Title

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Abstract

Phthalates are ubiquitous chemicals linked to hormonal disruptions that affect reproduction and development. Multiple antiandrogenic 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, 2001–2012), we developed a potency-weighted sum of daily intake (Σandrogen-disruptor; μg/kg/day) of di-*n*-butyl phthalate (DnBP), diisobutyl phthalate (DiBP), butyl benzyl phthalate (BBzP), and di(2-ethylhexyl) phthalate (DEHP) based on NAS recommendations, and included diethyl phthalate (DEP) and diisononyl phthalate (DiNP) in additional metrics (2005–2012). We compared racial/ethnic differences in Σandrogen-disruptor among 2842 reproductive-aged women. In sensitivity analyses, we assessed the influence of potency assumptions, alternate urine dilution adjustment methods, and weighting phthalate metabolites directly rather than daily intake estimates of parent compounds. We found that DEHP contributed most to Σandrogen-disruptor (48–64%), and that Σandrogen-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. Our approach provides a blueprint for combining chemical exposures linked to common adverse outcomes, and should be considered in future exposure, risk, and epidemiological studies.

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b00522.

> CHAP metric comparisons, Σandrogen-disruptor univariate statistics; sample calculations; urine dilution adjustment diagram and regression results; daily intake estimates over time (PDF)

Graphical abstract
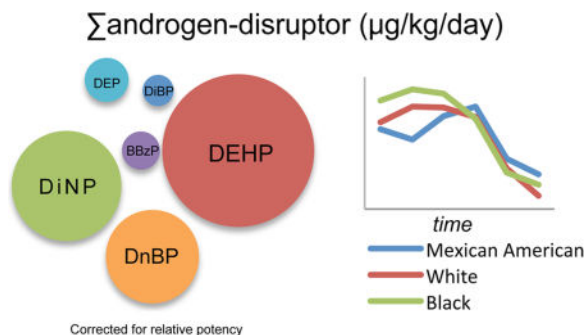
INTRODUCTION

Phthalate 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), low sperm count, and infertility.[1] Human studies support an association between developmental phthalate exposures and male reproductive effects.[2–6] Certain phthalates, such as di-*n*-butyl phthalate (DnBP), diisobutyl phthalate (DiBP), butyl benzyl phthalate (BBzP), di(2-ethylhexyl) phthalate (DEHP), and diisononyl phthalate (DiNP), exert toxicity primarily through androgen disruption.[1]

Phthalate exposures are ubiquitous due to their widespread use in myriad consumer and personal care products.[7,8] Biomonitoring studies show the U.S. population is exposed to multiple phthalates simultaneously.[9–12] Animal studies demonstrate that phthalate mixtures result in higher male reproductive risk than individual phthalates, especially during fetal development.[13–15] Human studies also find higher risks from multiple phthalates.[2,5] In 2008, the National Academy of Sciences (NAS) recommended phthalates and other antiandrogens for cumulative risk assessment based on their ability to cause common adverse outcomes (i.e., changes in testosterone concentration during development) 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.[16–19] However, advancements in the epidemiology of phthalate mixtures are limited by current methods for characterizing cumulative exposures.[20]

Identifying high-risk subpopulations for cumulative exposure warrants examination since individual phthalate profiles may not accurately represent the distribution of overall phthalate burden. Individual phthalate exposures have been shown to vary by race/ethnicity and socioeconomic status (SES), often in opposing directions.[9,21–24] For example, DnBP and BBzP are higher in low SES groups, while DEHP exposure is lower in the same subpopulation.[22,23] Characterizing cumulative exposure inequalities may help elucidate health disparities and identify solutions that better protect those at increased risk of exposure and disease.[25]

In this paper, we 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. We focus on women of reproductive age because in utero development has been identified as the most sensitive window for phthalate toxicity,[1] and previous work suggests that phthalate exposures among reproductive-aged and pregnant women are similar[12] but often distinct from other subpopulations.[26]

MATERIALS AND METHODS

Study Population

We pooled data from six cycles (2001–2012) of the National Health and Nutrition Examination Survey (NHANES), a nationally representative survey and physical examination of the civilian, noninstitutionalized U.S. population conducted by the U.S. Centers for Disease Control and Prevention (CDC) (http://www.cdc.gov/nchs/nhanes.htm). The study population was limited to females aged 15–44 years ($N = 3480$). Of these, we further excluded 544 women who self-identified as a race/ethnicity other than non-Hispanic white, non-Hispanic black, or Mexican American, who together comprise 86% of the total population. We also excluded participants who did not have at least one phthalate metabolite measurement ($N = 94$), resulting in a final sample size of 2842 participants.

