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A novel method for combining phthalates in a cumulative framework: Implications for exposure disparities and intervention opportunities

Author

Varshavsky, Julia Rachel

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A novel method for combining phthalates in a cumulative framework: Implications for exposure disparities and intervention opportunities

By

Julia Rachel Varshavsky

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Committee in charge:

Professor Rachel Morello-Frosch, Chair
Professor S. Katharine Hammond
Professor Tracey J. Woodruff
Professor Tyrone B. Hayes

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Abstract

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We are exposed to multiple chemicals throughout our lives from the air we breathe, the water we drink, the food we eat, the products we use, and the social context in which we live. These factors are interconnected and each affects our vulnerability and resilience in the face of cumulative exposures and their potential health implications. Yet we have little guidance on how to capture and quantify multiple chemicals in a construct that is truly relevant for human health.

In this dissertation, I address one piece of this complex picture by advancing a method that combines endocrine-disrupting chemicals into a cumulative framework for use in exposure science, risk assessment, and epidemiology research. The metric relies on benchmark doses and recommendations set forth by a 2008 National Academy of Sciences (NAS) report, *Phthalates and Cumulative Risk Assessment: The Tasks Ahead*.[1]

In Chapter 1, I describe the historical backdrop of cumulative methodologies in the United States, introduce the concept of endocrine-disrupting chemicals and anti-androgenic phthalates, and describe why they are suitable candidates for cumulative assessment. I develop a potency-weighted cumulative metric for phthalates exposure in Chapter 2, providing rationale, sample calculations, and an evaluation of the method's limitations by comparing the metric across racial/ethnic groups of U.S. reproductive-aged women. Chapter 3 includes another use of the metric to characterize phthalates exposure in a small cohort of Vietnamese immigrant nail salon workers. In Chapter 4, I use the metric to investigate dietary sources of cumulative phthalates exposure in U.S. children, adolescents, and adults; namely, by comparing consumption of food prepared away from home (i.e. dining out) to eating food prepared at home (i.e. purchased at a store). Chapter 5 concludes the dissertation by reflecting on the strengths and challenges of the cumulative exposure metric, providing recommendations for how to resolve some of its limitations and apply the method in future work.

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Chapter 1

Cumulative approach to characterizing endocrine-disrupting phthalate exposures

1.1 Conceptual overview of the cumulative approach

Traditional biomonitoring and health risk studies take a chemical-by-chemical approach in which hazards, exposures, and risk are evaluated one compound at a time. Realistically, humans are simultaneously exposed to multiple chemicals from diverse sources on a daily basis.[2–5] Therefore, it has become increasingly apparent over the last 30 years that advanced methodologies need to consider chemical combinations.[6] In 1986, the U.S. Environmental Protection Agency (EPA) released its first conceptual guidance document on the need to address the human health implications of exposure to chemical mixtures.[7] Since then, the initial conceptual ideas outlined by the EPA have evolved into several more concrete cumulative methodologies.[8,9,10,11]

The National Academy of Sciences (NAS) also supports moving from single-compound to chemical mixture assessments. It recommended the general paradigm shift in its 2008 report, *Phthalates and Cumulative Risk Assessment: The Tasks Ahead*,[1] which provides guidance for a cumulative approach to phthalates and other anti-androgens in particular. The report highlighted a variety of androgen-mediated mechanisms and additive effects of phthalates and other anti-androgens on male reproductive health. The authors stressed that phthalates and other androgen-disrupting agents should be combined under a dose-addition framework based on their collective ability to cause common adverse outcomes rather than on traditional criteria that requires common mechanism of action. This approach, they argued, is more "physiologically relevant" for overall human health risk.

This dissertation uses recommendations set forth by the NAS to combine phthalates in a potency-weighted cumulative exposure metric and subsequently applies the method by examining disparities in, and sources of, cumulative phthalates exposure. The findings presented here can be used by exposure scientists, risk assessors, epidemiologists, public health decision-makers, and environmental health and justice advocates to advance our collective understanding of cumulative methodologies, biologically-relevant exposures, associated health risks, and intervention opportunities for reducing cumulative phthalates exposure among vulnerable populations, as well as the broader U.S. population.

1.2 Phthalate properties, uses, and exposure

Phthalates are a class of 20 man-made hormonally-active chemicals that are produced in over 470 million pounds per year in the United States.[12] Globally, phthalates constitute 84% (or 6 million metric tons) of the plasticizer market.[13] High molecular weight (MW) phthalates, such as di(2-ethylhexyl) phthalate (DEHP) and di-isononyl phthalate (DiNP), are typically used to soften and impart resilience to polyvinyl chloride (PVC) plastic, for example, in children's toys, food packaging, construction materials, automobile interiors, and medical equipment.[3,5,14] Lower MW phthalates, such as diethyl phthalate (DEP) and di-*n*-butyl phthalate (DnBP), are more generally

used as solvents in personal care products, such as fragrances and cosmetics, but are also used in a wide variety of other products, including timed-release pharmaceuticals, lacquers, varnishes, and others.[5,14]

Phthalates, or phthalate acid esters, are diesters of benzenedicarboxylic acid that consist of a benzene ring and two ester side chains that vary in length, structure, and biologic activity. High MW phthalates tend to have longer side chains, longer half-lives, increased lipophilicity, decreased volatility, and increased toxicity.[14] Despite their relatively low volatility, phthalates are not chemically bound to plastic polymers, and can thus leach, migrate, and off-gas from products relatively easily, depending on the lipophilicity of the medium they encounter, the temperature to which they are exposed, and whether a solvent is present.[5,14] Their semi-volatile properties and short environmental half-lives (on the order of days at most) make them more difficult to track than highly persistent compounds in environmental media, such as soil, water, and air.[15–18] Indeed, they are not as commonly considered in environmental science methods that rely on traditional fate and transport modeling techniques, for example in life cycle assessments that are used to quantify and compare the cradle-to-grave energy, carbon, and pollution footprints of various materials and products (i.e. phthalates and non-phthalate plasticizer alternatives).[15–17]

The primary route of phthalate exposure is thought to be ingestion (predominantly through food and to a limited extent dust), but inhalation, dermal absorption, and intravenous intake (i.e. through medical tubing) also contribute to exposures.[5] Phthalates are absorbed and metabolized rapidly in the body and are efficiently transformed into hydrolyzed monoesters often before they reach the liver, where they are further hydrolyzed and/or oxidized through hepatic metabolism before they either travel to various tissues/organs throughout the body or are excreted directly into urine and feces.[1,14] Biomonitoring studies typically measure urinary metabolites because they are considered to be the biologically active form for phthalate toxicity. Measuring urinary metabolites also reduces potential contamination from parent compounds that are widely prevalent in indoor environments. Secondary oxidized metabolites are even less prone than primary hydrolyzed monoesters to laboratory contamination since they can be formed *in vivo* solely from hepatic metabolism.[1,14] The general process for phthalate metabolism is thought to be similar among humans and across species, but the rate of each metabolic step varies within and between species.[1,14] While researchers have developed several multi-compartmental physiologically-based pharmacokinetic (PBPK) models of phthalate absorption, distribution, metabolism, and elimination from animal studies,[19,20] many studies enlist simplified two-compartment models that compare administered parent compound doses with urinary metabolite concentrations in humans after a given time period (i.e. 24 hours).[21–23]

Although phthalates are not persistent in the environment or in peoples' bodies, with metabolic half-lives ranging in hours, studies indicate that human exposure is ubiquitous and continuous.[24–26] Consequently, multiple phthalate metabolites have been detected in the majority of the U.S. population, with nine or more metabolites measured simultaneously in 95% of U.S. pregnant women.[27] This is of particular concern for women of reproductive age since phthalates can cross the placenta during fetal development and are present in amniotic fluid and breast milk.[1]

1.3 Endocrine-disrupting effects

Phthalates are endocrine disrupting chemicals (EDCs) linked to a variety of adverse hormone-mediated effects across the life course, including pregnancy complications, preterm birth, reproductive tract malformations, neurodevelopmental problems, childhood asthma and allergies, metabolic disease, and breast and prostate cancers.[28–35] The U.S. economic burden of EDCs overall was recently estimated at more than $300 billion dollars, with phthalates alone contributing $56 billion to the total disease cost. Phthalate-attributable disorders included obesity, diabetes, infertility, and endometriosis, in addition to cardiovascular mortality associated with reduced testosterone levels.[36]

Since the endocrine system coordinates and controls numerous essential biological processes, including reproduction, growth/development, and metabolism, phthalates and other EDCs can impact lifelong health in numerous and complex ways.[32,37] EDCs can interfere with hormone synthesis, transport, target receptor action, and degradation across multiple tissues and organs. They can bind and activate receptors directly (agonists), block receptors from endogenous hormones (antagonists), and/or disrupt hormone production, metabolism, and elimination.[38] They can disrupt enzyme and hormone binding protein activities that support hormone regulation.[37] Hormone function is further complicated by multiple brain-organ signaling axes that interact with one another through multidimensional patterns.[38] For example, the hypothalamic-pituitary-gonadal (HPG) axis regulates reproduction and development by releasing luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the brain that travel to reproductive organs and stimulate androgen (i.e. testosterone) and estrogen production, respectively.[38] Depending on biological demand during reproduction, development, or aging, testosterone may further convert to estrogen to ensure an adequate supply. When sufficient levels are reached, estrogen directly suppresses its own production by signaling the brain to stop releasing FSH. However, it can also indirectly equalize itself through LH signal inhibition, which effectively reduces testosterone synthesis and subsequent conversion to estrogen. Endogenous estrogens can also inhibit thyroid hormone production at various points along the hypothalamic-pituitary-thyroid (HPT) axis, which can subsequently activate or suppress other endocrine axes that may further influence either or both the HPG and HPT.[32,38]

These interconnected pathways are sufficiently induced or suppressed by very low hormone levels, explaining why many EDCs are characterized by non-monotonic dose-response curves in which biological response varies with dose in a non-linear fashion.[39] Consequently, lower doses can produce greater (or different) effects than moderate or high doses.[37,39] Timing of exposure is a critical factor for low-dose endocrine-disrupting effects, since hormone levels and activity change across the life course, depending on developmental stage and concurrent physiological processes.[37] Fetal and early life are typically considered the most sensitive periods for EDC toxicity,[38] and subtle perturbations during key windows of fetal development can lead to overt adverse outcomes downstream.[40] For example, thyroxine (T4) and triiodothyronine (T3) are thyroid hormones that play key roles in metabolism and other vital functions throughout the life cycle, but they are especially critical for fetal brain development during the first trimester of pregnancy.[32,40] The HPT axis regulates T4 and T3 through a negative feedback system such that when levels decline, the pituitary gland secretes thyroid-stimulating hormone (TSH), which then stimulates the thyroid gland to secrete T4 and T3. While persistent T4 deficiencies during

pregnancy (i.e. clinical hypothyroidism) can lead to severe neurological problems such as mental retardation in children, modest T4 deficits (subclinical hypothyroidism) can also have lasting impacts on the brain, such as reduced Intelligence Quotient (IQ) scores or learning and developmental disorders.[32,40] In this context, HPT disruptions are upstream biomarkers of neurodevelopmental effects.[40]

Recent epidemiological studies on prenatal EDC exposures and neurodevelopment have associated phthalates with adverse cognitive and behavioral outcomes in children, such as impaired executive functioning and social awareness, particularly among boys.[31,35,41–43] Animal studies further demonstrate that phthalates can decrease T4 and T3 levels in rodents through multiple molecular and cellular mechanisms at each HPT axis level (hypothalamus, pituitary, and thyroid gland), including hormone biosynthesis, signaling, receptor activity, and clearance.[44–48] Human data is more ambiguous, though DEHP has been consistently associated with modest T4 reductions in multiple studies. Only one of these, however, included pregnant women (N = 76).[49–53] A consensus statement released by the Endocrine Society in 2015 called for more research regarding HPT effects from phthalates and other EDCs, due to their potential impacts on fetal brain development.[32]

Phthalates may also increase metabolic disease susceptibility later in life by promoting adipogenesis (fat cell differentiation) and hindering the body's ability to regulate lipids and sugars.[30,54] While they can reduce androgen and thyroid hormone levels associated with increased fat mass and distribution,[55–57] their affinity for binding the peroxisome proliferator-activated receptor (PPAR-γ) has gained more research interest in recent years. The PPAR-γ plays a central role in lipid metabolism, fat cell storage, and glucose regulation.[58,59] Pre- and post-natal phthalate activation of PPAR-γ causes adipogenesis, obesity, and other metabolic endpoints in animals.[58–66] In humans, early life and adult exposures are associated with increased body mass and insulin resistance among children and adults.[67–76] Associations with prenatal urinary metabolite concentrations are less consistent, with reports of both increased and decreased body mass among children,[67,68,77] but observed increases in placental PPAR-γ gene expression[78] indicate the potential for fetal programming of metabolic disease in humans.

Perhaps most notably, fetal exposure to certain anti-androgenic phthalates has been linked to a group of male reproductive outcomes known as the "phthalate syndrome" in animals, which includes birth defects of the penis (i.e. hypospadias), undescended testes (cryptorchidism), reduced anogenital distance (AGD), and infertility, among others.[1] An analogous syndrome has been proposed in humans, called the "testicular dysgenesis" or "androgen-insufficiency" syndrome, which is thought to consist of overlapping endpoints in addition to testicular cancer.[79,80] Rat models provide strong evidence that disruptions in androgen production and activity during a specific stage of male testis differentiation can lead to phthalate syndrome outcomes.[1] In humans, breast milk exposures can alter reproductive hormone levels in male babies,[81] and phthalates have been associated with reduced testosterone in men, women, and children.[82] Adult exposures are also associated with infertility and lower sperm production and quality.[83–86] Regarding prenatal exposures, several observational studies have associated urinary metabolite concentrations with genital malformations in male babies, such as reduced penis length and AGD.[87–92] Increased risks from co-exposures compared to individual phthalate exposures were reported in a subset of these studies.[87,88]

Although phthalates and other androgen-disrupting agents exert their toxic effects on male development through multiple mechanisms of action (i.e. by inhibiting testosterone synthesis or blocking insulin-like factor production, etc.), some of which are unclear, they are known to influence multiple androgen-mediated processes that give rise to common phthalate syndrome outcomes.[1,93] Moreover, phthalates and other anti-androgens can produce additive effects on male reproductive development when combined in laboratory studies, even when compounds in the anti-androgenic mixture are present at individually ineffective doses.[1,93]

Accordingly, the 2008 NAS report proposed phthalates and other anti-androgens for cumulative assessment under the dose addition principle, which was a novel approach given that dose addition traditionally assumes common mechanisms of action and/or similar chemical structures (with an additional assumption that structural similarities imply common mechanistic activity).[1,93] For example, polychlorinated dibenzo-p-dioxins (PCDDs) and other dioxin-like chemicals constitute a large group of persistent organic pollutants that can additively induce toxic effects, including birth defects, immunosuppression, and cancer, by competitively binding the aryl hydrocarbon (AhR) receptor. Thus, they are typically aggregated by their relative potencies for the AhR, which are known as toxic equivalence factors (TEF).[94] Other chemical families, such as polycyclic aromatic hydrocarbons (PAHs) and certain organophosphate pesticides, have also been combined based on common mechanistic activity, and the U.S. EPA specifically outlines this inclusion criteria in current guidance documents on cumulative assessment under the dose addition framework.[1,95,96]

Whereas dose addition involves the summation of chemical doses or concentrations weighted by their relative potencies, the response addition framework aggregates chemicals based on effect. Therefore, it is thought to be better suited for compounds with diverse toxicity mechanisms.[93] This "independent action" model precludes chemicals from cumulative assessment that do not individually induce effects (i.e. the dose present is below the individual chemical's "no observable effect" dose).[1,93] However, because certain anti-androgens collectively produce phthalate syndrome effects at individually ineffective doses, the response addition model may falsely exclude these chemicals at low doses, which in turn would underestimate overall risk.[1,93] Thus, NAS report authors argued for broader and more scientifically appropriate dose addition criteria regarding phthalates and other androgen-disrupting agents for which laboratory studies have empirically demonstrated their dose-additive properties.[1]

They further derived benchmark doses (BMDs) for five phthalates from a toxicological modeling study that predicted dose-additive effects based on individual phthalate dose response data.[1,97] Howdeshell et al. (2008) constructed a phthalates mixture by combining individual phthalates based on their ED50s (effective dose that results in a 50% reduction in fetal testosterone).[97] The NAS BMDs were estimated from these data by performing regression analyses on dose response curves for individual phthalates and extrapolating to lower doses at which no effect was observed for individual phthalates but was observed for the phthalates mixture. Though fetal testosterone production served as the singular endpoint for BMD estimates, the underlying dose response data used to construct the mixture have since been shown to accurately predict anti-androgenic mixture effects for a broader range of postnatal phthalate syndrome outcomes, including reduced anogenital distance and other reproductive malformations.[93,98,99] As such, the NAS-derived

BMDs are used in this dissertation to construct relative potency factors (RPFs) for phthalates combined in a biologically-weighted exposure metric based on common adverse outcomes rather than common mechanisms of toxicity.

A strong case can be made for aggregating EDCs based on risk of common adverse outcomes because the endocrine system's complexity makes it difficult to fully understand the array of mechanisms by which phthalates and other EDCs may act on the system. For example, while some anti-androgens can disrupt testosterone production and/or block androgens from binding to their receptors, consequently inhibiting biological "masculinization" during key developmental time points, it is conceivable they also act through estrogenic mechanisms that encourage "feminization" leading to similar physiological effects. Multiple phthalates promote estrogenic activity in laboratory and human studies,[91,100–106] which in turn can inhibit LH activity along the HPG axis.[38] Thus, it is plausible that phthalates may decrease androgen levels during critical stages of development by both their anti-androgenic and estrogenic properties. However, while parent compounds can be estrogenic, phthalate metabolites may not bind as readily to estrogen receptors.[107] Nevertheless, consideration of phthalates and other EDCs in the context of common adverse outcomes is warranted given the intricate nature of the endocrine system and our incomplete knowledge of the toxicological processes that involve EDCs.

1.4 Public and regulatory action

Though phthalates and other EDCs can impact development, they are not always considered or adequately evaluated in risk assessment frameworks that inform U.S. chemical policy, in part because traditional approaches rely on monotonic or linear dose-response relationships and clear disease endpoints such as cancer,[108] but also because federal regulation is relatively limited in the United States. The Endocrine Disruptor Screening Program was created in 1996 to identify high priority chemicals likely to cause adverse endocrine-disrupting effects in humans, but the program was never adequately funded or supported.[109] More recent efforts to advance EDC risk assessment have focused on standardizing systematic review methods that incorporate endocrinology principles into traditional toxicology frameworks.[108,109] Additionally, the U.S. EPA developed several management plans recently that address EDCs and other chemicals of concern, targeting phthalates as high priority with the release of the Phthalates Action Plan in 2009, the announcement of an Alternatives Assessment in 2011, and the addition of phthalates to the Toxic Substances Control Act (TSCA) Work Plan for risk evaluation, based on their high hazard and widespread exposure profiles.[12,110,111]

However, while the agency was granted authority to broadly regulate the chemical industry under TSCA in 1976, its power has been hindered by treatment of chemicals under the law as "innocent until proven guilty." TSCA established few industry requirements for pre-market testing to demonstrate chemical safety, yet placed several unrealistic expectations on the EPA to demonstrate known risk without adequate information or resources to conduct a sufficient toxicity review. The EPA's burden of proof was further challenged by the assessment of chemicals already circulating in commerce,[112] which conceivably might dissuade some chemical companies from cooperating with the review process, meanwhile increasing the potential for widespread exposure to potentially harmful compounds among the U.S. population. Recent TSCA reforms under the 2016 Chemical Safety for the 21st Century Act were expected to moderately improve the agency's ability to regulate hazardous chemicals and protect vulnerable

groups, such as pregnant women and disadvantaged communities. However, numerous loopholes that reflect industry interests combined with a lack of funding will likely hinder this newfound authority.[113] Additionally, with no requirement to consider endocrine-disrupting effects in toxicity assays, EDCs are not adequately addressed under the new chemical reform bill.[36]

Consequently, there is little to no toxicity information available for most of the tens of thousands of chemicals that have been registered for commercial use over the last 70-80 years,[114] many of which are high production volume chemicals like phthalates that are produced or imported at over one million pounds each year.[115] The vast majority of chemicals used in personal care products also have not been assessed for safety,[116] though the U.S. Food and Drug Administration (FDA) is responsible for regulating cosmetics, in addition to medical devices and certain foods. However, the agency also lacks the authority to require pre-market safety testing or full ingredient disclosure.[116,117] The FDA cites low exposure levels and a lack of human health data in determining that phthalates do not pose a significant safety risk,[117] though it recommends limiting phthalate exposures in the most sensitive populations (i.e. male newborns requiring medical intervention).[118]

To address heightened public health concerns over ineffective U.S. chemical policy, advocacy groups have engaged consumers in public campaigns over the last decade to urge large companies and local decision-makers to act on EDCs and other high profile chemicals. Several phthalates are currently included on the California EPA's *Proposition 65* list as reproductive toxicants.[119] Additionally, some states, including California, Washington, and Vermont, have restricted the sale and distribution of children's products containing phthalates, such as plastic toys.[120–122] While the federal government also took measures in 2008 to limit phthalates in children's toys,[123] the U.S. Product Safety Commission released a recent report recommending further protections than the law currently provides.[124]

Many companies appear to be shifting towards "greener" products in recent years, though a lack of transparency among U.S. industries makes this information difficult to ascertain.[125] Biomonitoring assessments of urinary metabolite concentrations among the U.S. population over the last decade have reported notable decreases in phthalates under scrutiny (i.e. DEHP, butyl-benzyl phthalate (BBzP), and DnBP) and marked increases in emerging phthalates, such as DiNP and di-isodecyl phthalate (DiDP).[3] Likewise, several studies have reported reductions in well-known phthalates, such as DEHP and DnBP, among common retail products sampled over time,[126–129] and evidence also suggests that less-studied phthalates of concern may be acting as replacements. For example, a recent analysis found higher DiBP than DnBP levels in nail polish, though DnBP is an established ingredient that is more commonly associated with nail polish.[130] Of the 200 consumer and personal care products they tested, Dodson et al. (2012) detected DiNP in products marketed as safer alternatives but not in those characterized as conventional.[130]

Although less potent than DEHP, DiNP also has an anti-androgenic toxicity profile and is considered a chemical of concern by governmental agencies worldwide.[13,124] Thus, replacing DEHP with DiNP may be leading to a "regrettable substitution" that contributes to risk rather than to less toxic alternatives.[3] As newer anti-androgenic phthalates replace those that are better characterized, a cumulative approach to monitoring and assessing risk becomes more important,

since tracking one compound at a time may ultimately underestimate overall risk to human health.

1.5 Environmental health disparities in vulnerable populations

Disadvantaged communities may be particularly vulnerable to cumulative chemical exposures from bearing disproportionate burdens of exposure in addition to experiencing non-chemical stressors that may influence health, including social and economic stress.[131] The U.S EPA recently outlined several key goals to assure the safety of chemicals and promote sustainability and human health in environmental justice communities,[132–134] releasing the Chemical Safety for Sustainability (CSS) research framework[135] and Plan EJ 2014.[136] The CSS framework discussed a strategy for designing and managing chemicals to promote public health, the environment, the economy, and the sustainability of future generations, while plan EJ 2014 outlined the need to protect health and the environment by engaging disadvantaged populations in community-research partnerships.[135,136]

Despite recent intentions to address chemical impacts among vulnerable populations, studies assessing social disparities in exposure and health risks are currently lacking, especially regarding chemical mixtures. Some research indicates that certain demographic groups have higher exposures to individual phthalate metabolites. For example, higher DEHP metabolites have been reported in children, and low socioeconomic status (SES) has been associated with elevated levels of DnBP and BBzP metabolites[137,138] and decreased levels of DEHP metabolites.[138] Biomonitoring studies have also shown that non-Hispanic black women have higher urinary levels of DEP and DnBP metabolites than their non-Hispanic white and Mexican American counterparts.[137–140] Some of these disparities may be related to structural racism associated with targeted marketing of personal care products like hair straighteners and feminine hygiene products to women of color.[140–142] For example, historical perceptions about body odor and targeted marketing have shaped vaginal douching preferences among U.S. reproductive-aged black women in the United States, which may contribute to higher DEP metabolite concentrations observed in this population[140,143]

The developmental effects of phthalate exposures may be of particular concern for reproductive-aged women who are also burdened by other social or environmental factors, such as poverty, social stress, and/or occupational exposures. For example, several studies have reported stronger effects of chemical exposures on low birth weight among black women compared to Hispanic or white women.[131,144,145] Environmental chemicals encompass only one set of factors that potentially influences health disparities across racial and ethnic groups, but studying them is important for identifying sensitive populations and creating effective intervention strategies. Additional research is needed to examine the extent to which certain demographic groups may experience higher phthalate exposures, especially to multiple phthalates simultaneously, and potentially higher susceptibility to adverse developmental effects from prenatal exposures.

1.6 Research objectives and chapter overview

In the following chapters, I construct and evaluate a cumulative exposure metric for anti-androgenic phthalates based on their relative abilities to cause common adverse outcomes related

to reduced fetal testosterone production. I apply the method to studies of exposure disparities and intervention opportunities among several populations in the United States.

In *Chapter 2*, I outline the cumulative methodology, providing background and rationale for the selection of phthalates, an explanation of how relative potency factors were constructed, and sample calculations for researchers who would like to use the method in their work. I apply the method to an examination of potential exposure disparities among U.S. reproductive-aged women using 2001-12 National Health and Nutrition Examination Survey (NHANES) data (N = 2842). Findings from multivariate linear regression models are highlighted, along with results from sensitivity analyses evaluating the metric's strengths and limitations.

In *Chapter 3*, I characterize individual urinary metabolite concentrations and cumulative phthalates daily intake in a pilot biomonitoring analysis of 17 Vietnamese nail salon workers in California, describing the 2011 data collection process, subsequent validated laboratory analysis, and statistical testing that I performed to compare exposures between nail salon workers and 2011-12 NHANES Asian Americans (n = 203).

In *Chapter 4*, I apply the method to identify dietary intake sources of phthalates exposure among age-specific subgroups in the United States, including children, adolescents, and adults, using 2005-14 NHANES data (N = 10,253). I describe results from multivariate regression models that estimated adjusted associations between consuming food prepared away from home (dining out), as opposed to eating food prepared at home only, and cumulative phthalates exposure.

