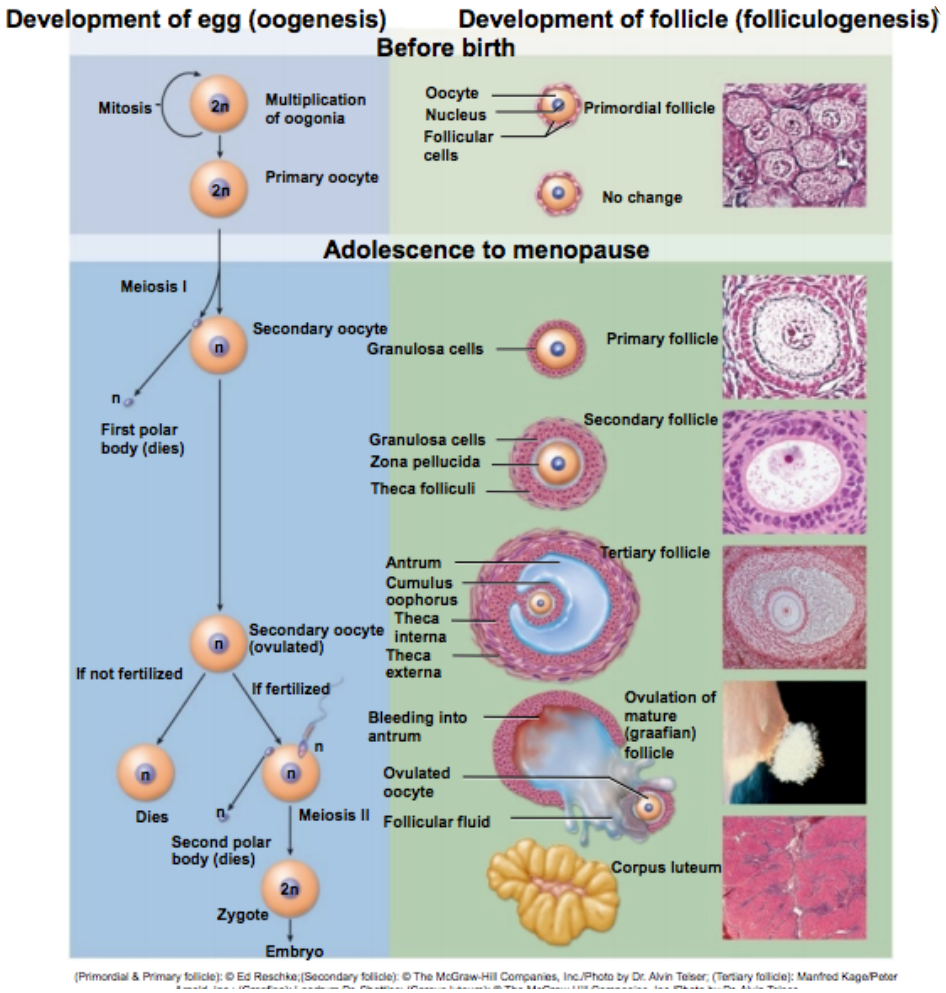


Figure 1 Imprinted (Inactivated) Genes and Metastic Alleles. Adapted from http://www.cell.com/trends/genetics/fulltext/S0168-9525(02)02709-9



(Primordial & Primary follicle): © Ed Reschke;(Secondary follicle): © The McGraw-Hill Companies, Inc./Photo by Dr. Alvin Telser; (Tertiary follicle): Manfred Kage/Peter

Figure 2 Oogenesis and Folliculogenesis descriptions. Adapted from https://classconnection.s3.amazonaws.com/576/flashcards/3146576/png/screen_shot_2013-12-07_at_55124_pm-142CF793A3830DB4CB1.png