Phthalate Metabolite Measurements

In each NHANES survey cycle, phthalate metabolites are measured in one-third of survey participants. Analytical methods are described elsewhere.[27] 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.[27,28] Laboratory files were downloaded from the NHANES web site in March 2015 and included necessary impurity corrections for some previously used analytical standards.[29] Not all phthalate metabolites are measured in every NHANES cycle, and limits of detection (LOD) for each metabolite can vary by cycle. Thus, we used the maximum LOD for each phthalate metabolite and substituted concentrations below the LOD with a value equal to the LOD divided by the square root of 2.[11,30]

Cumulative Exposure Metric

To characterize cumulative exposure to androgen-disrupting phthalates, we calculated a potency-weighted aggregate sum (Σandrogen-disruptor) of four phthalates (DnBP, DiBP, BBzP, and DEHP) that are recognized as antiandrogenic by the NAS and whose metabolites were measured in every cycle between 2001 and 2012 (Figure 1, model 1). The primary hydrolytic metabolites for DnBP, DiBP, BBzP, and DEHP, respectively, are mono-n-butyl phthalate (MnBP), monoisobutyl phthalate (MiBP), monobenzyl phthalate (MBzP), and mono-(2-ethylhexyl) phthalate (MEHP). MEHP further metabolizes to oxidative metabolites: mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP).

To calculate unitless relative potency factors (RPFs) for the four phthalates, we used 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 1).[1,31] BMDs were used rather than the lower limit of a one-sided 95% confidence interval (CI) on the BMD (BMDL) because we are comparing across chemicals.[31] In the NAS analysis, DnBP had the highest potency, or lowest BMD. Therefore, we derived RPFs by dividing the DnBP reference BMD by that of each phthalate.

$$\mathrm{RPF}_i = \mathrm{BMD}_{\mathrm{reference}} / \mathrm{BMD}_i; \qquad i = \text{individual phthlate} \quad (1)$$

Because BMDs are based on phthalate doses administered to laboratory animals and not metabolite concentrations, we estimated the daily intake of parent phthalate compounds (μg/kg/day) from measured urinary metabolites (ng/mL = μg/L) using a pharmacokinetic modeling equation adapted from previous studies.[32,33]

$$\text{daily intake}_i = \frac{(\mathrm{ME}_i \times \mathrm{CE}) \times (\mathrm{MW}_\mathrm{p})}{(F_{\mathrm{UE},i} \times 1000) \times (\mathrm{MW}_\mathrm{m})} \quad (2)$$

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), $F_{\mathrm{UE},i}$ 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, we initially assumed a uniform creatinine excretion rate of 18 mg/kg/day[34] for all participants. We calculated creatinine-adjusted concentrations (ME) for each participant by dividing their measured urinary concentrations (μg/L) by their measured urinary creatinine concentration (g/L). The fractional urinary excretion values, or F_{UE}, are 0.69, 0.69, and 0.73 for DnBP, DiBP, and BBzP, respectively.[35,36] The values for MEHP, MEHHP, and MEOHP are 0.062, 0.149, and 0.109, respectively. MECPP was not included as a DEHP metabolite for 2001–2012 analyses because it was not measured in all survey cycles, but it was included in 2005–2012 analyses with an F_{UE} of 0.132.[37] Potency-weighted daily intake estimates of Σandrogen-disruptor, expressed in μg/kg/day, were then calculated by summing the products of RPFs and daily intakes for each phthalate. (See Figure S1 for sample calculations.)

$$\sum \text{androgen-disruptor} = \sum (\text{daily intake}_i \times \mathrm{RPF}_i) \quad (3)$$

In addition to our original metric (Figure 1, model 1), we calculated a second version of Σandrogen-disruptor by adding diethyl phthalate (DEP) and DiNP to model 1 (Figure 1, model 2a). While DEP does not exhibit antiandrogenic properties in laboratory studies,[35] several epidemiologic studies report an association between MEP and male reproductive

health end points, although findings are inconsistent.[3,5,6] Therefore, we assumed DEP to have a low but nonzero potency equal to 1 order of magnitude less than the least potent phthalate in our model (RPF = 1/10 multiplied by 0.24, or 0.024). Monoethyl phthalate (MEP) is the primary metabolite of DEP, and DEP was assumed to have the same F_{UE} as DnBP.[32] We also added DiNP to the metric since it has been recognized as an antiandrogenic phthalate.[1,35] Since DiNP's primary metabolite, mono(carboxy-isooctyl) phthalate (MCOP), was not measured before 2005, model 2a was examined in 2005–2012 participants only ($N = 1723$). DiNP was assumed to be 2.3 times less potent than DEHP.[18] We used an F_{UE} value of 0.099 for MCOP.[37]

