ABSTRACT

Title of Thesis: ASSOCIATIONS BETWEEN URINARY PHTHALATES AND METABOLIC SYNDROME IN NHANES 2005-2010.

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Phthalates, commonly used to make plastics more durable, are a group of endocrine disrupting chemicals (EDC), with potential for adverse metabolic consequences. Associations between exposure to 13 phthalate metabolites and the prevalence of metabolic syndrome (MetS) were examined among 5,409 U.S adults ≥ 18 years of age, who participated in the National Health and Nutrition Examination Survey from 2005-2010. MetS was assessed using clinical and questionnaire data. Odds Ratio (OR) and 95% Confidence Intervals (CI) adjusting for age, creatinine and key confounders, were estimated with multivariable logistic regression. Positive associations were observed between individual phthalate metabolites and MetS: (MCOP OR=1.31, 95% CI=1.40, 1.64, p-trend<.01; MCPP OR=1.39, 95% CI=1.09, 1.77, p-trend=0.01). In gender stratified analyses, findings with MCOPP and MCPP were restricted to women only. Phthalate metabolites may increase the prevalence of MetS; however, further studies are needed to better understand the role of EDCs in the development of MetS.

ASSOCIATIONS BETWEEN URINARY PHTHALATES AND METABOLIC SYNDROME IN NHANES 2005-2010

By

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Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Masters in Public Health
2015

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Acknowledgements

I would like to express my deepest appreciation to those individuals who have made this thesis possible. First and foremost I would like to thank my advisor and committee chair, Dr. Cher Dallal. Her enthusiasm, diligent criticism and willingness to go above and beyond for her students has pushed me to complete my best work possible. Without her guidance and persistence this thesis would not have been possible.

Furthermore, I would like to thank my committee members, Dr. Paul Turner and Dr. Shuo Chen. Their expertise in their respective fields have helped me easily maneuver through this challenging process. I am greatly appreciative for the large amount of time my committee has already given me, and continues to give me during the research process.

I would also like to thank my mentor/coworkers at the Environmental Protection Agency, Integrated Risk Information System (IRIS) division. Their tireless work assessing adverse health effects of environmental chemicals, inspired this study.

In addition I would like to thank my fellow students, Ashley Woodall, Cherica Richmond, Diane Ng and Lisa Bowen for their knowledge, advice and emotional support. It was a pleasure completing my MPH alongside each of them. I also thank the department of Epidemiology and Biostatistics faculty for their help and support.

Finally I am grateful to my family and close friends: Kamilah, Hasannah, Sana and Sheima, for the unceasing encouragement, support, patience and attention they have provided me these past two years.

Table of Contents

Acknowledgements ii
Table of Contents iii
List of Tables iv
List of Figures v
List of Abbreviations vi
Chapter 1: Background 1
1.1 Phthalate Toxicities 2
1.2 Susceptible Populations 3
1.3 Exposure Assessment 4
1.4 Mechanistic Evidence 5
1. 5 Phthalate Exposure and Metabolic Syndrome 6
1.5.1 Waist Circumference 7
1.5.2 Insulin Resistance 8
1.5.3 Other Metabolic Syndrome Components 9
1.6 Public Health Significance 10
Chapter 2: Methods 12
2.1 Study Population 12
2.1.1 Analytic Sample 12
2.1.2 Data Collection 13
2.2 Variable Definitions 15
2.2.1 Independent Variable 15
2.2.2 Dependent Variable 17
2.2.3 Covariates 18
2.3 Statistical Analysis 19
2.4 Human Subjects Determination 21
Chapter 3: Results 22
Chapter 4: Discussion 32
4.1 Strengths and Limitations 35
4.2 Conclusion 37
Appendices 38
Bibliography 42

List of Tables

Table 2-1. Description of phthalate monoesters examined in NHANES 2005-2010 . 16
Table 3-1. Study population characteristics by gender NHANES (2005-2010)..... 22
Table 3-2. Distribution of phthalate metabolite in NHANES (2005-2010)..... 24
Table 3-3. Spearman correlation coefficients between phthalate metabolites NHANES 2005-2010 25
Table 3-4. Associations between urinary phthalate metabolite concentrations and metabolic syndrome 26
Table 3-5. Associations of phthalate metabolites with MetS by gender in NHANES (2005-2010)..... 29
Table 3-6. Associations of molar summed phthalate metabolites by gender in NHANES (2005-2010) 31
Table A-1. Summary of previous literature 38
Table A-2. Spearman correlation coefficients between phthalate metabolites among males in NHANES 2005-2010..... 40
Table A-3. Spearman correlation coefficients between phthalate metabolites among females in NHANES 2005-2010 40

List of Figures

Figure 1-1. Chemical structure of Di-ethyl Phthalate (DEP), a low molecular weight phthalate 2
Figure 1-2. Chemical structure of Di-2-ethylhexyl phthalate (DEHP), a high molecular weight phthalate 3
Figure A-1. Derivation of analytic sample 41

List of Abbreviations

ATP III	Adult Treatment Panel III
BBzP	butyl benzyl phthalate
BMI	body mass index
CM	centimeters
CI	confidence interval
DBP	dibutyl phthalate
DEP	diethyl phthalate
DEHP	di-2-ethlyhexyl phthalate
DiBP	di-isobutyl phthalate
DiDP	di-isodecyl phthalate
DiNP	di-isononyl phthalate
DMP	dimethyl phthalate
DOP	dioctyl phthalate
EDC	endocrine disrupting chemical
HDL	high density lipoprotein
HMW	high molecular weight
HOMA	homeostatic model assessment index
IQR	interquartile range
LMW	low molecular weight
LOD	limit of detection
MBP	monobutyl phthalate
MBzP	monobenzyl phthalate
MCNP	mono-carboxynonyl phthalate
MCOP	mono (carboxyisoctyl) phthalate
MCPP	mono-(3-carboxypropyl) phthalate
MEP	monoethyl phthalate
MECPP	mono (2-ethyl-5-carboxypentyl) phthalate
MEHP	mono 2-ethylhexyl phthalate
MEHHP	mono (2-ethyl-5-hydrohexyl) phthalate
MEOHP	mono (2-ethyl-5-oxyhexyl) phthalate
MetS	metabolic syndrome
MiBP	mono-isobutyl phthalate
MiNP	mono-isononyl phthalate
MMP	mono-methyl phthalate
mm Hg	millimeters of mercury
mmol	millimoles per liter
NCHS	National Center for Health Statistics
NHANES	National Health and Nutrition Examination Survey
OR	odds ratio
PPAR	peroxisome proliferator-activated receptor
PVC	polyvinyl chlorides
Std.dev	standard deviation
VIF	variance inflation factor
US	United States

Chapter 1: Background

Recent changes in the number and types of chemicals in the environment have been associated with increasing metabolic disorders[1–4]. Increasing exposure to phthalates, chemicals with endocrine-disrupting properties, may in part explain these observed associations. However, to date, the relationship between phthalates and metabolic syndrome has not been examined in epidemiological studies.

Phthalates are a large family of chemicals primarily used to make plastics more flexible and durable[5]. They are used to soften polyvinyl chlorides (PVCs), which are widely used in plastic materials, thus classifying this family of chemicals as a common plasticizer[5]. Additionally, phthalates are used in household cleaners, personal care products and children's toys. Phthalates, which include multiple parent compounds, are broken down into metabolites classified based on their molecular weight, including either low-molecular-weight (LMW) or high-molecular-weight phthalates (HMW)[5]. Phthalates within each category have different applications, and toxicological properties which are described in the following sections.

Phthalates have been associated with a number of adverse health outcomes, including obesity[6], diabetes[7], and heart disease[8] and cancer[9]. The following sections describe phthalates in terms of chemical properties, applications, toxicities and susceptible populations. Epidemiological and mechanistic evidence linking phthalates to metabolic syndrome[10] will also be reviewed in addition to the public health impact of the study findings

1.1 Phthalate Toxicities

Phthalates are divided into two distinct categories based on their molecular weight, as defined by the number of carbon atoms in the chemical structure[5]. Low-molecular-weight (LMW) phthalates (Figure 1-1) are composed of three to six carbons and high-molecular-weight (HMW) phthalates (Figure 1-2) are composed of more than six carbons[11]. Phthalate properties and applications differ according to their molecular weight whereby water solubility decreases with increasing length of the carbon chain, making LMW phthalates more water soluble than HMW phthalates[5]. Due to water solubility, LMW phthalates have a greater likelihood of being excreted quickly as its primary metabolite in contrast to HMW phthalates, which need to be oxidized further to their secondary metabolic form in order to be excreted[5]. As a result, HMW phthalates have a greater affinity of being stored longer then LMW phthalates. However, HMW phthalates, such as di-2-ethylhexyl phthalate (DEHP) and di-isononyl phthalate (DiNP), have greater volatility[5]. Researchers have documented these differences, as well as potency factors, in multiple in-vitro studies[3].

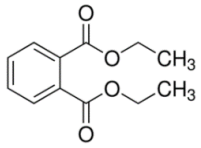


Figure 1-1. Chemical structure of Di-ethyl Phthalate (DEP), a low molecular weight phthalate

Mankidy et al. compared HMW and LMW phthalates in terms of cytotxocity, endocrine disruption and lipid peroxidation in fish embryos[3]. DEHP and di-ethyl phthalate (DEP) exhibited the strongest potency in terms of endocrine disruption and

lipid peroxidation, while dibutyl phthalate (DBP) documented the weakest overall potency[3]. A similar study conducted in rats found DEHP and DEP once again exhibiting the strongest level of potency[12]. However, butyl benzyl phthalate (BBzP) was found to be weaker in relation to DBP. The results of these studies suggest that in terms of endocrine disruption, DEHP and DEP exhibit the most potency while DBP and BBzP the weakest[13]. DBP and BBzP exhibited the strongest potency when examining estrogenic activity[14].

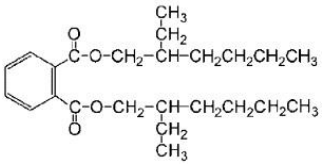


Figure 1-2. Chemical structure of Di-2-ethylhexyl phthalate (DEHP), a high molecular weight phthalate

1.2 Susceptible Populations

Humans may be exposed to phthalates through ingestion, inhalation and dermal contact[5]. In the general population, the most common route of exposure is through ingestion of contaminated food and water. The dietary intake of DEHP is estimated to be the highest in children[10], followed by adolescents, thus making children the population most vulnerable to being exposed[5]. However, several studies have also found phthalate levels to be higher in non-Caucasian racial groups[15] and females [16][17]. Women may also be more susceptible to dermal exposure of phthalates due to their larger use of phthalate containing personal care products and make-up[16]. Phthalate exposure is most prevalent among African Americans in comparison to other non-white minority groups[15]. Potential explanations for the racial differences in phthalate exposure remain unclear.

1.3 Exposure Assessment

The widespread use of phthalates results in human exposure via dietary ingestion of contaminated foods, dermal absorption of low molecular weight phthalates, inhalation of the more volatile phthalates and parenteral exposure from contaminated medical devices [18,19]. The presence of phthalates metabolites may be detected in serum and blood levels, human breast milk and urine [20]. These measured levels provide an estimate of exposure combined over all routes and sources. In a 2008 study comparing the suitability of detecting phthalate exposure for each of the aforementioned measures, analytic measurements using blood, serum and breast milk samples were found to have limited sensitivity[20]. In contrast, the study authors found that measuring phthalate metabolites in the urine gave more informative results. Overall, the availability and noninvasive nature of urine specimen collection makes it the most widely measured sample for phthalate exposure[21]. Despite this, there are inconsistencies regarding phthalate exposure characterization in urine, particularly in how accurately the measurement reflects true exposure level. Due to the short biologic half-lives of phthalates, urinary metabolite concentrations reflect exposures that occurred ≤1 day before the urine sample was collected[22]. It is due to this reason that single spot urine sample measurements of phthalate metabolites are often prone to exposure misclassification. Nonetheless, it is agreed by multiple researchers in the field that measurement of metabolites in the urine may be the golden standard of phthalate detection[20–22].