Table B Master MIAEH MPH program competency table

1. Evaluate and prioritize the direct and indirect human, ecological, and safety effects of major environmental and occupational agents.*
2. Identify and apply appropriate, state of the art, approaches for assessing, preventing, and controlling environmental and occupational hazards that pose risks to human health and safety.*
3. Incorporate the role of psychosocial factors that affect susceptibility to adverse health outcomes following exposure to environmental and occupational hazards into assessment, prevention, and control strategies.
4. Identify vulnerable populations and develop and apply risk management and risk communication approaches that address issues of environmental justice and equity.
5. Apply the concepts regarding genetic and physiologic factors and mechanisms of toxicity to evaluate and improve assessment, prevention, and control strategies.*
6. Evaluate policies and standards with respect to ethical considerations of and disparities in environmental and occupational health and use the evaluation to develop improved policies and standards.
7. Critique and apply current environmental risk assessment methods.*
8. Synthesize environmental-occupational health knowledge to design and evaluate environmental-occupational health policies, programs and research. Integrate, synthesize and apply theory to practice in the context of a research study, policy development, and public health systems development.*
9. Critique federal and state regulatory programs, guidelines, and authorities that control environmental-occupational health issues.

* denotes competencies which were fulfilled within the thesis solely (not included in Summer 2016 internship); all competencies were filled by thesis nonetheless

Table C Description of how competencies were met by thesis

1. Evaluation of the adverse health effects of phthalate exposure on women in the United States, both directly by personal care product use and ambient exposure.
2. Identified and applied novel public health methods, including biostatistics, to determine disparities in phthalate exposure and reproductive health outcomes and health care access.
3. Identified the role of psychosocial factors that affect susceptibility to infertility following exposure to environmental and occupational hazards into assessment, prevention, and control strategies.
4. Identified vulnerable populations of phthalates and develop and apply risk management and risk communication approaches that address issues of environmental justice and equity.
5. Literature review on phthalates identifies concepts regarding genetic and physiologic factors and modes of action to evaluate and improve assessment, prevention, and control strategies.
6. Evaluation of phthalate policies and standards with respect to ethical considerations of and disparities in environmental and occupational health and use the evaluation to develop improved policies and standards.
7. Critique and applied current phthalate environmental risk assessment methods from the EPA.
8. Synthesized environmental and occupational health knowledge from MPH curriculum to design and evaluate environmental-occupational health policies, programs and research for phthalate exposure. Integrated, synthesized and applied (health behavior) theory to practice in the context of a research study, policy development, and public health systems development.
9. Federal FDA, OSHA, FPLA agency regulations and other state regulations critiqued for limiting phthalate exposure among the general population and establishing occupational thresholds of exposure.

Table D Correlation coefficients and p values for phthalates in study population

	MEHHP	MEC(P)P	MEOHP	MEHP	MBP	MEP
MEHHP	1.00000	0.96766 <.0001	0.99595 <.0001	0.88636 <.0001	0.10706 0.0014	0.01839 0.5833
MEC(P)P	0.96766 <.0001	1.00000	0.97098 <.0001	0.91396 <.0001	0.11522 0.0006	0.01763 0.5990
MEOHP	0.99595 <.0001	0.97098 <.0001	1.00000	0.89096 <.0001	0.11956 0.0003	0.01839 0.5833
MEHP	0.88636 <.0001	0.91396 <.0001	0.89096 <.0001	1.00000	0.11524 0.0006	0.02478 0.4598
MBP	0.10706 0.0014	0.11522 0.0006	0.11956 0.0003	0.11524 0.0006	1.00000	0.06556 0.0503
MEP	0.01839 0.5833	0.01763 0.5990	0.01839 0.5833	0.02478 0.4598	0.06556 0.0503	1.00000

Table E Correlation coefficients and p values for covariates in study population

	Age	Race/ ethnicity	BMI	Smoking	PIR	Insurance
Age	1.00000	0.02324 0.22	0.16989 <.0001	0.10204 0.0013	0.17437 <.0001	-0.01752 0.3502
Race/ ethnicity	0.02324 0.22	1.00000	-0.10523 <.0001	-0.15690 <.0001	0.16566 <.0001	-0.13073 <.0001
BMI	0.16989 <.0001	-0.10523 <.0001	1.00000	0.08903 0.0063	-0.09616 <.0001	0.03813 0.0466
Smoking	0.10204 0.0013	-0.15690 <.0001	0.08903 0.0063	1.00000	0.25074 <.0001	-0.09649 0.0024
PIR	0.17437 <.0001	0.16566 <.0001	-0.09616 <.0001	0.25074 <.0001	1.00000	-0.28195 <.0001
Insurance	-0.01752 0.35	-0.13073 <.0001	0.03813 0.0466	-0.09649 0.0024	-0.28195 <.0001	1.00000