Data Analysis

We conducted analyses using SAS software, version 9.4 (SAS Institute Inc., Cary, NC). Since we combined multiple cycles of data, we calculated new sample population weights according to NHANES analytical guidelines.[38] All analyses were adjusted for sample population weights and the NHANES clustered sample design. We defined statistical significance at $p < 0.05$ on two-sided tests and considered $p < 0.10$ to be marginally significant. We natural-log-transformed Σandrogen-disruptor to account for its non-normal distribution. We calculated the geometric mean (GM) and 95% CI for Σandrogen-disruptor and for each phthalate parent compound as well as the percent contribution of each phthalate to the cumulative metric.

We used analysis of variance and linear regression to examine bivariate associations between Σandrogen-disruptor and sociodemographic, temporal, and biological variables. Socio-demographic characteristics included the following: age, 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), 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. We included sampling session (morning, afternoon, or evening) as a proxy variable for time of sample collection to help adjust for fasting time. Lastly, we considered the percent of total DEHP metabolites excreted as the primary metabolite (MEHP%). Hauser et al.[39] suggest that higher MEHP% may reflect less complete DEHP metabolism or excretion. Consequently, higher MEHP% would indicate greater susceptibility to DEHP exposure.

The core regression model included our main effect (race/ethnicity), educational attainment, income, age, and BMI. We included other covariates 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%. We also tested for statistical interaction between race/ethnicity and survey cycle, with survey cycle dichotomized as <2005 and 2005.

Sensitivity Analyses

We conducted three sensitivity analyses. First, we assessed the influence of DEP on our metric (Figure 1; model 2b) by increasing its potency to the least potent phthalate in our

model (RPF = 0.24). Second, we removed 342 pregnant women from our models to evaluate their influence on the original analysis.

Third, we examined the potential for exposure misclassification by comparing Σandrogen-disruptor to several other cumulative metrics, which were constructed with alternate urine dilution adjustment methods. We estimated urine flow rate (UFR) in milliliters per day for 2009–2012 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. We calculated an RPF-weighted sum (eq 3) based on UFR (Σurine-flow) with the following daily intake equation:

$$\text{daily intake}_i = \frac{(\text{UME}_i \times \text{UE}) \times (\text{MW}_\text{p})}{(F_{\text{UE},i} \times 1000) \times (\text{MW}_\text{m})} \quad (4)$$

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. This approach is equivalent to calculating creatinine excretion rates for each study participant (urinary creatinine concentration multiplied by UE) in eq 2, rather than using a uniform value for all participants. We then used UFR to calculate metabolite excretion rate, or the amount of metabolite excreted per day (μg/day) by multiplying UME by UFR. We used eq 3 to apply RPF weights directly to metabolite excretion rates (Σexrate-rpf), assuming that daily metabolite excretion is proportional to daily parent phthalate compound intake. For comparability, we also applied potency weights directly to measured urinary metabolite concentrations (Σmetab-rpf) (μg/L). See Figure S2 for a diagram of urine dilution adjustment methods.

We then assessed the correlation between metrics using Spearman's rank correlation (r_s) and compared regression results for race/ethnicity between 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) (Figure 1, models 3a–3e).[40] We also correlated our metric to that of other cumulative phthalate metrics used in previous studies, including molar sums of low and high molecular weight phthalates (Σlow-mw and Σhigh-mw),[24,41–45] and three approaches implemented by the Chronic Hazard Advisory Panel (CHAP) on Phthalates and Phthalate Alternatives.[35] We constructed Σchap-1, Σchap-2, and Σchap-3 using eqs 2 and 3, with relative potencies obtained from CHAP case studies. The CHAP calculates reference doses (RfDs) using uncertainty factors (UFs) and BMDLs, no observed adverse effect levels (NOAELs), and lowest observed adverse effect levels (LOAELs). Table S1 compares our approach to the three CHAP approaches.

RESULTS

Among all phthalates, DEHP and DEP had the highest daily intake (GM > 2.45 μg/kg/day) and DiBP and BBzP had the lowest (GM < 0.30 μg/kg/day) (Table 1). In both primary metrics (models 1 and 2a), DEHP contributed the largest percentage (48 and 64%) to the

cumulative androgen-disruption measure. The cumulative GM ranged from 2.58 (95% CI: 2.44, 2.72) to 4.22 (95% CI: 3.96, 4.51) μg/kg/day, depending on whether DEP and DiNP were included in the metric (Table S2).