In *Chapter 5*, I conclude with a discussion of the implications of my research from previous chapters, summarizing major findings, limitations, and future research needs.

Chapter 2

A novel method for calculating potency-weighted cumulative phthalates exposure with implications for identifying racial/ethnic disparities among U.S. reproductive-aged women[a]

2.1 Abstract

Phthalates are ubiquitous chemicals linked to hormonal disruptions that affect reproduction and development. Multiple anti-androgenic phthalates exposure during fetal development can have greater impacts than individual exposure; thus, the National Academy of Sciences (NAS) recommends them for cumulative assessment. Using National Health and Nutrition Examination Survey data (NHANES, 2001-12), I developed a potency-weighted sum of daily intake (∑androgen-disruptor; μg/kg/day) of di-*n*-butyl phthalate (DnBP), di-isobutyl phthalate (DiBP), butyl benzyl phthalate (BBzP), and di(2-ethylhexyl) phthalate (DEHP) based on NAS recommendations, and included diethyl phthalate (DEP) and di-isononyl phthalate (DiNP) in additional metrics (2005-12). Racial/ethnic differences in ∑androgen-disruptor were compared among 2842 reproductive-aged women. Sensitivity analyses were performed to assess the influence of potency assumptions, alternate urine dilution correction methods, and weighting phthalate metabolites directly rather than daily intake estimates of parent compounds. DEHP contributed most to ∑androgen-disruptor (48-64%), and ∑androgen-disruptor decreased over time. Black women generally had higher cumulative exposures than white women, although the magnitude and precision of the difference varied by model specification. This approach provides a blueprint for combining chemical exposures linked to common adverse outcomes, and should be considered in future exposure, risk, and epidemiological studies.

2.2 Background

Phthalates esters are hormonally active chemicals linked to a wide range of health outcomes. Fetal phthalate exposures cause a group of male reproductive problems known as the "phthalate syndrome" in animals, which includes birth defects of the testes and penis (e.g. cryptorchidism, hypospadias), and infertility.[1] Human studies support an association between developmental phthalate exposures and male reproductive effects.[87–90,146] Certain phthalates, such as DnBP, DiBP, BBzP, DEHP, DiNP, exert toxicity primarily through androgen disruption.[1]

Phthalate exposures are ubiquitous due to their widespread use in myriad consumer and personal care products.[33,139] Biomonitoring studies show the U.S. population is exposed to multiple phthalates simultaneously.[26,27,147,148] Animal studies demonstrate that phthalate mixtures pose higher male reproductive risk than individual phthalate exposures, especially during fetal development. Human studies also find higher risks from multiple compared to singular phthalate

[a] Portions of this chapter were published in Environmental Science and Technology as Varshavsky JR, Zota AR, and Woodruff TJ, "A Novel Method for Calculating Potency-Weighted Cumulative Phthalates Exposure with Implications for Identifying Racial/Ethnic Disparities among U.S. Reproductive-Aged Women in NHANES 2001-2002" *Environ Sci Technol* 50(19):10616-10624. doi: 10.1021/acs.est.6b00522

exposures.[88,90] In 2008, the NAS recommended phthalates and other anti-androgens for cumulative risk assessment based on their collective ability to cause common adverse outcomes (i.e. phthalate syndrome endpoints associated with decreased testosterone during critical windows of fetal sex differentiation) rather than shared mechanisms of action (i.e. how testosterone concentration is disrupted).[1] More recent mixture studies confirm NAS findings and further highlight the need to consider the joint effects of co-occurring phthalates.[98,99,149,150] However, advancements in the epidemiology of phthalate mixtures are limited by current methods for characterizing cumulative exposures.[151]

Identifying high-risk subpopulations for cumulative exposure warrants examination since individual phthalate exposure profiles may not accurately represent the distribution of overall phthalates burden. Individual phthalate exposures have been shown to vary by race/ethnicity and socioeconomic status (SES), often in opposing directions.[104,111–114] For example, DnBP and BBzP are higher in low SES groups, while DEHP exposure is lower in the same subpopulation.[137,138] Characterizing cumulative exposure inequalities may help elucidate health disparities and identify solutions that better protect those at increased risk of exposure and disease.[131]

In this chapter, I develop a potency-weighted metric of androgen-disrupting phthalates using 2008 NAS recommendations and examine demographic differences in cumulative phthalates exposure among U.S. reproductive-aged women. I focus on women of reproductive age because *in utero* development has been identified as a critical window for phthalate toxicity,[1] and previous work suggests that phthalate exposures among reproductive-aged and pregnant women are similar[27] but often distinct from other subpopulations.[153]

2.3 Methods

2.3.1 Study population

I pooled six cycles of data between 2001 and 2012 from NHANES, a nationally representative survey and physical examination of the civilian, non-institutionalized U.S. population that is administered by the U.S. Centers for Disease Control (CDC) to monitor health and nutrition over time in two-year cycles (http://www.cdc.gov/nchs/nhanes.htm). The study population was limited to females aged 15-44 years (n = 3480). Of these, 544 women were excluded from the analysis who self-identified as Asian American, other, or multi-racial, since the former category was not specified prior to 2011, and the latter category is not clearly defined. In contrast, women were included in the study population if they self-identified as non-Hispanic white, non-Hispanic black, or Mexican American, who together comprised 86% of the total population. Participants without at least one phthalate metabolite measurement were also excluded from the analysis (n = 94), resulting in a final sample size of 2842 participants.

2.3.2 Phthalate metabolite measurements

In each NHANES survey cycle, phthalate metabolites are measured in one-third of study participants. Analytical methods are described elsewhere.[154] Briefly, spot urine samples are collected as part of the NHANES medical examination and analyzed at the CDC's National

Center for Environmental Health (Atlanta, GA). Phthalate metabolites are quantified using high performance liquid chromatography coupled with tandem mass spectrometry.[154,155] I downloaded laboratory from the NHANES website in March 2015 and included necessary impurity corrections for some previously used analytical standards.[156] Because phthalate metabolites and detection limits can vary by survey cycle, I standardized the limit of detection for each phthalate metabolite across cycles by selecting the maximum (LOD_{max}) for each metabolite and substituting concentrations below that with a value equal to the LOD_{max} divided by √2 (**Table 2-1**).[3,26] Detection limits by NHANES survey cycle are provided in the Appendix (**Table A-1**).

Table 2-1 Percent below maximum limit of detection *(μg/L)* and substitution with $LOD_{max}/\sqrt{2}$ in NHANES 2001-12

Phthalate metabolites	LOD_{max}	$LOD_{max}/\sqrt{2}$	% <LOD_{max}
Mono-*n*-butyl phthalate (MnBP)	1.13	0.80	2.1
Mono-isobutyl phthalate (MiBP)	0.98	0.69	8.9
Monobenzyl phthalate (MBzP)	0.30	0.21	1.6
Mono(2-ethylhexyl) phthalate (MEHP)	1.20	0.85	28
Mono(2-ethyl-5-carboxypentyl) phthalate (MECPP)	0.60	0.42	0.1
Mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)	0.98	0.69	1.8
Mono(2-ethyl-5-oxohexyl) phthalate (MEOHP)	1.13	0.80	3.6
Monoethyl phthalate (MEP)	0.60	0.42	0.1
Mono(carboxy-isooctyl) phthalate (MCOP)	0.70	0.49	2.9

2.3.3 Cumulative exposure metric

To characterize cumulative exposure to androgen-disrupting phthalates, I calculated a potency-weighted sum (∑androgen-disruptor) of four phthalates (DnBP, DiBP, BBzP, and DEHP) that are recognized as anti-androgenic by the NAS and whose metabolites were measured in every cycle between 2001-12 (**Figure 2-1**; Model 1). The primary hydrolytic metabolites for DnBP, DiBP, BBzP, and DEHP, respectively, are mono-n-butyl phthalate (MnBP), mono-isobutyl phthalate (MiBP), monobenzyl phthalate (MBzP), and mono(2-ethylhexyl) phthalate (MEHP). MEHP further metabolizes to the following secondary oxidative metabolites: mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and mono(2-ethyl-5-carboxypentyl) phthalate (MECPP).

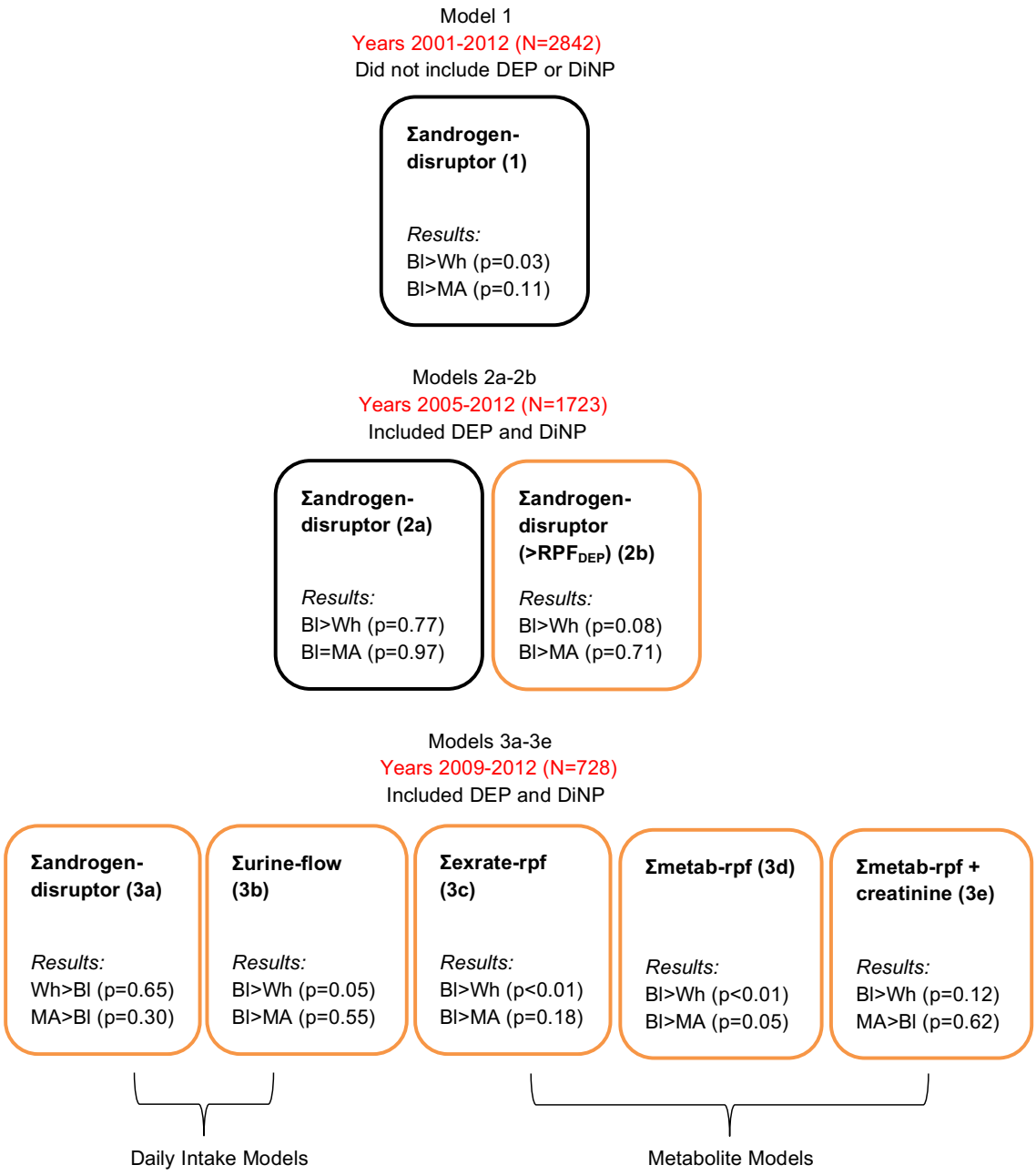

Figure 2-1 Overview of cumulative phthalates exposure models evaluated in Chapter 2. Primary analysis included Models 1 and 2a (2001−12 NHANES data). Model 1 included DnBP, DiBP, BBzP, and DEHP. Model 2a included two additional phthalates (DEP and DiNP), but fewer years (2005−12). Model 2b represents Model 2a with increased DEP potency, and Models 3a−3e represent urine dilution and daily intake estimation supplementary analyses (2009−12 data). Boxes include summarized results for adjusted percent difference across race/ethnicity (*p*-values). Black and orange colors indicate primary and secondary analyses, respectively. Potency-weighted daily intake estimates using creatinine excretion rate and urine flow rate are represented by ∑androgen-disruptor and ∑urine-flow, respectively; ∑exrate-rpf and ∑metab-rpf

represent RPF-weighted metabolite excretion rates and measured metabolites, respectively. Bl = Black; Wh = White; MA = Mexican American.

To calculate unitless relative potency factors (RPFs) for the four phthalates, NAS phthalate-specific benchmark doses (BMDs) (mg/kg/day), which are statistically derived doses related to a predefined change from controls in benchmark response (BMR) (in this case a 5% reduction in testosterone concentration, or a BMR equal to 5%) (**Table 2-2**).[1] BMDs were used rather than the lower limit of a one-sided 95% confidence interval (CI) on the BMD (BMDL) to facilitate comparison across chemicals.[157] Of the NAS phthalates included in this analysis, DnBP had the highest potency, or lowest BMD. Therefore, RPFs were calculated by dividing the DnBP reference BMD by that of each phthalate.

$$RPF_i = (BMD_{Reference} / BMD_i);\ i = individual\ phthalate \quad \textbf{(Eq. 2-1)}$$

Table 2-2 Estimated NAS benchmark doses (BMDs) and lower limit of one-sided 95% CI (BMDLs) associated with 5% reductions in testosterone concentration

Phthalate	**BMD** *mg/kg/day*	**BMDL** *mg/kg/day*
Di-*n*-butyl phthalate (DnBP)	30	20
Di-isobutyl phthalate (DiBP)	126	47
Butyl benzyl phthalate (BBzP)	116	66
Di(2-ethylhexyl) phthalate (DEHP)	49	31

Because BMDs are based on phthalate doses administered to laboratory animals and not metabolite concentrations, daily intake of parent phthalate compounds (μg/kg/day) were estimated from measured urinary metabolites (ng/mL = μg/L) using a pharmacokinetic modeling equation adapted from previous studies.[22,23]

$$Daily\ Intake_i = \frac{(ME_i \times CE) \times (MW_p)}{(F_{UE,i} \times 1000) \times (MW_m)} \quad \textbf{(Eq. 2-2)}$$

where ME_i is the urinary concentration of metabolite per gram of creatinine (μg/g) for each phthalate, CE is the creatinine excretion rate normalized by body weight (mg/kg/day), $F_{UE,i}$ is the molar fraction of urinary excreted metabolite related to parent compound for each phthalate (unitless), 1000 is a conversion factor (i.e. mg/g), and MW_p and MW_m are the molecular weights of the parent phthalates and metabolites, respectively. Although creatinine excretion rates vary across racial/ethnic groups, a uniform creatinine excretion rate of 18 mg/kg/day was initially assumed for all participants.[21] Creatinine-corrected concentrations (ME) were calculated for each participant by dividing their measured urinary concentrations (μg/L) by their measured urinary creatinine concentration (g/L). Previous human studies determined fractional urinary excretion values (F_{UE}) by comparing urinary metabolite concentrations with ingested parent compounds over a 24-hour period.[124,158,159] The values for MnBP/DnBP and MBzP/BBzP were 0.69 and 0.73, respectively, and DiBP metabolism was assumed to be similar to MnBP/DnBP (F_{UE} = 0.69). The values for MEHP, MEHHP, and MEOHP were 0.062, 0.149, and 0.109, respectively. I did not include MECPP in DEHP daily intake estimation for 2001-12 analyses because it was

not measured in all survey cycles, but I included MECPP in 2005-12 models with an F_{UE} of 0.132.[159] All F_{UE} values are listed in the Appendix (**Table A-1**).

A potency-weighted sum of phthalate compounds (∑androgen-disruptor, expressed in μg/kg/day) was then calculated by summing the products of RPFs and daily intake estimates for each phthalate.

$$\sum androgen\text{-}disruptor = \sum (Daily\ Intake_i \times RPF_i) \quad \textbf{(Eq. 2-3)}$$

In addition to the original metric (**Figure 2-1**; Model 1), I calculated a second version of ∑androgen-disruptor by adding DEP and DiNP to model 1 (**Figure 2-1**; Model 2a). Several epidemiologic studies have associated MEP with male reproductive health endpoints, [88,90,146] though findings are inconsistent and DEP does not exhibit anti-androgenic properties in laboratory studies.[124] To acknowledge the possibility that MEP might have anti-androgenic properties in humans while recognizing the absence of evidence in animals, I included DEP in model 2a with an assumed low but non-zero potency equal to one order of magnitude less than the least potent phthalate in the model (RPF = 1/10 multiplied by 0.24, or 0.024). Metabolism of DEP's primary metabolite, monoethyl phthalate (MEP), was assumed to be similar to that of DnBP (F_{UE} = 0.69).[22,124] DiNP was also added in model 2a since it has been recognized as anti-androgenic, though it is less potent than DEHP.[1,124] Because DiNP's primary metabolite, mono-isononyl phthalate (MiNP), was below the detection limit for most NHANES samples, the secondary metabolite, mono(carboxy-isooctyl) phthalate (MCOP), was the only measured metabolite used to estimate daily intake for DiNP.[124] Model 2a was restricted to 2005-12 survey cycles because MCOP was not measured before 2005 (N = 1723). DiNP was assumed to be 2.3 times less potent than DEHP, resulting in an RPF of 0.61/2.3 = 0.26.[124,150] An F_{UE} value of 0.099 was used to estimate DiNP from MCOP.[124,159]

A hypothetical female participant (SEQN = 1) has the following measured urinary metabolite concentrations (ng/mL = µg/L) and measured urinary creatinine concentration (g/L).

SEQN	MnBP	MiBP	MBzP	MEHP	MEHHP	MEOHP	Creatinine
1	34.4	11.7	15.192	5.6	88.5	58.4	2.65

1) **Calculate relative potency factors (RPF) using benchmark doses (BMD) (Eq. 2-1).** See Table 2-2 for BMDs.

$RPF_i = (BMD_{Reference} / BMD_i)$; i = individual phthalate;
Reference BMD = highest potency, or lowest BMD = DnBP BMD = 30 mg/kg/day

$RPF_{DnBP} = 30/30 = 1.00$
$RPF_{DiBP} = 30/126 = 0.24$
$RPF_{BBzP} = 30/116 = 0.26$
$RPF_{DEHP} = 30/49 = 0.61$

2) **Calculate daily intake estimates (Eq. 2-2).** Essentially, Eq. 2-2 calculates X moles of metabolite excreted per day using ME, CE, and MW_m; then uses F_{UE} to estimate the moles of parent compound intake required to excrete X moles of metabolite per day; and lastly, converts parent compound intake per day from moles to mass using MW_p. Values for F_{UE} are provided in the Appendix (**Table A-1**).

$$Daily\ Intake_i = \frac{(ME_i \times CE) \times (MW_p)}{(F_{UE,i} \times 1000) \times (MW_m)}$$

ME_i = Urinary concentration of metabolite per gram of creatinine (µg/g).
CE = Creatinine excretion rate normalized by body weight (mg/kg/day).
$F_{UE,i}$ = Molar fraction of urinary excreted metabolite related to parent compound (unitless).
1000 = Conversion factor (i.e. mg/g).
MW_p and MW_m = Molecular weights of parent phthalates and metabolites.

$$DnBP = \frac{((34.4\ \mu g/L) / (2.65\ g/L)) \times (18\ mg/kg/day) \times (278\ g/mol)}{(0.69 \times 1000\ mg/g) \times (222\ g/mol)} = 0.424\ \mu g/kg/day$$

$$DiBP = \frac{((11.7\ \mu g/L) / (2.65\ g/L)) \times (18\ mg/kg/day) \times (278\ g/mol)}{(0.69 \times 1000\ mg/g) \times (222\ g/mol)} = 0.144\ \mu g/kg/day$$

$$BBzP = \frac{((15.192\ \mu g/L) / (2.65\ g/L)) \times (18\ mg/kg/day) \times (312\ g/mol)}{(0.73 \times 1000\ mg/g) \times (256\ g/mol)} = 0.172\ \mu g/kg/day$$

Calculate metabolite molar sum for DEHP estimate (if including MECPP, $F_{UE} = 0.452$):

ΣDEHP metabolites = (MEHP/mw + MEHHP/mw + MEOHP/mw) = ((5.6 µg/L)/(278 g/mol)) + ((88.5 µg/L)/(294 g/mol)) + ((58.4 µg/L)/(292 g/mol)) = 0.521 (µg/L)(mol/g) = 0.521 µmol/L

$$DEHP = \frac{((0.521\ \mu g\text{-}mol/g\text{-}L)/(2.65\ g/L)) \times (18\ mg/kg/day) \times (390\ g/mol)}{(0.320 \times 1000\ mg/g)} = 4.31\ \mu g/kg/day$$

3) Calculate Σandrogen-disruptor (Eq. 2-3).

$\sum androgen\text{-}disruptor = \sum(Daily\ Intake_i \times RPF_i)]$

Σandrogen-disruptor = (DnBP x 1.00) + (DiBP x 0.24) + (BBzP x 0.26) + (DEHP x 0.61) = (0.424 x 1.00) + (0.144 x 0.24) + (0.172 x 0.26) + (4.31 x 0.61) = 3.13 µg/kg/day

4) Calculate daily intake for Σurine-flow metric (Eq. 2-4).

If NHANES reports a urine flow rate (UFR) of 0.415 mL/min and body weight of 72.3 kg, first estimate daily UFR and calculate UE (UFR normalized by body weight):

UFR = (0.415 mL/min) x (60 min/hr) x (24 hr/day) = 597.6 mL/day
UE = (597.6 mL/day) / (72.3 kg) = 8.27 mL/kg/day

$$Daily\ Intake_i = \frac{(UME_i \times UE) \times (MW_p)}{(F_{UE,i} \times 1000) \times (MW_m)}$$

UME_i = *Urinary metabolite concentration (µg/L).*

$$DnBP = \frac{(34.4\ \mu g/L) \times (8.27\ mL/kg/day) \times (278\ g/mol)}{(0.69 \times 1000\ mL/L) \times (222\ g/mol)} = 0.516\ \mu g/kg/day$$

$$DiBP = \frac{(11.7\ \mu g/L) \times (8.27\ mL/kg/day) \times (278\ g/mol)}{(0.69 \times 1000\ mL/L) \times (222\ g/mol)} = 0.176\ \mu g/kg/day$$

$$BBzP = \frac{(15.192\ \mu g/L) \times (8.27\ mL/kg/day) \times (312\ g/mol)}{(0.73 \times 1000\ mL/L) \times (256\ g/mol)} = 0.210\ \mu g/kg/day$$

Calculate metabolite molar sum for DEHP estimate (if including MECPP, Fue = 0.452):

ΣDEHP metabolites = (MEHP/mw + MEHHP/mw + MEOHP/mw) = ((5.6 µg/L)/(278 g/mol)) + ((88.5 µg/L)/(294 g/mol)) + ((58.4 µg/L)/(292 g/mol)) = 0.521 (µg/L)(mol/g) = 0.521 µmol/L

$$DEHP = \frac{((0.521\ \mu g\text{-}mol/g\text{-}L) \times (8.27\ mL/kg/day) \times (390\ g/mol)}{(0.320 \times 1000\ mL/L)} = 5.25\ \mu g/kg/day$$

Then Eq. 2-3 becomes $\sum urine\text{-}flow = \sum(Daily\ Intake_i \times RPF_i)]$

Σurine-flow = (DnBP x 1.00) + (DiBP x 0.24) + (BBzP x 0.26) + (DEHP x 0.61) = (0.516 x 1.00) + (0.176 x 0.24) + (0.210 x 0.26) + (5.25 x 0.61) = 3.82 µg/kg/day

Figure 2-2 Sample calculations for a hypothetical female with given measured urinary phthalate metabolite and creatinine concentrations.

2.3.4 Statistical analysis

Statistical analyses were conducted using SAS software, Version 9.4 (SAS Institute Inc., Cary, NC). Since multiple cycles of data were combined, new sample population weights were calculated according to NHANES analytical guidelines.[160] All analyses adjusted for sample population weights and the NHANES clustered sample design. Statistical significance was defined at $p < 0.05$ on two-sided tests, and $p < 0.10$ was considered to be marginally significant. Concentration of ∑androgen-disruptor was log-transformed to account for its non-normal distribution. Descriptive statistics were calculated for Σandrogen-disruptor and individual phthalate daily intake estimates, including geometric mean (GM), geometric standard error (GSE), range, and 25th, 50th, 75th, and 95th percentiles, in addition to the GM contribution of each phthalate to the cumulative metric.

Analysis of variance and linear regression were used to examine bivariate associations between ∑androgen-disruptor and socio-demographic, temporal, and biological variables. Socio-demographic characteristics included: Age (continuous), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American), educational attainment (less than high school diploma; high school graduate; or post-high school education, with women under 20 years old treated the same as women aged 20 and above), household income (below or above poverty level as defined by poverty to income ratio, or PIR), and body mass index (BMI) [underweight (< 18.5 kg/m^2), normal weight (18.5 – 25 kg/m^2), overweight (25 – 30 kg/m^2), and obese (≥ 30 kg/m^2)]. The NHANES survey cycle was used as a proxy for time. Sampling session (morning, afternoon, or evening) was included as a proxy variable for time of sample collection to help adjust for fasting time. Lastly, the percent of total DEHP metabolites excreted as the primary metabolite (MEHP%) was also considered. Hauser et al. (2006) have suggested that higher MEHP% may reflect less complete DEHP metabolism or excretion.[83] Consequently, higher MEHP% would indicate greater susceptibility to DEHP exposure.

The core multivariate regression models included the main effect of interest (race/ethnicity), in addition to educational attainment, PIR, age, and BMI. Other covariates were included if they were significant predictors of the outcome in bivariate analyses or if their inclusion changed the effect estimate for race/ethnicity by more than 20%. Statistical interaction was also tested between race/ethnicity and survey cycle, with survey cycle dichotomized as < 2005 and ≥ 2005.

2.3.5 Sensitivity analyses

Three sensitivity analyses were conducted in this chapter. First, the influence of DEP on the metric (**Figure 2-1**; Model 2b) was assessed by increasing its potency to the least potent phthalate in the model (RPF = 0.24). Second, 342 pregnant women were removed from multivariate models to evaluate their influence on the original analysis.

