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ENDOCRINE DISRUPTING CHEMICALS AND MALE REPRODUCTIVE HEALTH

FEIBY LABAN NASSAN TAWADROS

A Dissertation Submitted to the Faculty of

The Harvard T.H. Chan School of Public Health

in Partial Fulfillment of the Requirements

for the Degree of *Doctor of Science*

in the Department of *Environmental Health*

Harvard University

Boston, Massachusetts.

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Dissertation Advisor: Prof. Russ Hauser Feiby Laban Nassan Tawadros

Endocrine Disrupting Chemicals and Male Reproductive Health

Abstract

Phthalates and parabens are endocrine disrupting chemicals with ubiquitous human exposure. Medication coatings and personal care products (PCPs) are important exposure sources, however, their contribution to men's exposure as well as health effects are understudied.

Dibutyl phthalate (DBP) is used in some medication coatings e.g., mesalamine to treat inflammatory bowel disease. This work presents novel innovative methods to assess whether high-DBP exposure from mesalamine (>1000x background-exposure) altered serum reproductive hormones and semen quality. We took advantage of different mesalamine formulations with/without DBP to conduct a prospective crossover-crossback study (MARS study). We estimated crossover, crossback and carryover effects using multivariable linear mixed effect models.

We conclude that high-DBP exposure disrupted serum levels of pituitary-gonadal hormone levels that largely reversed after exposure removal, but only in men with no/short history (< 3 years) of previous high-DBP exposure. However, high-DBP exposure among men with no history of high-DBP mesalamine use led to a reduction, primarily in sperm motility that was not reversed but became more pronounced (carried-over) after removal of exposure for four months. Paradoxically, among men with longer duration of high-DBP exposure, removal of the exposure did not change hormone levels or semen quality, suggesting that long-term high-DBP exposure may alter the pituitary-gonadal axis and spermatogenesis making them insensitive to DBP-exposure changes. Based on this work, we offer recommendations for designing future crossover-crossback studies including longer crossover-crossback durations and different timing of sample collection.

In the prospective EARTH cohort study, we assessed PCPs' contribution to men's phthalate and paraben exposure. We estimated the changes in urinary concentrations associated with PCP-use using multivariable linear mixed and Tobit mixed regressions. We also estimated weights for each PCP in a weighted binary score regression and modeled the resulting composite weighted PCP-use. We identified ten PCPs of relevance and demonstrated that their use within six hours of urine collection strongly predicted specific phthalate and paraben urinary concentrations. We also offer concise PCP-use information by only asking specific questions about the use of the most relevant PCPs to help future research in decreasing missingness, improving recall, and decreasing misclassification while optimizing research cost and time.

Key words: crossover, crossback, endocrine disruptors, epidemiology, hormones, men, parabens, phthalates, personal care products, semen parameters.

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B_1HB_2-arm: B_1 represents background-exposure at baseline, **H** represents high-DBP exposure after crossover and **B_2** represents background-exposure after crossback; included 26 men taking mesalamine that did not contain DBP at baseline. **H_1BH_2-arm: H_1** represents high-DBP exposure at baseline, **B** represents background-exposure after crossover and **H_2** represents high-DBP exposure after crossback; included 47 men taking mesalamine-containing DBP at baseline. **Abbreviations: MARS**, Mesalamine And Reproductive health Study; **DBP,** dibutyl phthalate.

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Panel A shows the unadjusted means and 95% CI and **Panel B** shows the adjusted means and 95% CI [adjusted for abstinence time (categorical), age (continuous), season (warm versus cold) and duration on DBP-containing mesalamine medication at baseline (in years)]. For the log-transformed outcomes (sperm concentration, total sperm count, motile sperm count and morphologically normal sperm count), the geometric means and 95% CI are presented. **B_1HB_2-arm, B_1** represents background-exposure at baseline, **H** represents high-DBP exposure after crossover and **B_2** represents background-exposure after crossback; included 26 men taking mesalamine that did not contain DBP at baseline. **H_1BH_2-arm, H_1** represents high-DBP exposure at baseline, **B represents** background-exposure after crossover and **H_2** represents high-DBP exposure after crossback; included 47 men taking mesalamine-containing DBP at baseline. **Abbreviations: MARS**, Mesalamine And Reproductive health Study; **95% CI**, 95% Confidence Interval; **DBP**, dibutyl phthalate.

Supplementary Figure 1.1: Sample Size Flow Chart-Mesalamine And Reproductive health Study (MARS).
MARS Eligibility criteria: 18 to 55 years old males; diagnosed with mild IBD, visited gastroenterology clinics at Massachusetts General Hospital (MGH), Beth Israel Deaconess Medical Center (BIMC) or Brigham and Women's Hospital (BWH), Boston, MA, USA from October 2010 through October 2015. Participants must have had mild IBD score (5 or less for UC and 4 or less for CD) on the simple clinical colitis activity index, on oral mesalamine for at least the previous 3 months with at least 800 mg/3 times/ day. Men with history of infertility were not excluded. Men with history of vasectomy, steroid medication in the last 3 months, diabetes mellitus, hepatic or renal diseases were not eligible. **Potential eligible**: men who are eligible according to the medical records but we could not confirm eligibility due to failure to reach them. **Eligible**: eligibility confirmed after contacting the man.
B_1HB_2-arm, B_1 represents background-exposure at baseline, **H** represents high-DBP exposure after crossover and **B_2** represents background-exposure after crossback included 26 men taking mesalamine that did not contain DBP at baseline.
H_1BH_2-arm, H_1 represents high-DBP exposure at baseline, **B** represents background-exposure after crossover and **H_2** represents high-DBP exposure after crossback included 47 men taking mesalamine containing DBP at baseline.
Abbreviations: MARS, Mesalamine And Reproductive health Study; IBD, Inflammatory Bowel Disease; UC, ulcerative colitis; CD, Crohn's disease; DBP, dibutyl phthalate; B_1HB_2: $Background_1$-High-$Background_2$ DBP exposure; H_1BH_2; $High_1$-Background-$High_2$ DBP exposure.

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Confounders e.g., period under High-DBP before enrollment
Predictors e.g., abstinence time, age and season
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Abbreviations: DBP, dibutyl phthalate

Supplementary Figure 1.3. Means (95%CI) of semen parameters at baseline, crossover and crossback for the 3-arms (B1HB2--arm, H1BH2-arm with duration on DBP-containing mesalamine medication at baseline (period) <3 years and H1BH2-arm with period ≥ 3 years) –MARS.
Panel A shows the unadjusted means and 95%CI and **Panel B** shows the adjusted means and 95%CI (adjusted for abstinence time (categorical), age (continuous), season (warm versus cold) and duration on DBP-containing mesalamine medication at baseline (in years).
For the log transformed outcomes (sperm concentration, total sperm count, motile sperm count and morphologically normal sperm count), the geometric means and 95% CI are presented.
B_1HB_2-arm (26 men, 133 semen samples): included men taking mesalamine that did not contain DBP at baseline. **H_1BH_2-arm (47 men, 199 semen samples): included** men taking mesalamine containing DBP at baseline. **H_1BH_2-arm, <3ys**: 25 men, 100 semen samples and **H_1BH_2-arm, ≥3ys**: 22 men and 99 semen samples.
In B_1HB_2-arm, Background$_1$ represents background-DBP exposure at baseline, **H** represents high-DBP exposure after crossover and **Background$_2$** represents background-DBP exposure after crossback.
In H_1BH_2-arm, H_1 represents high-DBP exposure at baseline, **B** represents background-DBP exposure after crossover and **H_2** represents high-DBP exposure after crossback.
Abbreviations: MARS, Mesalamine And Reproductive health Study; **95% CI**, 95% Confidence Interval; **DBP**, dibutyl phthalate.

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Abbreviations: MARS, Mesalamine And Reproductive health Study; **95% CI**, 95% Confidence Interval; **DBP**, dibutyl phthalate; **SHBG**, sex hormone-binding globulin; **LH**, luteinizing hormone; **FSH**, follicle-stimulating hormone.

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MARS Eligibility criteria: 18 to 55 years old males; diagnosed with mild IBD, visited gastroenterology clinics at Massachusetts General Hospital (MGH), Beth Israel Deaconess Medical Center (BIMC) or Brigham and Women's Hospital (BWH), Boston, MA, USA from October 2010 through March 2016. Participants must have had mild IBD severity, on oral mesalamine for at least the previous 3 months. Men with history of infertility were not excluded. Men with history of vasectomy, steroid medication in the previous 3 months, diabetes mellitus, hepatic or renal diseases were not eligible. **Potentially eligible**: men who were eligible according to the medical records but we could not confirm eligibility due to failure to reach them. **Eligible**: eligibility confirmed after contacting the men.
B_1HB_2-arm, B_1 represents background-exposure at baseline, **H** represents high-DBP exposure after crossover and **B_2** represents background-exposure after crossback included 26 men taking mesalamine that did not contain DBP at baseline.