Between 2001 and 2012, Σandrogen-disruptor decreased by 54% ($p < 0.0001$) (Figure 2). This downward trend over time in cumulative phthalates exposure was evident for all races. 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 S3). 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 Σandrogen-disruptor, there was evidence of multiplicative interaction between race/ethnicity and time ($p_{\text{interaction}}$ = 0.025). Compared to 2001–2004, phthalate levels decreased in later years for black and white women ($p < 0.0001$ and $p = 0.0004$, respectively), but not in Mexican American women ($p = 0.177$).

In 2001–2012 adjusted models, we observed 12% higher levels of Σandrogen-disruptor in black women compared to white women ($p = 0.03$) (Table 2 and Figure 1, model 1). When we restricted the sample to 2005–2012 and included DEP and DiNP in the metric, the difference in Σandrogen-disruptor between black and white women changed substantially and was no longer significant ($p = 0.77$) (model 2a).

When we raised DEP's potency to the least potent phthalate in our models, the difference between black and white women increased from 2% in model 2a to 13% in model 2b and became marginally significant (from $p = 0.77$ to $p = 0.08$, respectively) (Table 2 and Figure 1). We did not observe a difference in any of our results when we removed pregnant women from the analysis (data not shown).

We found high correlation between Σandrogen-disruptor and Σurine-flow (2009–2012 data; Table 3, $r_s = 0.83$). The two metrics produced different but overlapping estimates for racial/ethnic disparities. There were no significant differences between 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 S3 and Figure 1, models 3a and 3b).

We observed high correlations between both daily intake metrics and the metric that weights metabolite excretion rates by RPFs (Σexrate-rpf) (Table 3, r_s range = 0.7–0.9), and moderate correlations between daily intake metrics and weighted urinary phthalate metabolites (Σmetab-rpf) (r_s range = 0.5–0.6). Racial/ethnic differences were significant in regression models when the outcomes were Σexrate-rpf and Σmetab-rpf without creatinine correction. We observed 32% (95% CI: –51, –13) higher exposures in black women than in white women in the Σexrate-rpf model (Table S3 and Figure 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 (models 3d and 3e).

Correlation of Σandrogen-disruptor and Σurine-flow with low molecular weight metabolites (Σlow-mw) was low (Table 3, $r_s < 0.20$), but the daily intake metrics were more highly correlated with high molecular weight metabolites (Σhigh-mw) ($r_s = 0.62$). We additionally

found that our Σandrogen-disruptor metric was highly correlated with CHAP metrics ($r_s >$ 0.80), with CHAP case 2 being the most highly correlated.

DISCUSSION

In this study, we 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. We created several alternate methods to assess the relative importance of potency estimates for individual phthalates (i.e., DEP) as well as approaches for urine dilution adjustment and daily intake estimation. In our assessment of racial/ethnic differences, we found that black women generally had higher exposure to multiple androgen-disrupting phthalates than white women, although the magnitude and precision of the difference varied by model specification.

To our knowledge, this is the first assessment using NAS recommendations to profile racial/ethnic differences in cumulative androgen-disrupting phthalates. When we pooled 2001–2012 NHANES data, black women had 12% higher cumulative body burden levels of androgen-disrupting phthalates than white women, thereby increasing their potential risk of adverse androgen-dependent outcomes. However, cumulative phthalate levels were similar across racial/ethnic groups when we restricted analysis to 2005–2012 data. In other models, raising DEP potency and using metabolite-based metrics generally increased exposure disparities between white and black women. For example, when we modeled measured metabolites without urine dilution adjustment, the difference was as high as 60%.

Reasons for racial/ethnic differences in cumulative exposure might include variations in health and behavior patterns that contribute to exposure, such as personal care product use, dietary habits, and medication intake.[8,21,35,46–48] For example, vaginal douching and fast food consumption have both been shown to vary by race and are associated with higher phthalate body burden levels.[21,46] Observed racial/ethnic differences in creatinine excretion, notably that black women have higher creatinine levels than other racial/ethnic groups,[40] may be one factor in this difference. Biological differences in phthalate metabolism/excretion and urine dilution may also contribute to racial differences of cumulative phthalates exposure.