Third, the potential for exposure misclassification was examined by comparing Σandrogen-disruptor to several other cumulative metrics, which were constructed with alternate urine dilution correction and daily intake estimation methods. Urine flow rate (UFR) in mL/day was calculated for 2009-12 NHANES participants (N = 728) by dividing the volume of urine collected by self-reported amount of time since last void. UFR is a more direct method to correct

for urine dilution than creatinine, which is considered a surrogate for urine dilution.[161,162] An RPF-weighted sum (**Eq. 2-3**) was calculated based on UFR (Σurine-flow) by adapting **Eq. 2-2** for daily intake equation.

$$Daily\ Intake_i = \frac{(UME_i \times UE) \times (MW_p)}{(F_{UE,i} \times 1000) \times (MW_m)} \quad \textbf{(Eq. 2-4)}$$

where UME_i is the measured urinary concentration of metabolite (μg/L) for each phthalate, UE is the UFR normalized by body weight (mL/kg/day), and all other variables are the same as in **Eq. 2-2**. This approach is equivalent to calculating creatinine excretion rates for each study participant (urinary creatinine concentration multiplied by UE) in **Eq. 2-2**, rather than using a uniform value for all participants. UFR was then used to calculate metabolite excretion rate, or the amount of metabolite excreted per day (μg/day) by multiplying UME by UFR. Assuming that daily metabolite excretion is proportional to daily parent phthalate compound intake, **Eq. 2-3** was used to apply RPF weights directly to metabolite excretion rates (Σexrate-rpf). For comparability, potency weights were also applied directly to measured urinary metabolite concentrations (Σmetab-rpf) (μg/L). See **Figure 2-3** for a diagram of urine dilution correction and daily intake estimation methods and equations.

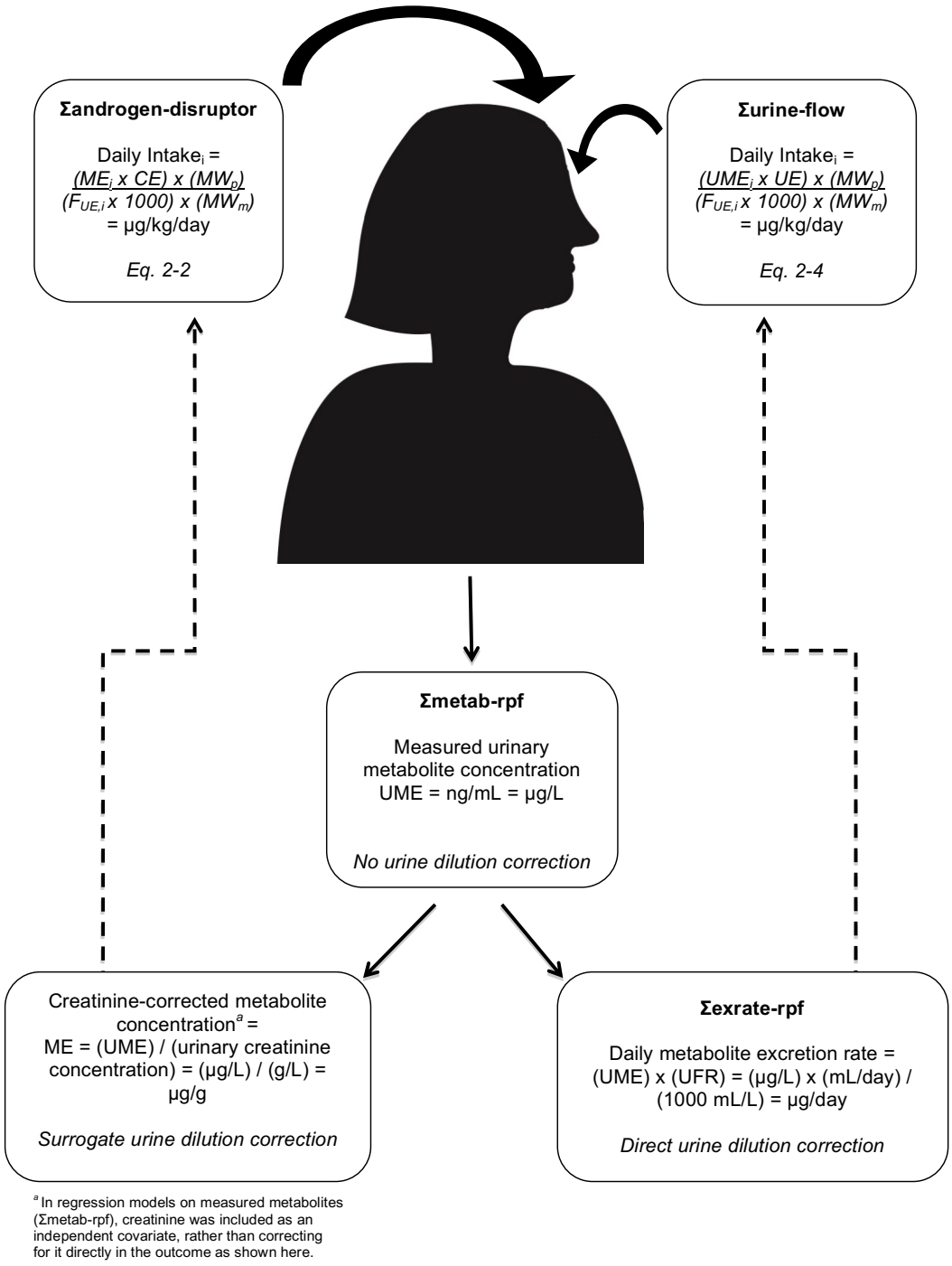

Figure 2-3 Urine dilution correction and daily intake method diagram and equations. Potency-weighted cumulative sums (**Eq. 2-3**) are bolded. Dotted arrows represent daily intake estimation from respective urine dilution-corrected metabolite measurements. ME_i = urinary concentration of metabolite per gram of creatinine (µg/g) for each phthalate, CE = creatinine excretion rate normalized by body weight (mg/kg/day), UME_i = measured urinary concentration of metabolite (µg/L) for each phthalate, UE = urine flow rate (UFR) normalized by body weight (mL/kg/day), $F_{UE,i}$ = molar fraction of urinary excreted metabolite related to parent compound for each phthalate, and MW_p and MW_m = molecular weights of parent phthalates and metabolites, respectively.

The resulting five cumulative metrics, including Σandrogen-disruptor, Σurine-flow, Σexrate-rpf, and Σmetab-rpf (with and without creatinine as an independent variable in the model as suggested by Barr et al. (2005)), were compared using Spearman's rank correlation (r_s) and multivariate regression models (**Figure 2-1**: Models 3a-3e).[161] Included in the correlation analysis were other cumulative phthalate metrics used in previous studies, including molar sums of low and high molecular weight phthalates (Σlow-mw and Σhigh-mw),[42,72,152,163–165] and three approaches implemented by the Chronic Hazard Advisory Panel (CHAP) on Phthalates and Phthalate Alternatives.[124] The CHAP conducted a cumulative risk assessment for DnBP, DiBP, BBzP, and DEHP based on elements of NAS report recommendations, namely by implementing a dose addition model for cumulative risk assessment known as the Hazard Index. Unlike the approach outlined in this chapter, in which BMDs from the NAS report were used directly as potency estimates, the CHAP determined three sets of anti-androgenic potency estimates (Case 1, Case 2, and Case 3) by calculating reference doses (RfDs) from a combination of uncertainty factors (UFs), NAS report BMDLs, no observed adverse effect levels (NOAELs), and lowest observed adverse effect levels (LOAELs). **Table 2-3** compares this study's approach to the three CHAP Case approaches.

To assess the influence of using CHAP's alternate potency estimates on the original cumulative exposure metric developed in this chapter (∑androgen-disruptor), which used creatinine excretion rate to estimate daily intake (**Eq. 2-2**) for DnBP, DiBP, BBzP, and DEHP (**Figure 2-1**; Model 1), **Eq. 2-1** was adapted to construct RPFs from the CHAP RfDs listed in **Table 2-3**. The reference RfD was selected as the lowest RfD, corresponding to highest potency.

$$RPF_{c,i} = (RfD_{Reference} / RfD_i);\ i = individual\ phthalate;\ c = chap\ case\ 1,\ 2,\ or\ 3 \quad \textbf{(Eq. 2-5)}$$

Next, potency-weighted cumulative metrics (μg/kg/day) for each CHAP Case ($\Sigma chap_1$, $\Sigma chap_2$, and $\Sigma chap_3$) were constructed by summing the products of newly calculated CHAP RPFs with previously calculated phthalate daily intake estimates (**Eq. 2-2)**.

$$\sum chap_c = \sum(Daily\ Intake_i \times RPF_{c,i}) \quad \textbf{(Eq. 2-6)}$$

Finally, $\Sigma chap_1$, $\Sigma chap_2$, and $\Sigma chap_3$ were compared to Σandrogen-disruptor as described using Spearman's correlation.

Table 2-3 Differences in relative potency determination between this study and the 2014 CHAP report[124]

	This study			CHAP Case 1			CHAP Case 2			CHAP Case 3		
Phthalate	**BMD** *mg/kg/d*	**RPF[a] Calc.**	**RPF**	**RfD[c]** *µg/kg/d*	**RPF[a] Calc.**	**RPF**	**RfD[b]** *µg/kg/d*	**RPF[a] Calc.**	**RPF**	**RfD[b]** *µg/kg/d*	**RPF[a] Calc.**	**RPF**
DnBP	30	30/30	1.00	100	30/100	0.30	50[c]	50/50	1.00	500	50/500	0.10
DiBP	126	30/126	0.24	200	30/200	0.15	50[c]	50/50	1.00	1250	50/1250	0.04
BBzP	116	30/116	0.26	330	30/330	0.09	50[c]	50/50	1.00	500	50/500	0.10
DEHP	49	30/49	0.61	30	30/30	1.00	50	50/50	1.00	50	50/50	1.00
DiNP	--	0.61/2.3	0.26	1500	30/1500	0.02	115	50/115	0.43	500	50/500	0.10
DEP[d]	--	--	0.024	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
RPF Rank	DnBP > DEHP > DiNP = BBzP > DiBP > DEP			DEHP > DnBP > DiBP > BBzP > DiNP			DnBP = DiBP = BBzP = DEHP > DiNP			DEHP > DnBP = BBzP = DiNP > DiBP		
Anti-androgenic endpoint	Testosterone production			Range of endpoints			Testosterone production			Range of endpoints		
Potency Determination	NAS report BMDs derived from Howdeshell et al. (2008)[1,97] toxicological mixture study, which found DnBP, DiBP, BBzP, and DEHP to have equivalent 50% effective dose (ED50) potencies. DiNP assumed to be 2.3 times less potent than DEHP based on Hannas et al. (2011).[150]			RfDs were calculated by dividing NAS report BMDLs (DnBP, DiBP, and BBzP)[1] and NOAELS/LOAELS (DEHP and DiNP) by UFs ranging from 100-500 based on study quality and *in-vivo* anti-androgenicity as determined by Kortenkamp and Faust (2010).[166]			RfDs calculated by dividing DEHP reference NOAEL of 5 mg/kg-d by UF of 100. DnBP, DiBP, and BBzP assumed to be equipotent to DEHP and DiNP assumed to be 2.3 times less potent based on Hannas et al. (2011).[150]			RfDs calculated by dividing NOAELs by UFs of 100 as determined in 2014 CHAP report.[124]		

RPF = Relative potency factor; BMD = Benchmark dose; Calc. = Calculation; BMDL = Lower confidence limit of benchmark dose; NOAEL = No observed adverse effect level; LOAEL = Lowest observed adverse effect level; RfD = Reference dose; UF = Uncertainty factor.

[a] RPFs were calculated using the highest potency BMD or RfD (the smallest BMD or RfD) divided by BMD or RfD of each individual phthalate in metric.

[b] BMDL/NOAEL/LOAEL units in mg/kg/day.

[c] Equivalent to CHAP Case 2 DEHP RfD.

[d] DEP relative potency selected arbitrarily as one order of magnitude lower than least potent phthalate in cumulative metric.

2.4 Results

In both primary cumulative exposure metrics (Models 1 and 2a), DEHP contributed the largest percentage (48% and 64%) to the cumulative androgen-disruption measure. The cumulative GM varied from 2.6 (GSE = 1.0, range = 0.18 - 520) to 4.2 (GSE = 1.0, range = 0.53 - 523) µg/kg/day, depending on whether DEP and DiNP were included in the metric (**Table 2-4)**. Among all phthalates, DEHP, DEP, and DiNP had the highest daily intakes (GM > 2.4 µg/kg/day) and DnBP, DiBP, and BBzP had the lowest (GM < 0.60 µg/kg/day) (**Table 2-5**).

Table 2-4 Univariate statistics for Σandrogen-disruptor *(µg/kg/day)* among reproductive-aged women in NHANES 2001-12 (*N* = 2842)

Σandrogen-disruptor	**N**	**GM (GSE)**	**Range**	**25th percentile**	**Median**	**75th percentile**	**95th percentile**
Model 1[a]	2842	2.6 (1.0)	0.18 - 520	1.4	2.3	4.1	14
Model 2a[b]	1723	4.2 (1.0)	0.53 - 523	2.3	3.8	6.8	20

[a] Model 1 included DnBP, DiBP, BBzP, and DEHP.
[b] Model 2a = Model 1 + DEP and DiNP and restricted to 2005-12 NHANES data.

Table 2-5 Individual phthalate daily intake and percent contribution to Σandrogen-disruptor *(µg/kg/day)* among U.S. reproductive-aged women in NHANES 2001-12 (*N* = 2842)

	2001-12 (N = 2842)		2005-12 (N = 1723)			
	Daily intake *µg/kg/day*		***Daily intake*** *µg/kg/day*		*GM % Contribution*	
Phthalates	**GM (GSE)**	**Range**	**GM (GSE)**	**Range**	**Model 1[a]**	**Model 2a[b]**
Di-*n*-butyl phthalate (DnBP)	0.60 (1.0)	0.02 - 520	0.54 (1.0)	0.04 - 520	29%	18%
Di-isobutyl phthalate (DiBP)	0.19 (1.0)	0.01 - 10	0.24 (1.0)	0.02 - 10	3%	2%
Butyl benzyl phthalate (BBzP)	0.26 (1.0)	0.01 - 14	0.22 (1.0)	0.01 - 8.9	4%	3%
Di(2-ethylhexyl) phthalate (DEHP)[c]	2.5 (1.0)	0.14 - 384	2.9 (1.0)	0.25 - 413	64%	48%
Diethyl phthalate (DEP)	--	--	2.5 (1.1)	0.10 - 1988	--	4%
Di-isononyl phthalate (DiNP)	--	--	2.4 (1.1)	0.11 - 158	--	25%

[a] Model 1 (*N* = 2842) included 2001-12 data.
[b] Model 2a (*N* = 1723) included 2005-12 data with DEP and DiNP.
[c] MECPP included in DEHP daily intake calculation for 2005-12 data.

Between 2001 and 2012, ∑androgen-disruptor decreased by 54% ($p < 0.0001$) (**Figure 2-4**). This downward trend over time in cumulative phthalates daily intake was evident for all race/ethnicities. Daily intake of DBnP, BBzP, DEHP, and DEP decreased by 40-70%, while DiBP and DiNP increased by 150-380% over the study period ($p < 0.0001$) (**Figure 2-5**). The relative ranking of racial/ethnic groups changed over time for most individual phthalates, although black women consistently had higher DEP exposures than white women. For ∑androgen-disruptor, there was evidence of multiplicative interaction between race/ethnicity and time ($p_{interaction} = 0.03$). Compared to 2001-04, phthalate concentrations decreased in later years for black and white women ($p < 0.0001$ and $p = 0.0004$, respectively); but not in Mexican American women ($p = 0.12$).

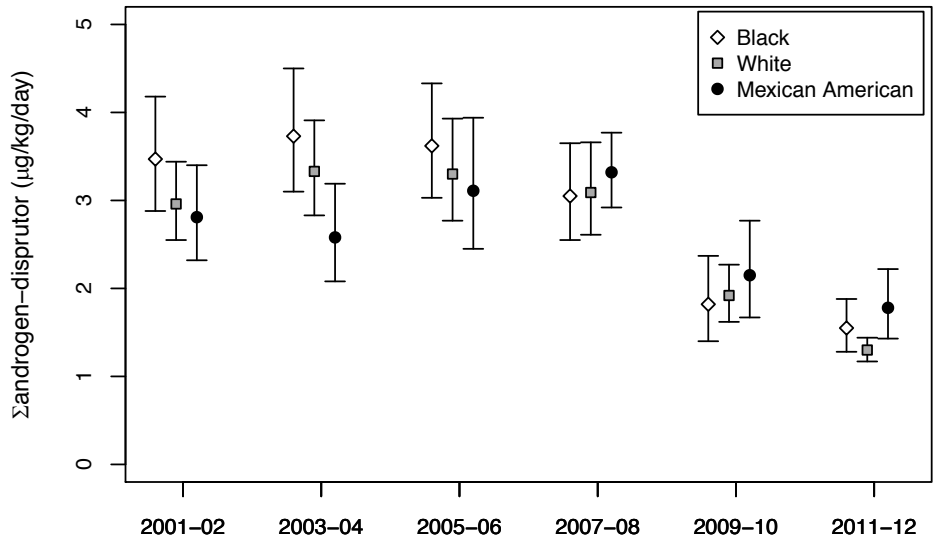

Figure 2-4 Unadjusted GM (95% CI) of cumulative phthalates daily intake (∑androgen-disruptor) over time by race/ethnicity among U.S. reproductive-aged women. The ∑androgen-disruptor metric included DnBP, DiBP, BBzP, and DEHP (Model1). Significant trends observed across NHANES survey cycles ($p < 0.0001$); n ranged from 56 to 262 (total $N = 2842$).

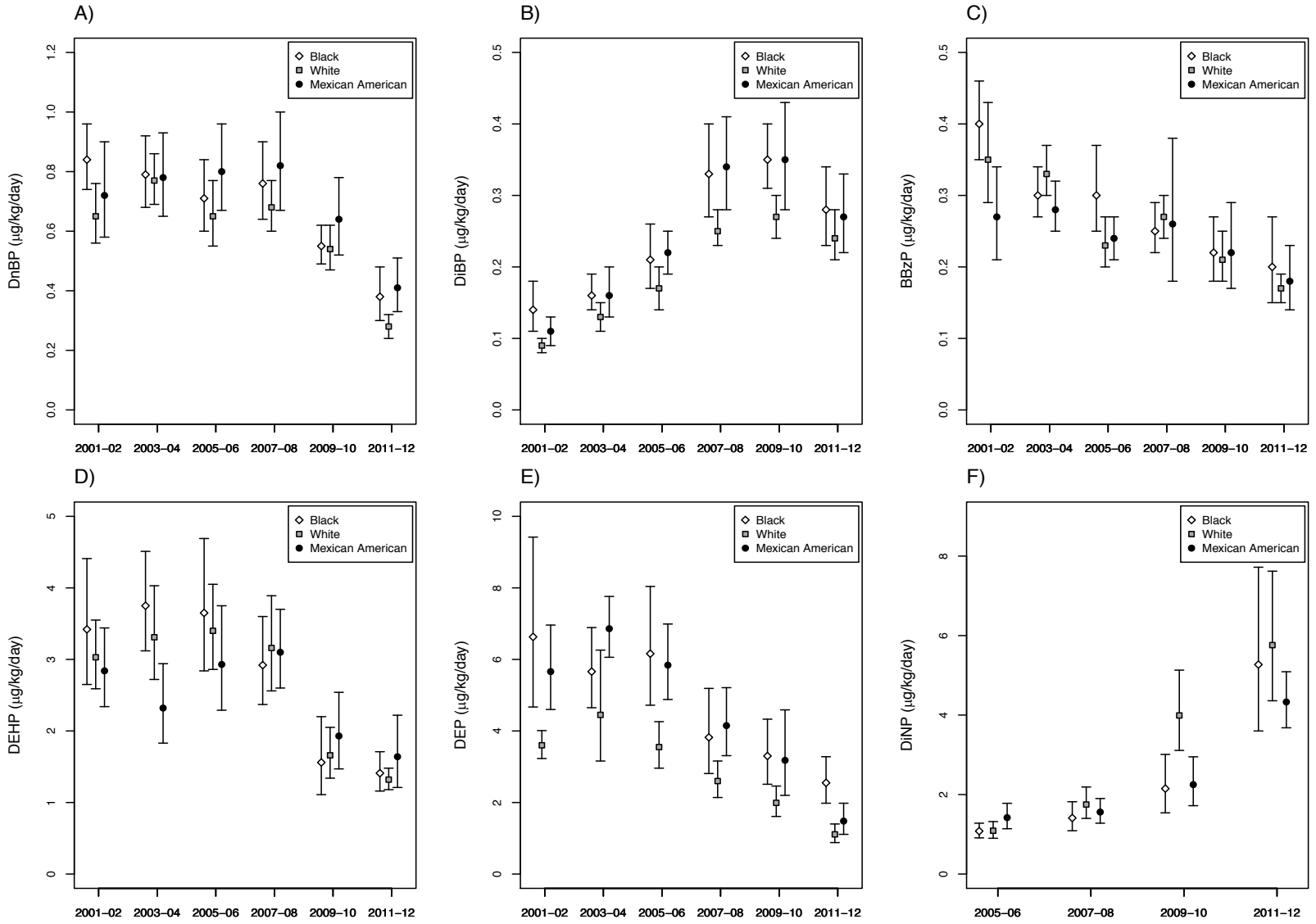

Figure 2-5 Unadjusted GM (95% CI) of individual phthalate daily intake over time by race/ethnicity among U.S. reproductive-aged women. Significant trends observed across NHANES survey cycles for each phthalate ($p < 0.0001$), n ranged from 56 to 262 (total $N = 2842$).

In 2001-12 adjusted models, 12% higher cumulative phthalates daily intake (∑androgen-disruptor) was observed in black women compared to white women ($p = 0.03$) (**Table 2-6** and **Figure 2-1**; Model 1). When the sample was restricted to 2005-12 and both DEP and DiNP were included in the metric, the percent difference in ∑androgen-disruptor between black and white women changed substantially and was no longer significant ($p = 0.77$) (**Figure 2-1**; Model 2a).

Table 2-6 Adjusted percent difference (% Δ) in Σandrogen-disruptor *(µg/kg/day)* across race/ethnicity[a] among reproductive-aged women in NHANES 2001-12 ($N = 2842$)

	Model 1[b] (N=2842)		*Model 2a[b] (N=1723)*		*Model 2b[b] (N=1723)*	
Race/ethnicity	**% Δ (95% CI)**	***p*-value**	**% Δ (95% CI)**	***p*-value**	**% Δ (95% CI)**	***p*-value**
Black[c]	--	--	--	--	--	--
White	-12% (-22, -1)	0.03	-2% (-16, 12)	0.77	-13% (-28, 2)	0.08
Mexican American	-9% (-21, 2)	0.11	0% (-14, 15)	0.97	-3% (-19, 13)	0.71

[a] Covariates: Age, BMI, MEHP%, education, poverty-to-income ratio (PIR), NHANES survey cycle, and time of sampling session.
[b] Model 1 included DnBP, DiBP, BBzP, and DEHP. Model 2a = Model 1 + DEP and DiNP and was restricted to 2005-12. Model 2b = Model 2a with increased DEP potency (RPF = 0.24).
[c] Reference group = Non-Hispanic black women.

When DEP's potency was raised to the least potent phthalate, the difference between black and white women increased from 2% in model 2a to 13% in model 2b and became marginally significant ($p = 0.77$ to $p = 0.08$, respectively) (**Table 2-6** and **Figure 2-1:** Models 2a and 2b). No difference was observed in any of the results when pregnant women were removed from the analysis and thus these data were not reported.

High correlation was observed between Σandrogen-disruptor and Σurine-flow (2009-12 data; **Table 2-7**: $r_s = 0.83$), and the metrics produced different but overlapping estimates for racial/ethnic disparities. There were no significant differences among racial/ethnic groups for Σandrogen-disruptor, whereas black women had 22% (95% CI: -45%, 0%) higher levels than white women in the Σurine-flow model (**Table 2-8** and **Figure 2-1**: Models 3a and 3b)

Table 2-7 Spearman's (r_s) correlation between cumulative exposure metrics[a] in NHANES 2009-12 (N = 728)

	Σandrogen-disruptor[b]	*Σurine-flow*[b,c]	*Σmetab-rpf*[d]	*Σexrate-rpf*[d]	*Σlow-mw*[e]	*Σhigh-mw*[f]	*Σchap$_1$*[g]	*Σchap$_2$*[g]	*Σchap$_3$*[g]
Σandrogen-disruptor[b]	-	**0.83**	**0.54**	**0.71**	**0.13**	**0.62**	**0.82**	**0.98**	**0.91**
Σurine-flow[b,c]	**0.83**	-	**0.56**	**0.86**	**0.18**	**0.62**	**0.64**	**0.80**	**0.73**
Σmetab-rpf[d]	**0.54**	**0.56**	-	**0.70**	**0.66**	**0.91**	**0.52**	**0.51**	**0.47**
Σexrate-rpf[d]	**0.71**	**0.86**	**0.70**	-	**0.38**	**0.65**	**0.66**	**0.67**	**0.64**
Σlow-mw[e]	**0.13**	**0.18**	**0.66**	**0.38**	-	**0.46**	0.09	0.06	0.02
Σhigh-mw[f]	**0.62**	**0.62**	**0.91**	**0.65**	**0.46**	-	**0.57**	**0.64**	**0.61**
Σchap$_1$[g]	**0.82**	**0.64**	**0.52**	**0.66**	0.09	**0.57**	-	**0.82**	**0.95**
Σchap$_2$[g]	**0.98**	**0.80**	**0.51**	**0.67**	0.06	**0.64**	**0.82**	-	**0.93**
Σchap$_3$[g]	**0.91**	**0.73**	**0.47**	**0.64**	0.02	**0.61**	**0.95**	**0.93**	-

[a] Log-transformed values used in correlation analysis. Significant correlations bolded.
[b] Σandrogen-disruptor and Σurine-flow included DnBP, DiBP, BBzP, DEHP, DEP, and DiNP. MECPP included in DEHP daily intake estimation.
[c] Σurine-flow restricted to n = 716 due to missing BMI data.
[d] Σmetab-rpf and Σexrate-rpf included MnBP, MiBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, MEP, and MCOP.
[e] Σlow-mw included MnBP, MiBP, and MEP.
[f] Σhigh-mw included MBzP, MEHP, MEOHP, MEHHP, MECPP, and MCOP.
[g] Σchap$_1$, Σchap$_2$, Σchap$_3$ included DnBP, DiBP, BBzP, DEHP, and DiNP. MECPP included in DEHP daily intake estimation.