1.4 Mechanistic Evidence

Substantial evidence shows the potential for phthalates to disrupt hormonal regulation and endocrine system functionality, affecting health and reproduction in both humans and animals[23]. This ability has earned phthalates the title of endocrine disrupting chemical (EDC)[24]. EDCs may target any hormonal system, however, a number of earlier observations have found that phthalates are more likely to impart anti-androgenic effects and disrupt reproduction[25,26]. More recently, studies in rodents have illustrated deregulation of cortisol, blood glucose, thyroid-stimulating hormone and serum insulin levels due to phthalate exposure[4]. These changes have been shown to increase abdominal obesity and insulin resistance in human studies, suggesting that phthalates, as endocrine disruptors, may also play a role in metabolic disorders[4][27].

There are three possible mechanisms by which EDCs, such as phthalates, may cause MetS[4]. The first mechanism is the inappropriate inactivation of hormonal receptors such as estrogen receptors, thyroid hormone receptors and glucocorticoid receptors[4]. Hormonal receptors are largely responsible for maintaining homeostasis and are only able to recognize select molecules with high affinity for receptor binding. These receptors are targeted by EDCs, which bind to them and subsequently result in reproductive and metabolic alterations [4]. Bisphenol A, another chemical plasticizer like phthalates, has a high affinity for glucocorticoid receptors and is able to stimulate lipid accumulation markers and increase expression of adipocyte-specific markers[28]. It is not yet recognized if phthalates are also able to act under the same mechanism.

The second mechanism by which EDCs act on MetS is through xenosensors, which regulate detoxification pathways[4]. The primary purpose of xenosensors is to protect the body from bioaccumulation of toxic chemicals. When activated, xenosensors disrupt estrogen receptor pathways, as well as other neuro-receptors. Inadvertently, the body's response to foreign chemicals disrupts the body's regular metabolic pathways.

The final mechanism that EDC's act on MetS is through the activation of peroxisome proliferator-activated receptors (PPARs)[4]. EDC's such as phthalates stimulate PPARs, which regulate adipogenesis in the body[29]. PPARs also have functions in lipid storage, insulin sensitivity control and inflammatory responses[30]. It is because of this mechanistic evidence that exposure to phthalates is examined in relation to obesity[31].

1. 5 Phthalate Exposure and Metabolic Syndrome

Given these suggested biological properties, a few epidemiological studies have assessed phthalate exposure in relation to obesity[32], heart disease[33] and stroke[34]. These chronic conditions are generally characterized by metabolic risk factors, including elevated levels of fasting glucose and cholesterol. The presence of multiple metabolic risk factors is referred to as metabolic syndrome (MetS)[35]. Individuals are diagnosed with MetS when they meet three of the following five criteria: blood pressure >130/85 mm Hg, fasting blood glucose > 5.6mmol/1, serum triglyceride level >1.7mmol/1, HDL cholesterol level <1.0mmol/1 in men and <1.3 in women and waist circumference >102cm in men and >88cm in women.

No prior studies have examined the relationship between phthalate exposure and MetS. However, a limited number of studies[36] have investigated the relationship between phthalate exposure and individual risk factors of MetS. A detailed overview of these studies can be found in Table A-1 in the appendix. All of the aforementioned studies measured phthalate exposure via individual metabolite concentrations in urine samples.

1.5.1 Waist Circumference

Seven studies have previously looked at waist circumference and body size in relation to phthalate exposures [6,37,32,38–41]. Four of the studies utilized a cross-sectional design[6,32,40,41], three of which were conducted in the U.S. using NHANES data in adults[6,40,41]. Overall detectable levels of metabolites of four phthalates: DEHP, DEP, DBP and BBP were primarily assessed in all of the NHANES studies[6,40,41] (Table A-1). All four of the studies found positive associations between urinary phthalates and increased waist circumference[6,32,40,41]. Additionally, Teitelbaum et al., conducted a longitudinal analysis to assess phthalate exposure in relation to waist circumference among children[39]. The authors reported a positive association with phthalate exposure and increased waist circumference; however, the results were inconsistent among the phthalate metabolites measured[39]. The strongest association was observed among girls exposed to monoethyl phthalate (MEP), in comparison to the other metabolites examined. However, the authors hypothesize that these results may be the result of the wide concentration range of metabolites in the study population[39]. Of the nine individual metabolites measured, MEP was the only metabolite with a concentration greater than the lower limit of detection in more than

10% of the study population[39]. Across all six studies[6,31,37–40], the strongest associations between waist circumference and phthalate exposure was found among DEP and DEHP metabolites. In one other longitudinal analysis which examined phthalate exposure in relation to waist circumference among 152 overweight/obese adults undergoing weight loss, the relationship was found to be null[37].

1.5.2 Insulin Resistance

Insulin resistance is another component of metabolic syndrome that has been widely studied in relation to phthalate exposure [7,40,42–44]. Five cross-sectional studies have found a positive correlation between exposure to phthalates and insulin resistance, as measured by the homeostatic model assessment index (HOMA), which is an index used to quantify insulin resistance and beta-cell function [40,45,42,46]; four studies used NHANES data[40,42,46] while one used an urban Chinese cohort[45]. Two studies focused on women and another sampled the elderly[43][47], limiting the generalizability to all adults. Huang et al., used a cross-sectional analysis to characterize diabetes prevalence across different racial groups exposed to phthalates[42] with results suggesting that associations between phthalates and insulin resistance may vary by race and gender, especially among Hispanics and Blacks. Three other cross-sectional studies examined phthalate exposure in relation to Type 2 diabetes among adults[47,7,44]. Type 2 diabetes was defined on the basis of a self-reported physician's diagnosis and fasting blood glucose[47] in one study and only self-reported physician's diagnoses in the others[7,44][48]. All three studies found borderline and/or statistically significant associations with urinary phthalate metabolites and diabetes.

1.5.3 Other Metabolic Syndrome Components

Additional MetS risk factors, including triglyceride level, HDL cholesterol and high blood pressure, are understudied in relation to phthalate exposure. Only two NHANES analyses by Shiue et al., and Trasande et al., examined these relationships, with positive associations reported between blood pressure and urinary phthalate metabolites[8,49]. Although prior studies have included these MetS components as adjustment factors in their analyses, they have not been included as outcome variables in studies of phthalate exposure[50]. Results from the two prior NHANES analyses[8,49] support a relationship between phthalate exposure and high blood pressure, however analyses were not conducted in relation to other MetS components.

There are limitations to the studies conducted above that should be considered. Firstly, there are inconsistencies among the outcome measures as several studies utilized a self-reported outcome while others used examination data. Secondly, issues related to co-exposures to confounding compounds, selection of control populations in the cohort studies and variability in data analysis complicate data interpretation. However, overall there is some evidence supporting the relationships between phthalate exposure and insulin resistance and waist circumference. Less evidence exists for the other three components of MetS and no prior study has examined metabolic syndrome as the overall outcome. Limited evidence also exists regarding differences by gender, among ethnically diverse populations and by type of phthalate. This study addressed these scientific gaps in the evidence.

1.6 Public Health Significance

Heart disease, stroke and type 2 diabetes have a large global impact on the lives of millions of people. Combined, these diseases affect 50% of the global population and accounted for 30% of the total mortality in 2012[51]. Diabetes and stroke are also included in the top ten leading causes of death in the U.S. Obesity rates continue to rise and it is projected that if the current trend continues, 86.3% of adults will be overweight or obese by 2030[52]. All of these diseases are considered to be metabolic disorders and individuals at risk of MetS are at greater risk of acquiring any of these diseases. Prevention initiatives and changes in national public health policies have reduced the risk of MetS within the last 10 years[53]. However, a fifth of the United States population still remain at high risk for MetS[54].

Sedentary behaviors, genetic factors and exposure to environmental chemicals such as phthalates promote the increasing risks of obesity, diabetes and cardiovascular diseases[55]. Widespread industrial use of phthalates in children's toys, make-up, perfumes, plastic food and storage containers as well as household cleaning supplies lead to a greater prevalence of human exposure[34]. Because the effects of phthalate exposure are only recently being studied, it is difficult to determine what the long-term effects of such a widespread exposure will be.

This study aims to evaluate the relationship between phthalate exposure and MetS among U.S adults and to examine differences in associations by gender. This will be evaluated through a cross sectional analysis of the 2005-2010 survey data from the U.S National Health and Nutrition Examination Survey (NHANES). This research is expected to contribute to the existing body of knowledge regarding

phthalate exposures and the independent risk factors that define MetS. Findings from this analysis will further our understanding of endocrine disruptors and their potential associations with MetS and other related chronic disease outcomes in the United States.

Chapter 2: Methods

2.1 Study Population

The study objectives were evaluated through a cross-sectional analysis of adults who participated in the NHANES 2005-2010 survey years. NHANES is a nationally representative sample of non-institutionalized U.S. citizens[56]. About 5000 people are surveyed each year and the data is released by the National Center for Health Statistics (NCHS) in two year cycles. The NHANES survey is a stratified, multistage probability sample. U.S. counties are partitioned into strata based on regional, economic and racial characteristics. Two primary sampling units are then selected from each strata. Within each sampling unit, neighborhoods are selected, from which random samples of households are selected. Neighborhoods that have a large proportion of oversampled age, ethnic and income groups have a greater likelihood of being chosen than others. Finally within the selected households, individuals within designated demographic sub-domains (i.e. gender, race, ethnicity and age) are randomly selected to be participants[56].

Because NHANES is a nationally representative sample, all demographic domains are examined. Hispanics, African Americans, Asians and individuals over the age of 60 are oversampled to ensure a suitable representation within the survey population.

2.1.1 Analytic Sample

The sample included participants of NHANES 2005-2010 (N=31034). Figure 2-1 in the appendix summarizes the derivation of the analytic population. Although

data from the NHANES survey conducted in 2011-2012 is available, this survey year was not included as phthalate measurements were derived from a different subsample then previous years. Only participants whose urine was measured for the presence of phthalate metabolites were retained in the sample (n=7901). Subjects were excluded if they were under the age of 18 (n=2307) since metabolic syndrome criteria for children is defined differently from adults[1]. Individuals undergoing dialysis (n=8) were excluded because these treatments may affect the biological mechanism of interest. Finally females identified as pregnant or possibly pregnant (determined by urine test at exam; n=177) were also excluded as pregnancy alters biomarker levels. After exclusions, the final analytic sample consisted of 5409 participants.

2.1.2 Data Collection

The following describes NHANES data collection procedures for the independent and dependent variables. In addition to the main variables of interest, NHANES collected data on relevant demographic, health and lifestyle factors via questionnaires administered during the home interview.

Urine samples were collected from all eligible participants ages 6 and above. Participants were asked to report their last urine void before coming to the mobile examination center. Urine samples were stored in frozen conditions until they were sent to NCHS to be tested. Phthalate metabolites were measured using high performance liquid chromatography-electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS). As phthalates have a short half-life, meaning the amount of time it takes for exposure concentration to reach half of its initial value, de-

conjugation efficiency was monitored to rapidly detect metabolites at the lowest limit of detection.

NHANES uses a combination of examination and laboratory data to examine the multiple components of metabolic syndrome. As part of the data collection procedures, participants provided a blood sample at the mobile examination center, after which the samples were frozen and stored in -20°C temperature until shipped to headquarters for testing. Serum triglyceride levels and HDL cholesterol levels were only assessed from blood draws conducted during morning examinations. Participants were asked to fast for 8.5 hours or more (but less than 24 hours) before giving a blood sample. Only participants over the age of 12 were asked to give fasting samples and measurements were conducted only among a subsample of participants that had received all other laboratory examinations. Fasting glucose levels were measured similarly to serum triglyceride and HDL cholesterol levels. Participants were given an initial blood test and were then asked to consume a glucose tolerance test beverage before providing another blood sample 2 hours later. An oral glucose tolerance test was also administered.