H_1BH_2-arm, H_1 represents high-DBP exposure at baseline, **B** represents background-exposure after crossover and **H_2** represents high-DBP exposure after crossback included 47 men taking mesalamine containing DBP at baseline.
H_1< 3 Yrs: represents high-DBP exposure at baseline for less than 3 years. **H_1≥ 3 Yrs:** represents high-DBP exposure at baseline for 3 years or more.
Note: 3 years was the median duration for men in the H_1BH_2-arm on high-DBP exposure at baseline (H_1).
Abbreviations: MARS, Mesalamine And Reproductive health Study; **IBD**, Inflammatory Bowel Disease; **UC**, ulcerative colitis; **CD**, Crohn's disease; **DBP**, dibutyl phthalate; **B_1HB_2**: $Background_1$-High-$Background_2$ DBP exposure; **H_1BH_2**; $High_1$-Background-$High_2$ DBP exposure.

Supplementary Figure 2.2: Mesalamine And Reproductive health Study (MARS) Design.
B_1HB_2-arm: B_1 represents background-exposure at baseline, **H** represents high-DBP exposure after crossover and **B_2** represents background-exposure after crossback; included 26 men taking mesalamine that did not contain DBP at baseline. **H_1BH_2-arm: H_1** represents high-DBP exposure at baseline, **B** represents background-exposure after crossover and **H_2** represents high-DBP exposure after crossback; included 47 men taking mesalamine containing DBP at baseline (25 men for less than 3 years and 22 men with 3 years or more).
H_1< 3 Yrs: represents high-DBP exposure at baseline for less than 3 years. **H_1≥ 3 Yrs:** represents high-DBP exposure at baseline for 3 years or more.
Note: 3 years was the median duration for men in the H_1BH_2-arm on high-DBP exposure at baseline (H_1).
Abbreviations: MARS, Mesalamine And Reproductive health Study; **DBP,** dibutyl phthalate.

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Figure shows the adjusted geometric means and 95% CI [adjusted for race (Caucasian or not), age (continuous), BMI (continuous), hour of sample draw (continuous), season (warm or cold), current smoking, age (continuous), and duration on DBP-containing mesalamine medication at baseline (in years)]. **B_1HB_2-arm, B_1** represents background-exposure at baseline, **H** represents high-DBP exposure after crossover and **B_2** represents background-exposure after crossback; included 26 men taking mesalamine that did not contain DBP at baseline. **H_1BH_2-arm, H_1** represents high-DBP exposure at baseline, **B represents** background-exposure after crossover and **H_2** represents high-DBP exposure after crossback; included 47 men taking mesalamine-containing DBP at baseline.
Abbreviations: MARS, Mesalamine And Reproductive health Study; **95% CI**, 95% Confidence Interval; **DBP**, dibutyl phthalate; **SHBG**, sex hormone-binding globulin; **LH**, luteinizing hormone; **FSH**, follicle-stimulating hormone.

Figure 3.1. Heatmap for adjusted % change in urinary phthalate metabolite and parabens concentrations associated with self-reported use of personal care products (PCPs) within 24 hours of urine sample collection among 400 men who contributed 1037 urine samples in the Environment and Reproductive Health (EARTH) Study.
Statistical significance indicated as *: if p-value < 0.05, **: if p-value < 0.01.
DEHP means: ∑DEHP metabolites (µmol/L)= sum of µmol/L of MEHP+ MEOHP+ MEHHP+ MECPP
Analysis was based on 10 imputed data using chained equations method.
Multiple imputing (MI) missing based on concordance of product use within persons. For any given PCP at any time point, the imputation model included PCP-use at other time points, urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no) and warm season (April through September) (yes/no).
Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon (>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).Total products: the crude sum of PCP used within 24 hours. The last column for the total products represents percent changes associated with each additional type of PCP used, regardless of which PCP.

Urinary concentrations were ordered according to the molecular weights within phthalates and within parabens. Combined other hair care products included mousse, hair bleach, relaxer, perm and straightener.

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Statistical significance indicated as *: if p-value < 0.05, **: if p-value < 0.01.
Analysis was based on 10 imputed data using chained equations method.
Multiple imputing missing based on concordance of product use within persons. Imputation model included urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no) and warm season (April through September) (yes/no).
Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).
Total products: the crude sum of PCP used within 24 hours.The total products' bars represent percent changes associated with each additional type of PCP used, regardless of which PCP. Combined other hair care products included mousse, hair bleach, relaxer, perm and straightener.

Figure 3.3. Weights of personal care products (PCPs) that contribute 100% and at least 70% to the overall urinary concentrations of biomarkers associated with PCPs-use within 24 hours and 6 hours of sample collection.
Panel 3.A. Weights of personal care products (PCPs) that contribute 100% to the overall urinary concentrations associated with PCPs-use within 24 hours and their overall weighted percent change.
Panel 3.B. Weights of personal care products (PCPs) that contribute 100% to the overall urinary concentrations associated with PCPs-use within 6 hours and their overall weighted percent change.
Panel 3.C. Weights of personal care products (PCPs) that contribute at least 70% to the overall urinary concentrations associated with PCPs-use within 24 hours and their overall weighted percent change.
Panel 3.D. Weights of personal care products (PCPs) that contribute at least 70% to the overall urinary concentrations associated with PCPs-use within 6 hours and their overall weighted percent change.
Numbers presented inside the stack bars represent the weights associated with PCP-use.
The weights of the 14 PCPs are summed to ~100 due to approximation.
The percent presented above the stacked bars represent the percent change associated with PCPs.
The weights presented are NOT summed to the percent change, instead summed to 100% for the 14 PCPs and 70 % or more for the PCPs presented above.
The percent changes are calculated by exponentiation of the weighted beta coefficient based on the PCPs used given the weights.
Analysis was based on 10 imputed data using chained equations method.
Multiple imputing missing based on concordance of product use within persons. For any given PCP at any time point, the imputation model included PCP-use at other time points, urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no) and warm season (April through September) (yes/no).
Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).
Combined other hair care products included mousse, hair bleach, relaxer, perm and straightener.

Product Use Questionnaire in the Environment and Reproductive Health (EARTH) Study

Figure 3.S1. Spearman correlation between urinary concentrations of phthalate metabolites and parabens among 965 samples provided by men in the Environment and Reproductive Health (EARTH) Study.
[a] 1037 urine samples were analyzed for phthalate metabolites and 965 for parabens.
[b] For Spearman correlations, data was restricted to 965 urine samples for which both phthalate metabolites and parabens were measured.

Figure 3.S2. Heatmap for adjusted % change in urinary concentrations of phthalate metabolites and parabens associated with self-report use of personal care products (PCPs) within 24 hours of urine sample collection among 400 men who contributed 1037 urine samples in the Environment and Reproductive Health (EARTH) Study in the complete case data.
[a] Significance indicated as *: if p-value < 0.05, **: if p-value < 0.01
[b] DEHP means: ∑DEHP metabolites= MEHP+ MEOHP+ MEHHP+ MECPP weighted by the molecular weight
[c] Analysis was based on complete case analyses.
[d] Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).
[e] Total products: the crude sum of PCP used within 24 hours
[f] The last column for the total products represents percent changes associated with each additional type of PCP used, regardless of which PCP.
[g] Urinary concentrations were ordered according to the molecular weights within phthalates and within parabens.
[h] Combined other hair care products included mousse, hair bleach, relaxer, perm and straightener.

Fgure 3.S3. (Panels A to E): Adjusted % change in urinary concentrations of parabens and phthalate metabolites associated with use of 14 personal care products within 24 and 6 hours of urine collection in the complete case analysis.
Panel 3.S3.A. Monoethyl phthalate (MEP) urinary concentration.
Panel 3.S3.B. Mono-isobutyl phthalate (MiBP) urinary concentration.
Panel 3.S3.C. Butylparaben urinary concentration.
Panel 3.S3.D. Methylparaben urinary concentration.
Panel 3.S3.E. Propylparaben urinary concentration.
[a] Statistical significance indicated as *: if p-value < 0.05, **: if p-value < 0.01
[b] Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).
[c] Total products: the crude sum of PCP used within 24 hours
[d] The total products' bars represent percent changes associated with each additional type of PCP used, regardless of which PCP.
[e] Combined other hair care products included mousse, hair bleach, relaxer, perm and straightener

Figure 3.S4. Heatmap for adjusted % change in urinary concentrations of phthalate metabolites and parabens associated with self-report use of personal care products (PCPs) within 24 hours of urine sample collection among 400 men who contributed 1037 urine samples in the Environment and Reproductive Health (EARTH) Study in the imputed missing as zero.
[a] Significance indicated as *: if p-value < 0.05, **: if p-value < 0.01
[b] Imputation for the missing values of PCP-use was assumed to be "0" i.e. no use.
[c] DEHP means: ∑DEHP metabolites= MEHP+ MEOHP+ MEHHP+ MECPP weighted by the molecular weight

[d] Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).
[e] Total products: the crude sum of PCP used within 24 hours
[f] The last column for the total products represents percent changes associated with each additional type of PCP used, regardless of which PCP.
[g] Combined other hair care products included mousse, hair bleach, relaxer, perm and straightener