Table 2-8 Adjusted percent difference (%Δ) in cumulative phthalates exposure using alternate urine dilution correction and daily intake estimation approaches[a] in NHANES 2009-12 (N = 728)

	Daily intake-based metrics				**Metabolite-based metrics**					
	Σandrogen-disruptor[b] *(μg/kg/day)*		***Σurine_flow***[b] *(μg/kg/day)*		***Σexrate_rpf***[c] *(μg/day)*		***Σmetab_rpf***[c] *Measured urinary metabolite concentration (μg/L)*			
	Daily intake with creatinine excretion rate		*Daily intake with urine flow rate*		*Metabolite excretion rate*		*No urine dilution correction*		*Creatinine-correction as independent covariate*	
	Model 3a		**Model 3b**		**Model 3c**		**Model 3d**		**Model 3e**	
Race/ethnicity	**% Δ (95% CI)**	***p***	**% Δ (95% CI)**	***p***	**% Δ (95% CI)**	***p***	**% Δ (95% CI)**	***p***	**% Δ (95% CI)**	***p***
Black[d]	--	--	--	--	--	--	--	--	--	--
White	4% (-14, 23)	0.65	-22% (45, 0)	0.05	-32% (-51, -13)	<0.01	-59% (-80, -38)	<.0001	-12% (-27, 3)	0.12
Mexican American	13% (-12, 37)	0.30	-9% (-37, 20)	0.55	-21% (-54, 11)	0.18	-30% (-61, 0)	0.05	6% (-19, 32)	0.62

[a] Models 3a-3e adjusted for age, BMI, MEHP%, education, poverty-to-income ratio (PIR), NHANES survey cycle, and time of sampling session. Model 3e additionally adjusted for log-transformed urinary creatinine concentration.
[b] Relative potency factors applied to daily intake of DnBP, DiBP, BBzP, DEHP, DEP, and DiNP. MECPP included in DEHP daily intake estimate.
[c] Relative potency factors applied to metabolites of MnBP, MiBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, MEP, and MCOP.
[d] Reference group = Non-Hispanic black women.

High correlations were observed between both potency-weighted daily intake metrics and the metric that weighted metabolite excretion rates by RPFs (Σexrate-rpf) (**Table 2-7**: r_s range = 0.7 to 0.9), and moderate correlations between daily intake metrics and RPF-weighted urinary phthalate metabolites (Σmetab-rpf) (r_s range = 0.5 to 0.6). Racial/ethnic differences were significant in regression models when the dependent variables were Σexrate-rpf and Σmetab-rpf without creatinine correction. Black women had 32% (95% CI: -51%, -13%) higher exposures than white women in the Σexrate-rpf model (**Table 2-8** and **Figure 2-1**: Model 3c). In the Σmetab-rpf model without creatinine correction, black women had 59% (95% CI: -80%, -38%) higher levels than white women, but the difference decreased to 12% (95% CI: -27%, 3%) when creatinine was added as an independent covariate (**Table 2-8** and **Figure 2-1**: Models 3d and 3e).

Correlation of Σandrogen-disruptor and Σurine-flow with low molecular weight metabolites (Σlow-mw) was low (**Table 2-7**: $r_s < 0.20$), but the daily intake metrics were more highly correlated with high molecular weight metabolites (Σhigh-mw) ($r_s = 0.62$). The ∑androgen-disruptor metric was highly correlated with CHAP metrics ($r_s > 0.80$), with CHAP Case 2 being the most highly correlated.

2.5 Discussion

In this chapter, I developed an approach for calculating potency-weighted cumulative exposure of co-occurring phthalates that contribute to common adverse outcomes using 2008 NAS recommendations and NHANES biomonitoring data. Several alternate methods were examined to assess the relative importance of potency estimates for individual phthalates (i.e. DEP) in addition to the impact of using alternate approaches for urine dilution correction and daily intake estimation. In this assessment of racial/ethnic disparities, black women generally had higher exposure to multiple androgen-disrupting phthalates than white women, although the magnitude and precision of the percent difference varied by model specification.

To my knowledge, this is the first assessment using NAS recommendations to profile racial/ethnic differences in cumulative anti-androgenic phthalates. When 2001-12 NHANES data was pooled, black women had 12% higher cumulative body burden concentrations of androgen-disrupting phthalates than white women, thereby increasing their potential risk of adverse androgen-dependent outcomes. However, cumulative phthalates daily intake were similar among racial/ethnic groups when the analysis was restricted to 2005-12 data. In other models, raising DEP's potency and using metabolite-based metrics generally increased aggregate exposure disparities between white and black women. For example, the difference was as high as 60% when measured metabolites were modeled without correcting for urine dilution.

Reasons for racial/ethnic differences in cumulative exposure might include variations in health and behavior patterns that contribute to phthalates exposure, such as personal care product use, dietary habits, and medication intake.[124,139,140,167–169] For example, vaginal douching and fast food consumption have both been shown to vary by race/ethnicity and are associated with higher phthalate body burden concentrations.[140,167] Observed racial/ethnic differences in creatinine excretion, notably that black women have higher creatinine levels than other racial/ethnic groups,[161] may be another factor in this difference. Biological differences in phthalate metabolism/excretion and urine dilution may also contribute to racial/ethnic differences in cumulative phthalates exposure.

The overall downward trend in cumulative phthalates daily intake among all racial/ethnic groups over time was consistent with temporal trends reported for the general U.S. population.[3] Zota et al. (2014) hypothesized that declines in DnBP, BBzP, DEHP, and DEP metabolites may be attributable to U.S. legislative activity enacted in 2008 to limit phthalates in children's products, including toys, in addition to public advocacy campaigns such as the Campaign for Safe Cosmetics.[3] The safe cosmetics campaign has targeted consumers, chemical companies, and product manufacturers over the last 15 years in a comprehensive effort to reduce harmful chemicals in personal care products.[3] Subsequent market shifts have been noted, with rapid growth reported for "green" personal care products in 2011 due to increased consumer demand.[125,127,128] Zota et al. (2014) further suggested the rise in DiBP and DiNP over time might reflect industry replacement strategies in response to public and regulatory pressure.[3] While some evidence suggests these compounds may be replacing traditional phthalates of concern in consumer and personal care products (i.e. higher DiBP than DnBP levels in nail polish and higher DiNP than DEHP levels in food contact materials),[130,167,170] quality U.S. data on product formulations and production rates is currently lacking.[3]

Although DEP's contribution to cumulative phthalates daily intake was minimal, inclusion of DEP nevertheless impacted racial/ethnic exposure disparities, in part due to its high exposure concentrations compared to other phthalates. When I increased DEP's potency in sensitivity models, the percent difference in cumulative exposure between white and black women increased because black women consistently had higher DEP exposures across the study period. The inclusion of DEP adds uncertainty to the cumulative exposure method since animal studies indicate that DEP is not anti-androgenic and human studies are equivocal.[88,90,124,146] However, NAS suggests that phthalates and other compounds be included in a cumulative assessment if there is reason to believe they may contribute to common adverse outcomes.[1] Furthermore, the toxicity of DEP in combination with other phthalates has not been sufficiently evaluated. Given this compound's widespread prevalence in humans, more research is needed to understand DEP's risk profile in mixtures and determine whether it should be included in a future cumulative exposure assessments.

The supplementary analysis evaluating alternate approaches to urine dilution correction and daily intake estimation revealed that regression modeling results vary with different approaches. Metabolite excretion rates and measured urine concentrations resulted in large and significant exposure disparities between white and black women, although including creatinine as a covariate in the latter model attenuated the difference. While cumulative daily intake that assumed a uniform creatinine excretion rate for the study population did not demonstrate a significant difference between racial/ethnic groups, urine flow rate correction (equivalent to using creatinine excretion rates for every participant) revealed a marginally significant aggregate exposure disparity between black and white women.

There is ongoing debate about the best approach for urine dilution correction in spot samples of non-persistent chemicals like phthalates. Several researchers suggest that urine flow rate is the best measure of urine dilution because it is a volume-based approach that directly calculates mass of metabolite excreted per day, which is assumed to be proportional to daily intake of parent phthalate.[161,162,171,172] Correction using creatinine or specific gravity, on the other hand, are considered imperfect proxies for urine dilution that rely on non-volume-based

assumptions.[161,162,171,172] For example, urinary metabolite concentration is assumed to be inversely proportion to urinary creatinine concentration, which varies with urine volume/dilution. Christensen et al. (2014) reported on phthalates specifically, demonstrating through simulation that metabolite excretion rates and urine metabolite concentrations resulted in the least biased associations between phthalate exposure and BMI, while creatinine correction and daily intake estimation resulted in the most biased associations.[171] This may be one reason why high correlation yet differing regression results were observed between daily intake estimates and metabolite excretion rates.

Inherent uncertainty exists when extrapolating from measured metabolites to daily intake of parent compounds. In particular, variability of F_{UE}, the molar amount of excreted metabolite relative to parent phthalate intake, can substantially impact regression results. For example, the percent contribution of DiNP to the cumulative metric becomes larger with daily intake calculation, due to a relatively small F_{UE}. On the other hand, the use of pharmacokinetic-based daily intake calculations is warranted since the proposed benchmark doses used to construct RPFs were derived from studies that administered parent compounds to animals.[1,97]

Ideally, I recommend constructing multiple cumulative metrics with alternate approaches for urine dilution and daily intake estimation, as demonstrated in this chapter, to compare the consistency of results across statistical models. However, many researchers may not have access to quality urine flow rate data, since at the very least, full urine void and time since last void are necessary to estimate daily excretion rates. In this case, researchers can either calculate daily intake using a uniform value for creatinine excretion rate or apply potency estimates to the metabolites directly and sum them. My sensitivity analysis positioned metabolite excretion rate modeling results between the more extreme (i.e. measured metabolites without urine dilution correction) and null (i.e. daily intakes, creatinine correction) disparity findings. However, a more rigorous analysis of potential error introduced by each approach is warranted in future studies. Additionally, future research should evaluate how this method might be applied to urine metabolite concentrations corrected with specific gravity, as many researchers prefer this approach to creatinine-correction, and these values are often reported in epidemiological studies that assess prenatal phthalate exposures and male reproductive outcomes.[90,146,173]

While there was high correlation between the original cumulative metric presented in this chapter and those of the CHAP, there were some differences. For one, DiNP potency varied between metrics. In this analysis, DiNP's relative potency was not based on NAS benchmark doses since these data were not available, but instead on findings from a separate study that assessed DiNP's potency with the same endpoint (fetal testosterone concentration).[150] CHAP Case 2 used an equivalent potency for DiNP and demonstrated the highest correlation with this study's metric. DiNP is one to two orders of magnitude less potent in CHAP Cases 1 and 3, based on older studies and other anti-androgenic endpoints,[124,166] which may be one possible reason for the lower correlation with my approach. All four approaches ranked DiNP as less potent than DEHP; however, other endpoints may be more sensitive to DiNP compared to DEHP. Thus, future research should include DiNP and use more recent potency estimates, such as the one assumed in this study.[150]

The CHAP approaches also differ in DEHP's potency determination. CHAP Cases 1 and 3 ranked DEHP as the most potent phthalate by at least one order of magnitude, while this study and CHAP Case 2 ranked DEHP relatively lower. One reason for this variation is that CHAP used a NOAEL for DEHP based on different anti-androgenic endpoints. Many problems have been identified with using NOAELs and LOAELs, including the values are influenced by experimental design.[1,157] Larger studies can result in lower NOAELs and LOAELs, and many studies comprise relatively few animals, which can decrease the statistical probability of finding effects at lower response levels (such as 1, 5, or 10%). Further, NOAELs and LOAELs may correspond to widely different response levels.[174] BMDs, on the other hand, provide more robust low dose extrapolations with consistent response levels.[1,157] Thus, using a BMD approach ensures the correct weighting of phthalates because exposures for the same response level (in this case 5%) are considered.[174] Furthermore, uncertainty factors used to obtain reference values in combination with NOAELs and LOAELs add an additional level of uncertainty as they are largely subjective.[1] Thus, forthcoming work on cumulative phthalates exposure and risk should use BMDs in weighted potency metrics, which is consistent with recommendations by the U.S. Environmental Protection Agency (EPA) for considering risks for non-cancer health effects.[157]

The most significant limitation to the estimation of cumulative exposure was information availability. For one, urine flow rate data was available only for 2009-12 NHANES cycles, which limited this study's statistical power. Also, the NAS recommended including other anti-androgens, such as polybrominated diphenyl ethers (PBDEs) and 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD).[1] However, comparable relative potency data for additional chemicals would be necessary to compute RPFs, and these chemicals were not measured in the same NHANES population with phthalate measurements. Moreover, NHANES does not currently measure metabolites of dipentyl phthalate,[3] which may be more potent than DEHP and DnBP for androgen-mediated endpoints.[1] Comparable relative potency data would also be useful to determine whether this method is applicable to other hormone-mediated outcomes, such as metabolic disease and impaired neurodevelopment in children, or whether the relative potencies would be different for these outcomes. Although the metric relies on only one anti-androgenic endpoint, fetal testosterone production effects have been shown to accurately predict phthalate mixture effects for phthalate syndrome outcomes in male rat offspring, suggesting the method may applicable to other androgen-mediated outcomes.[93,98,99]

Nevertheless, this method provides a blueprint for how to weight phthalates in a cumulative sum using relative potencies recommended by the NAS. This chapter additionally demonstrated how to include anti-androgenic compounds with more recent comparable relative potency data (i.e. DiNP) and addressed issues of uncertainty regarding chemicals without clear relative potency data (i.e. DEP). The analysis showed how issues of potential exposure misclassification might arise depending on which exposure metric and urine dilution correction method is used in regression modeling. Future studies should validate this method in exposure prediction, risk assessment, and epidemiologic models.

2.6 Conclusion

Because humans are continuously exposed to multiple phthalates and other anti-androgenic compounds, I present an approach that can be used in future cumulative exposure analyses, risk assessments, and epidemiologic studies. Efforts should be made to evaluate the combined effects

of phthalates and other anti-androgens since their co-occurrence and additive effects suggest that risk assessment approaches focused on one chemical at a time may underestimate risk. Cumulative assessment should also be more fully integrated into examinations of environmental chemical exposures in explaining racial/ethnic disparities in health outcomes, since multiple chemical exposures are more reflective of our modern environment. These approaches will contribute to more effective strategies to reduce exposures to potentially harmful chemicals and ultimately improve public health.

Chapter 3

Measurement of urinary phthalate metabolites and characterization of cumulative phthalates exposure in a pilot study of Vietnamese nail salon workers in California

3.1 Abstract

Many California nail salon workers are low-income Vietnamese immigrants who use nail products daily that contain phthalates, a group of reproductive toxicants that may additively disrupt androgen-dependent development during pregnancy. Yet, few studies have characterized exposures in this occupational group. Accordingly, post-work shift urine samples were collected in 2011 from 17 Vietnamese American workers at six San Francisco Bay Area nail salons and analyzed for four primary phthalate metabolites: mono-*n*-butyl-, mono-isobutyl-, mono(2-ethylhexyl)-, and monoethyl phthalates (MnBP, MiBP, MEHP, and MEP, respectively) (μg/L). A potency-weighted cumulative sum of parent compound daily intake (Σandrogen-disruptor, μg/kg/day) was constructed, and individual metabolite and Σandrogen-disruptor worker concentrations were compared to concentrations in 203 Asian Americans sampled in the 2011-12 National Health and Nutritional Examination Survey (NHANES). Creatinine-corrected MnBP, MiBP, MEHP (μg/g), and Σandrogen-disruptor (μg/kg/day) concentrations were 2.9 ($p < 0.0001$), 1.6 ($p = 0.015$), 2.6 ($p < 0.0001$), and 2.0 ($p < 0.0001$) times higher, respectively, in nail salon workers compared to NHANES. In some workers, concentrations exceeded the NHANES 95th and/or 75th percentiles. This pilot study suggests that nail salon workers may be disproportionately exposed to co-occurring anti-androgenic phthalates, a finding that warrants further investigation.

3.2 Background

The U.S. nail care industry expanded rapidly over the past 25-30 years, with estimated active nail technician licenses totaling over 400,000 in 2015.[175] Just over half of U.S. nail salon workers describe themselves as Vietnamese, and 97% are women, mostly of reproductive age. With the largest number of manicurists and salons in the country,[175,176] California experienced the most dramatic industry growth over the last few decades, in part due to an influx of Vietnamese workers. Between 1987 and 2002, Vietnamese nail technicians increased over 10 times in California, expanding the proportion of Vietnamese workers from 10% to 59%.[177,178]

Nail salon workers tend to be low-income with limited access to health care, labor rights protections, product ingredient disclosures, and chemical safety information.[179–181] Many stakeholders, including salon owners, workers, community-based organizations, and government agency officials, are concerned that nail salon workers constitute a disproportionately exposed and vulnerable occupational population to a variety of chemicals linked to acute and chronic health problems.[179,181,182] Attempts to address these concerns while maintaining the economic integrity of the nail salon worker community include county-based programs in Northern California that educate and reward nail salon workers and owners who prioritize safe working environments.[183] Additionally, several state-level and national initiatives have focused on increased commercial product labeling and the removal of hazardous chemicals from nail care

products.[184] Thus, characterizing chemical exposures in this workforce may inform practical workplace intervention strategies as well as upstream efforts to find safer alternatives.

Phthalates are one family of chemicals used in consumer and personal care products that have garnered significant public and scientific concern in recent years. They can disrupt the endocrine system and are associated with a wide range of health impacts, including infertility, pregnancy complications, neurodevelopmental effects, cancer, and metabolic disease.[29–32,34] Animal studies further demonstrate that combinations of anti-androgenic phthalates, such as di-*n*-butyl phthalate (DnBP) and di-(2-ethylhexyl) phthalate (DEHP), act cumulatively to disrupt male reproductive tract development during pregnancy, resulting in adverse male outcomes known as the phthalate syndrome, which range from birth defects to infertility.[1,93] Consequently, the U.S. Consumer Products Safety Commission and several states, including California, have limited the use of certain phthalates, such as DEHP and DnBP, in plastic toys and other children's products.[119–122,124]

Personal care products, on the other hand, have been given less regulatory attention in the United States, due to the U.S. Food and Drug Administration's (FDA) limited authority over cosmetic ingredients.[117] DnBP's common use as a hardener in nail polish to preserve shape and color is well known and has been under recent public scrutiny.[185] Prior research reported cross-shift increases of urinary DnBP metabolites among U.S. nail salon workers, as well as elevated concentrations compared to the general population.[186,187] Increased metabolite exposures were also reported for DiBP, which has similar properties and uses as DnBP.[3,187] Diethyl phthalate (DEP) is consistently associated with personal care product use among non-occupational groups, but not among nail salon workers.[168,169,187–189] Alternatively, DEHP is predominantly associated with diet among the general population, though elevated metabolite concentrations have been reported among nail salon workers.[124,187] Prior studies included nail salon workers of multiple race/ethnicities while no study has examined cumulative phthalates exposure in this workforce. Accordingly, the goal of this pilot study was to build on past research and for the first time characterize individual and cumulative phthalates exposure in an exclusive sample of Vietnamese manicurists in comparison to a national sample of Asian Americans.

3.3 Methods

3.3.1 Study population and sample collection

The study population comprises a subset of participants sampled in a 2011 pilot intervention study designed to educate nail salon workers and owners about how to reduce occupational exposures to various hazardous chemicals.[190] That study included 26 Vietnamese American workers at eight San Francisco Bay Area nail salons and measured each worker's personal breathing zone air for volatile organic compounds such as toluene and methyl methacrylate. During air monitoring sessions, each participant completed questionnaires on characteristics such as age, the number of other workers and customers on the day of sampling, types of nail care services performed, and individual protective behaviors. Prior to educating workers about how to reduce their exposures, post-shift urine samples were collected from 17 of these workers at six nail salons during busier days of the week (Thursday, Friday, and Saturday) and warmer months of the year (June and July). Written consent was obtained in Vietnamese, and human subjects

institutional review and approval from the Cancer Prevention Institute of California was received on May 11, 2011 (Protocol # 2010-013). Urine samples were collected in four-ounce glass containers, shipped on ice, and stored at -20 ^{o}C prior to analysis. To reduce potential effects of freeze-thaw cycles, samples were thawed and aliquoted (2-4 mL) in pre-cleaned amber vials. Water laboratory blanks were also prepared in the same type vials and frozen.

3.3.2 Laboratory analysis

To assess phthalate metabolite concentrations in nail salon workers, I worked with the Department of Toxic Substances Control (DTSC) in Berkeley, California to adapt and validate the Silva et al. (2004) method used by the Centers for Disease Control (CDC) to measure urinary MnBP, MiBP, MEHP, and MEP concentrations using liquid chromatography-tandem mass spectrometry (LC-MS/MS).[147] Isotopically-labeled internal standards (IS) were used for quantitation (MnBP IS was used for MiBP) and Agilent Nexus ABS Bond Elut cartridges for solid-phase extraction (SPE) clean-up. Samples were analyzed using a Varian 320MS LC-MS/MS with a Phenomenex Gemini C6-Phenyl column (100mm x 2.00mm, 3µm). Baseline peak resolution was achieved for all metabolites, and no significant laboratory background was detected. Relative recoveries of quality control standards spiked into the urine before SPE ranged between 80-95%. Inter- and intra-day reproducibility and precision (coefficient of variation < 15%) were demonstrated in addition to accuracy (< 15% difference between expected and observed concentrations). The limit of detection (LOD) was defined as the lowest calibration standard providing a signal-to-noise ratio > 6. Once the method was validated, all 17 nail salon worker urine samples were analyzed and aliquots were submitted to the clinical laboratory at San Francisco General Hospital for creatinine measurements.

3.3.3 Statistical analysis

Nail salon worker results were compared to the 2011-12 cycle of the CDC-administered NHANES data, a nationally representative questionnaire and physical survey of the U.S. civilian, non-institutionalized population. As part of the NHANES medical examination, spot urine samples are collected and shipped on dry ice to CDC's National Center for Environmental Health for laboratory analysis.[154] I downloaded demographic and laboratory files from the NHANES website in May 2015 (http://www.cdc.gov/nchs/nhanes.htm). Out of 9756 NHANES participants, 2489 were sampled for 14 urinary phthalate metabolites, quantified using high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS).[155] Primary comparison populations were restricted to pooled (men and women) and female-only NHANES Asian Americans 20-59 years old (n = 203 and n = 97, respectively). A secondary comparison population included all NHANES participants aged 20-59 years (N = 1175).

The data were analyzed using SAS software, Version 9.4 (SAS Institute Inc., Cary, NC, USA). Phthalate metabolite concentrations below the LOD were imputed with LOD divided by √2. To correct for urine dilution, metabolite concentration (µg/L) was divided by urinary creatinine concentration (g/L), which is often used as a surrogate for urine dilution.[161,162] A potency-weighted cumulative sum of phthalates daily intake (Σandrogen-disruptor, µg/kg/day) was constructed as described in Chapter 2.[191] Briefly, I summed daily intakes of DnBP, DiBP, DEHP,

and DEP that were each weighted by a relative potency factor (RPF) (**Eq. 2-3**) (**Table 2-3**). Daily intake was back-calculated from creatinine-corrected urinary metabolite concentrations (μg/g) using creatinine excretion rates and fractional excretion values of metabolites relative to parent compounds (F_{UE}) (**Eq. 2-2**). Because MEHP was the only DEHP metabolite included in this analysis, an F_{UE} value of 0.062 was used for DEHP estimation.[159] Average creatinine excretion rates of 23 mg/kg/day and 18 mg/kg/day were applied to men and women, respectively.[21] Concentrations of Σandrogen-disruptor and urinary metabolites were log-transformed prior to statistical testing to account for non-normal distributions, and statistical and marginal significance were defined at $p < 0.05$ and $p < 0.10$ for two-sided tests, respectively.

Descriptive statistics were calculated for worker and salon characteristics, such as average hours worked per week, number of services performed on the day of sampling, and salon volume in cubic meters of air (m^3). I calculated detection frequencies (% > LOD) of measured urinary metabolites and performed univariate statistics on creatinine-corrected concentrations, non-creatinine-corrected concentrations, and Σandrogen-disruptor. These included the arithmetic mean, geometric mean (GM), geometric standard deviation (GSD) or geometric standard error (GSE), range, and 25th, 50th, 75th, and 95th percentiles. Univariate statistics were calculated for all 17 nail salon workers and female-only workers ($n = 15$). This analysis was also conducted for NHANES comparison study populations, accounting for sample population weights and the complex NHANES survey design according to analytical guidelines.[154]

Phthalate concentrations were compared between nail salon workers and NHANES 2011-12 participants using a parametric t-test for MnBP, MEHP, MEP, and Σandrogen-disruptor. For statistical hypothesis testing, NHANES values were assumed to be the true population values. The Wilcoxon signed rank non-parametric t-test was used for the MiBP comparison, since several MiBP outliers were observed among nail salon workers. This analysis was performed separately for pooled (men and women) and female-only Asian Americans aged 20-59 years old, in addition to all race/ethnicities of the same age range.

Because urine dilution correction and daily intake estimation may both introduce uncertainty and variability into biological exposure metrics, as described in Chapter 2, I conducted a supplementary analysis to evaluate whether alternate approaches impacted results. Two additional cumulative exposure metrics were calculated by first applying RPF weights directly to non-creatinine-corrected concentrations measured in urine (μg/L), and then by applying RPF weights to creatinine-corrected urinary metabolite concentrations (μg/g).[191] Compared to the original metric (Σandrogen-disruptor), which both corrected for urine dilution and estimated daily intake, the first supplementary metric provided a comparison to an absence of both potential sources of uncertainty/variability, while the second provided a comparison to the absence of daily intake estimation only.

To facilitate interpretation of results, I additionally compiled a summary of urinary phthalate metabolite concentrations measured in relevant biomonitoring and health impact studies. These included two previous biomonitoring studies that reported creatinine-corrected values, one that collected 25 post-shift urine samples from predominantly Asian nail salon workers in Maryland, USA,[187] and another that collected 30 urine samples exclusively from the Vietnamese general population.[192] One other study that I know of measured urinary MnBP and MiBP in U.S. nail

salon workers, but only specific gravity-corrected concentrations were reported, and the study population was predominantly white.[186] Lastly, I included five epidemiology studies that reported significant associations between non-corrected concentrations of urinary metabolites measured in this study and adverse male developmental outcomes,[43,87,90,146,193] excluding studies that reported only specific-gravity corrected values, as they were not directly comparable.