Waist circumference and blood pressure were measured by NHANES personnel at examination centers. Waist circumference was measured for all participants. Examiners first marked the measurement site and then used a calibrated measuring tape to assess the circumference of the waist. Measures were recorded in centimeters. Blood pressure was measured in all examinees.

Participants were asked to sit quietly for 5 minutes before three consecutive blood pressures readings were taken. A fourth measurement was done if any of the

previous measures were disrupted due to movement, noise or incorrect readings. Blood pressure was measured using a mercury sphygmomanometer during the 2005-2008 survey cycle years. In the 2009-2010 survey, a new automated measurement protocol was introduced to supplement the sphygmomanometer measure.

2.2 Variable Definitions

2.2.1 Independent Variable

Individual phthalate metabolites were the main exposures assessed in this analysis. Only metabolites consistently detected in the urine across all three survey years were analyzed. 13 phthalate metabolites belonging to the following 9 parent compounds were included in this study: diisodecyl phthalate (DiDP), di(2-ethylhexyl) phthalate (DEHP), di-n-butyl phthalate (DBP), diethyl phthalate (DEP), diisononyl phthalate (DiNP), diisobutyl phthalate (DiBP), dimethyl phthalate (DMP), dioctyl phthalate (DOP) and butyl benzyl phthalate (BBP). For individuals assessed as having phthalate concentrations below the LOD, the LOD divided by the square root of two was reported by NHANES. A categorical indicator variable was created by NHANES for each metabolite to determine whether phthalate concentrations were above or below the LOD. Due to changes in LOD values across survey years, the highest LOD value for the years was used. A new indicator variable was created to determine whether concentrations remain above the new LOD value. Table 2-1 summarizes the LOD value and concentration amounts for each metabolite.

Urinary concentrations of phthalates were reported by NHANES on a continuous scale. However, each metabolites was analyzed as a categorical variable with the approximate quartiles of the distribution serving as the categories.

Distributions of each metabolite were reviewed through estimation of means, geometric means, and median values. Because metabolites were not normally distributed, metabolites were log transformed before geometric means could be calculated. For comparative analysis spearman correlation coefficients were calculated to assess metabolite relationships.

Table 2-1. Description of phthalate monoesters examined in NHANES 2005-2010

Metabolite	Abbreviation	Parent Compound	Molecular Weight	LOD[c1]
Mono-(carboxynonyl)	MCNP	Di-isodecyl phthalate	HMW[a]	0.6
Mono(carboxyisooctyl)	MCOP	Di-isononyl phthalate	HMW	0.7
Mono-isononyl	MiNP			1.2
Mono-(2-ethyl-5-carboxypentyl)	MECPP	Di-2-ethylhexyl phthalate	HMW	0.6
Mono-(2-ethyl-5-hydroxyhexyl)	MEHHP			0.7
Mono-2-ethylhexyl	MEHP			1.2
Mono-(2-ethyl-5-oxohexyl)	MEOHP			0.7
Mono-(3-carboxypropyl)	MCPP	Di-*n*-octyl phthalate	HMW	0.2
Mono-benzyl phthalate	MBzP	Butylbenzyll phthalate	HMW	0.2
Mono-*n*-butyl phthalate	MnBP	Dibutyl phthalates	LMW[b]	0.6
Mono-ethyl phthalate	MEP	Diethyl phthalate	LMW	0.5
Mono-methyl phthalate	MMP	Dimethyl phthalate	LMW	1.1
Mono-isobutyl phthalate	MiBP	Di-isobutyl phthalate	LMW	0.3

[a] HMW: High Molecular Weight
[b] LMW: Low Molecular Weight
[c] LOD: Limit of Detection
[1] Highest LOD for each metabolite across the three survey years was used for the lower limit of detection.

This analysis accounted for urine concentration and flow rate by adjusting for creatinine in the regression models. Previous studies have documented that creatinine-adjusted urinary metabolite concentrations better correlate with blood or plasma concentrations of the parent chemical then unadjusted values [57]. There are

two methods by which creatinine adjusted chemical values may be determined in a population. The first is that individual creatinine-adjusted urinary chemicals are analyzed as the independent variable used to determine whether the chemical of interest is associated with the outcome [58]. The second method involves adjusting for creatinine corrected chemical values as the dependent variable [58]. However, in both models the creatinine corrected chemical may be significantly associated with the outcome because the individual outcome or other model covariates were related to urinary creatinine. To overcome this limitation it is recommended to adjust for creatinine concentrations separately from the chemical as an independent variable, allowing creatinine to be adjusted for the urinary chemical of interest as well as other covariates included in the model[58].

2.2.2 Dependent Variable

The dependent variable was Metabolic Syndrome (MetS), defined by the presence of at least 3 of the following metabolic risk factors: blood pressure >130/85 mm Hg, fasting blood glucose > 5.6mmol/1, serum triglyceride level >1.7mmol/1, HDL cholesterol level <1.0mmol/1 in men and <1.3 in women and waist circumference >102cm in men and >88cm in women[59]. All 5 criteria were reported in NHANES as continuous variables. MetS was analyzed as a dichotomous variable (yes/no).

Cutoffs for the above mentioned MetS components were based off of the U.S National Cholesterol Education Program Adult Treatment Panel III (ATPIII)[59]. Each component was based on a single measurement taken at the examination portion except for blood pressure. Because 4 consecutive blood pressure measurements were

reported, the mean systolic and diastolic blood pressure measurements for each participant were used to define the variable. Individuals taking medication to lower their blood pressure were also considered to be hypertensive based on a cross-tabulation of the two variables.

2.2.3 Covariates

Gender, race, diet, socioeconomic status, total calorie intake, smoking behaviors and creatinine were assessed as possible covariates. Age was reported as the participant's age in years at the time of the interview. Imputed dates of birth were calculated by NHANES if missing. Adults over the age of 85 were all recorded as 85 to maintain anonymity. Race was recorded based on reported race and ethnicity and includes the following categories: non-Hispanic white, non-Hispanic black, Mexican American and other. The NHANES data also included "other Hispanic" and "other" categories. Other Hispanics were coded as other due to sample proportions lower than the actual U.S. population for that group as well as based on NHANES recommendations[60]. Asian Americans could not be differentiated as a racial group as they were not recognized as a separate racial category by NHANES until the 2011-2012 survey cycle. Gender was self-reported as either male or female.

Socioeconomic status was assessed by both education and income variables. Education was defined as some high school, high school diploma, some college or Associates degree and college degree or higher. Adult education information was only obtained by NHANES for participants greater than or equal to the age of 20. The income to poverty ratio was used to define individual's income. An individual was classified as impoverished if their income to poverty ratio was 0.99 or below. A value

of 1.0 is considered as the federal poverty threshold and values above 1.00 indicate income above poverty. Values above 5.0 were coded as 5.0 to protect participant's anonymity. In the present analysis income was categorized based on whether individuals were above or below the federal poverty threshold. Individuals who chose not to respond or did not know the correct answer were coded as missing.

Dietary questionnaires were used to assess participant's diet. Calorie intake was determined by total energy (kcal) and was assessed as a continuous variable. Finally, smoking behavior was determined by a combination of two variables asking whether participants had smoked at least 100 cigarettes in their life and whether they currently smoke cigarettes. Smoking status was categorized as never, former or current; non responses were coded as missing.

2.3 Statistical Analysis

All analyses conducted accounted for the NHANES complex survey sampling design. Univariable analyses including frequency distributions and calculations of mean (standard deviations) and median (interquartile range) values were estimated to evaluate the distribution of all covariates, phthalate concentrations and MetS variables by gender. Gender differences were assessed using t-tests and chi-square tests. Correlations between phthalate metabolites were estimated by Spearman correlation coefficients. Phthalate metabolites were divided into quartiles based on the overall distribution of each metabolite in the sample population. In addition to the 13 individual phthalates, metabolites were combined into molar sums that represent similar biologic activity and use (LMW, HMW and DEHP). The concentrations of LMW phthalate metabolites were expressed as the sum total of MEP, MBP, MMP

and MiBP. HMW phthalate concentrations were expressed as the sum of MCNP, MCOP, MiNP, MCPP, MEHP and MBzP. Finally, DEHP metabolite concentrations were calculated by adding the molarities of MEHP, MECPP, MEHHP and MEOHP. Bonferroni corrections were not applied as this was an exploratory analysis.

Odds ratios and 95% confidence intervals were estimated using multivariable logistic regression. Age-adjusted logistic regression and age and gender adjusted logistic regression models were conducted initially to assess associations between individual phthalate metabolites and MetS. Subsequently, multivariable regression was performed, adjusting for age, gender, race, education, poverty to income ratio and total calorie intake. Potential confounders were selected a priori based on previous literature. All of the aforementioned models adjusted for urinary creatinine.

Interaction by gender was tested by modeling an interaction term for each individual phthalate metabolite and gender. Likelihood ratio tests were used to compare multivariable models with and without the interaction term. Variance Inflation Factor (VIF) and tolerance tests were conducted to check for multicollinearity. Finally, model diagnostics tests were used to determine the overall fit of the model.

Previous studies have found prevalence of MetS to be higher among women[54]. To evaluate potential gender differences, final multivariable models were stratified by gender. Tests for trend were performed by modeling phthalate exposures as a linear variable. All analyses were conducted using SAS 9.3 (Cary, North Carolina).

2.4 Human Subjects Determination

The National Health and Nutrition Examination Survey was approved by the National Center for Health Statistics research ethics review board. The survey has received approval from the review board for every survey cycle since 1999. NHANES obtains informed human consent before any examinations are conducted. This analysis used de-identified human data from NHANES and obtained exemption from further review from the Institutional Review Board of the University of Maryland, College Park.

Chapter 3: Results

Table 3-1 describes the distribution of the study population based on key sociodemographic characteristics by gender. The mean age of the sample was approximately 47.84 years (std.dev: 19.1). There were slightly more men (n=2802) than women (2717) in the sample. The distribution of race/ethnicity was similar between men and women, with approximately 70% White, 12% Black, 10% Hispanic and 10% multiracial or other. Education was fairly evenly distributed across having a high school degree, some college education or having a college degree. However, fewer participants reported having less than a high school degree. The majority of the study population was above the federal poverty threshold (men=82% & women=78%). The mean calorie intake for the population was 2449 (std.dev=21.3) for men and 1769 (std.dev=16.4) for women and approximately 50% of men and women were never smokers. Calorie intake, poverty income ratio, smoking status and urinary creatinine concentrations were significantly different by gender ($p < 0.01$).

Table 3-1. Study population characteristics by gender NHANES (2005-2010)

Variable	**Men** N (%)[a]	**Women** N (%)[a]	**P-Value[c]**
Gender	2802 (49.04)	2717 (51.1)	
Age			0.43
18-27	542 (19.2)	505 (16.0)	
28-37	430 (17.9)	411 (16.5)	
38-47	444 (20.3)	465 (20.7)	
48-57	454 (19.6)	390 (18.2)	
≥58	932 (23.1)	946 (28.6)	
Race/ Ethnicity (%)			0.23
White	1363 (70.0)	1281 (70.3)	
Black	588 (10.7)	584 (12.2)	
Hispanic	518 (9.3)	487 (7.1)	

Variable	Men N (%)[a]	Women N (%)[a]	P-Value[c]
Other	333 (10.0)	365 (10.4)	
Education (%)			0.06
< High School	743 (19.1)	710 (18.0)	
High School Degree	648 (24.4)	633 (25.7)	
Some College or AA degree	669 (29.3)	720 (30.7)	
≥College Degree	536 (27.2)	489 (25.6)	
Poverty to Income Ratio (PIR)			< 0.01
Below poverty (PIR < 1.0)	707 (17.5)	826 (21.4)	
At or above poverty (PIR ≥1.0)	2095 (82.5)	1891 (78.6)	
Smoking Status			0.01
Never smoker	788 (53.0)	494 (51.0)	
Former smoker	120 (7.9)	74 (6.6)	
Current smoker	549 (39.0)	438 (42.4)	
	Mean (SD) [b]		
Body Mass Index	28.6 ± 0.13	29.03 ± 0.17	0.03
Total Calorie Intake	2449.9 ± 21.3	1769 ± 16.4	< 0.01
Urinary creatinine (mg/dL)	146.5 ± 2.0	112.4 ± 1.8	< 0.01
Metabolic Syndrome Components			
Metabolic Syndrome (n,%) [a]	521 (16.8)	628 (19.8)	< 0.01
Waist Circumference (cm)	100.6 ± 0.4	95.7 ± 0.3	< 0.01
Systolic blood pressure (mm of Hg)	125.3 ± 0.37	121.6 ± 0.5	< 0.01
Diastolic blood pressure (mm of Hg)	71.1 ± 0.37	68.2 ± 0.33	< 0.01
Serum triglycerides (mmol/1)	1.6 ± 0.03	1.4 ± 0.03	< 0.01
Serum HDL (mmol/1)	1.2 ± 0.01	1.5 ± 0.01	< 0.01
Serum glucose (mmol/1)	6.1 ± 0.05	5.9 ± 0.06	< 0.01

[a] Numbers are reported as total n and weighted sample percent
[b] Values are reported as weighted sample mean and standard deviation
[c] Gender differences calculated using t tests for continuous variables and chi square tests for categorical variables.