Figure 3.S5. (Panels A to E): Adjusted % change in urinary concentrations of parabens and phthalate metabolites associated with use of 14 personal care products within 24 and 6 hours of urine collection in the imputed missing as zero.
Panel 3.S5.A. Monoethyl phthalate (MEP) urinary concentration.
Panel 3.S5.B. Mono-isobutyl phthalate (MiBP) urinary concentration.
Panel 3.S5.C. Butylparaben urinary concentration.
Panel 3.S5.D. Methylparaben urinary concentration.
Panel 3.S5.E. Propylparaben urinary concentration.
[a] Statistical significance indicated as *: if p-value < 0.05, **: if p-value < 0.01
[b] Imputation for the missing values of PCP-use was assumed to be "0" i.e. no use.
[c] Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).
[d] Total products: the crude sum of PCP used within 24 hours
[e] The total products' bars represent percent changes associated with each additional type of PCP used, regardless of which PCP.
[f] Combined other hair care products included mousse, hair bleach, relaxer, perm and straightener

Figure 4.1 Mesalamine And Reproductive health Study (MARS) new proposed design.
B_1HB_2-arm: B_1 represents background-exposure at baseline, **H** represents high-DBP exposure after crossover and **B_2** represents background-exposure after crossback; includes men taking mesalamine that did not contain DBP at baseline. **H_1BH_2-arm: H_1** represents high-DBP exposure at baseline, **B** represents background-exposure after crossover and **H_2** represents high-DBP exposure after crossback; includes men taking mesalamine-containing DBP at baseline. **Abbreviations: MARS**, Mesalamine And Reproductive health Study; **DBP,** dibutyl phthalate; **Mo**, month.

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Table 1.1: Demographics of 73 men contributing 332 visits in the MARS Study by arm.
B_1HB_2-arm, B_1 represents background-exposure at baseline, **H** represents high-DBP exposure after crossover and **B_2** represents background-exposure after crossback; included 26 men taking mesalamine that did not contain DBP at baseline; 16 men (62%) on Lialda®, 8 men (31%) on Pentasa®, one man (4%) on Apriso® and one man (4%) was not on mesalamine medication at the time of recruitment.
H_1BH_2-arm, H_1 represents high-DBP exposure at baseline, **B** represents background-exposure after crossover and **H_2** represents high-DBP exposure after crossback; included 47 men taking mesalamine-containing DBP at baseline; 24 men (51%) on Asacol® and 23 men (49%) on Asacol®HD.
[a]**:**N (%) for categorical/binary variables and mean (SD), [Range] for continuous variables
[b]**:** P-values are based on Fisher exact test for categorical variables and Kruskal Wallis test for continuous variables.
[c]**:** Season of sample collection, Warm: April Through September
10 men missing information on education; for 2 semen samples missing abstinence times, we imputed values for their categories based on the other samples given by the same man (both men had the same abstinence time category for all their other samples).
[d]**:** IBD score: simple clinical colitis activity index, includes bowel frequency and urgency, presence of blood in the stool and general wellbeing. Mild IBD score: 5 or less for UC and 4 or less for CD.
Abbreviations: MARS, Mesalamine And Reproductive health Study; BMI, body mass index; Kg, Kilogram; m, meter; SD, standard deviation; IBD, Inflammatory Bowel Disease; UC, ulcerative colitis; CD, Crohn's disease; N, number of men; DBP, dibutyl phthalate; B_1HB_2, Background$_1$-High-Background$_2$ DBP exposure; H_1BH_2, High$_1$-Background-High$_2$ DBP exposure.

Table 1.2: Semen quality parameters for 73 men (332 semen samples) in the MARS study.
[a] Binary semen quality parameters less than WHO lower reference limits (2010)(1)**:** ejaculate volume<15 ml; sperm concentration <15 million/ml; total sperm count< 39 million; total motile sperm<40%; progressive motile sperm <32% and normal sperm morphology< 4% using "strict" Tygerberg method; **n:** # semen samples. There were 2 samples missing morphology **(n=330).**
[b] Motile count= Total Sperm Count x % Motile
[c] Morphologically normal count =Total Sperm Count x % normal morphology
Abbreviations: MARS, Mesalamine And Reproductive health Study; WHO: World health organization.

Table 1.3A: Adjusted effect (95%CI) of crossover, crossback and carryover on semen parameters among men starting on mesalamine medication not containing dibutyl phthalate (B_1HB_2-arm)[a]
Table 1.3B: Adjusted effect (95%CI) of crossover, crossback and carryover on semen parameters among men starting on mesalamine medication containing dibutyl phthalate (H_1BH_2-arm)[a]
[a]**:** Adjusted for abstinence time (categorical: < 2 days, 2≤days<4 and ≥ 4 days), age (continuous), season (warm (April through September) vs cold) and period (# years on DBP-containing mesalamine medication at baseline) in the mixed effect model.
[b]**:** Sperm concentration, total sperm count, motile sperm count and morphologically normal sperm count are presented as percentage change by exponentiating the log-transformed Beta coefficient. The sum of the percent changes for crossover and cross back do not equal the total carryover effect for the log-transformed variables because they are on the multiplicative scale. For the variables that are not log-transformed, the sum of the percent changes for crossover and cross back equal the total carryover effect as they represent the absolute difference on the additive scale
Motile sperm count (million) and morphologically normal sperm count (million) were log-transformed after adding 1 due to few zero values, they were back transformed and 1 was subtracted from the means.
B_1HB_2-arm: B_1 represents background low-DBP exposure at baseline, H represents high-DBP exposure after crossover and B_2 represents background low-exposure after crossback.
H_1BH_2-arm: H_1 represents high-DBP at baseline, B represents background low-DBP exposure after crossover and **H_2** represents high-DBP after crossback.

Note: 2 samples missing morphology; negative sign means a decrease or % decrease for the log-transformed variables compared to the measure in the previous period. **Abbreviations**: DBP, dibutyl phthalate; B_1HB_2: Background$_1$-High-Background$_2$ DBP exposure; H_1BH_2; High$_1$-Background-High$_2$ DBP exposure; 95%CI, 95% Confidence Interval.

Supplementary Table 1.1.A: Adjusted effect (95%CI) of crossover, crossback and carryover on semen parameters among men starting on mesalamine medication not containing dibutyl phthalate (B_1HB_2-arm)[a]
Supplementary Table 1.1.B: Adjusted effect (95%CI) of crossover, crossback and carryover on semen parameters among men starting on mesalamine medication containing dibutyl phthalate (H_1BH_2-arm)[a]
[a]: Adjusted for abstinence time (categorical: < 2 days, 2≤days<4 and ≥ 4 days), age (continuous), season (warm (April through September) vs cold) and period (# years on DBP-containing mesalamine medication at baseline) in the fixed effect model.
[b]: Sperm concentration, total sperm count, motile sperm count and morphologically normal sperm count are presented as percentage change by exponentiating the log transformed Beta coefficient. The sum of the percent changes for crossover and cross back do not equal the total carryover effect for the log transformed variables because they are on the multiplicative scale). For the variables that are not log transformed, the sum of the percent changes for crossover and cross back equal the total carryover effect as they represent the absolute difference on the additive scale.
Motile sperm count (million) and morphologically normal sperm count (million) were log transformed after adding 1 due to few zero values, they were back transformed and 1 was subtracted from the means.
B_1HB_2-arm: B_1 represents background-DBP exposure at baseline, H represents high-DBP exposure after crossover and B_2 represents background-exposure after crossback.
H_1BH_2-arm: H_1 represents high-DBP at baseline, B represents background-DBP exposure after crossover and H_2 represents high-DBP after crossback.
Note: 2 samples missing morphology
Note: Negative sign means a decrease or % decrease for the log transformed variables compared to the measure in the previous period
Abbreviations: DBP, dibutyl phthalate; B_1HB_2: Background$_1$-High-Background$_2$DBP exposure; H_1BH_2; High$_1$-Background-High$_2$ DBP exposure; 95%CI, 95% Confidence Interval.

Supplementary Table 1.2.A: Unadjusted effect (95% CI) of crossover, crossback and carryover on semen parameters among men starting on mesalamine medication not containing dibutyl phthalate (B_1HB_2-arm: B_1HB_2) -Mixed Model[a].
Supplementary Table 1.2.B: Unadjusted effect (95% CI) of crossover, crossback and carryover on semen parameters among men starting on mesalamine medication containing dibutyl phthalate (H_1BH_2-arm) - Mixed Model[a].
[a] Adjusted for abstinence time (categorical: < 2 days, 2≤days<4 and ≥ 4 days), age (continuous), season (warm (April through September) vs cold) and period (# years on DBP containing mesalamine medication at baseline).
[b] Sperm concentration, total sperm count, motile sperm count and morphologically normal sperm count are presented as percentage change by exponentiating the log transformed Beta coefficient. The sum of the percent changes for crossover and cross back do not equal the total carryover effect for the log transformed variables because they are on the multiplicative scale). For the variables that are not log transformed, the sum of the percent changes for crossover and cross back equal the total carryover effect as they represent the absolute difference on the additive scale
Motile sperm count (million) and morphologically normal sperm count (million) were log transformed after adding 1 due to few zero values, they were back transformed and 1 was subtracted from the means.
B_1HB_2-arm: Background$_1$ represents low exposure to DBP at baseline, **H** represents high DBP exposure after crossover and Background2 represents low DBP after crossback.
H_1BH_2-arm: H_1 represents high DBP at baseline, **B** represents Background exposure to DBP after crossover and **H_2** represents high DBP after crossback.
Note: 2 samples missing morphology
Note: Negative sign means a decrease or% decrease for the log transformed variables compared to the measure in the previous period

Abbreviations: DBP, dibutyl phthalate; **B_1HB_2:** Background-High-Background DBP exposure; **H_1BH_2;** High-Background-High DBP exposure**;95% CI**, 95% Confidence Interval.