We observed an overall downward trend in cumulative phthalates exposure among all racial groups across survey years, consistent with temporal trends reported for the general U.S. population.[30] Zota et al. hypothesize that declines in DnBP, BBzP, DEHP, and DEP may be attributable to legislative activity and public advocacy campaigns recently targeting consumers, chemical companies, and product manufacturers.[30] They further suggest that the rise in DiBP and DiNP over time might reflect industry replacement strategies in response to public and regulatory pressure.

DEP impacts cumulative phthalates exposure disparities due to its high concentration, even though its relative contribution to the cumulative metric is minimal. When we raised DEP's potency, the gap between white and black women increased because DEP exposure is consistently greater in black women compared to white women over time. The inclusion of

DEP adds uncertainty to the analysis since animal studies indicate that DEP is not antiandrogenic and human studies are equivocal.[3,5,6,35] 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 cumulative exposure metric.

Our sensitivity analysis revealed that regression modeling results differ depending on how adjustments for urine dilution are made and whether or not daily intake estimates or measured urinary metabolites are used in the outcome variable. Metabolite excretion rates and urine concentrations both resulted in large and significant exposure disparities between white and black women, although including creatinine as a covariate in the latter model attenuated this difference. While daily intake estimates that assumed a uniform creatinine excretion rate for the study population did not demonstrate a significant difference between racial/ethnic groups, urine flow rate adjustment (equivalent to using creatinine excretion rates for each individual) revealed a marginally significant exposure disparity between black and white women.

There is current debate about the best approach for urine dilution correction in spot samples of nonpersistent chemicals like phthalates. Several researchers suggest that metabolite excretion rate calculation minimizes exposure misclassification error because it provides for direct urine dilution adjustment, unlike other methods, including creatinine and specific gravity correction, which are imperfect proxies for urine dilution.[40,49–51] Christensen et al.[49] report on phthalates specifically, demonstrating through simulation that metabolite excretion rates and urine metabolite concentrations lead to the least biased associations between phthalate exposure and BMI, while creatinine correction and daily intake estimation lead to the most biased associations. This may be one reason why we observed high correlation yet differing regression results 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 greatly 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 our proposed benchmark doses were derived from studies administering parent compounds to animals.[1,14]

Ideally, we recommend constructing daily intake cumulative metrics from urine flow rate data and comparing regression results across weighted metabolite excretion rates and measured metabolite concentrations (with and without creatinine as an independent variable). 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. Our sensitivity analysis positioned metabolite excretion rate modeling results in between the more extreme (i.e., measured metabolites without urine dilution adjustment) and null (i.e., daily intakes, creatinine adjustment) 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 our method could be applied to metabolite levels adjusted for specific gravity.

While we found high correlation between our metric and those of the CHAP, there were some differences. DiNP potency varied between metrics. We did not base DiNP's relative potency on NAS benchmark doses since these data were not available, but instead used findings from a separate study that assessed DiNP's potency with the same end point (fetal testosterone concentration).[18] CHAP case 2 used an equivalent potency for DiNP and demonstrated the highest correlation with our metric. DiNP is 1–2 orders of magnitude less potent in CHAP cases 1 and 3, based on older studies and other antiandrogenic end points,[35,52] one possible reason for the lower correlation with our approach. All four approaches ranked DiNP as less potent than DEHP; however, other end points may be more or less sensitive to DiNP compared to DEHP. Thus, future cumulative assessments should include DiNP and use more recent potency assumptions such as ours.[18]

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 1 order of magnitude, while ours and CHAP case 2 ranked DEHP relatively lower. One reason for this variation is that CHAP used a NOAEL for DEHP based on different antiandrogenic end points. Many problems have been identified with using NOAELs and LOAELs, including the values are influenced by experimental design.[1,31] 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, NOEALs and LOAELs may correspond to widely different response levels.[53] BMDs, on the other hand, provide more robust low dose extrapolations with consistent response levels.[1,31] Thus, using a BMD approach ensures we are correctly weighting phthalates because we are considering exposures for the same response level (in this case 5%).[53] 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] Therefore, forthcoming work on cumulative phthalates exposure and risk should use BMDs in weighted potency metrics, which is consistent with recommendations by the Environmental Protection Agency (EPA) for considering risks for noncancer health effects.[31]

The most significant limitation to our estimation of cumulative exposures is data availability. For one, we had urine flow rate data for 2009–2012 NHANES cycles only, which limited our statistical power. Also, the NAS recommended including other antiandrogens, 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, which may be more potent than DEHP and DnBP for androgen-mediated end points.[1] Nevertheless, our method provides a blueprint for how to weight

phthalates in a cumulative sum using relative potencies recommended by the NAS. We additionally demonstrated how to include antiandrogenic 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). We also showed how issues of potential exposure misclassification may arise depending on which exposure metric and urine dilution adjustment method is used in regression modeling. Future studies should validate our method in risk assessment and epidemiologic models.