Table F Phthalate concentrations in products tested by 2010 Survey by FDA. Adapted from https://www.fda.gov/Cosmetics/ProductsIngredients/Ingredients/ucm128250.htm

Product and major Phthalate found	Brand	Phthalate concentration (ppm) (No entry = not found)
Nail Polish **DBP**	Hot Topic Nail Polish (Skull) Green	DMP: DEP: DBP: 4,800
	Hot Topic Nail Polish (Skull) Black	DMP: DEP: DBP: 4.4
	Borghese Nail Lacquer Vernic Botticelli Nude	DMP: DEP: DBP: 3.4
	Sally Hansen Salon Lacquer Nail Polish Orange You Cute? 450	DMP: DEP: DBP: 6.6
Skin Cream and Lotion **DEP**	Nivea Soft Refreshingly Soft Moisturizing Crème	DMP: DEP: 100 DBP:
	Bath, Body, etc… Organic Soothing Aloe Vera Body Lotion	DMP: DEP: 260 DBP:
	Jergens Original Scent Cherry-Almond Moisturizer	DMP: DEP: 110 DBP:
Fragrance **DEP**	Jovan Island Gardenia Cologne Spray	DMP: DEP: 14,000 DBP:
	Chantilly - Walmart Gift Pack	DMP: DEP: 7,300 DBP:
	Tabu - Walmart Gift Pack	DMP: DEP: 6,200 DBP:
	Heaven Sent - Walmart Gift Pack	DMP: DEP: 1,300 DBP:
	Navy - Walmart Gift Pack	DMP: DEP: 40,000 DBP:
	English Leather - Walmart Gift Pack	DMP: DEP: 3,900 DBP:

	British Sterling - Walmart Gift Pack	DMP: DEP: 480 DBP:
	Canoe - Walmart Gift Pack	DMP: DEP: 2,000 DBP:
	BOD Really Ripped Abs	DMP: DEP: 6,200 DBP:
Deodorant DEP (in women's products)	Brut 24-Hour Protection deodorant	DMP: DEP: 22 DBP:
	Secret Powder Fresh	DMP: DEP: 34 DBP:
	Degree Men Deodorant Silver Ion Intense Sport (for comparison purposes)	DMP: 2.9 DEP: DBP:
Hair Products **DEP**	Suave Professionals Styling Foam Extra Hold	DMP: DEP: 52 DBP:
	Rave 4X Mega Unscented Hair Spray	DMP: DEP: 16 DBP:
	White Rain Unscented Extra Hold Hair Spray	DMP: DEP: 61 DBP:
	TRESemme Tres Two Extra Hold Hair Spray	DMP: DEP: 37 DBP:
	American Crew Forming Cream	DMP: DEP: 50 DBP:
	Dep Sport Endurance Styling Gel	DMP: DEP: 6.8 DBP:
	Catwalk Extra Strong Mousse	DMP: DEP: 23 DBP:
Shampoo **DEP**	Ave Dual 2 in 1 Shampoo + Conditioner	DMP: DEP: 17 DBP:
	Big Sexy Hair Big Volume Shampoo	DMP: DEP: 210 DBP:

	VO5 Normal Balancing Shampoo	DMP: DEP: 440 DBP:
	Advance Techniques Color Reviving Shampoo	DMP: DEP: 82 DBP:
Body Wash **DEP**	Suave Men Body Wash Active Sport For comparison purposes	DMP: DEP: 10 DBP:
	Natural Concepts Sensitive Skin Body Wash	DMP: DEP: 340 DBP:
Face and Body Paint **DEP**	Claire's Cosmetics Vanilla Glitter Body Mist	DMP: DEP: 390 DBP:
Glitter Gel **DEP**	Claire's Club Scented Body Glitter	DMP: DEP: 167 DBP:
Baby Products **DEP**	Baby's Bliss Diaper Cream	DMP: DEP: 130 DBP:
	My Fair Baby Baby Wash with Camomile	DMP: DEP: 60 DBP:

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