Supplementary Table 1.3: Unadjusted effect (95% CI) of crossover, crossback and carryover on semen parameters among men starting on mesalamine medication not containing dibutyl phthalate (B_1HB_2-arm: B_1HB_2) and men starting on mesalamine medication containing dibutyl phthalate (H_1BH_2-arm) -Fixed Model[a].

[a] Unadjusted fixed effect model.

[b] Sperm concentration, total sperm count, motile sperm count and morphologically normal sperm count are presented as percentage change by exponentiating the log transformed Beta coefficient. The sum of the percent changes for crossover and cross back do not equal the total carryover effect for the log transformed variables because they are on the multiplicative scale). For the variables that are not log transformed, the sum of the percent changes for crossover and cross back equal the total carryover effect as they represent the absolute difference on the additive scale

Motile sperm count (million) and morphologically normal sperm count (million) were log transformed after adding 1 due to few zero values, they were back transformed and 1 was subtracted from the means.

B_1HB_2-arm: Background$_1$ represents low exposure to DBP at baseline, **H** represents high DBP exposure after crossover and Background2 represents low DBP after crossback.

H_1BH_2-arm: H_1 represents high DBP at baseline, **B** represents Background exposure to DBP after crossover and **H_2** represents high DBP after crossback.

Note: 2 samples missing morphology

Note: Negative sign means a decrease or% decrease for the log transformed variables compared to the measure in the previous period

Abbreviations: DBP, dibutyl phthalate; **B_1HB_2:** Background-High-Background DBP exposure; **H_1BH_2;** High-Background-High DBP exposure**;95% CI**, 95% Confidence Interval.

Supplementary Table 1.4: Adjusteda effect (95% CI) of crossover, crossback and carryover on semen parameters among men starting on mesalamine medication not containing dibutyl phthalate (B_1HB_2-arm) and men starting on mesalamine medication containing dibutyl phthalate (H_1BH_2-arm with duration on DBP-containing mesalamine medication at baseline (period) <3 years and H_1BH_2-arm with period ≥ 3 years)-Mixed model-3-arms[a].

[a] Adjusted for abstinence time (categorical: < 2 days, 2≤days<4 and ≥ 4 days), age (continuous), season (warm (April through September) vs cold) and period (# years on DBP containing mesalamine medication at baseline).

[b] Sperm concentration, total sperm count, motile sperm count and morphologically normal sperm count are presented as percentage change by exponentiating the log transformed Beta coefficient. The sum of the percent changes for crossover and cross back do not equal the total carryover effect for the log transformed variables because they are on the multiplicative scale. For the variables that are not log transformed, the sum of the percent changes for crossover and cross back equal the total carryover effect as they represent the absolute difference on the additive scale

Motile sperm count (million) and morphologically normal sperm count (million) were log transformed after adding 1 due to few zero values, they were back transformed and 1 was subtracted from the means.

B_1HB_2-arm: Background$_1$ represents low exposure to DBP at baseline, **H** represents high DBP exposure after crossover and Background2 represents low DBP after crossback. 26 men, 133 semen samples.

H_1BH_2-arm: H_1 represents high DBP at baseline, **B** represents Background exposure to DBP after crossover and **H_2** represents high DBP after crossback. **H_1BH_2-arm, <3ys**: 25 men, 100 semen samples and **H_1BH_2-arm, ≥3ys**: 22 men and 99 semen samples.

Note: 2 samples missing morphology

Note: Negative sign means a decrease or% decrease for the log transformed variables compared to the measure in the previous period

Abbreviations: DBP, dibutyl phthalate; **B_1HB_2:** Background-High-Background DBP exposure; **H_1BH_2;** High-Background-High DBP exposure; **95% CI**, 95% Confidence Interval

Supplementary Table 1.5: Adjusted effect (95% CI) of crossover, crossback and carryover on semen parameters among men starting on mesalamine medication not containing dibutyl phthalate (B_1HB_2-arm) and men starting on mesalamine medication containing dibutyl phthalate (H_1BH_2-arm with duration on DBP-containing mesalamine medication at baseline (period) <3 years and H_1BH_2-arm with period ≥ 3 years)-Fixed model-3arms[a].

[a] Adjusted for abstinence time (categorical: < 2 days, 2≤days<4 and ≥ 4 days), age (continuous), season (warm (April through September) vs cold) and period (# years on DBP containing mesalamine medication at baseline).

[b] Sperm concentration, total sperm count, motile sperm count and morphologically normal sperm count are presented as percentage change by exponentiating the log transformed Beta coefficient. The sum of the percent changes for crossover and cross back do not equal the total carryover effect for the log transformed variables because they are on the multiplicative scale. For the variables that are not log transformed, the sum of the percent changes for crossover and cross back equal the total carryover effect as they represent the absolute difference on the additive scale

Motile sperm count (million) and morphologically normal sperm count (million) were log transformed after adding 1 due to few zero values, they were back transformed and 1 was subtracted from the means.

B_1HB_2-arm: Background$_1$ represents low exposure to DBP at baseline, **H** represents high DBP exposure after crossover and Background2 represents low DBP after crossback. 26 men, 133 semen samples.

H_1BH_2-arm: H_1 represents high DBP at baseline, **B** represents Background exposure to DBP after crossover and **H_2** represents high DBP after crossback. **H_1BH_2-arm, <3ys**: 25 men, 100 semen samples and **H_1BH_2-arm, ≥3ys**: 22 men and 99 semen samples.

Note: 2 samples missing morphology **Note:** Negative sign means a decrease or% decrease for the log transformed variables compared to the measure in the previous period**. Abbreviations: DBP,** dibutyl phthalate; **B_1HB_2:** Background-High-Background DBP exposure; **H_1BH_2;** High-Background-High DBP exposure; **95% CI**, 95% Confidence Interval.

Table 2.1. Demographics of 73 men contributing 341 blood samples in the MARS Study by arm.

B_1HB_2-arm, B_1 represents background-exposure at baseline, H represents high-DBP exposure after crossover and B_2 represents background-exposure after crossback; included 26 men taking mesalamine that did not contain DBP at baseline; 16 men (62%) on Lialda®, 8 men (31%) on Pentasa®, one man (4%) on Apriso® and one man (4%) was not on mesalamine medication at the time of recruitment.

H_1BH_2-arm, H_1 represents high-DBP exposure at baseline, B represents background-exposure after crossover and H_2 represents high-DBP exposure after crossback; included 47 men taking mesalamine-containing DBP at baseline; 24 men (51%) on Asacol® and 23 men (49%) on Asacol®HD.

[a] N (%) for categorical/binary variables and mean (SD), [Range] for continuous variables

[b] P-values are based on Fisher exact test for categorical variables and Kruskal Wallis test for continuous variables.

[c] Season of sample collection, Warm: April Through September

[d] IBD score: included bowel frequency and urgency, presence of blood in the stool and general wellbeing. Mild IBD score: 5 or less on the simple clinical colitis activity index for UC and 4 or less on the Harvey-Bradshaw index for CD.

Abbreviations: MARS, Mesalamine And Reproductive health Study; BMI, body mass index; Kg, Kilogram; m, meter; SD, standard deviation; IBD, Inflammatory Bowel Disease; UC, ulcerative colitis; CD, Crohn's disease; N, number of men; DBP, dibutyl phthalate; B_1HB_2, Background$_1$-High-Background$_2$ DBP exposure; H_1BH_2, High$_1$-Background-High$_2$ DBP exposure.

Table 2.2. Hormone concentrations for 73 men (341 blood samples) in the MARS study.

Abbreviations: MARS, Mesalamine And Reproductive health Study; **SD**, standard deviation; **Perc**, percentile; **QR**: quartile range; **SHBG**, sex hormone-binding globulin; **LH**, luteinizing hormone; **FSH**, follicle-stimulating hormone.

Table 2.3A. Adjusted percent changes (95%CI) of crossover, crossback and carryover on hormone concentrations among men starting on mesalamine medication not containing dibutyl phthalate (B_1HB_2-arm)[a]
Table 2.3B. Adjusted percent changes (95%CI) of crossover, crossback and carryover on hormone concentrations among men starting on mesalamine medication containing dibutyl phthalate (H_1BH_2-arm)[a]
[a] Adjusted for race (Caucasian or not), age (continuous), BMI (continuous), hour of sample draw (continuous), season (warm or cold), current smoking, age (continuous), and duration on DBP-containing mesalamine medication at baseline (in years) in the mixed effect model.
[b] Percentage changes are presented by exponentiating the beta coefficient. Negative sign means % decrease for the corresponding outcome compared to the measure in the previous period.
[c] **B_1HB_2-arm:** B_1 represents background low-DBP exposure at baseline, H represents high-DBP exposure after crossover and B_2 represents background low-exposure after crossback.
[d] **H_1BH_2-arm: H_1** represents high-DBP at baseline, **B** represents background-DBP exposure after crossover and **H_2** represents high-DBP after crossback.
Abbreviations: MARS, Mesalamine And Reproductive health Study; N, number of men; **B_1HB_2**, Background$_1$-High-Background$_2$ DBP exposure; **H_1BH_2**, High$_1$-Background-High$_2$ DBP exposure; **95% CI**, 95% Confidence Interval; **DBP**, dibutyl phthalate; **SHBG**, sex hormone-binding globulin; **LH**, luteinizing hormone; **FSH**, follicle-stimulating hormone.