3.4 Results

All 17 nail salon workers were born in Vietnam and most preferred to speak Vietnamese at home (**Table 3-1**). The average age was 40 years. There was a wide range of hours worked per week at the salon (10 to 60 hours), with a mean of 34.5 hours per week, and number of services performed on the day of sampling (1 to 17 services), with an average of 8 services. The average number of other workers in the salon on the day of sampling was 4 and ranged from 2 to 8. The estimated number of total salon customers on the day of sampling was around 22, with a range between 7 and 40. Most workers reported using gloves and a metal trash bin. The most popular ventilation practice was leaving the doors of the salon open.

Table 3-1 Characteristics of Vietnamese nail salon workers in California (*n* = 17)

Worker and salon characteristics	**Mean (range)* or *n* (%)**
Age	40 (23-57)*
Sex	
Female	15 (88%)
Male	2 (12%)
Birthplace	
Vietnam	17 (100%)
Preferred language(s)	
Vietnamese	17 (100%)
Average work hours per week[a]	34.5 (10-60)*
Salon volume (m^3)	274 (42-437)*
Number of services performed that day[a]	8 (1-17)*
Number of other workers that day[a]	4 (2-8)*
Number of salon customers that day[a]	22 (7-40)*
General reported glove use[a]	10 (67%)
Metal trash bin with tight fitting lid use[a]	9 (60%)
Ventilation practices that day[a]	
Used a table fan	7 (47%)
Left windows open	5 (33%)
Left doors open	14 (93%)
Other ventilation practices	10 (59%)
Total number of ventilation practices	3 (0-5)*

[a] Restricted to *n* = 15 due to missing survey data for two workers.

Detection frequencies for urinary phthalate metabolites were similar or higher in nail salon workers (93 - 100%) compared to NHANES Asian Americans (80 - 100%) (**Tables 3-2** and **3-3**). In all workers (*n* = 17), MEP had the highest creatinine-corrected GM of 38 μg/g (GSD = 3.3, range = 8.1 - 497 μg/g), followed by 23 μg/g for MnBP (1.7, 9.9 - 61 μg/g), 13 for MiBP (2.3,

3.6 - 121 μg/g), and 5.9 for MEHP (1.8, 2.2 - 16 μg/g) (**Table 3-2**). The same rank order was observed among female-only workers and NHANES Asian Americans (pooled and female participants) (**Tables 3-2** and **3-3**), as well as for non-creatinine-corrected values (**Table 3-4**).

Table 3-2 Comparison of creatinine-corrected phthalate metabolite concentrations *(μg/g)* between all nail salon workers and 2011-12 NHANES Asian Americans[a]

Phthalate metabolites	All nail salon workers (*n* = 17)				All NHANES Asian Americans (*n* = 203)			
	GM (GSD)	Range	% >LOD[b]	% >NH_{p95}	GM (GSE)	Range	% >LOD[b]	NH_{p95}
Mono-*n*-butyl phthalate (MnBP)	23 (1.7)	9.9 – 61	94%	18%	8.2 (1.1)	0.44 - 930	90%	42
Mono-isobutyl phthalate (MiBP)	13 (2.3)	3.6 - 121	100%	12%	7.6 (1.1)	0.36 - 90	98%	31
Mono(2-ethylhexyl) phthalate (MEHP)	5.9 (1.8)	2.2 - 16	94%	6%	2.3 (1.1)	0.18 - 155	80%	14
Monoethyl phthalate (MEP)	38 (3.3)	8.1 - 497	100%	6%	301 (1.1)	0.75 - 1876	100%	355

NH_{p95} = NHANES 95th percentile. LOD = Limit of detection.

[a] *P*-values of statistical tests of difference between all nail salon workers and NHANES Asian Americans: MnBP (<0.0001), MiBP (0.015), MEHP (<0.0001), and MEP (0.445).

[b] Nail salon worker LOD = 1.20 μg/L for MnBP, MiBP, and MEHP, and 3.56 μg/L for MEP. NHANES LOD = 0.40, 0.20, 0.50, and 0.60 μg/L for MnBP, MiBP, MEHP, and MEP, respectively.

Table 3-3 Comparison of creatinine-corrected phthalate metabolite concentrations *(μg/g)* between female nail salon workers and 2011-12 NHANES Asian American females[a]

Phthalate metabolites	Female nail salon workers (*n* = 15)				Female NHANES Asian Americans (*n* = 97)			
	GM (GSD)	Range	% >LOD[b]	% >NH_{p95}	GM (GSE)	Range	% >LOD[b]	NH_{p95}
Mono-*n*-butyl phthalate (MnBP)	22 (1.6)	9.9 - 55	93%	13%	9.3 (1.1)	0.28 - 371	90%	44
Mono-isobutyl phthalate (MiBP)	11 (1.9)	3.6 – 62	100%	7%	7.5 (1.1)	0.14 - 66	98%	28
Mono(2-ethylhexyl) phthalate (MEHP)	5.6 (1.9)	2.2 – 16	93%	7%	2.6 (1.2)	0.35 - 160	80%	15
Monoethyl phthalate (MEP)	44 (3.3)	8.1 – 497	100%	7%	34 (1.1)	0.42 - 1293	100%	298

NH_{p95} = NHANES 95th percentile. LOD = Limit of detection.

[a] *P*-values of statistical tests of difference between female nail salon workers and NHANES Asian American females: MnBP (<0.0001), MiBP (0.041), MEHP (0.0002), and MEP (0.455).

[b] Nail salon worker LOD = 1.20 μg/L for MnBP, MiBP, and MEHP, and 3.56 μg/L for MEP. NHANES LOD = 0.40, 0.20, 0.50, and 0.60 μg/L for MnBP, MiBP, MEHP, and MEP, respectively.

Table 3-4 Comparison of non-creatinine-corrected phthalate metabolite concentrations *(μg/L)* between nail salon workers and 2011-12 NHANES Asian Americans[a]

	All nail salon workers (n = 17)	All NHANES Asian Americans (n = 203)		Female nail salon workers (n = 15)	Female NHANES Asian Americans (n = 97)		Male nail salon workers (n = 2)
Phthalate metabolites	***GM (GSD)***	***GM (GSE)***	***p-value[b]***	***GM (GSD)***	***GM (GSE)***	***p-value[b]***	***Observed values***
MnBP	16 (2.4)	6.1 (1.2)	0.007	14 (2.1)	5.6 (1.2)	0.014	20, 105
MiBP	8.5 (3.0)	5.6 (1.1)	0.147	7.0 (2.2)	4.4 (1.1)	0.056	7.1, 209
MEHP	3.9 (2.2)	1.7 (1.1)	<0.001	3.5 (2.0)	1.6 (1.1)	0.001	5.9, 16
MEP	26 (3.5)	23 (1.1)	0.681	27 (3.7)	21 (1.1)	0.425	15, 16

[a] Nail salon worker limit of detection (LOD) = 1.20 μg/L for MnBP, MiBP, and MEHP, and 3.56 μg/L for MEP. NHANES LOD = 0.40, 0.20, 0.50, and 0.60 μg/L for MnBP, MiBP, MEHP, and MEP, respectively.

[b] *P*-values from statistical tests of difference between nail salon workers and NHANES.

Creatinine-corrected phthalate metabolite concentrations were higher among nail salon workers compared to NHANES Asian Americans, and some of the differences were statistically significant (**Table 3-2**). MnBP, MiBP, MEHP, and MEP were 186% ($p < 0.0001$), 66% ($p = 0.015$), 158% ($p < 0.0001$), and 25% ($p = 0.445$) higher, respectively. For some nail salon workers, creatinine-corrected metabolite concentrations exceeded the NHANES 95th percentile in adult Asian Americans. The highest worker concentrations were 44%, 293%, 20%, and 40% above the NHANES 95th percentile for MnBP, MiBP, MEHP, and MEP, respectively. Additionally, two nail salon workers had more than one urinary metabolite concentration greater than the NHANES 95th percentile (**Table 3-2**). Female nail salon workers had similar but attenuated results, with 132% ($p < 0.0001$), 46% ($p = 0.041$), 120% ($p = 0.0002$), and 27% ($p = 0.455$) higher MnBP, MiBP, MEHP, and MEP concentrations, respectively, compared to NHANES Asian American females (**Table 3-3**).

Non-creatinine-corrected results for pooled (men and women) and female participants were also similar and/or slightly attenuated, though MiBP was not significantly higher in pooled nail care workers compared to NHANES Asian Americans, and only marginally significant when I restricted the analysis to female participants (**Table 3-4**). Because urinary concentrations among all NHANES race/ethnicities ($N = 1175$) were lower than those measured in NHANES Asian Americans for each phthalate metabolite except MEP, this study population was not included in further analyses.

Among pooled study populations (men and women), the GM of cumulative phthalates daily intake (Σandrogen-disruptor) was 97% higher in nail salon workers (GM = 2.6; GSD = 1.7 μg/kg/day) compared to NHANES (GM = 1.3; GSE = 1.1 μg/kg/day) ($p < 0.0001$) (**Figure 3-1**). Among women, the percent difference between workers and NHANES Asian Americans was 81% ($p = 0.0004$). However, the maximum NHANES cumulative exposure of 39 μg/kg/day was over five times greater than the maximum nail salon worker cumulative exposure of 6.7 μg/kg/day. The Σandrogen-disruptor distributions for pooled and female-only NHANES Asian Americans were very similar, except for at the upper tail end of the distribution, where most extreme cumulative exposure concentrations belonged to women. Thus, the NHANES 95th percentile for women (8.5 μg/kg/day) was substantially higher than the pooled NHANES 95th percentile (5.2 μg/kg/day). None of the nail salon workers exceeded the female-only NHANES 95th percentile, but two nail salon workers (both women) exceeded the pooled NHANES 95th percentile. Twelve workers (70%), including both men, were above the NHANES 75th percentile for female-only and pooled Asian Americans (**Figure 3-1**).

In a supplementary analysis exploring alternate approaches to urine dilution correction and daily intake estimation, I observed similar cumulative exposure differences between nail salon workers and NHANES when I applied relative potency weights directly to non-corrected and creatinine-corrected urinary metabolite concentrations. Thus, I reported cumulative exposure results only for daily intake estimates of parent compounds (Σandrogen-disruptor), and not for urinary metabolite concentrations.

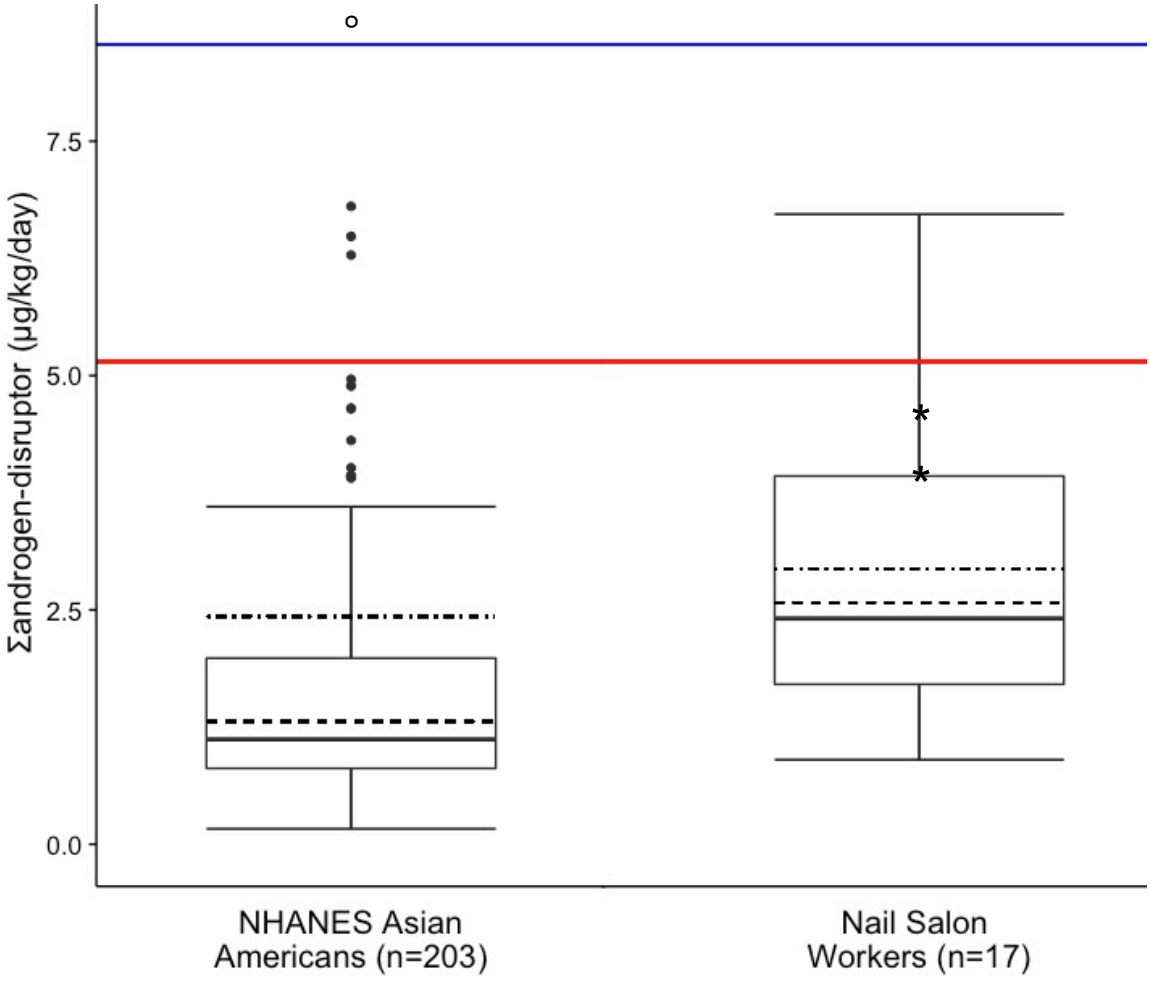

Figure 3-1 Comparison of cumulative phthalates daily intake (Σandrogen-disruptor) between nail salon workers and NHANES 2011-12 Asian Americans. Boxes represent interquartile range (IQR: 25th to 75th percentiles). Dark lines represent medians. Dashed lines represent geometric means. Dot-dashed lines represent arithmetic means. Whiskers extend to min and max (Max = most extreme values within 1.5 • IQR of the median for NHANES). NHANES outliers are represented by dark points, and hollow point denotes outliers off the y-axis scale (a total of seven, five of which were women). Red line represents 95th percentile for pooled NHANES Asian Americans (n = 203). Blue line represents 95th percentile for female NHANES Asian Americans (n = 97). P-value of statistical tests of difference between nail salon workers and NHANES Asian Americans < 0.0001. * Indicates male nail salon worker observations (n = 2).

All creatinine-corrected urinary phthalate metabolite concentrations measured among nail salon workers in this study were similar to or higher than concentrations reported in a previous biomonitoring study of the Vietnamese general population (**Table 3-5**).[192] However, lower creatinine-corrected urinary concentrations were observed in this study compared to nail salon workers sampled in 2003-4, except for MiBP, which was twice as high in this study nail salon worker population.[187] Except for MEP, non-creatinine corrected metabolite concentrations in this study were similar to or higher than those measured in epidemiology studies that reported a significant association with adverse male developmental endpoints, such as decreased anogenital

distance (AGD), reductions in penile length, and reduced masculine play among boys (**Table 3-6**).[43,87,89,90,193]

Table 3-5 Comparison of creatinine-corrected GM (range) of phthalate metabolite concentrations *(μg/g)* measured in this study to previous biomonitoring studies

Metabolites	**This Study**	**Hines et al. (2009)**[187]	**Guo et al. (2011)**[192]
MnBP	23 (3.0 - 105)	34 (<LOD - 199)	17 (8.0 - 47)
MiBP	13 (1.7 - 209)	6.3 (<LOD - 27)	12 (5.7 - 34)
MEHP	5.9 (<LOD - 16)	19 (<LOD - 1480)	2.4 (<LOD - 5.3)
MEP	38 (5.7 - 399)	119 (17 - 1580)	6.5 (1.8 - 42)
Study details	17 post-shift samples collected from 100% Vietnamese nail salon workers in California, USA, 2011	25 post-shift urine samples collected from 88% Asian nail salon workers in Maryland, USA, 2003-4	30 samples collected from general population in Vietnam, 2006-7

Table 3-6 Comparison of non-creatinine-corrected phthalate metabolite concentrations *(μg/L)* measured in this study to previous epidemiology studies that reported significant associations between prenatal exposures and male developmental endpoints[a]

	This Study					*Swan et al. (2005)*[87]	*Swan et al. (2010)*[43]	*Suzuki et al. (2012)*[89]	*Bustamante-Montes et al. (2013)*[193]	*Swan et al. (2015)*[90]
Metabolites	**GM**	**Mean**	**Range**	**Median**	**p75**	**Median (p75)**			**Mean (Range)**	**GM (p75)**
MnBP	14	17	3.0 – 38	13	28	14 (31)**	13 (28)**	47 (65)	0.65 (0.25 - 1.6)	6.4 (17)
MiBP	7.0	9.1	1.7 - 26	8.5	13	2.5 (5.1)**	2.4 (5.1)**	n/a	n/a	4.0 (11)
MEHP	3.5	4.5	0.85 - 13	3.2	6.9	3.3 (9.0)	2.9 (6.2)	3.7 (7.1)**	4.0 (0.4 - 20)**	1.9** (4.7)
MEP	27	66	4.7 - 399	18	68	128 (437)**	n/a	7.8 (32)	7.6 (0.27 - 27)	28 (81)
Sample size (*N*)	*17*					*85*	*74*	*111*	*73*	*753*

p75 = 75th percentile. Green color indicates associated metabolite concentrations that were similar or below those measured in this study.

[a] Developmental endpoints examined in each study: Reduced anogenital distance *[Swan et al. (2005, 2015),*[87,90] *Suzuki et al. (2012)*,[89] and *Bustamante-Montes et al. (2013)*[193]*]*, reduced penile size *[Bustamante-Montes et al. (2013)*[193]*]*, and reduced masculine play in young boys *[Swan et al. (2010)*[43]*]*.

** Denotes significant association.

3.5 Discussion

To my knowledge, this is the first biomonitoring assessment of phthalates exposure in Vietnamese nail salon workers residing in California, who constitute a vulnerable occupational group with limited English proficiency.[177] This pilot study of 17 Vietnamese-born nail salon workers provides evidence of elevated individual metabolite and cumulative phthalates exposure in comparison to a national sample of the U.S. Asian American population. Specifically, nail salon workers had significantly higher exposure concentrations of creatinine-corrected MnBP, MiBP, and MEHP, and of a potency-weighted sum of parent phthalate daily intake (Σandrogen-disruptor), though non-creatinine-corrected MiBP results were attenuated and less significant. This pilot study highlights the possibility that nail salon workers are exposed to phthalates at higher concentrations than the general population, a finding that warrants further examination.

While this study did not assess direct connections between phthalates and health impacts, MnBP, MiBP, and MEHP concentrations were similar to or above those measured in studies reporting adverse developmental endpoints in male offspring.[43,87,89,90,193] For example, in a large prospective cohort study across four major U.S. cities ($N = 753$) that found a significant association between MEHP and reduced anogenital distance among baby boys, Swan et al. (2015) reported a non-creatinine corrected GM of 1.9 ng/mL (95% CI: 1.8, 2.1), which was >1.5 times lower than the GM of 3.5 ng/mL (95% CI: 2.4, 5.3) observed among female nail salon workers in this study. These exposure levels are of potential concern for women in the nail salon workforce who are of reproductive age and may become pregnant.

Individual metabolite concentrations exceeded the NHANES 95th percentile in several nail salon workers, and for more than one metabolite in two participants, indicating that some workers may be simultaneously exposed to phthalate combinations at high levels relative to the general Asian American population. Most nail salon workers had higher potency-weighted cumulative phthalates daily intake than the NHANES 75th but not 95th percentiles. However, the NHANES Asian American comparison population might conceivably include Vietnamese nail salon workers, which could have skewed the distribution (and 95th percentile) towards the upper end of extreme values. Additionally, cumulative exposure may be underestimated, as some phthalates were excluded from the cumulative metric because the lab did not analyze all relevant metabolites. Due to existing evidence that co-exposures may have greater adverse health effects than individual phthalates, these finding should be further investigated.[1,87,88]

MnBP, MEHP, and MEP concentrations measured in this study were lower than those reported for predominantly Asian nail salon workers sampled 7-8 years prior to 2011.[187] This difference mirrors U.S. biomonitoring trends that indicate declines for all three metabolites between 2001 and 2012.[3] Similarly, MiBP concentrations were two times higher in the current nail salon worker population, which is consistent with a reported > 100% increase in urinary MiBP concentrations over the last decade.[3] Though personal care product ingredients are not fully disclosed in the United States, these temporal trends may be due to product reformulations over time.[125] For example, researchers have reported decreased DnBP and increased DiBP detection in nail polish over time, indicating that industry might be replacing the better-known DnBP with DiBP.[3,126–128,130] Recent public pressure may be a factor. Over the last 10-15 years, a widespread safe cosmetics campaign persuaded leading nail and beauty companies, such as OPI, Sally

Hansen, Revlon, and L'Oreal, to remove phthalates, including DnBP, from their products.[194] There is no way to be certain about whether these companies are actually reformulating their products, but there are market incentives to so. Green beauty products are the fastest growing sector of the cosmetics industry, which includes nationwide spending of up to $8.5 billion and $768 million on nail care services and nail polish, respectively.[195,196] However, phthalates have been detected in nail polish and other cosmetics that claim to be phthalate-free.[130,197] Continued product testing and biomonitoring research are required to monitor these trends going forward.

Though metabolite concentrations have changed over time, exposure differences between nail salon workers and the general population remained relatively constant between a 2003-4 study (in which Hines et al. (2009) compared worker concentrations to NHANES 2001-2)[187] and this 2011 analysis (in which I compared to NHANES 2011-12). Both studies found higher MnBP and MiBP concentrations among nail salon workers, which is consistent with common use of their parent compounds in personal care products.[3,186] Also observed in both studies, increased MEHP exposure is more surprising, given that diet is thought to be the primary source of DEHP exposure.[124] Hines et al. (2009) reported MEHP concentrations almost five times higher in manicurists relative to NHANES. MEHP concentrations were higher than every other occupational group examined, including phthalate and polyvinyl chloride (PVC) manufacturing workers.[187] The authors attributed this finding to metabolic differences among their predominately Asian American study population or some unknown source of DEHP exposure in nail salons. One possibility may be that MEHP is an ester derivative of benzoic acid, a metabolite of benzoyl peroxide, which is a catalyst found in acrylic nail powder.[198,199]

Consistent with previous findings, MEP concentrations among nail salon workers in this study were higher than NHANES, though not statistically significant.[187] Prior epidemiological studies have associated MEP with personal care product use in non-occupational populations.[168,169,200] It may be that MEP exposures at the nail salon are negligible relative to high background exposures in the general population. Indeed, MEP concentrations were the highest among metabolites evaluated in this study.

While this chapter suggests that nail salon workers may be disproportionately exposed to phthalates, this pilot study had several limitations that should be addressed in future work. First, the sample size was too small to predict phthalate metabolite and cumulative exposure concentrations from questionnaire data on worker and salon characteristics, thus limiting statistical power to identify specific occupational sources of phthalates exposure. Additionally, DEHP's secondary oxidative metabolites (i.e. mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP)) were not measured in this study, yet they constitute a larger fraction of the absorbed parent compound dose than the monoester, and measuring MEHP alone may underestimate DEHP daily intake. Additionally, oxidative metabolites are less sensitive than primary monoesters to laboratory contamination because they are formed solely from phthalate metabolism in the liver.[14] Future biomonitoring studies that evaluate DEHP exposure should aim to include at least one of the oxidized metabolites.

Forthcoming research on nail salon exposures should also include the collection of pre-shift urine samples in addition to air and skin samples collected during the shift, which would help differentiate between occupational and non-occupational (environmental) exposures. Including

questionnaire data about non-occupational exposure sources, such as personal care product use and dietary behaviors, could also facilitate the delineation between occupational and environmental exposures. Moreover, future studies should aim to collect longitudinal data for each participant, as spot samples, while convenient and cost-effective, typically are not ideal for evaluating phthalate metabolites over time. Spot samples for some metabolites, including MEP and MnBP, have demonstrated moderate long-term reliability in adults, but variability studies assessing their reproducibility over a given time period in various subpopulations are somewhat equivocal.[173,201–204] Furthermore, intra-person variability is likely to be higher in the workplace due to short-term spikes in exposure from certain job tasks, since the metabolic half-lives of phthalates are typically short (less than 24 hours).[159]

Lastly, although nail salon workers in this analysis were compared to NHANES Asian Americans of the same age range sampled within a year of workers in this study, some non-occupational differences between the groups could potentially explain exposure disparities. For example, workers in this study were Vietnamese-born, while the NHANES Asian American population may include second or third generation immigrants. Vietnamese immigrants might use different personal care products or eat different foods than those born in the United States, which could result in observed disparities between workers and the NHANES sample that are not occupationally based. However, Guo et al. (2011) biomonitored 30 people in Vietnam and reported equal or lower creatinine-corrected phthalate metabolite levels than workers in this study,[192] suggesting these findings are not likely to due to cultural differences in personal care product use or diet originating from the country of origin. Other differences between the groups, such as location, sampling strategy (convenience rather than multistage probability), data collection time of day (post-shift compared to random), and month or season of sampling (summer rather than throughout the year), might also partially explain observed exposure disparities.

Despite these limitations, comparing Vietnamese nail salon workers to a national sample of similarly aged Asian Americans provides useful insight and supporting evidence that higher concentrations of individual metabolites and cumulative phthalates may be due to workplace exposures. Further research should more broadly characterize workplace exposures and their health implications in this occupational subpopulation. The addition of pre-shift urine samples may be the best way to assess phthalate exposures in nail salon workers, given the short half-lives of phthalates.

3.6 Conclusion

This pilot study provides suggestive evidence that nail salon workers may be disproportionately exposed to anti-androgenic phthalates and their combinations. Larger longitudinal studies could better characterize workplace phthalate exposures, identify sources, and inform strategies (i.e. education, product replacement, local ventilation, personal protective equipment, etc.) for reducing occupational sources of phthalates exposure in the nail care services industry.