Statistically significant gender differences were found for MetS as well as its individual components ($p < 0.01$). Although mean levels of the individual MetS components were higher among men, the prevalence of MetS was greater among women (19.8%) than men (16.8%). Mean values for each MetS components were significantly higher among men versus women ($p < 0.01$).

Table 3-2. Distribution of phthalate metabolite in NHANES (2005-2010)

Metabolite	**Mean** (SD)	**Median** (IQR[a])	Geometric Mean	(95% CI)	% below LOD
MEP	403.4 ± 22.6	83.2 (31.5, 251.1)	4.5	(4.5, 4.6)	0.1
MECPP	73.8 ± 5.5	25.9 (12.4, 57.1)	3.3	(3.2, 3.4)	0.1
MEHHP	55.7± 4.1	17.1 (7.8, 39.8)	2.9	(2.8, 3.0)	0.4
MnBP	41.9 ± 5.5	17.3 (8.4, 32.2)	2.8	(2.7, 2.6)	0.5
MEOHP	32.1 ± 2.4	10.5 (4.7, 23.0)	2.4	(2.3, 2.5)	0.9
MCOP	21.5 ± 1.5	6.5 (3.0, 16.9)	2.0	(1.9, 2.1)	2.4
MBzP	17.9 ± 3.1	6.8 (2.9, 15.4)	1.9	(1.8, 1.9)	1.4
MiBP	15.4 ± 2.7	6.9 (3.1, 13.3)	1.8	(1.7, 1.9)	1.2
MCNP	5.7 ± 0.4	2.5 (1.3, 4.9)	0.9	(0.9, 1.0)	6.2
MCPP	5.5 ± 0.4	2.4 (1.1, 4.9)	0.9	(0.8, 0.9)	2.7
MEHP	8.0 ± 0.7	1.9 (0.9, 4.9)	0.8	(0.8, 0.9)	28.6
MMP	6.2 ± 2.0	0.8 (0.8, 2.3)	0.2	(0.2, 0.3)	52.1
MiNP	2.3 ± 0.2	0.87 (0.9, 0.9)	0.1	(0.01, 0.1)	78.5

[a] IQR: Interquartile Range

Distributions of individual phthalate metabolites were evaluated in the study population (Table 3-2). Of the 13 metabolites, two metabolites MMP and MiNP had concentrations below the LOD, 52.1% and 78.5% respectively, for more than half of the sample population. Concentrations for MEP (geometric mean = 4.5, 95% CI: 4.5, 4.6), MECPP (geometric mean = 3.3, 95% CI: 3.2, 3.4), MEHHP (geometric mean = 2.9, 95% CI: 2.8, 3.0) and MnBP (geometric mean = 2.8, 95% CI: 2.7, 2.9) were the highest among all participants. MinP (geometric mean = 0.05, 95% CI: 0.01, 0.08), MMP (geometric mean = 0.25, 95% CI: 0.18, 0.32) and MEHP (geometric mean= 0.8, 95% CI: 0.8, 0.9) had the lowest concentrations. Strong statistically significant correlations were found between MEHHP and MECPP (r=0.94), MEOHP and MEHHP (r=0.98) and MEOHP and MECPP (r=0.94). MBzP (r=0.69) and MiBP

(r=0.74) were also strongly correlated with MnBP. Correlations between other metabolites were not as strong; however, all correlations were found to be statistically significant (p<.001 for all) (Table 3-3). Observed correlations were generally stronger for women than men. (Table A-2 & A-3).

Table 3-3. Spearman correlation coefficients between phthalate metabolites NHANES 2005-2010

	mcnp	mcop	mecpp	mnbp	mcpp	mep	mehhp	mehp	mmp	minp	meohp	mbzp	mibp
MCNP	1	0.61	0.50	0.36	0.72	0.18	0.45	0.34	0.11	0.35	0.46	0.35	0.43
MCOP		1	0.46	0.39	0.59	0.24	0.41	0.33	0.20	0.28	0.43	0.36	0.40
MECPP			1	0.54	0.56	0.30	0.94	0.74	0.22	0.34	0.94	0.47	0.48
MnBP				1	0.56	0.42	0.57	0.46	0.30	0.23	0.58	0.69	0.74
MCPP					1	0.25	0.55	0.42	0.19	0.38	0.57	0.50	0.54
MEP						1	0.31	0.26	0.26	0.15	0.32	0.32	0.37
MEHHP							1	0.79	0.23	0.36	0.98	0.51	0.51
MEHP								1	0.20	0.42	0.79	0.41	0.42
MMP									1	0.16	0.23	0.24	0.27
MiNP										1	0.35	0.18	0.18
MEOHP											1	0.52	0.52
MBzP												1	0.60
MiBP													1

Correlations were statistically significant at the p < 0.001 level.

Associations with MetS across quartiles of 13 phthalate metabolites are presented in Table 3-4. Age was significantly associated with MetS (p< 0.01) and was adjusted for in the first model along with creatinine. A statistically significant linear trend was observed for MCOP (p trend: 0.02), MECPP (p trend: 0.03) and MCPP (p trend: 0.03) in the age-adjusted models. No other statistically significant associations were observed between individual phthalate metabolites and MetS in age and creatinine adjusted models.

In models additionally adjusted for gender, similar linear trends were observed with MCOP, MECPP and MCPP. However, individual estimates were statistically significant in this model.

Table 3-4. Associations between urinary phthalate metabolite concentrations and metabolic syndrome

Phthalate Metabolite	MetS (y/n)	Age-adjusted model[1]		Gender-adjusted model[2]		Multivariable-adjusted model[3]	
		OR (95% CI)	P trend	OR (95% CI)	P trend	OR (95% CI)	P trend
MEP							
Q1	295/1058	REF		REF		REF	
Q2	283/1070	1.02 (0.79, 1.32)		1.05 (0.81, 1.37)		0.86 (0.64, 1.17)	
Q3	273/1078	0.91 (0.69, 1.18)		0.92 (0.70, 1.21)		0.90 (0.68, 1.21)	
Q4	275/1077	1.04 (0.32, 0.82)[a]		1.04 (0.82. 1.32)		0.89 (0.66, 1.19)	
			0.88		0.94		0.43
MCOP							
Q1	285/1098	REF		REF		REF	
Q2	277/1050	0.74 (0.58, 0.95)[a]		0.75 (0.59, 0.96)[a]		1.31 (1.40, 1.64)[a]	
Q3	291/1056	0.93 (0.74, 1.17)		0.93 (0.74, 1.18)		1.43 (1.06, 1.92)[a]	
Q4	273/1079	1.04 (0.81, 1.33)		1.04 (0.81, 1.34)		1.30 (1.16, 1.89)[a]	
			0.02		0.02		0.004
MECPP							
Q1	263/1091	REF		REF		REF	
Q2	281/1071	0.76 (0.57, 1.02)		0.78 (0.59, 1.03)		1.16 (0.91, 1.48)	
Q3	310/1042	0.84 (0.64, 1.11)		0.86 (0.65, 1.12)		1.43 (1.10, 2.13)[a]	
Q4	272/1079	1.11 (0.87, 1.42)		1.12 (0.88, 1.43)		1.48 (0.96, 1.77)	
			0.03		0.04		0.05
MnBP							
Q1	286/1070	REF		REF		REF	
Q2	307/1043	0.97 (0.68, 1.40)		1.06 (0.73, 1.53)		1.19 (0.86, 1.63)	
Q3	272/1083	1.15 (0.85, 1.55)		1.24 (0.92, 1.68)		1.02 (0.75, 1.38)	
Q4	261/1087	1.03 (0.78, 1.35)		1.07 (0.82. 1.40)		0.87 (0.58, 1.29)	
			0.94		0.59		0.35
MBzP							
Q1	312/1047	REF		REF		REF	
Q2	289/1067	1.09 (0.85, 1.40)		0.86 (0.61, 1.23)		1.02 (0.80, 1.30)	
Q3	265/1078	1.06 (0.79, 1.45)		0.94 (0.70, 1.27)		0.99 (0.72, 1.36)	
Q4	260/1091	1.20 (0.85, 1.70)		0.91 (0.67, 1.23)		1.05 (0.72, 1.53)	
			0.36		0.48		0.85
MMP							
Q1	667/2423	REF		REF		REF	
Q2	187/780	1.21 (0.95, 1.55)		1.23 (0.96, 1.57)		0.88 (0.69, 1.13)	
Q3	272/1080	1.10 (0.79, 1.52)		1.12 (0.81, 1.54)		0.83 (0.65, 1.07)	
			0.10		0.09		0.11
MEHP							
Q1	438/1379	REF		REF		REF	
Q2	205/696	1.17 (0.88, 1.57)		1.18 (0.89, 1.57)		0.99 (0.75, 1.31)	
Q3	276/1963	1.17 (0.88, 1.56)		1.18 (0.88, 1.58)		1.03 (0.77, 1.38)	
Q4	207/1145	1.22 (0.94, 1.60)		1.24 (0.95, 1.62)		0.84 (0.63, 1.12)	

Phthalate Metabolite	MetS (y/n)	Age-adjusted model[1]		Gender-adjusted model[2]		Multivariable-adjusted model[3]	
		OR (95% CI)	P trend	OR (95% CI)	P trend	OR (95% CI)	P trend
			0.42		0.41		0.35
MCNP							
Q1	283/1070	REF		REF		REF	
Q2	313/1059	0.98 (0.78, 1.22)		0.97 (0.78, 1.22)		1.19 (0.95, 1.49)	
Q3	277/1058	1.17 (0.89, 1.53)		1.17 (0.90, 1.53)		1.17 (0.94, 1.47)	
Q4	253/1096	1.09 (0.8, 1.39)		1.09 (0.86, 1.40)		1.07 (0.87, 1.33)	
			0.88		0.90		0.71
MCPP							
Q1	287/1066	REF		REF		REF	
Q2	271/1087	0.78 (0.60, 1.01)		0.79 (0.61, 1.02)		1.03 (0.76, 1.38)	
Q3	278/1079	0.74 (0.54, 1.00)		0.75 (0.55, 1.01)		1.12 (0.84, 1.50)	
Q4	290/1051	0.85 (0.65, 1.11)		0.85 (0.66, 1.11)		1.39 (1.09, 1.77)[a]	
			0.03		0.04		0.01
MEHHP							
Q1	280/1076	REF		REF		REF	
Q2	276/1077	0.79 (0.59, 1.05)		0.80 (0.60, 1.06)		1.14 (0.88, 1.48)	
Q3	299/1049	0.85 (0.30, 1.13)		0.86 (0.64, 1.15)		1.27 (0.93, 1.74)	
Q4	271/1081	1.01 (0.79, 1.29)		1.01 (0.79, 1.29)		1.25 (0.92, 1.70)	
			0.08		0.09		0.61
MiNP							
Q1	942/3381	REF		REF		REF	
Q2	184/902	1.00 (0.80, 1.26)		0.99 (0.79, 1.25)		1.05 (0.83, 1.34)	
			0.98		0.95		0.68
MEOHP							
Q1	288/1066	REF		REF		REF	
Q2	270/1081	0.91 (0.68, 1.22)		0.92 (0.69, 1.24)		0.97 (0.74, 1.28)	
Q3	307/1046	0.84 (0.64, 1.09)		0.86 (0.66, 1.12)		1.27 (0.92, 1.77)	
Q4	261/1090	1.15 (0.89, 1.49)		1.16 (0.90, 1.49)		1.08 (0.81, 1.46)	
			0.22		0.28		0.35
MiBP							
Q1	288/1069	REF		REF		REF	
Q2	284/1076	0.83 (0.63. 1.10)		0.88 (0.67, 1.16)		1.08 (0.81, 1.42)	
Q3	275/1065	0.92 (0.72, 1.17)		0.96 (0.75, 1.24)		1.00 (0.73, 1.37)	
Q4	279/1073	0.87 (0.68, 1.10)		0.89 (0.69, 1.15)		1.03 (0.77, 1.37)	
			0.29		0.50		0.99

[a] Statistically Significant ($p<0.05$)

[1] All analyses adjusted for age and creatinine

[2] All analyses adjusted for age, gender and creatinine

[3] All analyses adjusted for age, gender, race, education, income, calorie intake and creatinine.