Supplementary Table 2.1. Demographics of 73 men contributing 341 blood samples in the MARS Study by arm.
B_1HB_2-arm, B_1 represents background-exposure at baseline, H represents high-DBP exposure after crossover and B_2 represents background-exposure after crossback; included 26 men taking mesalamine that did not contain DBP at baseline; 16 men (62%) on Lialda®, 8 men (31%) on Pentasa®, one man (4%) on Apriso® and one man (4%) was not on mesalamine medication at the time of recruitment.
H_1BH_2-arm, H_1 represents high-DBP exposure at baseline, B represents background-exposure after crossover and H_2 represents high-DBP exposure after crossback; included 47 men taking mesalamine-containing DBP at baseline; 24 men (51%) on Asacol® and 23 men (49%) on Asacol®HD.
H_1< 3 Yrs: represents high-DBP exposure at baseline for less than 3 years. **$H_1\geq$ 3 Yrs:** represents high-DBP exposure at baseline for 3 years or more.
Note: 3 years was the median duration for men in the H_1BH_2-arm on high-DBP exposure at baseline (H_1).
[a] N (%) for categorical/binary variables and mean (SD), [Range] for continuous variables
[b] P-values are based on Fisher exact test for categorical variables and Kruskal Wallis test for continuous variables.
[c] Season of sample collection, Warm: April Through September
[d] IBD score: included bowel frequency and urgency, presence of blood in the stool and general wellbeing. Mild IBD score: 5 or less on the simple clinical colitis activity index for UC and 4 or less on the Harvey-Bradshaw index for CD.
[e] Alcohol: the average number of days per week the men drank alcoholic beverages, over the previous 3 months.
Abbreviations: MARS, Mesalamine And Reproductive health Study; BMI, body mass index; Kg, Kilogram; m, meter; SD, standard deviation; IBD, Inflammatory Bowel Disease; UC, ulcerative colitis; CD, Crohn's disease; N, number of men; DBP, dibutyl phthalate; B_1HB_2, Background$_1$-High-Background$_2$ DBP exposure; H_1BH_2, High$_1$-Background-High$_2$ DBP exposure.

Supplementary Table 2.2. Adjusteda percent changeb (95%CI) of crossover, crossback and carryover on reproductive hormone parameters among men starting on mesalamine medication not containing dibutyl phthalate (B1HB2-arm)[c]
[a] Adjusted for race (Caucasian or not), age (continuous), BMI (continuous), hour of sample draw (continuous), season (warm or cold), current smoking, age (continuous), and duration on DBP-containing mesalamine medication at baseline (in years) in the mixed effect model.

[b] Percentage changes are presented by exponentiating the Beta coefficient. Negative sign means % decrease for the corresponding outcome compared to the measure in the previous period.
[c] **B_1HB_2-arm:** B_1 represents background low-DBP exposure at baseline, H represents high-DBP exposure after crossover and B_2 represents background low-exposure after crossback.
[d] **H_1BH_2-arm, period<3 yrs: H_1** represents high-DBP at baseline for duration less than 3 years, **B** represents background low-DBP exposure after crossover and **H_2** represents high-DBP after crossback.
[e] **H_1BH_2-arm, period≥3 yrs: H_1** represents high-DBP at baseline for duration 3 years or more, **B** represents background low-DBP exposure after crossover and **H_2** represents high-DBP after crossback.
Abbreviations: MARS, Mesalamine And Reproductive health Study; BMI, body mass index; Kg, Kilogram; m, meter; SD, standard deviation; IBD, Inflammatory Bowel Disease; UC, ulcerative colitis; CD, Crohn's disease; N, number of men; DBP, dibutyl phthalate; B_1HB_2, $Background_1$-High-$Background_2$ DBP exposure; H_1BH_2, $High_1$-Background-$High_2$ DBP exposure.

Supplementary Table 2.3. Unadjusted Mixed model: 2 arms
Supplementary Table 2.3.A. Unadjusted percent change (95%CI) of crossover, crossback and carryover on hormone concentrations among men starting on mesalamine medication not containing dibutyl phthalate (B1HB2-arm)[a].
Supplementary Table 2.3.B. Unadjusted percent change (95%CI) of crossover, crossback and carryover on hormone concentrations among men starting on mesalamine medication containing dibutyl phthalate (H_1BH_2-arm)[a].

Supplementary Table 2.4. Fixed model: 2 arms.
Supplementary Table 2.4.A. B_1HB_2 Arm.
Supplementary Table 2.4.B. Fixed: H_1BH_2 Arm.

Supplementary Table 2.5. Sensitivity: Mixed adjusted model with empirical standard error (sandwich estimator).

Supplementary Table 2.6. Unadjusted Mixed model: 3 arms.

Supplementary Table 2.7. Adjusted Fixed model- 3 arms.
Supplementary Table 2.7.A. Adjusted percent change (95%CI) of crossover, crossback and carryover on reproductive hormone parameters among men starting on mesalamine medication not containing dibutyl phthalate (B_1HB_2-arm)[a]
Supplementary Table 2.7.B. Adjusted percent change (95%CI) of crossover, crossback and carryover on reproductive hormone parameters among men starting on mesalamine medication containing dibutyl phthalate (H_1BH_2-arm)[a]
Supplementary Table 2.7.C. Adjusted percent change (95%CI) of crossover, crossback and carryover on reproductive hormone parameters among men starting on mesalamine medication containing dibutyl phthalate (H_1BH_2-arm)[a]

Supplementary Table 2.8. Unadjusted Fixed model- 3 arms
Supplementary Table 2.8.A. Unadjusted percent change (95%CI) of crossover, crossback and carryover on reproductive hormone parameters among men starting on mesalamine medication not containing dibutyl phthalate (B_1HB_2-arm)[a]
Supplementary Table 2.8.B. Unadjusted percent change (95%CI) of crossover, crossback and carryover on reproductive hormone parameters among men starting on mesalamine medication containing dibutyl phthalate (H_1BH_2-arm)[a]
Supplementary Table 2.8.C. Unadjusted percent change (95%CI) of crossover, crossback and carryover on reproductive hormone parameters among men starting on mesalamine medication containing dibutyl phthalate (H_1BH_2-arm)[a]

Supplementary Table 2.9. Empirical Adjusted 3 arms analyses.

Table 3.1. Demographics for 400 men who contributed 1,037 urine samples in the Environment and Reproductive Health (EARTH) Study.
Abbreviations: N, men's number; n, urine samples' number; SD, standard deviation; BMI, body mass index; Kg, Kilogram; m, meter.
N or n (%) for categorical/binary variables and mean± SD [Range] for continuous variables**.**
[a] 2 men missing information on BMI and 83 missing education.

Table 3.2. Self-reported use of 14 personal care products (PCPs) within 24 and 6 hours of collection of urine sample among 400 men in the EARTH Study
Abbreviations: EARTH, Environment and Reproductive Health Study; n, number of visits/ urine samples; PCPs, personal care products.
[a] Concordant: same answer for product use within 24 hours over different visits for the same man, only includes observations for men who completed at least 2 visits.
[b] Combined other hair care products included mousse, hair bleach, relaxer, perm and straightener.

Table 3.3. Distributions of urinary concentrations of phthalate metabolites and parabens measured in 400 men (n=1,037 urine samples)[a] in the Environment and Reproductive Health (EARTH) Study
Abbreviations: SD, standard deviation; LOD, limit of detection; GM, geometric mean; NHANES, the National Health and Nutrition Examination Survey; Perc, percentile.
[a] Phthalate metabolites were measured in 1037 urine samples from 400 men and parabens in 965 samples from 383 men.
[b] Urinary concentrations were ordered according to the molecular weights within phthalates and within parabens.
[c] Concentrations in µg/L (except for ∑DEHP) not corrected for urine dilution; concentrations < LOD were replaced by LOD/√2.
[d] LOD range for [MnBP: 0.4, 0.6], [MCPP: 0.18, 0.2],[MEHHP: 0.2, 0.7], [MEOHP: 0.2, 0.7], [MEHP: 0.5, 1.2], [MiBP: 0.2, 0.3], [MBzP: 0.2, 0.3], (M_pb: 1),[B_pb: 0.1, 0.2], [B_pb: 0.1, 0.2]µg/L.
[c] Concentrations from NHANES (2005-2006), (2009-2010), and (2011-2012) because our data encompassed the years 2004-2015.
[f] ∑DEHP metabolites (µmol/L)= µmol/L sum of MEHP + MEOHP + MEHHP + MECP.