Chapter 4

Dietary sources of cumulative phthalates exposure among the U.S. general population: Food prepared away from home compared to food prepared at home

4.1 Abstract

Anti-androgenic phthalates are associated with various health impacts across the life course, and cumulative prenatal exposures may have additive effects on male reproductive tract development. Diet is the primary source of high molecular weight phthalates, such as di(2-ethylhexyl) phthalate (DEHP) and di-isononyl phthalate (DiNP), which can contaminate food supplies through various industrialized production practices. Previous research links fast food and packaged meals with DEHP and DiNP exposure. Accordingly, this study examined dietary intake sources ascertained from 24-hour recall surveys and their associations to cumulative phthalates exposure among 10,253 children, adolescents, and adults ≥ 6 years old sampled in the National Health and Nutrition Examination Survey (NHANES 2005-14). To construct a potency-weighted sum of cumulative exposure (∑androgen-disruptor, μg/kg/day), relative potency factors were applied to six phthalate daily intake estimates, including DEHP and DiNP, that were calculated from urinary metabolite concentrations. Multivariate linear regression was used to compare ∑androgen-disruptor concentrations between consumers of food prepared away from and at home, calculated as the percent of total energy intake (TEI), as well as by intake of particular foods the prior day. A consistent positive association was observed between dining out and Σandrogen-disruptor concentrations among all age-specific subgroups, with 55% (95% CI: 35%, 78%) increased phthalates exposure in high adolescent consumers of food prepared away from home compared to adolescents who consumed only food prepared at home. Particular foods prepared away from home but not those prepared at home were also associated with elevated exposure. These findings suggest that eating more at home may reduce cumulative phthalates exposure. Future studies should evaluate the efficacy of this behavior change and elucidate modifiable food production practices that contribute to these exposures.

4.2 Background

Endocrine disrupting chemicals (EDCs) are associated with hormone-mediated health outcomes, such as reproductive issues, metabolic disease, and neurodevelopmental problems.[32,205] In the United States alone, researchers recently quantified the disease cost of EDCs at $340 billion and identified phthalates as the second-leading driver of this burden.[36] Anti-androgenic phthalates are risk factors for adverse effects on male sex differentiation that are associated with reduced testosterone concentration during fetal development. Prenatal androgen disruption has been associated with genital abnormalities and reduced anogenital distance in humans and animals.[1,87,89–92,146,193] Laboratory studies further demonstrate that combined prenatal exposures can have additive effects. Co-exposures during pregnancy have also been associated with increased risks in humans,[87,88] suggesting that cumulative phthalate assessments are more biologically relevant for human health than chemical-by-chemical approaches.[1] Though pregnancy is one critical stage of toxicity, phthalates are associated with health impacts across the life course, including reduced semen quality, increased obesity, diabetes, and

cancer.[30,36,74,83,206,207] Low testosterone is also linked to adult mortality and metabolic disease, indicating that anti-androgenic phthalates may play a specific role in these health endpoints.[208] Thus, efforts to identify opportunities for exposure reductions may have significant implications for preventing metabolic and other hormone-mediated illnesses and decreasing the economic burden of EDCs.

Phthalates have multiple uses in commerce, including in food contact materials (i.e. plastic food packaging), personal care products, medical tubing, and/or any material containing polyvinyl chloride (PVC).[3] Consequently, human exposure to phthalates is ubiquitous, with multiple phthalates simultaneously detected in the vast majority of the U.S. population.[3,27,148] Although limited evidence exists identifying specific sources of exposure, the dietary pathway is thought to dominate across different populations, especially for high molecular weight phthalates such as DEHP and DiNP.[124,170,209,210] These phthalates are predominantly found in fatty foods such as meat and dairy,[211,212] although they've also been linked to grains and spices,[167,170,213] and are thought to enter the food supply through packaging, processing, and handling.[214–219] Thus, it is plausible that a significant source of phthalate exposure may come from foods prepared outside the home which undergo substantial industrialized production practices, such as in the fast food and restaurant industries, school cafeterias, food trucks, and sports and entertainment facilities.

Consumption of food prepared away from home, rather than food purchased in a store and prepared at home, has grown steadily over the last few decades in the United States. Between 1970 and 2014, household food expenditures devoted to dining out increased from 25.9% to 43. 7%, respectively, and over half of total U.S. food dollars are currently spent on foods purchased outside the home.[220] Like adults, children 2-17 years old are dining out more, with 35% of their total calories sourced from food prepared away from home in 2003-6 compared to 20% in 1977-8.[221] Among children, younger kids are more likely to eat meals offered by their school cafeterias, while adolescents are more likely to eat from competing vendors, such as fast food chains.[221] Although public health programs geared towards preventing obesity and improving American diets have predominantly focused on the nutritional aspect of foods offered from these establishments,[220,222] it is also important to consider the potential impact of chemical exposures introduced through increased packaging and industrial food processing, especially regarding EDCs that contribute to weight gain and related health conditions.

Several studies have investigated the connection between food prepared outside the home and phthalates. Zota et al. (2016) recently reported a consistent, positive association between fast food consumption and measured urinary metabolites of DEHP and DiNP in the U.S. general population,[167] and fast food intake has also been associated with DiNP and butyl benzyl phthalate (BBzP) metabolites in the urine of a cohort of young children.[223] Additionally, smaller studies have linked some phthalates to takeout food containers and packed school lunches and hospital meals in Italy and Japan.[215,219,224–226] However, a broader analysis across the U.S. population is warranted to assess the extent to which these and other dietary intake sources are associated with cumulative phthalates exposure.[1] Accordingly, this chapter compares cumulative phthalates exposure between consumption of food prepared at home and food prepared away from home, such as from fast food chains, full-service restaurants, and cafeterias, across age groups in the United States, including children, adolescents, and adults.

4.3 Methods

4.3.1 Study population

I combined five cycles of laboratory, questionnaire, and dietary NHANES data between 2005 and 2014 for this study (http://www.cdc.gov/nchs/nhanes.htm). The U.S. Centers for Disease Control and Prevention (CDC) administers NHANES as a nationally representative survey and physical examination of the civilian, non-institutionalized general population. The original study population included all participants ≥ 6 years old for which metabolite data, urinary creatinine measurements, kilocalorie and dietary intake source information were available (N = 12,134). Participants with missing information on income and education (n = 920) as well as those who did not self-identify as Hispanic, Mexican American, non-Hispanic white, or non-Hispanic black (n = 961) were excluded from the analysis due to racial/ethnic ambiguity (i.e. other or multi-racial classifications) and data availability. In particular, Asian American information was not specifically ascertained prior to 2011. The final sample size included 10,253 study participants.

4.3.2 Phthalates exposure assessment

The NHANES survey provides phthalate metabolite measurements for approximately one-third of study participants in each survey cycle. Spot urine samples are collected as part of the medical examination and analyzed at the CDC in Atlanta, GA., with analytical methods detailed elsewhere.[154,155] In summary, phthalate metabolites are quantified using high performance liquid chromatography coupled with tandem mass spectrometry. Laboratory files for 2005-12 survey cycles were downloaded from the NHANES website in March 2015 and included necessary impurity corrections for certain previously used analytical standards.[156] NHANES 2013-14 data was downloaded in January 2017. The maximum LOD was used to standardize variable detection limits across survey cycles[3] and concentrations below the LOD_{max} were substituted with LOD_{max} divided by √2 (**Table 4-1**). Detection limits by NHANES survey cycle are reported in the Appendix (**Table A-1**).

Table 4-1 Percent below maximum limit of detection *(μg/L)* and substitution with $LOD_{max}/\sqrt{2}$ in NHANES 2005-14

Phthalate metabolites	LOD_{max}	$LOD_{max}/\sqrt{2}$	% <LOD_{max}
Mono-*n*-butyl phthalate (MnBP)	0.60	0.42	1.8
Mono-isobutyl phthalate (MiBP)	0.80	0.57	3.9
Monobenzyl phthalate (MBzP)	0.30	0.21	1.8
Mono(2-ethylhexyl) phthalate (MEHP)	1.20	0.85	39
Mono(2-ethyl-5-carboxypentyl) phthalate (MECPP)	0.60	0.42	0.2
Mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)	0.70	0.49	1.1
Mono(2-ethyl-5-oxohexyl) phthalate (MEOHP)	0.70	0.49	2.2
Monoethyl phthalate (MEP)	1.20	0.85	0.1
Mono(carboxy-isooctyl) phthalate (MCOP)	0.70	0.49	1.9

A potency-weighted sum of daily intake (∑androgen-disruptor, μg/kg/day) for the following six phthalates was calculated according to the cumulative method developed in Chapter 2: di-*n*-butyl phthalate (DnBP), di-isobutyl phthalate (DiBP), BBzP, DEHP, diethyl phthalate (DEP), and DiNP.[191] In summary, relative potency factors (RPFs) for each phthalate were constructed using

benchmark doses (BMDs) associated with a 5% reduction in fetal testosterone concentration (**Eq. 2-1**). The phthalate with the lowest BMD and highest potency (DnBP) was used as the reference BMD to which all other BMDs were compared. The RPFs were applied to daily intake estimates of parent compounds (**Eq. 2-3**), which were back-calculated from measured urinary metabolite concentrations using published excretion fractions that relate urinary metabolites to parent compound intake (F_{UE}) (**Eq. 2-2**). Values for F_{UE} are provided in the Appendix (**Table A-1**). Average creatinine excretion rate values of 23 mg/kg/day and 18 mg/kg/day were used for men and women, respectively. For boys and girls < 20 years old, I calculated average creatinine excretion rates of 21 mg/kg/day and 19 mg/kg/day, respectively, from previously published literature.[21,227]

4.3.3 Dietary exposure assessment

As part of NHANES, the CDC also collects 24-hour dietary recall data from study participants that includes extensive information about what foods were eaten, time of eating occasion, and food source (where obtained or purchased).[228] I used self-reported information about each participant's dietary behavior the day prior to urine sample collection because phthalates have short metabolic half-lives (12-24 hours).[159,229] It is therefore reasonable to assume that urinary metabolite concentrations measured within 24 hours of parent compound exposure would appropriately reflect dietary intake the previous day.[159,229] Participants 12 years and older completed the survey independently unless they chose otherwise while proxy-assisted interviews were automatically provided for children 6-11 years old. Energy and nutrient intake were then calculated and made available by the CDC and the U.S. Department of Agriculture (USDA).[228,230,231]

Based on Zota et al. (2016) methods, I calculated TEI in kilocalories (kcals) over the past 24 hours for each participant by summing the NHANES-provided kcals for all foods recorded during each participant's dietary interview.[167] I additionally calculated total fat in grams by summing NHANES-provided grams of fat for all recorded food items, since dining out is positively associated with total caloric and fat intake among the U.S. general population, and consumption of high-fat foods has been linked to phthalate exposures in prior studies.[211,212,221,232] Total fat in kcals was calculated using nine fat grams per calorie as the conversion factor, and the percent of TEI from fat was then determined by dividing fat kcals by TEI.

Additionally, I categorized all foods reported by each participant as either food prepared away from home or food prepared at home, ascertained from the NHANES survey question regarding where the food (or the majority of its ingredients) was obtained or purchased. Seventeen mutually exclusive responses were provided by NHANES. I defined food prepared away from home as follows: 1) Fast food, defined by NHANES as food obtained from restaurants without table service, pizza restaurants regardless of waiter/waitress service, and all carryout and delivery food; 2) Full-service restaurants, or restaurants with table service, including bars, taverns, and lounges; 3) Cafeterias, including K-12, workplace, etc.; and 4) All other marginal sources of food prepared away from home that each contributed < 2% to TEI (and together contributed < 5% to TEI), which included child/family care centers, soup kitchen/shelter/food pantry, Meals on Wheels, community food programs, vending machines, sport/recreation/entertainment facilities, street vendor/vending trucks, residential dining facilities, and fundraiser sales. I defined food

prepared at home as food purchased at a store, anything home grown or caught, and "from someone else/gift", which the USDA has characterized as dinner cooked by a friend.[221]

I then calculated total kcals consumed by each participant from food prepared away from home and food prepared at home by summing NHANES-provided kcals for all foods that were reportedly obtained from either source. The percent of TEI from food prepared away from home and food prepared at home were then calculated by dividing total kcals from each source by TEI. I additionally calculated the percent of TEI from fat that was consumed from sources of food prepared away from home by summing NHANES-provided grams of fat for all foods reportedly prepared away from home, then converting to kcals and dividing by TEI.

4.3.4 Statistical analysis

Study population characteristics were assessed by performing analysis of variance (ANOVA) to test statistical differences in continuous log-transformed Σandrogen-disruptor concentrations across the following categorical variables: Age (children 6-11, adolescents 12-19, adults 20-59, and older adults ≥ 60 years old); sex (male or female); race/ethnicity [non-Hispanic white, non-Hispanic black, or Hispanic]; body mass index (BMI) [underweight (< 18.5 kg/m^2), normal weight (18.5 - 25 kg/m^2), overweight (25 - 30 kg/m^2), or obese (≥ 30 kg/m^2)]; poverty-to-income ratio, or PIR [ratio of household income to poverty threshold adjusted to family size and inflation: < 1 (beneath poverty threshold), 1 - 2.99, or ≥ 3]; educational attainment (less than high school, high school graduate, or any post high school education) in adults only; NHANES survey cycle (2005-6, 2007-8, 2009-10, 2011-12, or 2013-14); time of sampling session (morning, afternoon, or evening); and dietary intake of food prepared away from home [any (those who reported dining out within the last 24 hours) or none (those who reported consuming only food prepared at home the prior day)]. Univariate statistics, including geometric mean (GM), 95% confidence intervals (95% CI), range (min – max), and 25^{th}, 50^{th}, and 75^{th} percentiles were additionally calculated for cumulative phthalates daily intake (Σandrogen-disruptor).

I examined dining out behavior across the study population by comparing the weighted percent of participants who consumed any or none of their calories from food prepared away from home (total combined and divided into fast food, full-service restaurants, cafeterias, and other marginal sources) using the Rao Scott chi-square test of independence, first across age (children, adolescents, and adults 20-39, 40-59, and ≥ 60 years old); then by sex; and finally, across NHANES survey cycles. The remainder of statistical analyses focused on age-specific subgroups, since cumulative phthalates daily intake and behavioral differences across age were more pronounced than between men and women or across time.

Core multivariate linear regression models were then performed separately for children, adolescents, adults, and older adults to estimate adjusted associations between Σandrogen-disruptor (continuous dependent variable) and dietary intake of food prepared away from home, calculated as the percent of TEI (categorical independent variable), divided into none (participants who consumed 100% of calories from food prepared at home the prior day), low, and high (with low and high divided at the weighted median of the exposed group, which comprised all participants who consumed any calories from food prepared away from home the prior day). Covariates included all variables evaluated in the original ANOVA analysis (with age

modeled continuously) except for BMI, which was excluded because it was either a "collider" or on the causal path of an exposure prediction model visualized through a directed acyclic graph (DAG) (**Figure 4-1**).[233] As part of this analysis, however, I added BMI into core regression models as an independent covariate to evaluate its influence on adjusted dining out associations.

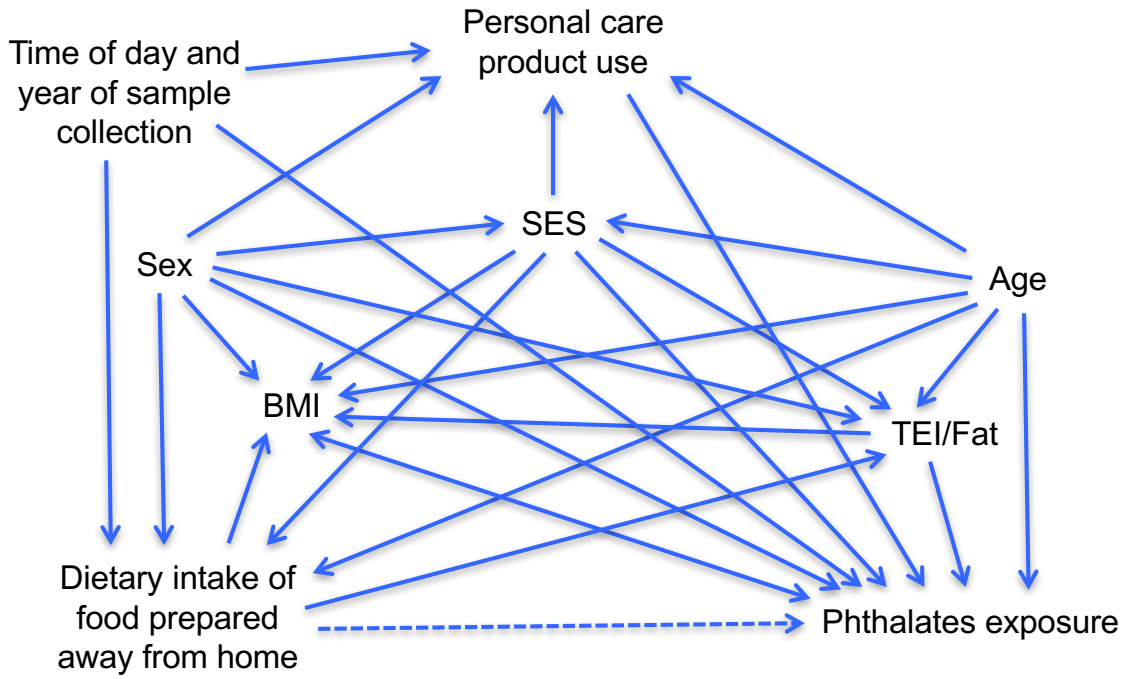

Figure 4-1 Directed acyclic graph (DAG) of the potentially causal association between dietary intake of food prepared away from home and phthalates exposure. Dashed arrow represents association of interest. TEI = Total energy intake (TEI).

Following the Zota et al. (2016) approach,[167] multivariate models were also performed for children, adolescents, adults, and older adults to estimate adjusted associations between Σandrogen-disruptor and 1) TEI, 2) Total fat intake (% TEI), and 3) Food prepared away from home-derived fat intake (% TEI), each modeled separately as categorical independent variables that were divided into low, middle, and high tertiles calculated from age-specific weighted distributions. This analysis was conducted to assess whether and by how much TEI and/or fat intake might be independently associated with phthalates exposure, which would indicate they might mediate observed associations with dining out association.

I also constructed age-specific multivariate models for specific dietary intake sources of food prepared away from home, including fast food, full-service restaurants, and cafeterias (children, adolescents, and all adults combined). Each specific away-from-home food source was modeled separately as a binary independent variable and grouped as any or none, where none was defined as a standard reference group consisting of participants who did not consume any food prepared away from home the prior day (i.e. those who consumed all their calories from food prepared at home), with 31% of children and 43% of adolescents and adults meeting this criteria.

Additionally, I performed multivariate regression to estimate adjusted associations between Σandrogen-disruptor (continuous dependent variable) and dietary intake of particular foods (including fruits, vegetables, sandwiches, fried potatoes, and pizza, each modeled separately as binary independent variables). First, I summed the USDA-converted cup-equivalent data for fruit and vegetable intake from each participant's 24-hour food record.[234] Participants were then grouped into yes/no categories based on whether or not they met their daily dietary recommendations for either fruit or vegetable intake.[235] Sandwich, fried potato, and pizza consumption over the last 24 hours was divided into any or none (where none indicates no sandwich consumption the prior day), first for those prepared at home and then for those prepared away from home. Sandwiches included all those containing meat/poultry/fish, cheese, and/or egg; fried potatoes included French fries; and pizza excluded "no-cheese" items for which meat was also not specified. Similar to Sebastian et al. (2014),[236] I avoided underestimating sandwich consumption by counting sandwiches recorded as singular "mixtures" (one food record with a unique "sandwich" food code) as well as those recorded as combinations of individual ingredients (multiple food records with their own unique food codes). For example, if cheese, beef patty, bun, and tomato each had a food record but were eaten during the same meal, they were counted as a sandwich, just as one food record described as "cheeseburger" was counted as a sandwich. In addition to adjusting for independent covariates described previously, for this analysis I separately added TEI and total fat intake (% TEI) to multivariate models to evaluate them as potential confounders.

From regression models, the percent difference (change) in cumulative phthalates exposure and 95% CI were estimated as $(e^{(\beta)} - 1) * 100$ and $(e^{(\beta \pm \text{critical value x SE})} - 1) * 100$, respectively, where β and SE are the beta coefficient and standard error, respectively. Additionally, I tested for linear trends in percent differences in phthalates exposure by modeling categorical dietary intake variables as ordinal terms in multivariate models. All statistical analyses were performed in SAS version 9.4 (SAS Institute Inc., Cary, NC) with new sample weights calculated according to analytical guidelines for combining multiple cycles of data.[154] Models adjusted for population weights as well as the stratified multi-stage sample design, and statistical and marginal significance were respectively defined at $p < 0.05$ and $p < 0.10$ for two-sided tests. Degrees of freedom for variance estimation were determined by subtracting the number of strata by the number of unique clusters. All phthalate exposure metrics, including individual daily intake estimates, cumulative daily intake, and metabolite-based cumulative metrics, were converted to logarithms prior to statistical testing to account for their non-normal distributions.

4.3.5 Supplementary analyses

Because urine dilution correction and daily intake estimation may introduce uncertainty and variability into regression models,[161,162,171] I assessed alternate measures of urine dilution (i.e. urine flow rate rather than creatinine) and/or whether applying potency-weights directly to metabolites impacted results. Adjusted associations were compared between Σandrogen-disruptor and four other potency-weighted cumulative metrics as described in Chapter 2 and Varshavsky et al. (2016): 1) Parent compound daily intake metric calculated with individual urine flow rate rather than average creatinine excretion rate (Σurine-flow); 2) Metabolite-based analyte excretion rate (mass/time) calculated using urine flow rate (Σexrate-rpf); 3) Metabolite concentration-based metric without urine dilution correction (Σmetab-rpf); and 4) Metabolite

concentration-based metric correcting for creatinine as an independent variable in the regression model (Σmetab-rpf + creat).[191] These models were restricted to 2009-14 data because NHANES did not collect urine flow rate data prior to 2009. I selected the largest age-specific subgroup for this analysis (adults 20-59 years old, $N = 2695$).

Lastly, the percent GM contribution to cumulative phthalates daily intake (Σandrogen-disruptor) was calculated for individual phthalate daily intake estimates (μg/kg/day). Adjusted associations with dining out were additionally evaluated for each phthalate daily intake separately.

4.4 Results

The majority of the study population was non-Hispanic white, above normal weight, between 20-59 years old, and in the middle income category. Most adults over 20 years old had at least some post-high school education (**Table 4-2**). More participants reported dining out than eating only at home the prior day, and unadjusted daily intake of cumulative phthalates (Σandrogen-disruptor) was 35% (95% CI: 29%, 41%) higher among consumers of food prepared away from home ($p < 0.0001$). Higher Σandrogen-disruptor concentrations were observed in earlier NHANES cycles, with a 50% total decrease in exposure between 2005 and 2014 ($p < 0.0001$). Cumulative phthalates were also elevated in males; Hispanic and white compared to black participants; underweight compared to normal weight participants; those in the highest (≥ 3) compared the middle (1 – 3) PIR; adults with the least (< high school) compared to the most (> high school) education; and in evening rather than morning sample collection times. Unadjusted cumulative phthalates exposure was fairly similar for adolescents, adults, and older adults (GM range: 3.9 to 4.3 μg/kg/day), while the GM for children (GM = 6.6 μg/kg/day; 95% CI: 6.2, 6.9) was 50-70% higher ($p < 0.0001$) (**Table 4-2** and **Figure 4-2**).

Table 4-2 Unadjusted percent difference (% Δ) in cumulative phthalates daily intake (Σandrogen-disruptor, *μg/kg/day*) across population characteristics among the U.S. general population in NHANES 2005−14 (*N* = 10,253)

Population Characteristics	*n (%)*	*% Δ (95% CI)*	*GM (GSE)*	*p-value*
Age (years)				<0.0001
≥ 60	2244 (22)	Referent	3.9 (3.7, 4.2)	
20-59	4601 (45)	9.2 (2.4, 17)**	4.3 (4.1, 4.5)	
12-19	1887 (18)	9.2 (1.3, 18)*	4.3 (4.0, 4.6)	
6-11	1521 (15)	70 (56, 81)***	6.6 (6.2, 6.9)	
Sex				0.032
Male	5089 (50)	Referent	4.5 (4.3, 4.6)	
Female	5164 (50)	-4.4 (-8.2, -0.4)*	4.3 (4.1, 4.5)	
Race/ethnicity				0.0003
Black	2633 (26)	Referent	3.9 (3.7, 4.1)	
White	4592 (45)	15 (7.1, 26)**	4.4 (4.2, 4.8)	
Mexican American/Hispanic	3028 (30)	16 (7.1, 26)**	4.5 (4.2, 4.8)	
Body mass index (BMI)[a]				<0.0001
Normal (18.5-25 kg/m^2)	3211 (32)	Referent	4.3 (4.1, 4.5)	
Underweight (< 18.5 kg/m^2)	1230 (12)	39 (29, 49)***	6.0 (5.6, 6.3)	
Overweight (25-30 kg/m^2)	2751 (27)	-0.8 (-6.2, 4.8)	4.3 (4.0, 4.5)	
Obese (≥ 30 kg/m^2)	2985 (29)	-2.6 (-8.1, 3.2)	4.3 (4.1, 4.5)	
Poverty:income ratio (PIR)				0.035
≥ 3 (Highest income)	3389 (33)	Referent	4.5 (4.3, 4.8)	
1-3 (Moderate income)	4260 (42)	-7.4 (-13, -1.8)*	4.2 (4.0, 4.4)	
< Poverty line	2604 (25)	-5.2 (-12, 1.8)	4.3 (4.1, 4.5)	
Education[b]				0.023
> High School	3420 (50)	Referent	4.3 (4.1, 4.5)	
= High School	1652 (24)	-4.0 (-10, 2.3)	4.1 (3.9, 4.4)	
< High School	1773 (26)	-9.3 (-15, -2.8)**	3.9 (3.7, 4.1)	
Survey cycle				<0.0001
2013-14	1873 (18)	Referent	3.5 (3.3, 3.7)	
2011-12	1735 (17)	16 (3.4, 30)*	4.1 (3.7, 4.5)	
2009-10	2241 (22)	21 (10, 33)**	4.3 (4.0, 4.6)	
2007-8	2193 (21)	39 (25, 55)***	4.9 (4.5, 5.3)	
2005-6	2211 (22)	49 (34, 66)***	5.2 (4.8, 5.7)	
Sampling session				<0.0001
Morning	4833 (47)	Referent	4.2 (4.0, 4.4)	
Afternoon	3629 (35)	-2.0 (-7.0, 3.2)	4.1 (4.0, 4.4)	
Evening	1791 (18)	22 (15, 30)***	5.2 (4.9, 5.4)	
Dietary intake of food prepared away from home (prior 24 hours)				<0.0001
None	4024 (39)	Referent	3.6 (3.5, 3.8)	
Any	6229 (61)	35 (29, 41)***	4.9 (4.7, 5.1)	

[a] Sample size restricted to 10,177 due to missing BMI data.
[b] Educational attainment restricted to adults only (*N* = 6845).
*p<0.05. **p<0.01. ***p<0.0001.