Results from the multivariable analyses are also summarized in Table 3-4. This model included adjustments for age, gender, race, education, income, calorie intake and creatinine. Increasing levels of MCOP, MECPP and MCPP were significantly associated with an increase in the prevalence of MetS (p trend: 0.004, 0.05, and 0.1, respectively). More specifically, when comparing those in the highest to lowest quartiles, the odds of MetS was elevated: MCOP (1.3, 95% CI: 1.06, 1.92), MECPP (1.4, 95% CI: 1.10, 2.13) and MCPP (1.4, 95% CI: 1.09, 1.77). .

Table 3-5 presents results from the gender stratified multivariable models examining the associations of phthalate metabolites with MetS. Overall, the odds of MetS was higher among women exposed to phthalate metabolites than men. No statistically significant associations were observed for men. However, select metabolites demonstrated an increase in the odds of MetS for women. Similar to the findings from the overall multivariable analyses, MCOP (1.7, 95% CI: 1.3, 2.2) and MCPP (1.7, 95% CI: 1.3, 2.4) demonstrated an increased odds of MetS when comparing women in quartile 4 to those in quartile 1. In contrast, a significant inverse association was observed among women in the highest quartile of MnBP (0.57, 95% CI: 0.37, 0.87) and MEHP (0.57, 95% CI: 0.34, 0.96) compared to those in the lowest. A statistically significant p-trend was observed for MCOP, MCPP, MnBP and MEHP, suggesting that among women, there may be evidence of a potential dose response relationship with MetS for these metabolites. However, tests for interaction between each metabolite and gender were not statistically significant ($p>0.05$ for all).

Table 3-5. Associations of phthalate metabolites with MetS by gender in NHANES (2005-2010)

Metabolite	**Men** (n=2802)		**Women** (n=2717)	
	Age-Adjusted model[1]	**Multivariable-adjusted model[2]**	**Age-Adjusted model[1]**	**Multivariable-adjusted model[2]**
	OR (95% CI)			
MCNP				
Q1	REF	REF	REF	REF
Q2	1.26 (0.94, 1.70)	1.05 (0.76, 1.44)	1.28 (0.93, 1.78)	1.25 (0.85, 1.84)
Q3	1.02 (0.74, 1.40)	1.14 (0.80, 1.62)	1.07 (0.78, 1.47)	1.09 (0.78, 1.53)
Q4	0.96 (0.69, 1.33)	1.06 (0.40, 1.52)	1.12 (0.79, 1.59)	1.23 (0.86, 1.76)
P-trend	0.55	0.70	0.83	0.44
MCOP				
Q1	REF	REF	REF	REF
Q2	1.17 (0.92, 1.50)	1.41 (0.98, 2.04)	1.15 (0.86, 1.52)	1.20 (0.89, 1.60)
Q3	1.44 (0.97, 2.14)	1.35 (0.92, 1.99)	1.33 (0.93, 1.89)	1.46 (0.99, 2.16)
Q4	1.43 (1.09, 1.88)[a]	1.26 (0.82, 1.94)	1.44 (1.10, 1.88)	1.72 (1.33, 2.21)[a]
P-trend	0.01	0.43	0.01	< 0.01
MECPP				
Q1	REF	REF	REF	REF
Q2	0.94 (0.69, 1.30)	1.09 (0.77, 1.56)	0.97 (0.67, 1.41)	0.99 (0.68, 1.46)
Q3	1.28 (0.85, 1.41)	1.38 (0.97, 1.97)	1.23 (0.82, 1.85)	1.24 (0.79, 1.94)
Q4	1.04 (0.71, 1.51)	1.49 (0.98, 2.25)	1.06 (0.72, 1.56)	1.08 (0.75, 1.56)
P-trend	0.53	0.05	0.56	0.50
MnBP				
Q1	REF	REF	REF	REF
Q2	1.05 (0.78, 1.43)	1.22 (0.74, 1.99)	0.97 (0.73, 1.28)	0.94 (0.70, 1.26)
Q3	1.15 (0.86, 1.55)	0.90 (0.60, 1.34)	1.17 (0.82, 1.66)	1.15 (0.81, 1.63)
Q4	0.76 (0.53, 1.09)	1.22 (0.67, 2.22)	0.62 (0.40, 0.95)	0.57 (0.37, 0.87)[a]
P-trend	0.23	0.76	0.10	0.05
MCPP				
Q1	REF	REF	REF	REF
Q2	1.02 (0.74, 1.41)	0.93 (0.63, 1.39)	0.91 (0.62, 1.32)	1.08 (0.75, 1.56)
Q3	1.23 (0.83, 1.83)	1.04 (0.71, 1.52)	1.27 (0.84, 1.93)	1.42 (0.97, 2.07)
Q4	1.43 (1.02, 1.99)[a]	1.06 (0.71, 1.59)	1.42 (1.01, 2.01)[a]	1.73 (1.27, 2.38)[a]
P-trend	0.04	0.65	0.02	< 0.01
MEP				
Q1	REF	REF	REF	REF
Q2	1.17 (0.80, 1.70)	0.65 (0.40, 1.05)	1.14 (0.78, 1.63)	1.08 (0.76, 1.53)
Q3	1.06 (0.74, 1.51)	0.88 (0.57, 1.37)	1.07 (0.74, 1.55)	0.91 (0.63, 1.33)
Q4	1.04 (0.70, 1.56)	0.85 (0.58, 1.24)	1.03 (0.68, 1.54)	0.85 (0.53. 1.36)
P-trend	0.95	0.73	0.97	0.39
MEHHP				
Q1	REF	REF	REF	REF
Q2	0.96 (0.72, 1.28)	1.24 (0.84, 1.82)	0.86 (0.63, 1.19)	0.91 (0.67, 1.24)
Q3	1.41 (0.98, 2.02)	1.26 (0.85, 1.88)	1.35 (0.93, 1.97)	1.41 (0.98, 2.03)
Q4	0.99 (0.66, 1.49)	1.59 (1.02, 2.48)[a]	0.93 (0.61, 1.42)	0.97 (0.66, 1.43)
P-trend	0.58	0.04	0.73	0.62
MEHP				

Metabolite		**Men** (n=2802)		**Women** (n=2717)	
		Age-Adjusted model[1]	**Multivariable-adjusted model[2]**	**Age-Adjusted model[1]**	**Multivariable-adjusted model[2]**
		OR (95% CI)			
	Q1	REF	REF	REF	REF
	Q2	0.96 (0.65, 1.41)	1.13 (0.76, 1.70)	0.94 (0.62, 1.42)	0.93 (0.60, 1.44)
	Q3	0.78 (0.55, 1.12)	1.41 (0.96, 2.07)	0.77 (0.51, 1.15)	0.77 (0.50, 1.16)
	Q4	0.61 (0.39, 0.96)[a]	1.16 (0.75, 1.80)	0.60 (0.37, 0.99)[a]	0.57 (0.34, 0.96)[a]
P-trend		0.02	0.32	0.03	0.02
MMP					
	Q1	REF	REF	REF	REF
	Q2	0.76 (0.50, 1.14)	1.03 (0.76, 1.41)	0.76 (0.50, 1.14)	0.76 (0.48, 1.18)
	Q3	0.83 (0.55, 1.24)	0.84 (0.61, 1.14)	0.82 (0.55, 1.23)	0.82 (0.54, 1.26)
P-trend		0.19	0.41	0.19	0.22
MiNP					
	Q1	REF	REF	REF	REF
	Q2	1.01 (0.69, 1.47)	1.01 (0.75, 1.37)	1.01 (0.69, 1.47)	1.09 (0.73, 1.63)
P-trend		0.73	0.95	0.98	0.66
MEOHP					
	Q1	REF	REF	REF	REF
	Q2	0.88 (0.67, 1.16)	1.06 (0.72, 1.32)	0.80 (0.58, 1.09)	0.81 (0.60, 1.10)
	Q3	1.21 (0.85, 1.73)	0.91 (0.60, 1.38)	1.11 (0.75, 1.64)	1.10 (0.74, 1.63)
	Q4	0.84 (0.56, 1.28)	0.91 (0.60, 1.36)	0.74 (0.47, 1.17)	0.76 (0.49, 1.17)
P-trend		0.73	0.06	0.41	0.40
MBzP					
	Q1	REF	REF	REF	REF
	Q2	1.05 (0.75, 1.51)	1.08 (0.76, 1.52)	1.04 (0.71, 1.51)	0.96 (0.67, 1.36)
	Q3	1.29 (0.90. 1.85)	0.75 (0.49, 1.13)	1.42 (0.95, 2.14)	1.33 (0.88, 2.02)
	Q4	1.28 (0.81, 2.04)	0.84 (0.50, 1.40)	1.37 (0.83, 2.26)	1.33 (0.80, 2.23)
P-trend		0.19	0.27	0.12	0.15
MiBP					
	Q1	REF	REF	REF	REF
	Q2	1.23 (0.85, 1.77)	0.97 (0.71, 1.32)	1.24 (0.87, 1.78)	1.20 (0.83, 1.74)
	Q3	1.16 (0.83, 1.62)	0.91 (0.60, 1.38)	1.21 (0.83, 1.75)	1.10 (0.76, 1.60)
	Q4	1.24 (0.83, 1.85)	0.91 (0.60, 1.36)	1.28 (0.80, 2.07)	1.31 (0.70, 1.83)
P-trend			0.59	0.31	0.65

[a] Statistically Significant ($p<0.05$)

[1] All analyses adjusted for age and creatinine

[2] All analyses adjusted for age, gender, race, education, income, calorie intake and creatinine.

Table 3-6 presents the associations of LMW, HMW and DEHP metabolites in relationship to MetS by gender. Among women, the molar sum of HMW metabolites was significantly associated with an increased prevalence of MetS in multivariable models (OR_{Q4vsQ1}=1.55, 95% CI: 1.1, 2.2, p-trend=0.01). In contrast, results from multivariable adjusted analyses suggest that the odds of MetS was greater in men for DEHP metabolites (OR_{Q4vsQ1}1.5, 95% CI: 0.9, 2.4); however, these results were not statistically significant. No statistically significant results were observed for the molar sum of LMW metabolites.