Table 3.S1. Adjusted [a] percent change associated with the weighted score: (based on binary use up-to 24 hours use and 6 hours) using the weighted binary score (WBS) in the 10 imputed data.
Abbreviations**:** PCP; personal care product, 95% CI; 95% confidence intervals, MEP; Monoethyl phthalate, MnBP; Mono-n-butyl phthalate, MiBP; Mono-isobutyl phthalate, MBzP; Monobenzyl phthalate, MEHP; Mono(2-ethylhexyl) phthalate, MEOHP; Mono(2-ethyl-5-oxohexyl) phthalate, MEHHP; Mono(2-ethyl-5-hydroxyhexyl)

phthalate, MECPP; Mono(2-ethyl-5-carboxypentyl) phthalate, DEHP; (Di(2-ethylhexyl) phthalate) metabolites, MCPP; Mono(3-carboxypropyl) phthalate.

[a] Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).

[b] ∑DEHP metabolites= MEHP+ MEOHP+ MEHHP+ MECPP weighted by the molecular weight.

Table 3.S2. Adjusted [a] odds ratio for detected versus undetected urinary butylparaben associated with last 24 hours personal care product use in the 10 imputed data.

Abbreviations: OR; odds ratio, 95% CI; 95% confidence intervals, MEP; Monoethyl phthalate, MnBP; Mono-n-butyl phthalate, MiBP; Mono-isobutyl phthalate, MBzP; Monobenzyl phthalate, MEHP; Mono(2-ethylhexyl) phthalate, MEOHP; Mono(2-ethyl-5-oxohexyl) phthalate, MEHHP; Mono(2-ethyl-5-hydroxyhexyl) phthalate, MECPP; Mono(2-ethyl-5-carboxypentyl) phthalate, DEHP; (Di(2-ethylhexyl) phthalate) metabolites, MCPP; Mono(3-carboxypropyl) phthalate.

[a] Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).

[b] Generalized estimating equations (GEE) was used for modeling butylparaben as binary variable: detected vs undetected in the 10 imputed data.

Table 3.S3. Adjusted [a] odds ratio for detected versus undetected urinary butylparaben associated with last 24 hours personal care product use in the complete case data.

Abbreviations: OR; odds ratio, 95% CI; 95% confidence intervals, MEP; Monoethyl phthalate, MnBP; Mono-n-butyl phthalate, MiBP; Mono-isobutyl phthalate, MBzP; Monobenzyl phthalate, MEHP; Mono(2-ethylhexyl) phthalate, MEOHP; Mono(2-ethyl-5-oxohexyl) phthalate, MEHHP; Mono(2-ethyl-5-hydroxyhexyl) phthalate, MECPP; Mono(2-ethyl-5-carboxypentyl) phthalate, DEHP; (Di(2-ethylhexyl) phthalate) metabolites, MCPP; Mono(3-carboxypropyl) phthalate.

[a] Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).

[b] Generalized estimating equations (GEE) was used for modeling butylparaben as binary variable: detected vs undetected in the complete case data.

Table 3.S4. Adjusted [a] percent change associated with the weighted score: (based on binary use up-to 24 hours use and 6 hours) using the weighted binary score (WBS) in the complete case data.

Abbreviations: 95% CI; 95% confidence intervals, MEP; Monoethyl phthalate, MnBP; Mono-n-butyl phthalate, MiBP; Mono-isobutyl phthalate, MBzP; Monobenzyl phthalate, MEHP; Mono(2-ethylhexyl) phthalate, MEOHP; Mono(2-ethyl-5-oxohexyl) phthalate, MEHHP; Mono(2-ethyl-5-hydroxyhexyl) phthalate, MECPP; Mono(2-ethyl-5-carboxypentyl) phthalate, DEHP; (Di(2-ethylhexyl) phthalate) metabolites, MCPP; Mono(3-carboxypropyl) phthalate.

[a] Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).

[b] ∑DEHP metabolites= MEHP+ MEOHP+ MEHHP+ MECPP weighted by the molecular weight.

Table 3.S5. Adjusted [a] odds ratio for detected versus undetected urinary butylparaben associated with last 24 hours personal care product use in the imputed missing as zero.

Abbreviations: OR; odds ratio, 95% CI; 95% confidence intervals, MEP; Monoethyl phthalate, MnBP; Mono-n-butyl phthalate, MiBP; Mono-isobutyl phthalate, MBzP; Monobenzyl phthalate, MEHP; Mono(2-ethylhexyl) phthalate, MEOHP; Mono(2-ethyl-5-oxohexyl) phthalate, MEHHP; Mono(2-ethyl-5-hydroxyhexyl) phthalate, MECPP; Mono(2-ethyl-5-carboxypentyl) phthalate, DEHP; (Di(2-ethylhexyl) phthalate) metabolites, MCPP; Mono(3-carboxypropyl) phthalate.

[a] Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).

[b] Generalized estimating equations (GEE) was used for modeling butylparaben as binary variable: detected vs undetected in the imputed missing as zero.0.

Table 3.S6. Adjusted [a] percent change associated with the weighted score: (based on binary use up-to 24 hours use and 6 hours) using the weighted binary score (WBS) in the imputed missing as zero.

Abbreviations: 95% CI; 95% confidence intervals, MEP; Monoethyl phthalate, MnBP; Mono-n-butyl phthalate, MiBP; Mono-isobutyl phthalate, MBzP; Monobenzyl phthalate, MEHP; Mono(2-ethylhexyl) phthalate, MEOHP; Mono(2-ethyl-5-oxohexyl) phthalate, MEHHP; Mono(2-ethyl-5-hydroxyhexyl) phthalate, MECPP; Mono(2-ethyl-5-carboxypentyl) phthalate, DEHP; (Di(2-ethylhexyl) phthalate) metabolites, MCPP; Mono(3-carboxypropyl) phthalate.

[a] Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).

[b] $\sum$DEHP metabolites= MEHP+ MEOHP+ MEHHP+ MECPP weighted by the molecular weight.

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Introduction

Because of their widespread use, exposure to ortho-phthalates (hereto referred to as phthalates) is ubiquitous in the general population[1]. Phthalates are high production volume synthetic chemicals with global production of 11 billion pounds per year[2] and more than 470 million pounds produced or imported into the United States (US) annually[3]. Phthalates are a family of chemicals commonly used as plasticizers in polyvinyl chloride plastics and in consumer products, including personal care products (PCPs), medications, and food processing and packaging materials[1].

Concomitant with the widespread use of phthalates and other synthetic chemicals, there is accumulating evidence that male testosterone levels have decreased during the last several decades[4,5] as well as a downward trend[6-8] and geographic variability in semen quality[9], surrogates for male fertility. These trends raise public and scientific concerns that lifestyle or environmental exposures may affect the endocrine system, semen quality, and hence male fertility and reproductive health[8,10-13].

In experimental studies, several phthalates, including dibutyl phthalate (DBP), have adverse impacts on male reproductive health[14-18]. Primarily for this reason, DBP was banned in toys and child care products in the European Union (EU) in 2005[19] and in the US in 2008[20]. In the EU, DBP was also banned in cosmetics in 2004[21]. However, despite these bans and restrictions, DBP is still used in the enteric coatings of some medications including specific mesalamine formulations[22-25].

The use of DBP in enteric coatings represents a source of very high exposure to DBP [22,23,25-31]. Recently, the U.S. Food and Drug Administration (FDA) recommended but did not require that certain phthalates including DBP should not be used as excipients in medications[32]. Mesalamine or 5-aminosalicylic acid (5-ASA), the active ingredient in Asacol®, Asacol®HD, Lialda®, Pentasa®, Apriso®, and Delzicol®, is a common anti-inflammatory maintenance therapy prescribed for inflammatory bowel diseases (IBD),

including ulcerative colitis (UC) and Crohn's disease (CD). The enteric coating of Asacol®, and Asacol®HD contains DBP as an excipient[33], whereas DBP is not used in the other mesalamine formulations such as Lialda®, Pentasa®, Apriso®, and Delzicol®[22,34]. Asacol® and Asacol®HD are prescribed as six to eight tablets per day leading to high-DBP exposure as measured by urinary monobutyl phthalate (MBP) concentrations, the primary DBP metabolite[23-25] with urinary concentrations approximately1000 times higher than the median concentrations reported for men in the U.S. general population (National Health and Nutrition Examination Survey (NHANES))[35]. Therefore, patients with IBD taking DBP-containing mesalamine will have chronic high exposure to DBP because the medication is taken daily to treat IBD.

Experimental studies have shown that DBP was anti-androgenic and a reproductive toxicant, however, most studies focused on the *in utero* exposure[36-38] rather than postnatal or adult exposures[39,40]. There is limited evidence from epidemiologic studies on the association of general population DBP-exposure with semen quality[41,42] and with serum reproductive hormones[43-47]. Most of the studies were conducted in men recruited from infertility clinics[48-56], and although some studies found associations of DBP with lower semen quality[50,52] and reproductive hormonal changes specifically decreased testosterone[48,49,56], others did not[41,42,51,57].