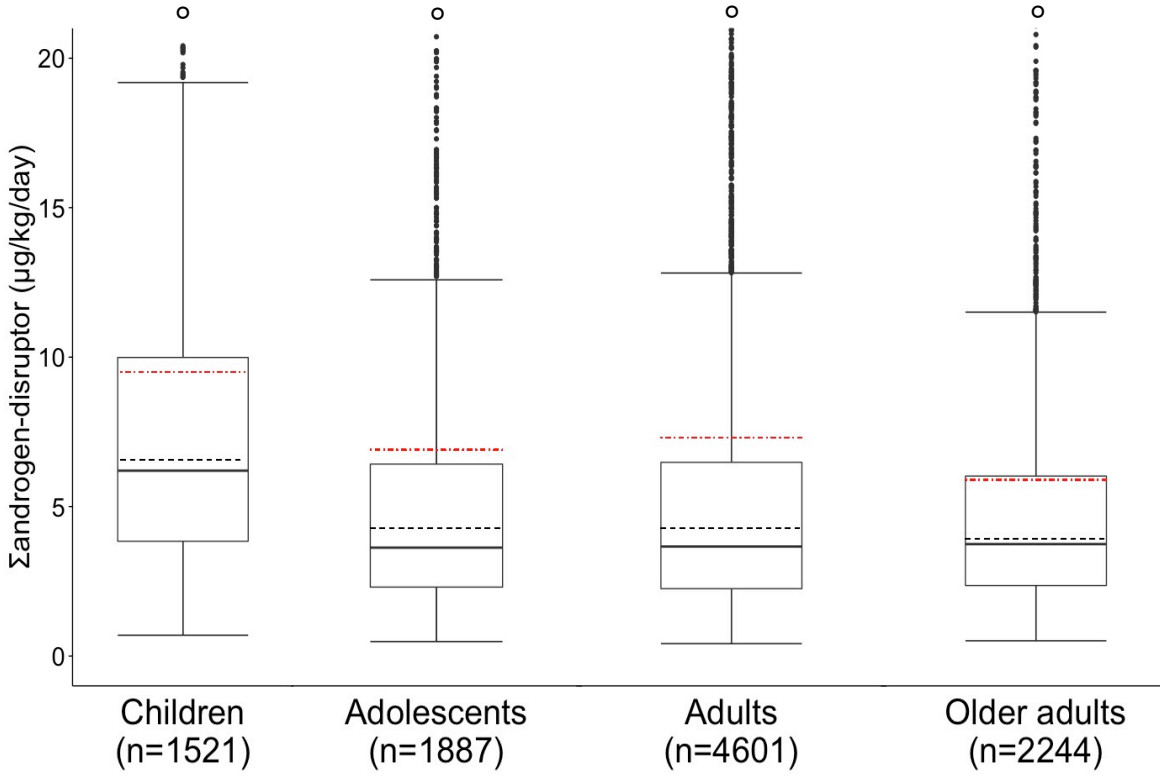

Figure 4-2 Distribution of unadjusted cumulative phthalates daily intake (Σandrogen-disruptor) among age-specific subgroups in NHANES 2005-14 (N = 10,253). Boxes represent interquartile range (IQR: 25th to 75th percentiles). Dark lines represent medians. Red dashed lines represent geometric means. Dot-dashed lines represent arithmetic means. Whiskers extend to min and max (Max = most extreme values within 1.5 • IQR of the median). Outliers are represented by dark points, and hollow points denote outliers off the y-axis scale. A total of 127 (8.3%), 205 (11%), 469 (10%), and 200 (8.9%) outliers were observed among children, adolescents, adults, and older adults, respectively. P-value from statistical test of difference between age-specific subgroups was < 0.0001.

Dietary consumption patterns varied significantly by age and sex ($p < 0.0001$) (**Table 4-3**). All food consumed in the previous 24 hours was prepared at home for 52% of older adults compared to 33% of adults 20-39 years old. A higher percentage of children ate cafeteria food compared to adolescents, but a higher proportion of adolescents than children consumed calories from fast food and full-service restaurants. Adults 20-39 years old were the highest percent eaters of fast food and full-service restaurants in the entire population. More men proportionally consumed food prepared outside the home than did women, but the differences were less pronounced than those observed across age (**Table 4-3**). Similarly, dining out differences were less pronounced over time. Though a temporary decrease to 58% was observed for 2009-10 ($p = 0.03$), just under 65% reported consumption of food prepared away from home across most NHANES survey cycles, and thus these data were not reported.

Table 4-3 Percent of participants who reported consumption of food prepared away from home in the past 24 hours across age and by sex among the U.S. general population[a] in NHANES 2005-14 (N = 10,253)

	Age (years)						***Sex (Adults ≥ 20 years)***		
	6-11	**12-19**	**20-39**	**40-59**	**≥ 60**		**Men**	**Women**	
	n=1521	*n = 1887*	*n = 2407*	*n = 2194*	*n = 2244*		*n = 3337*	*n = 3508*	
Dietary Intake[b] *Food prepared away from home*	**% (SE)**	**% (SE)**	**% (SE)**	**% (SE)**	**% (SE)**	***p*-value**	**% (SE)**	**% (SE)**	***p*-value**
NONE *Only food prepared at home*	31 (1.7)	34 (1.3)	33 (1.2)	37 (1.4)	52 (1.5)	<0.0001	35 (1.1)	43 (1.0)	<0.0001
TOTAL *Any food prepared away from home*	69 (1.7)	67 (1.3)	67 (1.2)	63 (1.4)	48 (1.5)	<0.0001	65 (1.1)	57 (1.0)	<0.0001
Fast food *Any*	36 (1.7)	42 (1.2)	44 (1.1)	34 (1.4)	21 (1.5)	<0.0001	38 (1.1)	33 (0.8)	<0.0001
Full-service restaurant *Any*	14 (1.2)	18 (1.5)	28 (1.2)	27 (1.0)	22 (1.1)	<0.0001	27 (0.9)	22 (0.8)	<0.0001
Cafeteria *Any*	28 (1.8)	16 (1.1)	4.0 (0.6)	4.0 (0.6)	3.0 (0.5)	<0.0001	8.0 (0.5)	7.0 (0.5)	0.420
Other (marginal) *Any*	17 (1.2)	12 (1.0)	15 (1.0)	18 (1.2)	12 (1.0)	<0.0001	17 (0.7)	13 (0.7)	<0.0001

[a] Percentages are weighted. Differences evaluated using Rao Scott chi-square test for independence.
[b] None indicates participants who did not consume any food prepared away from home the prior day (i.e. 100% of their calories came from food prepared at home). Total indicates participants who consumed any food prepared away from home (including from fast food, full-service restaurants, cafeterias, and/or "other" marginal sources that together contributed less than 5% to total energy intake, or TEI).

Consuming food prepared away from home was associated with increased cumulative phthalates daily intake (∑androgen-disruptor) across age-specific subgroups in multivariate models (*p* for trend < 0.0001) (**Figure 4-3** and **Table 4-4**). While associations were significant among all age groups, the magnitude of association was largest among adolescents, with high adolescent consumers of food prepared away from home having 55% (95% CI: 35%, 78%) higher concentrations of ∑androgen-disruptor, respectively, compared to adolescents who consumed all their calories from food prepared at home. The weakest associations were observed among children, with high consumers of food prepared away from home having 30% (95% CI: 16%, 45) higher ∑androgen-disruptor concentrations than children who consumed all their calories from food prepared at home. Similar positive associations were observed across age-specific subgroups between food away from home-derived fat intake and ∑androgen-disruptor (*p* for trend < 0.0001). TEI and total fat intake in particular were modestly associated with ∑androgen-disruptor among adults, but these baseline associations were substantially smaller than those observed for dining out. Additionally, BMI did not influence dining out associations with phthalates exposure when added to core multivariate models (**Table 4-4**).

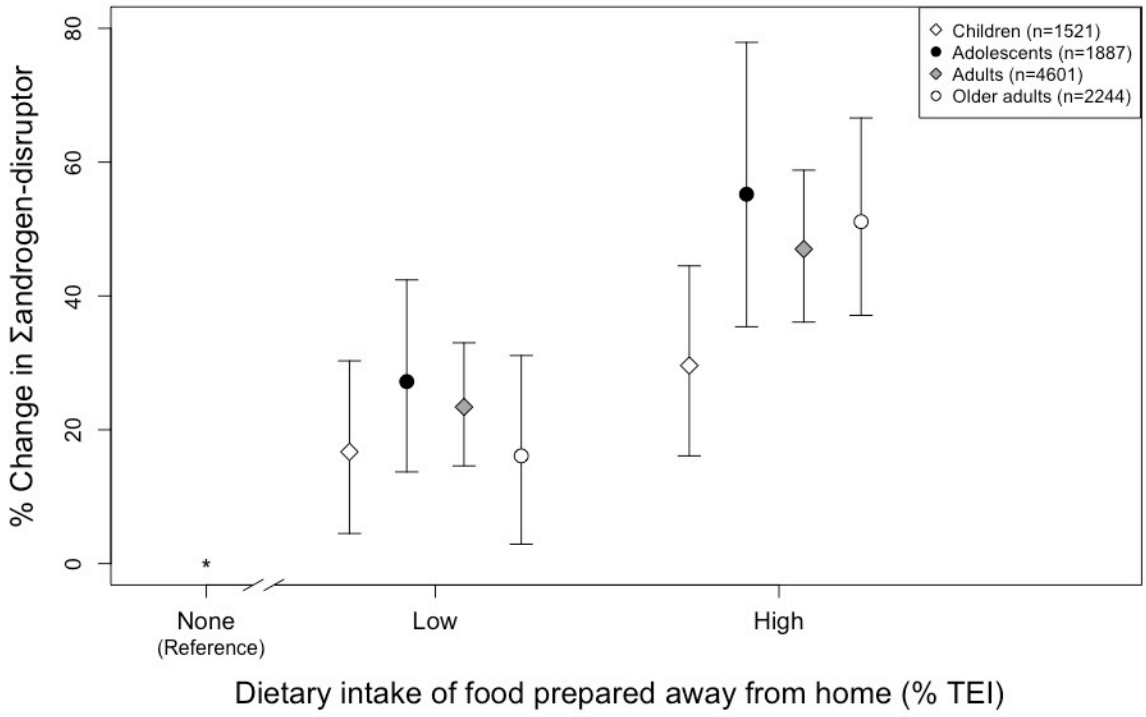

Figure 4-3 Adjusted percent difference (% change) and 95% CI of Σandrogen-disruptor *(µg/kg/day)* among age-specific subgroups in NHANES 2005-14 (*N* = 10,253). Covariates: Sex, age, race/ethnicity, poverty-to-income (PIR), education (adults only), NHANES survey cycle, and time of sampling session. *P* for trend was < 0.0001 for all age subgroups. Low and high intake divided at weighted median of participants who consumed any food prepared away from home the prior day. TEI = Total energy intake (kcals).

Table 4-4 Adjusted percent difference (% Δ) in Σandrogen- disruptor *(μg/kg/day)*[a] in NHANES 2005−14 (*N* = 10,253)

	Children 6-11 yrs (n = 1521)		*Adolescents 12-19 yrs (n = 1887)*		*Adults 20-59 yrs (n = 4601)*		*Adults ≥ 60 yrs (n = 2244)*	
Dietary Intake	***n***	**% Δ (95% CI)**	***n***	**% Δ (95% CI)**	***n***	**% Δ (95% CI)**	***n***	**% Δ (95% CI)**
Away from home (% TEI)[b]								
None	471	Referent	630	Referent	1714	Referent	1209	Referent
Low	504	17 (4.5, 30)*	650	27 (14, 42)**	1462	23 (15, 33)**	526	16 (2.9, 31)*
High	546	30 (16, 45)**	607	55 (35, 78)**	1425	47 (36, 59)**	509	51 (37, 67)*
p for trend		<0.0001		<0.0001		<0.0001		<0.0001
Away from home (% TEI) + BMI[b,c]								
None	470	Referent	624	Referent	1709	Referent	1191	Referent
Low	504	16 (3.3, 30)*	647	27 (14, 42)**	1456	24 (15, 33)**	510	16 (2.5, 31)*
High	546	29 (16, 44)**	600	56 (36, 79)**	1416	48 (37, 60)**	504	50 (36, 65)**
p for trend		<0.0001		<0.0001		<0.0001		<0.0001
Away from home-derived fat (% TEI)[b]								
None	477	Referent	638	Referent	1756	Referent	1221	Referent
Low	506	18 (4.9, 32)*	647	27 (14, 42)**	1438	23 (14, 32)**	525	18 (5.1, 31)*
High	538	28 (14, 44)**	602	54 (34, 78)**	1407	46 (34, 58)**	498	51 (36, 68)**
p for trend		<0.0001		<0.0001		<0.0001		<0.0001
Total fat (% TEI)[d]								
Low	497	Referent	634	Referent	1634	Referent	795	Referent
Mid	498	-0.16 (-11, 12)	603	3.6 (-8.1, 17)	1465	3.1 (-4.0, 11)	759	-9.3 (-18, 0.8)
High	526	0.93 (-9.0, 12)	650	9.8 (-2.9, 24)	1502	14 (6.6, 22)*	690	10 (-1.4, 23)
p for trend		0.86		0.13		0.0002		0.09
Total energy intake (TEI) (kcals)[d]								
Low	528	Referent	626	Referent	1570	Referent	848	Referent
Mid	494	4.5 (-6.7, 17)	615	-7.5 (-17.0, 3.0)	1526	1.9 (-5.8, 10)	672	-8.1 (-18, 2.3)
High	499	2.6 (-8.2, 15)	646	12 (-4.6, 30)	1505	9.5 (1.0, 19)*	724	-1.1 (-13, 12)
p for trend		0.64		0.18		0.03		0.90

[a] Covariates: Sex, age, race/ethnicity, poverty-to-income ratio (PIR), education (adults only), NHANES survey cycle, and time of sampling session.
[b] Low/high divided at weighted median of participants who consumed any food prepared away from home the prior day (% TEI). Weighted median for away from home intake *[+ BMI]*: 40.3, 48.5, 44.3 *[44.5]*, and 37.8 *[37.3]* (Range for all age subgroups = 0 – 100). Weighted median (range) for away from home-derived fat intake (% TEI): 14.0 (0 - 42.4), 17.9 (0 – 62.3), 16.7 (0 – 55.7), and 15 (0 – 61.7) for children, adolescents, adults, and older adults, respectively.
[c] BMI added as additional covariate. Sample sizes were 1520, 1871, 4581, and 2005 for children, adolescents, adults, and older adults, respectively, due to missing BMI data.
[d] Low/mid/high calculated from weighted distributions of age-specific subgroups. Tertile divisions (range) for children, adolescents, adults, and older adults, respectively: 30.1, 35.9 (3.8 – 61.5), 30.2, 36.5 (1.8 – 69.2), 29.9, 37.2 (1.5 – 74.7), and 30.7, 38.3 (4.1 – 64.6) for total fat intake (% TEI); 1613, 2112 (171 - 6992), 1650, 2379 (193 - 9363), 1746, 2570 (89 – 13,133), and 1496, 2004 (188 - 6305) for TEI (kcals).
*p<0.05. **p<0.0001.

Each specific source of food prepared away from home was also significantly associated with ∑androgen-disruptor across all age subgroups (**Table 4-5**). However, fast food and full-service restaurant consumption were more highly associated with ∑androgen-disruptor than was cafeteria food intake among children. Children who consumed food from cafeterias had 15% (95% CI: 4.0%, 28%) higher ∑androgen-disruptor concentrations compared to those who consumed all their calories from home, whereas fast food and restaurant food intake were associated with 29% (95% CI: 16%, 43%) and 46% (95% CI: 22%, 73%) higher concentrations, respectively, compared to the same reference group. On the other hand, the cafeteria had a relatively stronger association with cumulative phthalates exposure in adolescents and adults, with adult cafeteria consumers having 64% (95% CI: 40%, 92%) higher cumulative phthalates daily intake than adults who consumed all their calories from home **(Table 4-5**).

Table 4-5 Adjusted percent difference (% Δ) in Σandrogen-disruptor *(μg/kg/day)* from specific sources of food prepared away from home[a] in NHANES 2005−14 (*N* = 10,253)

	Children 6-11 yrs old (*N* = 1521)		Adolescents 12-19 yrs old (*N* = 1887)		Adults ≥ 20 years (*N* = 6845)	
Dietary Intake (% TEI)	***n***	**% Δ (95% CI)**	***n***	**% Δ (95% CI)**	***n***	**% Δ (95% CI)**
Fast food restaurant						
None[b]	471	Referent	630	Referent	2923	Referent
Any	545	29 (16, 43)**	798	47 (32, 64)**	2313	39 (31, 48)**
Full-service restaurant						
None[b]	471	Referent	630	Referent	2923	Referent
Any	165	46 (22, 73)**	251	52 (24, 86)**	1503	41 (31, 51)**
Cafeteria						
None[b]	471	Referent	630	Referent	2923	Referent
Any	488	15 (4.0, 28)*	381	45 (24, 68)**	223	64 (40, 92)**

[a] Covariates: Sex, age, race/ethnicity, poverty-to-income ratio (PIR), education (adults only), NHANES survey cycle, and time of sampling session.
[b] Reference group = Participants who did not eat any calories from food prepared away from home, i.e. 100% of their calories came from home.
*p<0.01. **p<0.0001.

Among all subgroups, consuming sandwiches, fried potatoes, and pizza prepared at home was not associated with increased ∑androgen-disruptor, but consuming these items prepared outside the home was associated with elevated cumulative phthalates concentrations across the study population (with the exception of pizza, which was associated with phthalates in children only (**Table 4-6**). For example, children who consumed sandwiches prepared away from home had 35% (95% CI: 20%, 51%) higher ∑androgen-disruptor concentrations than children who did not consume any sandwiches the prior day ($p < 0.0001$). Meeting daily requirements for fruit or vegetable intake was not significantly associated with ∑androgen-disruptor in most subgroups. However, ∑androgen-disruptor concentrations were decreased by 13% (95% CI: 1.9%, 23%) among adolescents who did not meet either requirement, compared to adolescents who met at least one of the daily recommendations ($p = 0.03$), although the difference was no longer significant when the adjusted model included TEI as an additional independent covariate, indicating that TEI might be a confounder of the association between fruit or vegetable intake and phthalates exposure (**Table 4-6**).

Table 4-6 Adjusted percent difference (% Δ) in Σandrogen-disruptor *(μg/kg/day)* by particular food[a] with total fat and energy intake added separately as potential confounders in NHANES 2005−14 (*N* = 10,253)

		Children 6-11 yrs (*N* = 1521)		Adolescents 12-19 yrs (*N* = 1887)		Adults ≥ 20 yrs (*N* = 6845)	
Dietary Intake		*n*	% Δ (95% CI)	*n*	% Δ (95% CI)	*n*	% Δ (95% CI)
≥ Fruit/veggie guideline[a]	Yes	498	--	415	Referent	1890	Referent
	No[b]	751	3.0 (-8.0, 15)	1158	-13 (-23, -1.9)*	3668	4.1 (-1.7, 10)
	No[c]	751	3.8 (-7.4, 16)	1158	-14 (-24, -2.4)*	3668	3.2 (-2.5, 9.2)
	No[d]	751	3.9 (-7.2, 16)	1158	-9.9 (-20, 1.7)	3668	4.9 (-1.1, 11)
Sandwich[e]							
At home	None	821	Referent	974	Referent	3801	Referent
	Any[b]	394	6.8 (-2.1, 17)	467	3.2 (-9.9, 18)	1895	-1.2 (-7.0, 5.0)
	Any[c]	394	7.0 (-1.8, 17)	467	3.1 (-9.9, 18)	1895	-1.5 (-7.3, 4.6)
	Any[d]	394	6.4 (-2.4, 16)	467	2.5 (-11, 17)	1895	-1.3 (-7.2, 5.0)
Away from home	None	821	Referent	974	Referent	3801	Referent
	Any[b]	364	35 (20, 51)***	530	35 (15, 59)**	1413	22 (12, 33)***
	Any[c]	364	35 (20, 51)***	530	35 (15, 58)**	1413	21 (11, 32)***
	Any[d]	364	35 (20, 51)***	530	34 (14, 57)**	1413	22 (12, 33)***
Fried potatoes[e]							
At home	None	1170	Referent	1437	Referent	5696	Referent
	Any[b]	87	-6.5 (-21, 10)	83	-12 (-26, 3.7)	318	-2.6 (-15, 12)
	Any[c]	87	-6.5 (-21, 11)	83	-13 (-26, 3.8)	318	-3.2 (-16, 11)
	Any[d]	87	-7.0 (21, 9.6)	83	-12 (-26, 3.2)	318	-2.7 (-15, 12)
Away from home	None	1170	Referent	1437	Referent	5696	Referent
	Any[b]	271	26 (11, 44)**	375	28 (8.3, 51)*	855	16 (5.6, 27)*
	Any[c]	271	27 (11, 45)**	375	27 (7.4, 51)*	855	14 (4.1, 25)*
	Any[d]	271	26 (11, 44)**	375	27 (6.8, 50)*	855	16 (5.3, 27)*
Pizza[e]							
At home	None	1139	Referent	1492	Referent	6166	Referent
	Any[b]	88	0.9 (-18, 25)	97	-8.4 (-22, 7.5)	244	0.3 (-13, 16)
	Any[c]	88	0.6 (-19, 25)	97	-8.5 (-22, 7.4)	244	-0.7 (-14, 15)
	Any[d]	88	0.1 (-19, 24)	97	-9.9 (-23, 5.7)	244	-0.1 (-14, 15)
Away from home	None	1139	Referent	1492	Referent	6166	Referent
	Any[b]	303	12 (0.8, 25)*	304	5.8 (-6.3, 20)	442	5.3 (-4.6, 16)
	Any[c]	303	12 (0.7, 26)*	304	5.8 (-6.5, 20)	442	4.8 (-5.1, 16)
	Any[d]	303	12 (0.0, 25)*	304	3.7 (-7.8, 17)	442	4.7 (-4.9, 15)

[a] Sample sizes restricted due to data unavailability for 2013-14.
[b] Covariates: Sex, age, race/ethnicity, poverty-to-income ratio (PIR), education (adults only), NHANES survey cycle, time of sampling session, and either total energy intake (TEI) in kcals or total fat intake (% TEI).
[c] Original adjusted core model[b] with additional total fat intake (% TEI) as independent covariate.
[d] Original adjusted core model[b] with additional TEI as independent covariate.
[e] Reference groups for at home and away from home comparisons = Participants who did not consume any sandwiches, fried potatoes, or pizza, respectively.
*p<0.05. **p<0.001. ***p<0.0001.

Consumption of food prepared away from home was positively associated with phthalates exposure across all supplementary cumulative metrics that evaluated alternate approaches to urine dilution correction and daily intake estimation (**Table 4-7**). However, the magnitudes of association were somewhat varied. Adjusted associations for daily intake metrics (Σandrogen-disruptor and Σurine-flow) were more similar to each other and larger than those observed for metabolite-based metrics. Of the three metabolite-based metrics, the metric that applied RPF weights directly to non-creatinine-corrected urinary metabolite concentrations (Σmetab-rpf) generally produced the strongest associations, followed by the metric combining RPF-weighted metabolite excretion rates (Σexrate-rpf). The metric that applied RPF weights directly to urinary metabolite concentrations and adjusted for creatinine as an independent covariate in the multivariate model (Σmetab-rpf + creat) generally produced the weakest associations (**Table 4-7**).

Table 4-7 Adjusted percent difference (%Δ) in cumulative phthalates exposure across alternate approaches to urine dilution correction and daily intake estimation[a] among adults 20-59 years old in NHANES 2009-14 (N = 2695)

	Daily intake-based metrics			Metabolite-based metrics		
	Σandrogen-disruptor[b] *(μg/kg/day)*		***Σurine_flow***[b] *(μg/kg/day)*	***Σexrate_rpf***[c] *(μg/day)*	***Σmetab_rpf***[c] *Measured urinary metabolite concentration (μg/L)*	
Dietary intake (%TEI)	*Daily intake with creatinine excretion rate*		*Daily intake with urine flow rate*	*Metabolite excretion rate*	*No urine dilution correction*	*Creatinine-correction as independent covariate*
Away from home	***n***	**% Δ (95% CI)**	**% Δ (95% CI)**	**% Δ (95% CI)**	**% Δ (95% CI)**	**% Δ (95% CI)**
None (0)[d]	1023	--	--	--	--	--
Low (0.07 – 44.1)	841	27 (16, 39)***	29 (15, 43)***	14 (3.1, 26)*	19 (8.1, 32)**	12 (4.2, 21)*
High (44.2 - 100)	832	55 (40, 71)***	58 (39, 80)***	29 (16, 44)***	40 (24, 58)***	25 (14, 36)***
p for trend		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a] Covariates: Sex, age, race/ethnicity, poverty-to-income (PIR), education, NHANES survey cycle, and time of sampling session.
[b] Relative potency factors applied to daily intake estimates of DnBP, DiBP, BBzP, DEHP, DEP, and DiNP.
[c] Relative potency factors applied directly to urinary metabolite concentrations for MnBP, MiBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, MEP, and MCOP.
[d] Reference group = Adults who did not eat any food prepared away from home the prior day (i.e. 100% of their calories came from food prepared at home).
*p<0.05. **p<0.001. ***p<0.0001.

When evaluated separately, daily intake of DEHP and DiNP were each positively associated with dining out ($p < 0.0001$), with high consumers of food prepared away from home having 74% more DiNP concentrations than participants who consumed all their calories from food prepared at home (**Table 4-8**). These high molecular weight (MW) phthalates were the top contributors to Σandrogen-disruptor (45% and 30%, respectively), while DnBP ranked third (16%). However, DnBP was negatively associated with dining out ($p = 0.056$), and all other phthalates were not individually associated with dietary intake of food prepared away from home. Thus, dining out associations were largely driven by DEHP and DiNP (**Table 4-8**).