Table 3-6. Associations of molar summed phthalate metabolites by gender in NHANES (2005-2010)

Metabolite	**Men** (n=2802)		**Women** (n=2717)	
	Age-Adjusted model[1]	**Multivariable-adjusted model[2]**	**Age-Adjusted model[1]**	**Multivariable-adjusted model[2]**
	OR (95% CI)			
∑ LMW				
Q1	REF	REF	REF	REF
Q2	1.03 (0.70, 1.51)	0.78 (0.54, 1.13)	1.02 (0.71, 1.48)	1.00 (0.73, 1.38)
Q3	0.75 (0.53, 1.05)	0.84 (0.51, 1.36)	1.12 (0.74, 1.69)	0.76 (0.54, 1.07)
Q4	0.84 (0.58, 1.23)	1.02 (0.68, 1.52)	0.50 (0.68, 1.46)	0.72 (0.47, 1.10)
P-trend	0.95	0.87	0.77	0.08
∑ HMW				
Q1	REF	REF	REF	REF
Q2	1.09 (0.70, 1.69)	0.98 (0.68, 1.42)	0.67 (0.45, 0.99)[a]	1.23 (0.92, 1.67)
Q3	1.03 (0.67, 1.59)	1.06 (0.74, 1.53)	0.80 (0.57, 1.13)	1.71 (1.14, 2.58)[a]
Q4	1.17 (0.80, 1.71)	0.94 (0.60, 1.48)	1.07 (0.69, 1.64)	1.55 (1.08, 2.21)[a]
P-trend	0.85	0.87	0.02	0.01
∑ DEHP				
Q1	REF	REF	REF	REF
Q2	0.67 (0.42, 1.06)	1.20 (0.77, 1.86)	1.04 (0.67, 1.62)	1.02 (0.71, 1.48)
Q3	0.77 (0.55, 1.08)	1.44 (0.95, 2.20)	1.00 (0.67, 1.49)	1.47 (0.94, 2.30)
Q4	0.92 (0.67, 1.27)	1.50 (0.90, 2.49)	1.46 (0.97, 2.17)	0.97 (0.61, 1.55)
P-trend	0.55	0.08	0.72	0.76

[a] Statistically Significant ($p<0.05$)

[1] All analyses adjusted for age and creatinine

[2] All analyses adjusted for age, gender, race, education, income, calorie intake and creatinine.

Chapter 4: Discussion

This cross-sectional analysis of thirteen phthalate metabolites revealed that select metabolites, specifically MCOP and MCPP, are positively associated with the prevalence of metabolic syndrome, with variations by gender and molecular weight, after adjustment for key confounders

Of the 13 phthalate metabolites analyzed in this study, MCOP and MCPP consistently exhibited the strongest significant associations with MetS. MCOP has not previously been studied and MCPP is widely understudied in relation to individual MetS components. Both MCOP and MCPP are HMW metabolites and are widely understudied in relation to individual MetS components. However, despite the limited amount of existing evidence [7,8,39,42], our study results were consistent with the few epidemiologic that actually examined those two metabolites. Previous studies by Huang[42], Tetielbaum[39] and Shiue[8] have found MCPP to be positively associated with increased fasting blood glucose/ insulin resistance, increased waist circumference and increased blood pressure respectively. Compared to MCPP, MCOP has not been studied in relation to individual MetS components. However, results from two prior studies found that individuals exposed to MCOP were at greater risk of experiencing oxidative stress and having inflammation markers [61].

The prevalence of MetS was significantly higher among women in the study population than men. This is consistent with current national trends, which report that MetS rates are increasing faster among women than men [62]. The strongest positive associations with MetS were seen in women, especially for MCOP and MCPP. Women exposed to MEHP and MnBP exhibited inverse associations with MetS,

which is consistent to results from a previous study examining phthalate exposure in relation to waist circumference[6]. A possible explanation for this finding is that DEHP and DBP are anti-androgens, meaning that individuals with higher exposures to these compounds will have lower levels of androgens[63]. Females exposed to androgenic compounds, such as MCPP, have higher androgen levels and are at greater risk of polycystic ovarian disease, higher waist circumference and metabolic syndrome[64]. Therefore, women with higher levels of MEHP and MBP may have lower levels of androgens or a higher androgen/estrogen ratio, which could possibly explain the inverse association seen for these two metabolites and MetS.

In addition to the observed gender differences, the odds of MetS differed by molecular weight group. The grouped molar sum of HMW metabolites showed a significantly increased odds of MetS among women whereas the grouped molar sums of DEHP metabolites and LMW phthalates were not associated with MetS among women. Prior studies have also derived indices for HMW, LMW and DEHP metabolites according to the individual molecular weight of each metabolites, under the presumptive hypothesis that molecular weight is related to potency[39]. However, current epidemiologic evidence [39,42,46] has demonstrated mixed associations between HMW and LMW metabolites when examining individual MetS components. Stronger associations were seen for LMW metabolites (MEP, MBP) in studies examining obesity/waist circumference [39,49]. In contrast, studies examining insulin resistance/diabetes in relation to phthalates found stronger odds among HMW metabolites (MCPP, MEHP)[42]. Despite, the mixed results, HMW metabolites have

been shown to have a greater affinity for metabolic disruption, oxidative stress and lipid accumulation in previous mechanistic studies[3].

Previous exposure assessments on phthalates have determined that routes and sources of exposure are more varied among HMW metabolites in comparison to LMW metabolites[34]. In general, exposure to phthalates occur largely through dermal and oral routes. However, because HMW phthalates demonstrate greater volatility than other phthalate metabolites, exposure may also occur via inhalation routes. HMW phthalates are used more widely in industrial settings then LMW metabolites, especially in the production of PVC and other construction materials. Because of these differences, it is not surprising that exposures to HMW metabolites are generally higher in studies using nationally representative data, MEP being the only exception.

Overall, this study found that individuals exposed to specific phthalate metabolites had an increased odds of MetS and this association was mainly observed among women. While it was hypothesized that individuals with elevated levels of phthalate exposure would have increased odds of MetS, this did not hold true for some of the individual metabolites. For example, MEP had the highest urinary concentrations of the 13 phthalates metabolites analyzed yet this metabolite was not significantly associated with MetS. However, MCOP, and MCNP showed stronger odds of MetS, despite having lower mean concentrations than MEP. One possible explanation for this result is that phthalate exposure was measured at only one time point for this analysis, meaning that these results reflect only current exposure instead of chronic. Differences in excretion rates for LMW and HMW allow LMW

metabolites to be better represented in urine measurements. Typically phthalate metabolites have a half-life of 12-48 hours[65]. However, fat deposition may lengthen the half-lives of metabolites due to the lipid soluble nature of phthalate compounds[66]. Of note, this study found that the average waist circumference values in the sample population were much higher than the MetS cutoff value of 88cm for women, as defined by ATP III. This, in combination with the increase in metabolite half-life for individuals with greater body fat accumulation, offers perspective into why stronger odds of MetS were seen for women in this study.

This present study adds to the growing body of literature showing phthalates to be positively associated with diseases such as diabetes and obesity, which are characterized by metabolic disturbances. Evidence from animal studies suggest a number of potential mechanistic pathways through which phthalates may affect the body's metabolism including the inactivation of hormonal receptors and the binding and inappropriate activation of PPARs[4]. When activated by phthalates PPARs reduce insulin sensitivity and increase inflammatory responses resulting in greater fat accumulation and insulin resistance[4]. Despite the growing body of supporting animal evidence it remains unclear whether phthalates alter these same mechanisms in humans. Further research is needed examining PPAR and its response to phthalates in humans.

4.1 Strengths and Limitations

This study had several limitations that should be noted. First, given the cross-sectional study design, a temporal relationship between phthalate exposure and MetS could not be established. Because phthalates metabolites are quickly metabolized and

excreted, the single point measurements utilized in this study may not accurately reflect long term phthalate exposures. Metabolites with short or long half-lives may be easily over or underrepresented when measuring exposure over an interval [67].

There are a number of notable strengths to this study. This is the first study to examine phthalate exposures in relation to MetS. Given the large sample size, differences in associations with MetS by type of phthalate and gender were examined which revealed significant associations by HMW metabolites among women. This study used clinical examination and laboratory data from NHANES in combination with self-reported data to assess all variables, instead of relying only on self-reported data, which minimizes the potential for outcome misclassification. Additional strengths of using NHANES data are that the sample population is well characterized allowing for the adjustment of several key confounders, that the study sample is nationally representative, rendering the findings generalizable to the U.S. population and that the study findings can be compared to those from prior studies of phthalate metabolites and individual MetS components.

4.2 Conclusion

Phthalates are widely included in industrial, household and personal care products. The widespread use of contaminated products coupled with sedentary behaviors and genetic predispositions promote the increasing prevalence of obesity and cardiovascular disease in the U.S. Establishing a relationship between phthalate exposure and metabolic syndrome grants insight into the risk factors of this disorder as well as other metabolic diseases. Because this is the first study to examine this relationship, study findings help identify susceptible populations and give direction to future analyses. Replication by longitudinal studies is needed to establish temporality and further assess the potential association between phthalate exposure and metabolic syndrome

Appendices

Table A-1. Summary of previous literature

MetS	Study	Study Topic	Study Design	Study Demographics	Sample Size	# of Metabolites Type of Phthalate	Results
Waist Circumference	Dirtu, 2013[37]	Exposure and weight loss	Cohort	Overweight/ obese adults	195	9 metabolites DMP, DEP, DEHP, DiBP, DBP, BBP	Null
	Wang, 2013[32]	BMI & Waist Circumference	Cross-Sectional	Chinese, Children (8-15)	259	12 metabolites DEHP, BBP, DBP, DiBP, DMP, DEP	+
	Lind, 2012[38]	Abdominal fat distribution	Cohort	Women, 70 years	1016	4 metabolites DEHP, DMP, DiBP, DEP	+
	Teitelbaum, 2012[39]	BMI & Body Size	Cohort	Children (6-8), Hispanic & Black	387	9 metabolites DEP, DBP, BBP, DiBP, DEHP	+
	Hatch, 2010[41]	BMI & Waist Circumference	Cross-Sectional	NHANES (6-80)	4369	6 metabolites DEHP, DBP, DEP, BBP	+
	Hatch, 2008[6]	BMI & Waist Circumference	Cross-Sectional	NHANES (6-80)	4369	6 metabolites DEHP, DEP, DBP, BBP	+
	Stahlhut, 2007[40]	Waist Circumference	Cross-Sectional	NHANES male	1451	6 metabolites DBP,DEHP,BBP,DEP	+
High Blood Pressure	Shiue, 2014[8]	Phthalate and High Blood Pressure	Cross-Sectional	NHANES	10,537	15 metabolites DnOP, DiDP, DEHP, DBP, DCHP, DEP, DiBP, DMP, DiNP, BBP, DOP	+
	Trasande, 2013[49]	Phthalate and High Blood Pressure	Cross-Sectional	NHANES, children (6-19)	2838	8 metabolites DEP, DiBP, DEHP, DBP	+
Fasting Blood Sugar	Huang, 2014[42]	Gender/racial differences& diabete	Cross-Sectional	NHANES Women (12-80)	3083	8 metabolites BBP, DBP, DiBP, DEHP, DOP	+
	James Todd, 2012[7]	Phthalate & diabetes risk factors	Cross-Sectional	NHANES Women (20-79)	2350	7 metabolites BBP, DBP, DiBP, DEHP, DOP	+
	Svensson, 2011[44]	Phthalates and self-reported diabetes	Cross-sectional	Mexican Women	221	9 metabolites DEHP, BBP,	+
	Trasande, 2013[46]	Phthalate and insulin resistance	Cross-Sectional	NHANES Adolescents (12-19)	766	6 metabolites BBP,DEP, DiBP, DBP, DOP, DEHP	+

MetS	Study	Study Topic	Study Design	Study Demographics	Sample Size	# of Metabolites Type of Phthalate	Results
	Lind, 2013[47]	Phthalates and type 2 diabetes	Cross-sectional	Swedish Elderly (70)	1016	10 metabolites DEP, DEHP, DBP, BBP, DiBP, DMP	+
	Stahlhut, 2007[40]	Insulin Resistance	Cross-Sectional	NHANES male	1451	6 metabolites DBP,DEHP,BBP,DEP	+
	Kim, 2013[43]	Insulin Resistance via Oxidative Stress	Cross-Sectional	Korean Elderly Environmental Panel (60+)	560	3 metabolites DEHP, DBP	+
	Hong, 2009[45]	Insulin Resistance via Oxidative Stress	Cross-Sectional	Chinese Adults	960	3 metabolites DEHP, DBP	+
Triglyceride Level	Trasande, 2013[49]	Phthalate and High Blood Pressure	Cross-Sectional	NHANES, children (6-19)	2838	8 metabolites DEP, DiBP, DEHP, DBP	Null