There are no studies that we are aware of on very high-DBP exposure from medications and their potential health effects. Therefore, we took advantage of the difference in DBP-exposure from different mesalamine formulations and conducted a crossover-crossback prospective study (Mesalamine And Reproductive health Study (MARS)) to examine the associations in adult men between the high-DBP exposure from mesalamine medications and semen quality in Chapter 1 and serum reproductive hormones in Chapter 2.

Another overlooked exposure source to endocrine disrupting chemicals in men is PCPs. In addition to phthalates, PCP is an important exposure source to parabens[31,58]. Parabens are a family of chemicals with antimicrobial preservative properties that are also widely used in PCPs, pharmaceuticals, food, and beverages to increase the shelf life of the product[58-60]. Exposure to phthalates and parabens from PCP use occurs through direct dermal application[61,62], inhalation, oral ingestion or even transdermal exposure from air[63]. Phthalates and parabens have short biological half-lives (6-24 hours)[61,64], and urinary concentrations of phthalate metabolites and parabens are the preferred exposure biomarkers[1,65].

In addition to phthalates, general population exposure to parabens is ubiquitous[1]. Diethyl phthalate (DEP) is the most commonly used phthalate in PCPs[66] and methylparaben, propylparaben and butylparaben are commonly used in PCPs[58,67,68]. Phthalates and parabens are endocrine disrupting chemicals[13,69,70] with potential adverse health effects including adverse reproductive outcomes[10,71,72], and metabolic disorders[72-74].

Prior studies on the contribution of PCP use to exposure to phthalates and parabens focused primarily on women and children[67,68,75-81] with limited epidemiological studies in men[67,82]. Therefore, we examined in Chapter 3 the association between self-reported use of fourteen PCPs and urinary concentrations of nine urinary phthalate metabolites and three parabens in men recruited as part of a prospective cohort study (Environment And Reproductive Health (EARTH) Study).

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Chapter 1

A crossover–crossback prospective study of dibutyl-phthalate exposure from mesalamine medications and semen quality in men with inflammatory bowel disease

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Abstract

Background: Phthalates are widely used chemicals with ubiquitous exposure. Dibutyl-phthalate (DBP), a male reproductive toxicant in animals, is understudied in humans. Some mesalamine medications used to treat inflammatory bowel disease (IBD) have DBP in their coating, whereas other mesalamine formulations do not.

Objectives: Taking advantage of differences in mesalamine formulations, we investigated whether high-DBP exposure from mesalamine medications was associated with decreased semen parameters.

Methods: 73 men with IBD taking mesalamine participated in a crossover-crossback prospective study. Men taking non-DBP containing mesalamine at baseline i.e., background exposure, crossed-over for four months to high-DBP mesalamine and then crossed-back for four months to their non-DBP mesalamine (B_1HB_2-arm;$Background_1$-$High$-$Background_2$) and vice versa for men taking high-DBP mesalamine at baseline (H_1BH_2-arm;$High_1$-$Background$-$High_2$). Men provided up to six semen samples (2: baseline, 2: crossover and 2: crossback).

Results: We estimated crossover, crossback and carryover effects using linear mixed models adjusted for abstinence time, age, season and duration on high-DBP mesalamine at baseline. Semen parameters in B_1HB_2-arm (26 men, 133 samples) decreased after high-DBP mesalamine exposure (crossover versus baseline), especially motility parameters, and continued to decrease further even after crossback to non-DBP mesalamine (crossback versus crossover).The cumulative carryover effect of high-DBP (crossback versus baseline) was a decrease of % total sperm motility by 7.61(CI:-13.1, -2.15), % progressive sperm motility by 4.23(CI:-8.05, -0.4) and motile sperm count by 26.0% (CI:-46.2%, 1.7%). However, H_1BH_2-arm (47 men, 199 samples) had no significant change during crossover or crossback.

Conclusions: Men newly exposed to high-DBP mesalamine for four months had a cumulative reduction in several semen parameters, primarily sperm motility, that was more pronounced and statistically significant even after exposure ended for four months.

Keywords: Phthalates, Mesalamine, Inflammatory Bowel Disease (IBD), Semen Quality

Abbreviations

DBP, dibutyl phthalate; FDA, Food and Drug Administration; 5-ASA, 5-aminosalicylic acid; IBD, Inflammatory Bowel Disease; UC, ulcerative colitis; CD, Crohn's disease; MBP, monobutyl phthalate; NHANES, National Health and Nutrition Examination Survey; MARS, Mesalamine And Reproductive health Study; BIMC, Beth Israel Deaconess Medical Center; BWH, Brigham and Women's Hospital; MGH, Massachusetts General Hospital; IRB, institutional review board; B_1HB_2, $Background_1$-High-$Background_2$ DBP exposure; H_1BH_2, $High_1$-Background-$High_2$ DBP exposure; QC, quality control; BMI, body mass index; Kg, Kilogram; m, meter; SD, standard deviation; LMEM, mixed effects models; FEM, fixed effect models; N, number of men; WHO, World Health Organization; EPA, Environmental Protection Agency.

Introduction

Over the last several decades, accumulating evidence suggests a downward trend(7-9) and geographic variability in semen quality(10), a surrogate for male fertility. These trends raise concern that lifestyle or environmental exposures may affect semen quality and male fertility(13). One class of environmental chemicals for which there is concern about potential adverse male reproductive health effects are phthalates(12). In experimental animal studies, several phthalates including dibutyl-phthalate **(**DBP) were anti-androgens and male reproductive toxicants, adversely affecting testicular function(15-19). The most studied window of exposure is *in-utero* exposure which led to male reproductive tract malformations in rats(37-39). Less well-studied are puberty and adulthood exposure. Studies in rats have shown effects of postnatal exposure to DBP on the male reproductive tract(41,84-86) and to butyl benzyl phthalate (BBzP)(87). There are several epidemiologic studies in adult men that explored cross-sectional associations between background low-DBP environmental exposure, and other phthalates, with semen quality. Most of these studies were conducted in men recruited from infertility clinics(49-56), and although some studies found associations of DBP(51,53) and other phthalates(49,50,53,54,88) with lower semen quality, others did not(42,43,52).

In addition to widespread general population DBP exposure from personal care and consumer products(32,89), some medications such as specific mesalamine formulations have enteric coatings that contain DBP(23,24,27,28,32) despite the recent US Food and Drug Administration (FDA) recommendation against the use of phthalates in drug delivery vehicles(33). Mesalamine or 5-aminosalicylic acid (5-ASA) is a commonly prescribed maintenance therapy for inflammatory bowel diseases (IBD), specifically ulcerative colitis (UC) and Crohn's disease (CD)(90). Our research and others have shown that mesalamine medications with coatings that contain DBP contribute to high-DBP exposure as measured by concentrations of urinary monobutyl phthalate (MBP), the primary DBP metabolite(25,26). Specifically, in individuals taking mesalamine medications that contain DBP, their

urinary levels of MBP were approximately 1000 times higher than the median levels reported for men in the US general population (National Health and Nutrition Examination Survey (NHANES))(36). Therefore, patients with IBD taking DBP-containing mesalamine will have chronic high exposure to DBP because the medication is taken daily to treat IBD.

Mesalamine is the active ingredient in Asacol® and Asacol®HD and DBP is an excipient in their enteric coating(34). Other mesalamine formulations such as Pentasa®, Lialda®, Apriso®, and Delzicol® do not contain DBP(23,35). Asacol®, widely used to treat IBD in adults and children, was a first line of therapy for patients with UC and often used in pregnant women with IBD(35,91). The aim of the study was to investigate the effect of high-DBP exposure on semen quality, taking advantage of the difference in mesalamine formulations to conduct a crossover-crossback prospective study in adult men with IBD.

Materials and Methods

Study population

We conducted a crossover-crossback prospective study in adult men with IBD (Mesalamine And Reproductive health Study (MARS)). Eligibility for participants in the MARS was 18 to 55 years of age and taking oral mesalamine for at least the past three months. All men must have had a mild IBD score on the simple clinical colitis activity index(92) (five or less for UC) or Harvey-Bradshaw index(93) (four or less for CD). Men were recruited from gastroenterology clinics at three Boston hospitals; Beth Israel Deaconess Medical Center (BIMC), Brigham and Women's Hospital (BWH) and Massachusetts General Hospital (MGH) from October 2010 through October 2015. MARS was approved by the institutional review boards (IRBs) of Harvard T.H. Chan School of Public Health, BIMC, BWH and MGH. All men signed informed consents.

Study design

Eligible men were invited to participate in up to six visits; to account for within-person variability in semen parameters. Each man was asked to participate in two visits at baseline, after crossover and after crossback. At each of the two baseline visits, participants provided semen, urine and blood samples collected two weeks apart (visits 1 and 2). Men were then asked to crossover to another formulation of mesalamine; men who were taking non-DBP mesalamine at baseline crossed-over to DBP-containing mesalamine medication (i.e., Asacol®) and vice versa for men prescribed DBP-containing mesalamine at baseline crossed-over to non-DBP mesalamine. After crossover for four months, two sets of semen, urine and blood samples were collected two weeks apart (visits 3 and 4). Participants were then asked to crossback for four months to their original mesalamine medications after which two sets of semen, urine and blood samples were collected two weeks apart (visits 5 and 6) (Figure 1.1).