Table 4-8 Adjusted percent difference (%Δ) in daily intake of individual phthalates (*μg/kg/day*)[a] and percent contribution to ∑androgen-disruptor among the general population in NHANES 2005-14 (*N* = 10,253)

Dietary intake of food away from home (% TEI)	***n***	**DEHP**	**DiNP**	**DnBP**	**DEP**	**BBzP**	**DiBP**
None (0)	4024	Referent	Referent	Referent	Referent	Referent	Referent
Low (0.04 - 43.3)	3178	15 (9.1, 21)**	37 (27, 47)**	-1.3 (-5.9, 3.5)	-2.4 (-10, 6.1)	-2.3 (-8.3, 4.1)	-0.8 (-5.4, 3.9)
High (43.4 - 100)	3051	31 (22, 40)**	74 (60, 901)**	-5.8 (-11, 0.1)*	-6.9 (-14, 1.0)*	-3.0 (-8.7, 3.0)	-2.7 (-7.8, 2.8)
p for trend		<0.0001	<0.0001	0.056	0.088	0.314	0.333
Contribution *(%)*		*45*	*30*	*16*	*4*	*2*	*2*

[a] Covariates: Sex, age, race/ethnicity, poverty-to-income ratio (PIR), education (adults only), NHANES survey cycle, and time of sampling session.
*p<0.10. **p<0.0001.

4.5 Discussion

This is first study to my knowledge that examines specific dietary sources associated with cumulative phthalates exposure among the U.S. general population. In this cross-sectional analysis of NHANES study participants sampled between 2005 and 2014, consuming food prepared outside the home, particularly dining out, was positively associated with cumulative phthalates exposure across age groups in the United States, with as much as 55% increased cumulative daily intake in high adolescent consumers of food away from home compared to consumers of food prepared at home only. Moreover, particular foods including sandwiches and French fries, were not significantly associated with cumulative exposure unless the items were prepared away from home, indicating that food source may matter more than food type.

While DEHP and DiNP metabolites are associated with fast food intake among the U.S. general population,[167,223] this study demonstrates that other sources of food prepared away from home beyond fast food, such as full-service restaurants and cafeterias, may also be important contributors to phthalates exposure. However, though the proportion of children who consumed cafeteria food was seven times that of adults and double that of adolescents, the cafeteria had a less substantial relative role in phthalates exposure among children, suggesting that teenagers and adults might be consuming more phthalate-contaminated foods in the cafeteria setting. Indeed, adolescents have greater autonomy than children in their cafeteria food choices, and high-school meals are generally richer in fat and lower in nutritional quality than those prepared for younger kids.[221,237] Additionally, while adults make better decisions when provided with healthy cafeteria food options than they might otherwise,[238] many workplace cafeterias predominantly offer high-calorie, fatty foods, and recent studies indicate that healthier options alone are not sufficient for improving dietary behavior among U.S. workers.[239–243]

Though more adolescents and adults proportionally consumed restaurant and/or fast food in this study, children had substantially higher cumulative daily intake of anti-androgenic phthalates (70% higher than older adults), suggesting that sources beyond food prepared outside the home may be important considerations for this age group. These results are consistent with past studies that reported higher concentrations of DEHP and DiNP metabolites in children.[139,244] Several age-dependent biological and behavior differences may be possible factors, as phthalate metabolism varies with age, children consume a higher proportion of food to body size, and younger kids may disproportionately ingest phthalates that settle in house dust from consumer and personal care products by playing on the floor and engaging in hand-to-mouth activity.[139,244–249] Children also eat more snack foods than older groups[250,251] and conceivably might be consuming more phthalates from processed or packaged foods at home.

Phthalates have been detected in processed foods, such as cereals and cookies, among numerous other food products sold in European markets.[252] They have also been linked to prepared school and hospital meals, as well as takeout containers made from both plastic and paper or cardboard (i.e. pizza delivery boxes) in studies outside the United States.[215,219,225,226,252] Though clear contamination pathways are difficult to ascertain, phthalates

can migrate into foods from industrialized processing equipment, such as conveyer belts or plastic tubing, from gloves used to handle food along factory assembly lines, and from diverse food contact materials, including plastic wrap, metal gaskets in glass jar lids, and printed ink food labels.[211,214,219,224,252,253] Their migration potential depends on MW and lipophilicity of foods they contact (with high MW phthalates readily attracted to foods higher in fat), among other factors like temperature and duration of storage and transport.[4]

Though prior U.S. research has identified important at-home sources of dietary phthalates exposure, such as store-bought packaged foods, olive oil, milk, poultry, cooking spices, and bread,[213,217,254–257] my findings suggest that eating at home may actually reduce exposure relative to dining out. Yet data regarding phthalate uses across dining out supply chains is relatively lacking in the United States. Further efforts that target dietary sources of exposure should focus on U.S. fast food, restaurant, and cafeteria food production lines. Future studies should also more broadly characterize food contamination pathways for emerging anti-androgens of concern. For example, relatively little is known about DiNP despite its replacement of DEHP in the global plasticizers market and increased detection in European food contact materials.[13,170] DiNP was more highly associated than DEHP with dining out in this study and with fast food in previous research.[167,223] I additionally observed marked DEHP decreases and DiNP increases between 2001 and 2012 in Chapter 2 (**Figure 2-5**) that mirror urinary metabolite trends among the U.S. population.[3] While DiNP may have increased, overall cumulative phthalates exposure decreased across the study period, which is consistent with cumulative exposure trends noted in Chapter 2 (**Table 4-2** and **Figure 2-4**). This decrease over time is likely due to DiNP's reduced relative potency, which corresponds to a relatively low "weight" in the cumulative exposure metric. Nevertheless, DiNP is a recognized anti-androgen of concern.[13,124] Furthermore, if dining out trends remain constant or increase, food prepared outside the home will likely continue to be an important source of anti-androgenic exposure going forward, due in large part to DiNP's strong associations.

Alternatively, DnBP, DiBP, BBzP, and DEP were not individually associated with dining out in this study, indicating that dietary intake of food prepared away from home is not a substantial source of exposure to these phthalates. Although lower MW phthalates have been used in food contact materials and/or detected in food itself, high MW phthalates, including DEHP and DiNP, are much more clearly and consistently linked to dietary intake.[4,217] Nevertheless, diet is thought to be a significant source of BBzP and DnBP exposure.[217] Fast food intake has been associated with urinary BBzP metabolite concentrations among young children in the United States,[223] and a U.S. study recently reported DnBP contamination of supermarket foods from paper food packaging labels made from printed ink that contained DnBP.[257] Continued efforts should be made to evaluate these phthalates in future dietary assessments, especially regarding DnBP, which constituted the third largest portion of the anti-androgenic metric (~16%), and for which past studies of dietary and non-dietary sources have not adequately explained its widespread exposure profile.[3]

Though evaluating individual phthalate sources is useful for identifying opportunities to modify specific phthalate exposures, cumulative daily intake is more relevant for overall risk. Future research should continue to examine anti-androgenic phthalates singularly and collectively. Continued monitoring of racial/ethnic differences is also warranted, given that lower cumulative exposures were observed in black compared to Hispanic and white participants in this study, whereas reproductive-aged black women had higher cumulative concentrations than white women in Chapter 2. The difference in observed disparities is likely attributable to study population differences, as this chapter included men, older women, adolescents, and children, as well as a broader definition of the Hispanic racial/ethnic category. The opposing results are also likely attributable to decreased racial/ethnic disparities over time that were noted in Chapter 2, since this study examined later survey cycles than did the prior analysis. Additionally, while associations in this study were robust across different approaches to urine dilution correction and daily intake estimation, previous work suggested that daily intake estimation and creatinine-correction may introduce bias when modeling phthalate effects on a BMI-related outcome.[171] Findings from Christensen et al. (2014) may be specific to that model, but comparable simulation studies would be a useful future step in determining which cumulative metric minimizes bias specifically when modeling dietary or other sources of phthalates exposure.[171]

This study was limited by its cross-sectional design, which makes it difficult to determine the causal direction of the observed association between consumption of food prepared away from home and cumulative phthalates exposure. Conceptually, it is more likely that dining out increases phthalates exposure rather than phthalates exposure affecting dining out behavior. However, a longitudinal study could more appropriately delineate the causal direction of the association. Additionally, NHANES dietary data is based on self-reported behavior recalled from memory, which may be prone to exposure misclassification. The error is likely to be non-systematic (non-differential) and would bias results towards the null hypothesis (no association). However, the weaker associations I observed in children might be partially attributable to differential misclassification from proxy-assisted interviews. For example, children who have high phthalates exposure may not be completely honest about foods they consumed away from home the prior day when interviewed in front of their parents.

Another limitation of this study is that the cumulative phthalates exposure metric is based on the relative ability of phthalates to decrease testosterone levels during pregnancy, which is most applicable to reproductive outcomes in male offspring.[1,97] The underlying dose response data that produced NAS benchmark doses, which I used to construct RPFs in Chapter 2, were recently shown to predict mixture effects for multiple postnatal male developmental endpoints, including reproductive malformations and reduced anogential distance. Future work is needed to determine whether the method is appropriate for other hormone-mediated outcomes. For example, though the metric is relevant for adult obesity and diabetes with regard to low testosterone effects,[207,208] current data gaps preclude my ability to compare RPFs calculated in Chapter 2 with potency estimates reported in laboratory studies for metabolic outcomes. Existing studies on the competitive ability of phthalates to bind the peroxisome proliferator-activated receptor activity (PPAR-γ)

receptor, an important component of the metabolic disease pathway, vary widely in terms of whether experiments were performed with phthalate metabolites or parent compounds and whether they used mouse or human cells.[60,62–65,258] Future research to resolve these issues and provide relevant data on prenatal phthalates PPAR-γ activity could help determine whether relative potency factors used in this method may apply to metabolic disease, or whether a cumulative metric specific to metabolic disease would require different relative potency estimates.

Despite these limitations, this chapter identifies a potential intervention opportunity for reducing cumulative phthalates exposure among the U.S. general population. Namely, that eating more at home may reduce overall exposure and associated health risks to androgen-disrupting phthalates. The effectiveness of this behavior change should be examined in future studies. Efforts should also be made to identify modifiable production practices that may provide opportunities for upstream solutions that reduce dietary phthalates exposure.

4.6 Conclusion

This study demonstrates that food prepared outside the home may be a significant source of cumulative phthalates exposure across all age groups in the U.S. population. Future efforts to reduce cumulative phthalates exposure should consider dietary interventions that encourage increased intake of food prepared at home and opportunities to remove phthalates from food production lines.

Chapter 5

Evaluation and future application of the cumulative exposure metric

My overarching dissertation goals were to 1) advance cumulative environmental methodologies that better characterize biologically relevant chemical mixtures, and 2) identify exposure disparities and intervention opportunities that may ultimately protect at-risk populations and the U.S. general population from potentially harmful endocrine disrupting chemical (EDC) exposures.

To meet these goals, I developed and evaluated a potency-weighted sum of anti-androgenic phthalates based on National Academy of Sciences (NAS) recommendations. I then compared cumulative phthalates exposure ascertained from National Health and Nutrition Examination Survey (NHANES) data across racial/ethnic groups of U.S. reproductive-aged women using multivariate linear regression. I also used the cumulative metric in a pilot biomonitoring assessment of phthalates exposure among Vietnamese nail salon workers in California. The third application examined dietary sources of cumulative exposure across age groups in the U.S. general population, again using NHANES data and linear regression models. The sentinel findings of this work are outlined below, along with potential limitations that became evident when I operationalized the method as outlined in Chapters 2 through 4. This chapter concludes with a discussion about future research needs and potential applications of the method going forward.

5.1. Summary of major findings

I used benchmark doses (BMD) recommended by the NAS in 2008 to develop a potency-weighted cumulative metric for co-occurring phthalates that is biologically relevant for human health because it is based on a sensitive endocrine endpoint associated with common adverse outcomes (Chapter 2). This study included sample calculations and an evaluation of how alternate approaches to urine dilution correction and daily intake estimation may influence exposure modeling results. I also demonstrated how to use new relative potency data to incorporate emerging compounds into the metric that have known anti-androgenic toxicity profiles (i.e. di-isononyl phthalate (DiNP)). In addition, I showed how indeterminate anti-androgens (for which there may be some suggestive data, i.e. diethyl phthalate (DEP)), may be considered and evaluated under this framework.

Once developed, I used the method to examine disparities in cumulative phthalates exposure across racial/ethnic groups of U.S. reproductive-aged women by aggregating daily intake of individual phthalates that were estimated from measured metabolites in urine (Chapter 2). Black women generally had higher cumulative phthalates exposure than white women, although the magnitude and precision of the percent difference varied by model specification (i.e. alternate approaches to urine dilution correction and daily intake estimation) (Chapter 2). However, percent differences between racial/ethnic groups decreased over time, as did the overall cumulative exposure burden among all race/ethnicities. Additionally, opposing racial/ethnic disparities for the general population were later revealed in Chapter 4, which were likely due to temporal trends in cumulative

phthalates exposure and study population differences. Researchers should continue to assess racial/ethnic differences in cumulative and individual phthalate exposures to improve our understanding of disparities and the factors that drive them.

I also applied the cumulative exposure method to a pilot biomonitoring study of predominantly female Vietnamese immigrant nail salon workers in California and compared their aggregated daily intake estimates with those calculated for Asian Americans in the U.S. general population who were of similar age and sampled around the same year. I concluded that nail salon workers may be disproportionately exposed to phthalate mixtures (Chapter 3), but efforts should be made to assess whether these findings are reproducible in larger studies that collect both pre- and post-shift samples. Larger studies should also identify viable workplace intervention opportunities and product reformulation strategies that mitigate exposure among this occupational group.

Finally, I used the metric to examine dietary sources of cumulative phthalates exposure across age groups in the U.S. general population, including children, adolescents, adults, and older adults (Chapter 4). Across all groups, I found that dining out (i.e. at fast food, full-service restaurants, and school or workplace cafeterias) may be a significant source of cumulative phthalates exposure, and that eating at home may substantially reduce daily intake of combined phthalates. Thus, encouraging people to eat out less may be an effective strategy for reducing dietary sources of phthalates exposure, though efficacy of this behavior change will need to be evaluated in future research. In addition, efforts to identify the most important U.S. food production practices that give rise to phthalates contamination of the food supply could inform upstream opportunities that reduce dietary exposure.

5.2. Limitations and future research needs

5.2.1 Resolving uncertainty and variability

From the first and third study, I concluded that black women generally had higher cumulative phthalates exposure levels than other reproductive-aged women (when earlier NHANES cycles were included in the analysis) and that eating food prepared outside of the home was associated with elevated phthalates exposure. However, I could not definitively report on the magnitude or precision of the differences due to the inherent uncertainty and variability introduced when correcting measured urinary metabolite concentrations for urine dilution and estimating parent compound daily intake.

The urine dilution issue is not exclusive to this cumulative method and is a topic of ongoing debate in studies examining individual phthalate metabolite exposures and related health effects.[161,162,171] Because we do not yet have definitive scientific consensus or guidance on which urine dilution measure is most appropriate for urinary phthalate metabolite correction, I recommend collecting multiple urine dilution measures (i.e. urine flow rate, creatinine, and specific gravity) in future studies to improve our understanding of this problem and to systematically assess the extent to which these measures influence study results. Regardless of the urine dilution-correction approach, estimating parent

compound daily intake from urinary metabolite concentrations introduces an additional level of uncertainty and variability to the cumulative method because the applied pharmacokinetic equation essentially assumes identical phthalate metabolism for everyone in the study (**Eq. 2-2**). On the other hand, estimating daily intake is appropriate because relative potency factors (RPFs) derived from BMDs are based on toxicology studies of phthalate diesters for anti-androgenic effects **(Table 2-3)**. While I recommend using multiple measures of urine dilution and applying RPFs directly to daily intake estimates and metabolite-based metrics to evaluate the consistency of results, more rigorous studies that delineate the best approaches to urine dilution-correction and daily intake estimation could potentially resolve these issues and improve the accuracy and precision of the method going forward.

Another possible source of uncertainty and variability in daily intake estimation may arise from a lack of data availability on multiple measured urinary phthalate metabolites. For example, for this analysis, mono(carboxy-isooctyl) phthalate (MCOP) was the only DiNP metabolite available for daily intake estimation, yet it constitutes less than 10% of the parent compound dose. Thus, future research efforts should be made in biomonitoring studies to measure other DiNP metabolites and improve DiNP's daily intake estimation.

5.2.2 Method applicability

The biggest factors regarding method applicability are the assumptions that relative potencies are accurately predicted from toxicology studies, including those determined for less-studied phthalates like DiNP, and that they are subsequently appropriate for multiple endpoints. Though this cumulative metric weights phthalates by relative potencies determined specifically for inhibition of fetal testosterone production, this upstream endpoint was recently shown to accurately predict additive effects on a broader range of androgen-mediated male reproductive and developmental outcomes in laboratory studies, such as nipple retention and reduced anogenital distance.[98]

Applicability to other hormone-mediated outcomes associated with prenatal phthalates exposure, such as neurodevelopmental problems and metabolic disease, is less studied. However, using the same relative potencies may be a reasonable approach for these endpoints, depending on knowledge about phthalate toxicity along the disease pathway. For example, we know that thyroid hormone disruption during critical stages of fetal brain development can be considered an upstream biomarker of effect for neurodevelopmental outcomes.[40] If existing mixture toxicology data provided accurate relative potency estimates for thyroid hormone level reductions during pregnancy from exposure to phthalates included in this method (i.e. di-*n*-butyl phthalate (DnBP), di(2-ethylhexyl) phthalate (DEHP), etc.), cumulative metrics could be constructed using both sets of relative potencies and compared. A similar analysis could be performed with quality potency data on relative affinities for binding the peroxisome proliferator-activated receptor activity (PPAR-γ), which is critical for metabolic regulation and has received much research attention in recent years. However, inconsistencies in PPAR-γ studies make it difficult to accurately determine relative potency.

Another important limitation of the cumulative exposure metric is the assumption that BMDs associated with decreased fetal testosterone concentrations during prenatal development in rats is appropriate for humans, since phthalate mechanisms and effects vary across species. For example, while researchers have demonstrated phthalate-induced testosterone reductions in marmosets and rats, findings from mouse and human xenograft studies are more equivocal. Thus, a comparative endocrinology and toxicology approach to phthalates activity is critical for determining the overall weight of scientific evidence regarding their endocrine-disrupting properties.

Some have suggested that the rat model is appropriate for humans because reductions of fetal testosterone levels during a very specific window of male sex differentiation is thought to produce overlapping outcomes encompassed by the phthalate syndrome and its proposed analogue in humans.[1] Indeed, while endocrine signaling is generally thought to be highly conserved across mammals, some evidence suggests that certain species may have differential abilities to compensate for phthalate-induced effects on androgen levels (i.e. by increasing luteinizing hormone secretion to restore androgen levels),[124] thereby increasing their resilience to phthalate exposure.

While species specificity is important to consider and is currently a matter of ongoing scientific debate and research, many of the inconsistent findings reported across animal and *in-vitro* studies are likely attributable to study variability in timing of exposure and statistical power to observe an effect. For example, most human cell transplants were not relevant to the critical window of phthalate toxicity, likely because the age of available fetal tissue is difficult to control, and multiple study repetitions with large sample sizes are difficult to perform.[124] Additionally, it has been suggested that without definitive evidence to the contrary, it is reasonable to assume that findings from rat experiments are relevant to humans, especially considering existing observational data that relates phthalates to developmental and reproductive health outcomes.[124]

5.2.3 Masking individual phthalate exposure disparities and intervention opportunities

Although the anti-androgenic metric is more relevant for human health than a single-phthalate framework, evaluating the cumulative metric alone can mask exposure disparities and intervention opportunities for specific phthalates of concern. For example, if DEHP is higher in one group and DEP is higher in the other, the disparity between the two groups might go unnoticed when comparing their cumulative exposure differences. This may have potential implications for exposure reduction opportunities, since targeting DEHP (i.e. through dietary interventions) may be an effective strategy in the first group, while targeting DEP (i.e. through personal care product use interventions) may be more appropriate for the second group.

Similarly, a cumulative analysis might conceal exposure sources specific to individual phthalates. In the analysis on dietary sources of phthalates exposure (Chapter 4), I observed a significant association between dining out and cumulative phthalates exposure, but DnBP was not individually associated with dietary intake of food prepared away from home, even though it constituted the third largest portion of the cumulative

metric (~16%). This indicates that highly processed, packaged, or handled foods are not probable sources of DnBP exposure, which is informative given that primary DnBP sources remain poorly understood, and a recent U.S. study found that store-bought foods contaminated with DnBP from the printing ink used to label the food's paper packaging material.[3,257]

Though modeling the cumulative metric alone would not reveal these findings, investigating sources and intervention opportunities of potency-weighted aggregate exposures remains a health relevant approach. Moreover, tracking one phthalate at a time can potentially underestimate cumulative exposure and associated health risks since new anti-androgenic compounds are replacing those under public and regulatory scrutiny.[3,167] Thus, depending on the research question, I recommend coupling cumulative and individual phthalate analyses to gain a more comprehensive understanding of exposure disparities, health implications, sources, and intervention opportunities.

5.2.4 Expanding the scope and impact of the method

Future efforts to expand this cumulative approach should aim to collect data on dietary behaviors and personal care product use in both occupational and non-occupational study populations. The nail salon study was too small to ascertain exposure sources from questionnaire data, and NHANES does not systematically collect information about personal care product use from study participants. However, future data collection on both diet and personal care product use could help delineate the relative contribution of each to cumulative phthalates exposure in these different populations. For example, information about consumption of food prepared away from home coupled with potential sources of phthalates exposure among nail salon workers (i.e. number of manicures performed) could provide a useful comparison between non-occupational and occupational sources of phthalates exposure. Likewise, information about personal care product use among NHANES participants might provide more insight into non-dietary phthalates exposure sources, especially for individual phthalates like DnBP, for which relative potency is high (**Table 2-3**), exposure source information is incomplete, and food prepared away from home is an unlikely source (**Table 4-8**).

Researchers should also consider broadening the scope of this metric to include additional androgen-disrupting chemicals, since the NAS recommended including all compounds for which there is any evidence of anti-androgenic effects, and my studies were limited by data availability. For example, dipentyl phthalate (DPP) is twice as potent as DnBP (the most potent phthalate currently included in the cumulative metric) but is not currently measured in NHANES. Other non-phthalate anti-androgens, such as 2,3,7,8-tetrachloro-dibenzo-p -dioxin (TCDD) and polybrominated diphenyl ethers (PBDEs), are measured by NHANES but in different subpopulations or survey cycles. Moreover, existing data gaps about their relative potencies need to be addressed before these compounds can be appropriately included. Research that identifies new androgen-disrupting compounds and/or more conclusively discerns the anti-androgenic profile of chemicals for which the data are inconsistent, such as DEP, is also warranted.

While some epidemiologists have begun to use this cumulative method in their work,[259] future observational studies on combined health impacts associated with phthalates and other anti-androgens would help identify limitations and benefits of the approach in this particular context. Risk experts, alternatives assessors, green chemists, and industry leaders that seek to reduce the overall exposure burden of phthalates and other anti-androgens from high production materials, food processing, consumer goods, and personal care products should consider integrating this cumulative method in future efforts to avoid risk underestimation and regrettable substitutions. Lastly, public health professionals and environmental health and justice activists can also use findings from this dissertation in their efforts to educate and advocate for reducing the totality of risk associated with anti-androgenic phthalates and other chemicals that cause common adverse outcomes; for example, by highlighting the benefits of eating at home and the need for product reformulations and safer alternatives.

5.3. Conclusion

Although improvements should be made to this cumulative method in future work, I have shown that a biologically weighted exposure metric is a viable approach for evaluating exposure disparities and identifying sources of and intervention opportunities for phthalates exposure. This cumulative approach is more relevant for human health than a chemical-by-chemical approach, since anti-androgenic phthalates display additive properties and may cause common adverse outcomes. Methods such as this one should be used in future exposure research, risk evaluation, and epidemiology studies to identify opportunities for reducing cumulative exposures and their associated health risks.

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APPENDIX

Table A-1 Fractional excretion values (F_{UE}) and limits of detection (LOD) by survey cycle for urinary phthalate metabolites measured in 2001-14 NHANES

Phthalate Parent Compound Measured urinary metabolite[a]	F_{UE}[b]	*NHANES survey cycle* **2001-2** *µg/L*	**2003-4** *µg/L*	**2005-6** *µg/L*	**2007-8** *µg/L*	**2009-10** *µg/L*	**2011-12** *µg/L*	**2013-14** *µg/L*
Di-*n*-butylphthalate (DnBP)								
Mono-n-butyl phthalate (MnBP)	0.69	1.13	0.40	0.60	0.60	0.40	0.40	0.40
Di-isobutyl phthalate (DiBP)								
Mono-isobutyl phthalate (MiBP)	0.69	0.98	0.26	0.30	0.30	0.20	0.20	0.80
Butyl benzyl phthalate (BBzP)								
Monobenzyl phthalate (MBzP)	0.73	0.22	0.07	0.22	0.22	0.22	0.30	0.30
Di(2-ethylhexyl) phthalate (DEHP)								
Mono(2-ethylhexyl) phthalate (MEHP)	0.062	0.98	0.90	1.20	1.10	0.50	0.50	0.80
Mono(2-ethyl-5-carboxypentyl) phthalate (MECPP)	0.132	--[c]	0.25	0.60	0.50	0.20	0.20	0.40
Mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)	0.149	0.98	0.32	0.70	0.70	0.20	0.20	0.40
Mono(2-ethyl-5-oxohexyl) phthalate (MEOHP)	0.109	1.13	0.45	0.70	0.60	0.20	0.20	0.20
Diethyl phthalate (DEP)								
Monoethyl phthalate (MEP)	0.69	0.60	0.26	0.53	0.46	0.46	0.60	1.20
Di-isononyl phthalate (DiNP)								
Mono(carboxy-isooctyl) phthalate (MCOP)	0.099	--[c]	--[c]	0.70	0.70	0.20	0.20	0.30
Mono-isononyl phthalate (MiNP) [c]	0.03	1.23	1.54	1.23	1.23	0.77	0.50	0.90

[a] All metabolites listed were evaluated in this dissertation, except for MiNP, which was not detected in most NHANES study participants.
[b] Molar fraction of ingested parent compound excreted as metabolies in urine (F_{UE}) was determined in humans over a 24-hour period, except for MEP/DEP and MiBP/DiBP, which were assumed to be similar to MnBP/DnBP.[124]
[c] Not measured in this NHANES survey cycle.