In conclusion, because humans are continuously exposed to multiple phthalates and other antiandrogenic compounds, we 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 antiandrogens since their co-occurrence and potential for biological interaction means that risk assessment approaches that only focus on one chemical at a time will 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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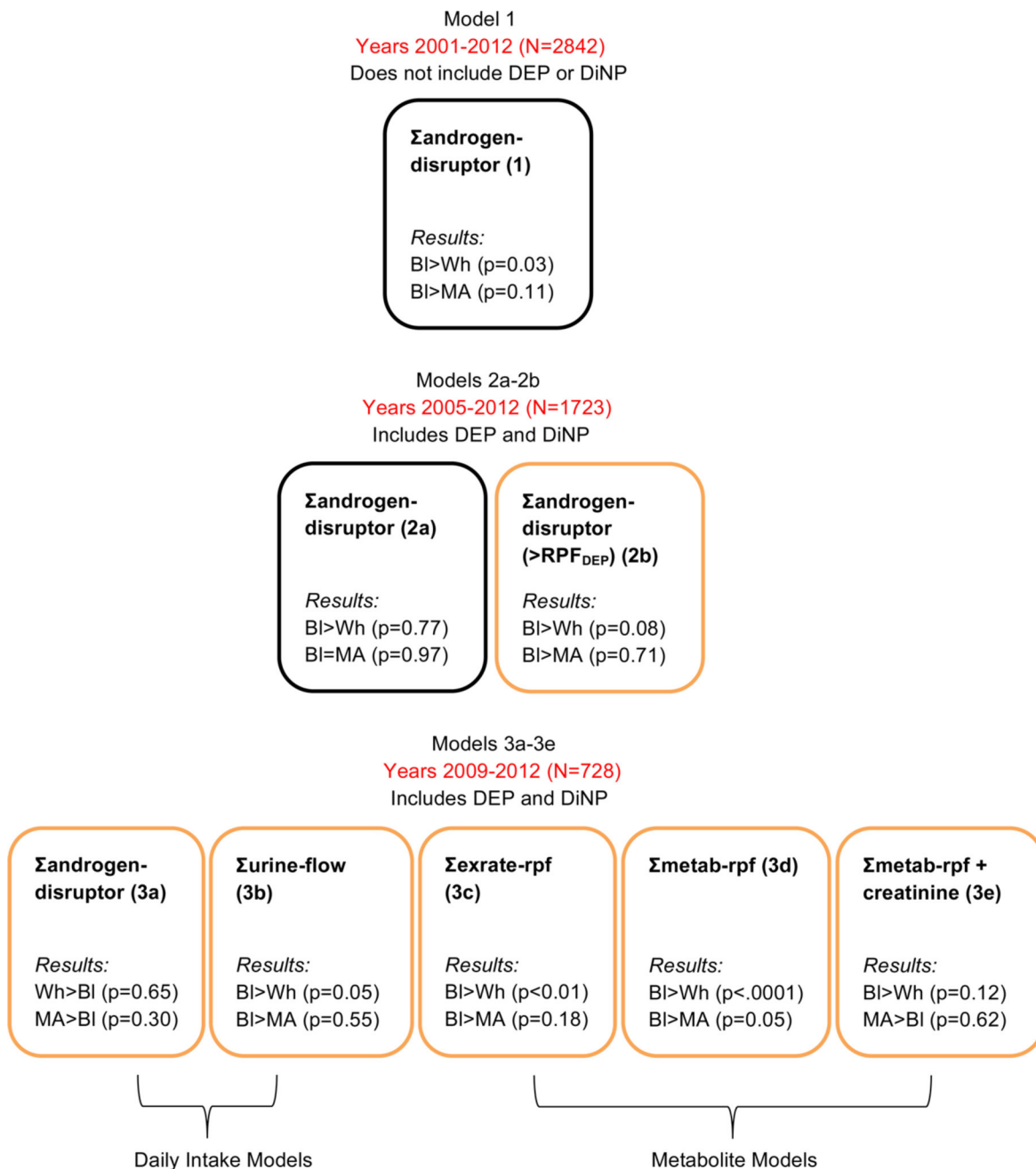
Model 1
Years 2001-2012 (N=2842)
Does not include DEP or DiNP

Σandrogen-
disruptor (1)

Results:
Bl>Wh (p=0.03)
Bl>MA (p=0.11)