Table A-2. Spearman correlation coefficients between phthalate metabolites among males in NHANES 2005-2010

	mcop	mcnp	mecpp	mnbp	Mcpp	mep	mehhp	mehp	mmp	minp	meohp	mbzp	mibp
MCOP	1	0.58	0.46	0.34	0.70	0.14	0.43	0.35	0.09	0.39	0.44	0.31	0.39
MCNP		1	0.43	0.39	0.57	0.22	0.39	0.31	0.18	0.31	0.40	0.34	0.37
MECPP			1	0.50	0.53	0.27	0.94	0.74	0.18	0.36	0.94	0.42	0.45
MnBP				1	0.54	0.37	0.54	0.44	0.29	0.24	0.54	0.69	0.72
MCPP					1	0.21	0.52	0.40	0.16	0.40	0.53	0.46	0.49
MEP						1	0.28	0.23	0.28	0.15	0.28	0.30	0.33
MEHHP							1	0.79	0.19	0.38	0.98	0.46	0.48
MEHP								1	0.16	0.43	0.78	0.37	0.40
MMP									1	0.14	0.19	0.21	0.26
MiNP										1	0.37	0.18	0.18
MEOHP											1	0.47	0.48
MBzP												1	0.56
MiBP													1

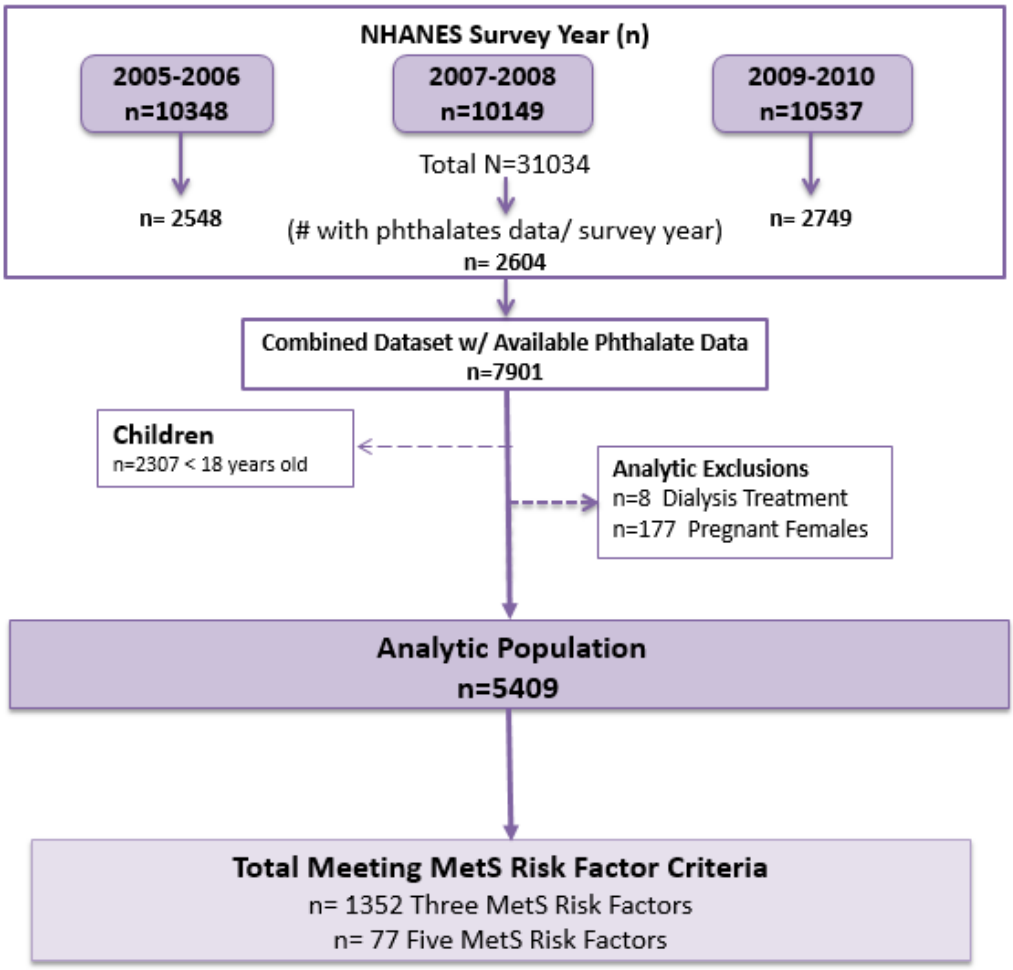
Correlations were statistically significant at the $p < 0.001$ level.

Table A-3. Spearman correlation coefficients between phthalate metabolites among females in NHANES 2005-2010

	mcop	mcnp	mecpp	mnbp	mcpp	mep	mehhp	mehp	mmp	minp	meohp	mbzp	mibp
MCOP	1	0.63	0.52	0.38	0.73	0.21	0.47	0.33	0.13	0.31	0.48	0.38	0.48
MCNP		1	0.49	0.39	0.61	0.26	0.43	0.33	0.21	0.24	0.44	0.38	0.44
MECPP			1	0.57	0.60	0.34	0.94	0.74	0.25	0.32	0.94	0.52	0.52
MnBP				1	0.60	0.46	0.61	0.49	0.31	0.23	0.62	0.70	0.75
MCPP					1	0.30	0.58	0.43	0.22	0.35	0.59	0.54	0.58
MEP						1	0.35	0.29	0.24	0.14	0.35	0.35	0.42
MEHHP							1	0.79	0.26	0.33	0.98	0.56	0.55
MEHP								1	0.24	0.41	0.78	0.44	0.44
MMP									1	0.18	0.27	0.25	0.28
MiNP										1	0.32	0.19	0.17
MEOHP											1	0.57	0.56
MBzP												1	0.63
MiBP													1

Correlations were statistically significant at the $p < 0.001$ level.

Figure A-1. Derivation of analytic sample



Bibliography

1. Kaur J. A comprehensive review on metabolic syndrome. Cardiology Research and Practice. 2014.

2. Mottillo S, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, et al. The metabolic syndrome and cardiovascular risk: A systematic review and meta-analysis. Journal of the American College of Cardiology. 2010. p. 1113–32.

3. Mankidy R, Wiseman S, Ma H, Giesy JP. Biological impact of phthalates. Toxicol Lett [Internet]. 2013 Feb 13 [cited 2014 Oct 13];217(1):50–8. Available from: http://www.sciencedirect.com/science/article/pii/S0378427412014051

4. Casals-Casas C, Desvergne B. Endocrine disruptors: from endocrine to metabolic disruption. Annu Rev Physiol [Internet]. 2011 Jan [cited 2014 Jul 12];73:135–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21054169

5. Lyche JL. Phthalates. In: Gupta RC, editor. Reproductive and Developmental Toxicology. Amsterdam: Elsevier; 2011. p. 637–55.

6. Hatch EE, Nelson JW, Qureshi MM, Weinberg J, Moore LL, Singer M, et al. Association of urinary phthalate metabolite concentrations with body mass index and waist circumference: a cross-sectional study of NHANES data, 1999-2002. Environ Health [Internet]. 2008 Jan [cited 2014 Oct 13];7:27. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2440739&tool=pmcentrez&rendertype=abstract

7. James-Todd T, Stahlhut R, Meeker JD, Powell S-G, Hauser R, Huang T, et al. Urinary phthalate metabolite concentrations and diabetes among women in the National Health and Nutrition Examination Survey (NHANES) 2001-2008. Environ Health Perspect [Internet]. 2012 Sep [cited 2014 Oct 23];120(9):1307–13. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3440117&tool=pmcentrez&rendertype=abstract

8. Shiue I, Hristova K. Higher urinary heavy metal, phthalate and arsenic concentrations accounted for 3-19% of the population attributable risk for high blood pressure: US NHANES, 2009-2012. Hypertens Res [Internet]. 2014 Jul 31 [cited 2014 Oct 31]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/25077919

9. López-Carrillo L, Hernández-Ramírez RU, Calafat AM, Torres-Sánchez L, Galván-Portillo M, Needham LL, et al. Exposure to phthalates and breast cancer risk in northern Mexico. Environ Health Perspect [Internet]. 2010 Apr [cited 2015 Apr 28];118(4):539–44. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2854732&tool=pmcentrez&rendertype=abstract

10. Wittassek M, Koch HM, Angerer J, Brüning T. Assessing exposure to phthalates - The human biomonitoring approach. Molecular Nutrition and Food Research. 2011. p. 7–31.

11. Frederiksen H, Skakkebæk NE, Andersson AM. Metabolism of phthalates in humans. Mol Nutr Food Res. 2007;51:899–911.

12. Seo KW, Kim KB, Kim YJ, Choi JY, Lee KT, Choi KS. Comparison of oxidative stress and changes of xenobiotic metabolizing enzymes induced by phthalates in rats. Food Chem Toxicol [Internet]. 2004 Jan [cited 2014 Oct 26];42(1):107–14. Available from: http://www.sciencedirect.com/science/article/pii/S027869150300245X

13. Jobling S, Reynolds T, White R, Parker MG, Sumpter JP. A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. Environ Health Perspect. 1995;103:582–7.

14. Harris CA, Henttu P, Parker MG, Sumpter JP. The estrogenic activity of phthalate esters in vitro. Environ Health Perspect. 1997;105:802–11.

15. Silva MJ, Barr DB, Reidy JA, Malek NA, Hodge CC, Caudill SP, et al. Urinary levels of seven phthalate metabolites in the U.S. population from the National Health and Nutrition Examination Survey (NHANES) 1999-2000. Environ Health Perspect. 2004;112:331–8.

16. Kobrosly RW, Parlett LE, Stahlhut RW, Barrett ES, Swan SH. Socioeconomic factors and phthalate metabolite concentrations among United States women of reproductive age. Environ Res. 2012;115:11–7.

17. Witorsch RJ, Thomas JA. Personal care products and endocrine disruption: A critical review of the literature. Crit Rev Toxicol [Internet]. 2010 Nov [cited 2015 Jan 27];40 Suppl 3:1–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20932229

18. ATSDR - Toxicological Profile: Di(2-ethylhexyl)phthalate (DEHP). [cited 2014 Dec 5]; Available from: http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=684&tid=65

19. ATSDR - Toxicological Profile: Di-n-butyl Phthalate. [cited 2014 Dec 5]; Available from: http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=859&tid=167

20. Högberg J, Hanberg A, Berglund M, Skerfving S, Remberger M, Calafat AM, et al. Phthalate diesters and their metabolites in human breast milk, blood or serum, and urine as biomarkers of exposure in vulnerable populations. Environ Health Perspect [Internet]. 2008 Mar [cited 2014 Dec 5];116(3):334–9. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2265037&tool=pmcentrez&rendertype=abstract

21. Christensen K, Sobus J, Phillips M, Blessinger T, Lorber M, Tan Y-M. Changes in epidemiologic associations with different exposure metrics: A case study of phthalate exposure associations with body mass index and waist circumference. Environ Int [Internet]. 2014 Dec [cited 2014 Nov 29];73:66–76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25090576

22. Hauser R, Meeker JD, Park S, Silva MJ, Calafat AM. Temporal variability of urinary phthalate metabolite levels in men of reproductive age. Environ Health Perspect [Internet]. 2004 Dec [cited 2014 Dec 5];112(17):1734–40. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1253667&tool=pmcentrez&rendertype=abstract

23. Diamanti-Kandarakis E, Bourguignon J-P, Giudice LC, Hauser R, Prins GS, Soto AM, et al. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr Rev [Internet]. 2009 Jun [cited 2014 Jul 10];30(4):293–342. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2726844&tool=pmcentrez&rendertype=abstract

24. Rogan WJ, Ragan NB. Some evidence of effects of environmental chemicals on the endocrine system in children. Int J Hyg Environ Health. 2007;210:659–67.