Models 2a-2b
Years 2005-2012 (N=1723)
Includes DEP and DiNP

Σandrogen-
disruptor (2a)

Results:
Bl>Wh (p=0.77)
Bl=MA (p=0.97)

Σandrogen-
disruptor
(>RPF$_{DEP}$) (2b)

Results:
Bl>Wh (p=0.08)
Bl>MA (p=0.71)

Models 3a-3e
Years 2009-2012 (N=728)
Includes DEP and DiNP

Σandrogen-
disruptor (3a)

Results:
Wh>Bl (p=0.65)
MA>Bl (p=0.30)

Σurine-flow
(3b)

Results:
Bl>Wh (p=0.05)
Bl>MA (p=0.55)

Σexrate-rpf
(3c)

Results:
Bl>Wh (p<0.01)
Bl>MA (p=0.18)

Σmetab-rpf (3d)

Results:
Bl>Wh (p<.0001)
Bl>MA (p=0.05)

Σmetab-rpf +
creatinine (3e)

Results:
Bl>Wh (p=0.12)
MA>Bl (p=0.62)

Daily Intake Models

Metabolite Models

Figure 1.
Overview of antiandrogen models and phthalates evaluated in the study. Primary analysis includes models 1 and 2a (2001–2012 NHANES data). Model 1 includes DnBP, DiBP, BBzP, and DEHP. Model 2a includes two additional phthalates (DEP and DiNP), but fewer years (2005–2012). Model 2b represents model 2a with increased DEP potency, and models 3a–3e represent urine dilution sensitivity analyses (2009–2012 data). Boxes include racial/ethnic difference results (*p*-values). Black and orange colors indicate primary and secondary analyses, respectively. Σandrogen-disruptor and Σurine-flow represent daily intake estimates using average creatinine excretion rate and urine flow rate, respectively; Σexrate-rpf and

Σmetab-rpf represent RPF-weighted metabolite excretion rates and measured metabolites, respectively. Bl = black; Wh = white; MA = Mexican American.
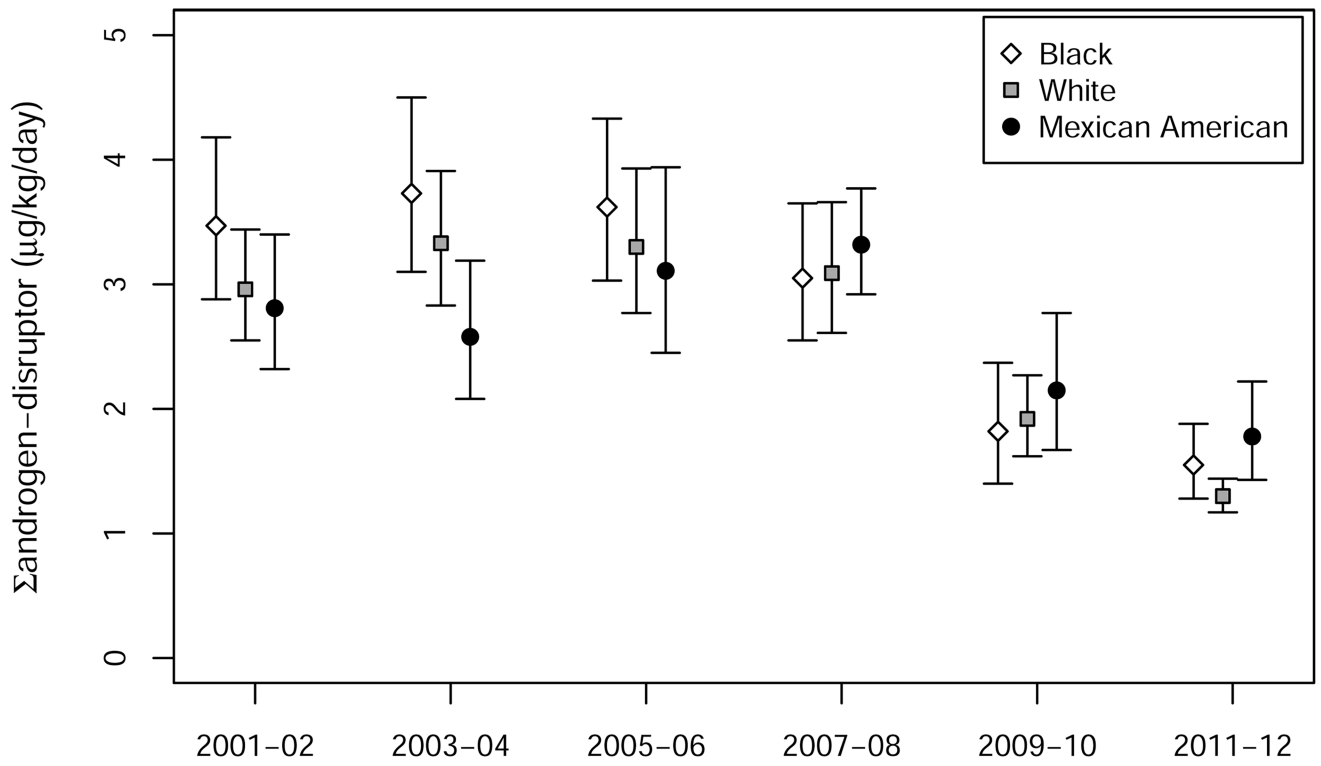
Figure 2.
Crude/unadjusted geometric means of cumulative phthalates exposure (Σandrogen-disruptor) over time for each race. Phthalates included in metric: DnBP, DiBP, BBzP, and DEHP (model 1). Sample sizes ranged from 56 to 262 (total $N = 2842$). Significant trend observed across the study period ($p < 0.0001$).

Table 1

Phthalate Daily Intake Estimates, Benchmark Doses (BMDs), Relative Potency Factors (RPFs), and Geometric Mean (GM) Contribution of Phthalates to Σandrogen-disruptor

phthalate	daily intake (µg/kg/day) [2001–2012, N = 2842]			daily intake (µg/kg/day) [2005–2012, N = 1723]			BMD (mg/kg/day)	RPF	mean contribution to Σandrogen-disruptor	
	GM	95% CI		GM	95% CI				model 1[a]	model 2a[b]
di-*n*-butyl phthalate (DnBP)	0.60	0.57	0.63	0.54	0.51	0.57	30	1.000	0.29	0.18
diisobutyl phthalate (DiBP)	0.19	0.18	0.20	0.24	0.23	0.26	126	0.240	0.03	0.02
butyl benzyl phthalate (BBzP)	0.26	0.25	0.27	0.22	0.21	0.24	116	0.260	0.04	0.03
di(2-ethylhexyl) phthalate (DEHP)[c]	2.52	2.36	2.69	2.88	2.65	3.13	49	0.610	0.64	0.48
diethyl phthalate (DEP)	–	–	–	2.47	2.25	2.72	–	0.024	–	0.04
diisononyl phthalate (DiNP)	–	–	–	2.40	2.14	2.68	–	0.260	–	0.25

[a]Model 1 (*N* = 2842) includes 2001–2012 data.

[b]Model 2a (*N* = 1723) includes 2005–2012 data with DEP and DiNP.

[c]MECPP included in DEHP daily intake calculation for 2005–2012 data.

Table 2

Percent Change (%), 95% Confidence Intervals (CI), and *p*-Values on Race/Ethnicity from Linear Regression Models Predicting Σandrogen-disruptor[a] (μg/kg/day) for NHANES 2001–2012

race/ethnicity	model 1[b] (N = 2842)			model 2a[b] (N = 1723)			model 2b[b] (N = 1723)		
	%	(95% CI)	*p*-value	%	(95% CI)	*p*-value	%	(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: race/ethnicity, age, BMI, survey cycle, MEHP%, education, poverty, and sampling session (morning/afternoon/evening). Outcome variable logged to account for non-normal distributions.

[b] Model 1 Includes DnBP, DiBP, BBzP, and DEHP. Model 2a = model 1 + DEP and DiNP and is restricted to 2005–2012. Model 2b = model 2a with increased DEP potency (RPF = 0.24).

[c] Reference group = black women.

Table 3

Spearman's (r_s) Correlation between Metrics (NHANES 2009–2012, $N = 728$)[a]

	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] Logged values used in correlation analysis. Significant correlations bolded.

[b] Σandrogen-disruptor and Σurine-flow include DnBP, DiBP, BBzP, DEHP, DEP, and DiNP. MECPP included in DEHP daily intake estimate.

[c] Σurine-flow restricted to $N = 716$ due to missing body weight data.

[d] Σmetab-rpf and Σexrate-rpf include MnBP, MiBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, MEP, and MCOP.

[e] Σlow-mw includes MnBP, MiBP, and MEP.

[f] Σhigh-mw includes MBzP, MEHP, MEOHP, MEHHP, MECPP, and MCOP.

[g] Σchap-1, -2, and -3 include DnBP, DiBP, BBzP, DEHP, and DiNP. MECPP included in DEHP daily intake estimate.