25. Sharpe RM, Skakkebaek NE. Testicular dysgenesis syndrome: mechanistic insights and potential new downstream effects. Fertility and Sterility. 2008.

26. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod. 2001;16:972–8.

27. Wittassek M, Angerer J. Phthalates: Metabolism and exposure. International Journal of Andrology. 2008. p. 131–6.

28. Wei J, Lin Y, Li Y, Ying C, Chen J, Song L, et al. Perinatal exposure to bisphenol A at reference dose predisposes offspring to metabolic syndrome in adult rats on a high-fat diet. Endocrinology [Internet]. 2011 Aug [cited 2014 Sep 26];152(8):3049–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21586551

29. Desvergne B, Feige JN, Casals-Casas C. PPAR-mediated activity of phthalates: A link to the obesity epidemic? Mol Cell Endocrinol [Internet]. 2009 May 25 [cited 2014 Nov 18];304(1-2):43–8. Available from: http://www.sciencedirect.com/science/article/pii/S030372070900149X

30. Eveillard A, Lasserre F, de Tayrac M, Polizzi A, Claus S, Canlet C, et al. Identification of potential mechanisms of toxicity after di-(2-ethylhexyl)-phthalate (DEHP) adult exposure in the liver using a systems biology approach. Toxicol Appl Pharmacol [Internet]. 2009 May 1 [cited 2014 Oct 20];236(3):282–92. Available from: http://www.sciencedirect.com/science/article/pii/S0041008X09000751

31. Thayer KA, Heindel JJ, Bucher JR, Gallo MA. Role of environmental chemicals in diabetes and obesity: a National Toxicology Program workshop review. Environ Health Perspect [Internet]. 2012 Jun [cited 2014 Dec 5];120(6):779–89. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3385443&tool=pmcentrez&rendertype=abstract

32. Wang H, Zhou Y, Tang C, He Y, Wu J, Chen Y, et al. Urinary phthalate metabolites are associated with body mass index and waist circumference in Chinese school children. PLoS One [Internet]. 2013 Jan [cited 2014 Oct 13];8(2):e56800. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3577690&tool=pmcentrez&rendertype=abstract

33. Olsén L, Lind L, Lind PM. Associations between circulating levels of bisphenol A and phthalate metabolites and coronary risk in the elderly. Ecotoxicol Environ Saf [Internet]. 2012 Jun [cited 2014 Oct 20];80:179–83. Available from: http://www.sciencedirect.com/science/article/pii/S0147651312000632

34. National Research Council (US) Committee on the Health Risks of Phthalates. Phthalates and Cumulative Risk Assessment: The Tasks Ahead. [Internet]. National Academies Press; 2008. Available from: http://www.ncbi.nlm.nih.gov/books/NBK215044/

35. Institute NHL and B. What is Metabolic Syndrome [Internet]. Available from: http://www.nhlbi.nih.gov/health/health-topics/topics/ms/

36. Lind PM, Risérus U, Salihovic S, Bavel B van, Lind L. An environmental wide association study (EWAS) approach to the metabolic syndrome. Environ Int [Internet]. 2013 May [cited 2014 Oct 20];55:1–8. Available from: http://www.sciencedirect.com/science/article/pii/S0160412013000378

37. Dirtu AC, Geens T, Dirinck E, Malarvannan G, Neels H, Van Gaal L, et al. Phthalate metabolites in obese individuals undergoing weight loss: Urinary levels and estimation of the phthalates daily intake. Environ Int [Internet]. 2013 Sep [cited 2014 Oct 13];59:344–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23892227

38. Lind PM, Roos V, Rönn M, Johansson L, Ahlström H, Kullberg J, et al. Serum concentrations of phthalate metabolites are related to abdominal fat distribution two years later in elderly women. Environ Health [Internet]. 2012 Jan [cited 2014 Oct 23];11:21. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3379932&tool=pmcentrez&rendertype=abstract

39. Teitelbaum SL, Mervish N, Moshier EL, Vangeepuram N, Galvez MP, Calafat AM, et al. Associations between phthalate metabolite urinary concentrations and body size measures in New York City children. Environ Res [Internet]. 2012 Jan [cited 2014 Oct 23];112:186–93. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3267869&tool=pmcentrez&rendertype=abstract

40. Stahlhut RW, van Wijngaarden E, Dye TD, Cook S, Swan SH. Concentrations of urinary phthalate metabolites are associated with increased waist circumference and insulin resistance in adult U.S. males. Environ Health Perspect [Internet]. 2007 Jun [cited 2014 Oct 23];115(6):876–82. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1892109&tool=pmcentrez&rendertype=abstract

41. Hatch EE, Nelson JW, Stahlhut RW, Webster TF. Association of endocrine disruptors and obesity: perspectives from epidemiological studies. Int J Androl [Internet]. 2010 Apr [cited 2014 Oct 10];33(2):324–32. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3005328&tool=pmcentrez&rendertype=abstract

42. Huang T, Saxena AR, Isganaitis E, James-Todd T. Gender and racial/ethnic differences in the associations of urinary phthalate metabolites with markers of diabetes risk: National Health and Nutrition Examination Survey 2001-2008. Environ Health [Internet]. 2014 Jan [cited 2014 Oct 23];13(1):6. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3922428&tool=pmcentrez&rendertype=abstract

43. Kim JH, Park HY, Bae S, Lim Y-H, Hong Y-C. Diethylhexyl phthalates is associated with insulin resistance via oxidative stress in the elderly: a panel study. Wang M, editor. PLoS One [Internet]. Public Library of Science; 2013 Jan [cited 2014 Oct 19];8(8):e71392. Available from: http://dx.plos.org/10.1371/journal.pone.0071392

44. Svensson K, Hernández-Ramírez RU, Burguete-García A, Cebrián ME, Calafat AM, Needham LL, et al. Phthalate exposure associated with self-reported diabetes among Mexican women. Environ Res [Internet]. 2011 Aug [cited 2014 Oct 31];111(6):792–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21696718

45. Hong Y-C, Park E-Y, Park M-S, Ko JA, Oh S-Y, Kim H, et al. Community level exposure to chemicals and oxidative stress in adult population. Toxicol Lett [Internet]. 2009 Jan 30 [cited 2014 Oct 23];184(2):139–44. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19049859

46. Trasande L, Spanier AJ, Sathyanarayana S, Attina TM, Blustein J. Urinary phthalates and increased insulin resistance in adolescents. Pediatrics [Internet]. 2013 Sep [cited 2014 Oct 9];132(3):e646–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23958772

47. Lind PM, Zethelius B, Lind L. Circulating levels of phthalate metabolites are associated with prevalent diabetes in the elderly. Diabetes Care [Internet]. 2012 Jul [cited 2014 Oct 23];35(7):1519–24. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3379584&tool=pmcentrez&rendertype=abstract

48. Kuo C-C, Moon K, Thayer KA, Navas-Acien A. Environmental chemicals and type 2 diabetes: an updated systematic review of the epidemiologic evidence. Curr Diab Rep [Internet]. 2013 Dec [cited 2014 Oct 17];13(6):831–49. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24114039

49. Trasande L, Sathyanarayana S, Spanier AJ, Trachtman H, Attina TM, Urbina EM. Urinary phthalates are associated with higher blood pressure in childhood. J Pediatr [Internet]. 2013 Sep [cited 2014 Oct 30];163(3):747–53.e1. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4074773&tool=pmcentrez&rendertype=abstract

50. Wiberg B, Lind PM, Lind L. Serum levels of monobenzylphthalate (MBzP) is related to carotid atherosclerosis in the elderly. Environ Res [Internet]. 2014 Aug [cited 2014 Oct 8];133:348–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25036990

51. WHO | The top 10 causes of death [Internet]. World Health Organization; [cited 2014 Oct 21]. Available from: http://www.who.int/mediacentre/factsheets/fs310/en/

52. Wang Y, Beydoun MA, Liang L, Caballero B, Kumanyika SK. Will all Americans become overweight or obese? estimating the progression and cost of the US obesity epidemic. Obesity (Silver Spring) [Internet]. 2008 Oct [cited 2014 Oct 21];16(10):2323–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18719634

53. Grundy SM. Metabolic syndrome pandemic. Arteriosclerosis, Thrombosis, and Vascular Biology. 2008. p. 629–36.

54. Beltrán-Sánchez H, Harhay MO, Harhay MM, McElligott S. Prevalence and trends of metabolic syndrome in the adult U.S. population, 1999-2010. J Am Coll Cardiol [Internet]. Journal of the American College of Cardiology; 2013 Aug 20 [cited 2014 Oct 18];62(8):697–703. Available from: http://content.onlinejacc.org/article.aspx?articleid=1709463

55. Portha B, Fournier A, Kioon MDA, Mezger V, Movassat J. Early environmental factors, alteration of epigenetic marks and metabolic disease susceptibility. Biochimie [Internet]. 2014 Feb [cited 2014 Sep 24];97:1–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24139903

56. NHANES - About the National Health and Nutrition Examination Survey. [cited 2014 Oct 31]; Available from: http://www.cdc.gov/nchs/nhanes/about_nhanes.htm

57. Cline RE, Hill RH, Phillips DL, Needham LL. Pentachlorophenol measurements in body fluids of people in log homes and workplaces. Arch Environ Contam Toxicol [Internet]. [cited 2015 Apr 28];18(4):475–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2774665

58. Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect [Internet]. 2005 Feb [cited 2015 Mar 23];113(2):192–200. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1277864&tool=pmcentrez&rendertype=abstract

59. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Final Report. Circulation [Internet]. 2002 Dec 17 [cited 2014 Oct 27];106(25):3143 – . Available from: http://circ.ahajournals.org/cgi/content/long/106/25/3143

60. Johnson C, Paulose-Ram R, Ogden C. National Health and Nutrition Survey: Analytic Guidelines, 1999-2010. 2013.

61. Ferguson KK, Loch-Caruso R, Meeker JD. Urinary phthalate metabolites in relation to biomarkers of inflammation and oxidative stress: NHANES 1999-2006. Environ Res [Internet]. 2011 Jul [cited 2015 Apr 28];111(5):718–26. Available from: http://www.sciencedirect.com/science/article/pii/S0013935111000570

62. Bray GA, Bellanger T. Epidemiology, trends, and morbidities of obesity and the metabolic syndrome. Endocrine [Internet]. 2006 Feb [cited 2015 Apr 28];29(1):109–17. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16622298

63. G P, T H, M Y, S Z, P W, H T, et al. Decreased serum free testosterone in workers exposed to high levels of di-n-butyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP): a cross-sectional study in China. [Internet]. Environmental health perspectives. 2006 [cited 2015 Apr 28]. p. 1643–8. Available from: http://europepmc.org/articles/PMC1665432

64. Barber TM, McCarthy MI, Wass JAH, Franks S. Obesity and polycystic ovary syndrome. Clin Endocrinol (Oxf) [Internet]. 2006 Aug [cited 2015 Feb 18];65(2):137–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16886951

65. Hoppin JA, Brock JW, Davis BJ, Baird DD. Reproducibility of urinary phthalate metabolites in first morning urine samples. Environ Health Perspect [Internet]. 2002 May [cited 2015 Apr 28];110(5):515–8. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1240840&tool=pmcentrez&rendertype=abstract

66. Mes J, Coffin DE, Campbell DS. Di-n-butyl-and Di-2-ethylhexyl phthalate in human adipose tissue. Bull Environ Contam Toxicol [Internet]. 1974 Dec [cited 2015 Apr 28];12(6):721–5. Available from: http://link.springer.com/10.1007/BF01685921

67. Lorber M, Angerer J, Koch HM. A simple pharmacokinetic model to characterize exposure of Americans to di-2-ethylhexyl phthalate. J Expo Sci Environ Epidemiol [Internet]. Nature Publishing Group; 2010 Jan 7 [cited 2015 Apr 28];20(1):38–53. Available from: http://dx.doi.org/10.1038/jes.2008.74