In brief, men who were prescribed non-DBP mesalamine with background exposure from other sources crossed-over to high-DBP mesalamine then crossed-back to non-DBP mesalamine (B_1HB_2-arm: $Background_1$-High-$Background_2$). Men who were prescribed high-DBP mesalamine crossed-over to non-DBP mesalamine then crossed-back to high-DBP mesalamine (H_1BH_2-arm: $High_1$-Background-$High_2$). The 'wash-in' and 'wash-out' periods between crossover and crossback were four months to extend beyond the 70 days average period of spermatogenesis(94). Questionnaires about lifestyle factors, medical history and ejaculation abstinence time were administered at every visit.

Among the 47 men in the H_1BH_2-arm, 13 men participated only in a short protocol defined as up to four visits. These 13 men did not want to change medication but because the manufacturer was reformulating Asacol® to remove DBP, we anticipated that they would be 'switched' to a non-DBP mesalamine when this came to market. Warner Chilcott discontinued Asacol® in 2013 and introduced Delzicol® (non-DBP mesalamine) to the market. However, Asacol®HD (containing DBP) remained on the market. For 10 of the 13 men, their physician changed their medications to Asacol®HD, thus they never crossed-over to

non-DBP mesalamine and only contributed to the baseline visits while three men changed medication to Delzicol® i.e. crossed-over. However, by design none of the men in the short protocol crossed-back.

Men were asked to abstain from ejaculation for 2-5 days before providing semen samples, collected by masturbation at the MGH andrology laboratory into a sterile container and analyzed using standardized clinical protocols and quality control (QC) as described previously(51,95). Briefly, semen was allowed to liquefy at 37° C for 20 minutes. The physical properties of the semen were reported, including the sample volume, pH, color and viscosity. Ejaculate volume was measured using a graduated serological pipet. Sperm concentration and motility were assessed with a computer-aided semen analysis (CASA; 10HTM-IVOS, Hamilton-Thorne Research, Beverly, MA) which is used for routine diagnostic applications(1). For semen concentration and motility assessment, 5 µl of semen from each sample was placed into a pre-warmed (37°C) Makler counting chamber (Sefi-Medical Instruments, Haifa, Israel). Minimum of 200 sperm cells from at least four different fields were analyzed from each specimen. Sperm motility was expressed as the percentage of total motile (progressive + non-progressive) and percentage of progressive motile spermatozoa and defined as World Health Organization (WHO-2010) grade "a" sperm (rapidly progressive with a velocity ≥25 µm/sec) plus "b" grade sperm (slow/sluggish progressive with a velocity of ≥5 µm/sec but < 25 µm/sec)(1). Sperm morphology was measured on two slides for each specimen (with at least 200 cells assessed per slide) with a microscope using an oil-immersion 100× objective (Nikon, Tokyo, Japan). Sperm morphology was assessed using Kruger's strict criteria (96). To minimize variability, the laboratory followed a constant analysis set-up including play-back and QC plots if sperm counts < 20 or > 50 million/mL. Unusual values were re-evaluated. In addition to a quarterly competency technicians' evaluation, an outside evaluator performed proficiency testing, biannually. The technicians performing the semen assays were blinded to the study group.

Statistical Analysis

We created a six-level indicator variable cross-classifying each observation according to medication type (high-DBP or non-DBP mesalamine) and period (baseline, crossover and crossback) for the two study arms (H1BH2 and B1HB2). We considered for analysis, ejaculate volume, sperm concentration, motility (% motility and % progressive motility) and morphology. We also calculated total sperm count, motile sperm count, and morphologically normal sperm count. We used natural log-transformed sperm concentration, total sperm count, motile sperm count and morphologically normal count to satisfy model's normality assumption.

We performed descriptive statistics for participants' baseline and time-varying characteristics in both study arms. We also tested for any differences between the two arms using Kruskal-Wallis test for continuous variables and Fisher's exact test for categorical variables.

We used linear mixed effects models (LMEM) with a random intercept to account for within-person correlation among longitudinal measures of a given outcome arising from person-to-person heterogeneity across the study population. We estimated the DBP mesalamine crossover, crossback and carryover effects on semen parameters as absolute mean differences for the non-transformed semen parameters and as percent change for the log-transformed parameters.

Selection of covariates was based on directed acyclic graphs (Supplementary Figure 1.2) and statistical considerations (>10 % change in the effect estimate). The final model included abstinence time (< 2 days, 2≤ days <4 and ≥ 4 days), age at baseline (continuous), the season of the sample collection (warm and cold) and duration on high-DBP containing mesalamine at baseline (continuous in years). There were two samples missing abstinence times and we imputed the category of abstinence time based on the other

semen samples provided by the same man (both men had the same abstinence time category for all their other semen samples).

In our analysis, we also considered that the duration of IBD as a chronic inflammatory condition may affect semen quality. In preliminary models, we considered adjustment for duration of IBD, severity score of the disease, IBD condition (UC/CD), race, history of reproductive diseases or surgeries, BMI and smoking but they were not confounders and thus not retained in the final models.

We assessed model sensitivity to the covariance structure implied by the random intercept model, using empirical standard errors that are robust to misspecification of the covariance structure(97). As a sensitivity analysis, we further adjusted for body mass index (BMI <18.5, 18.5 ≤BMI<25, 25≤BMI<30 or ≥30)[98] and smoking status (never, former and current) in addition to the above covariates. As a secondary analysis, we applied fixed effect models (FEM) that, rather than assume a random distribution for the person-specific intercepts, estimate these terms as ordinary fixed regression coefficients in the model. These models isolate the longitudinal within-person effect of exposure, adjusted for the same covariates as above(97).

Although not a primary aim of our study, we explored the cross-sectional differences between the two arms at baseline by restricting the data to the average of the first two visits, applying linear regression adjusted for the covariates above. We further explored the effect of the duration under high-DBP mesalamine at enrollment on baseline semen parameters in the H_1BH_2-arm.

We conducted all statistical analyses using SAS version 9.4 (SAS Institute Inc., Cary, NC) and considered two-sided alpha <0.05 as statistically significant.

Results

Out of 215 confirmed eligible men, 73 agreed to participate (34%) (Supplementary Figure 1.1) and provided 332 semen samples with an average of 4.6 semen samples per man [range: 1 to 6]. The B_1HB_2-arm included 26 men (133 semen samples) with an average 5.1 samples per man. The H_1BH_2-arm included 47 men (199 samples) with an average of 4.9 samples per man. The two study arms had comparable baseline fixed and time-varying characteristics (Table 1.1). The semen parameter distributions are presented (Table 1.2). Men enrolled in the short protocol provided 31 samples (26 baseline and 5 after crossover samples).

the 60 full-protocol enrolled men, 51 (85%) men crossed-over medications, 23 (89%) in B_1HB_2-arm and 28 (82%) in H_1BH_2-arm; 43 (72%) men crossed-back medications, 19 (73%) in B_1HB_2-arm and 24 (71%) in H_1BH_2-arm. During follow-up, 9 (15%) men dropped out, 3 (12%) in B_1HB_2-arm and 6 (18%) in H_1BH_2-arm (p-value= 0.64). The median period between the second baseline visit (visit-2) and collection of crossover semen samples at visit-3 and similarly between visit-4 and visit-5 for crossback was 119 days. The median time period between visits on the same medication (visits-1&2), (visits-3&4) and (visits-5&6) was 16 days. The H_1BH_2-arm men were on their high-DBP mesalamine medication at entry for median 2.9 years and up to 24.1 years. The B_1HB_2-arm men were on their non-DBP mesalamine medication at baseline for median 1.0 year and up to 10.3 years.

None of the semen samples were azoospermic **(**Table 1.2); 188 (55%) samples were above the reference limits of the World Health Organization (WHO-2010)(1) with regard to sperm concentration, total count, percent motile and percent normal morphology.

Although not statistically significant, in a cross-sectional analysis of the baseline visits, sperm motility and morphology parameters for men in H_1BH_2-arm were higher than among men in B_1HB_2-arm (Figure

1.2). However, among men in the H_1BH_2-arm at the baseline, there was a negative association between the number of years on high-DBP containing mesalamine and semen parameters, steeper in the early years as compared to the later years (data not shown).

In the adjusted analyses (Table 1.3A & Figure 1.2), on average among men in the B_1HB_2-arm who were newly-exposed to high-DBP mesalamine after crossover had decreased (though non-statistically significant) semen parameters, apart from ejaculate volume and % normal morphology. Their % total sperm motility decreased by 4.19 (95% confidence interval (CI): -9.25, 0.86), % progressive sperm motility by 1.76 (CI: -5.29, 1.78) and motile sperm count by 13.7% (CI: -35.7%, 15.8). After crossback to their non-DBP mesalamine, all semen parameters (except sperm concentration) continued to decrease *further*. The % total sperm motility further decreased by 3.41 (CI: -8.69, 1.86), % progressive sperm motility by 2.47 (CI: -6.16, 1.22) and motile sperm count by 14.3% (CI: -36.9%, 16.5%). The *cumulative carryover* effect of high-DBP exposure (i.e., crossback compared to baseline) was a decrease in *all* semen parameters; % total sperm motility decreased by 7.61 (CI: -13.1, -2.15), % progressive sperm motility by 4.23 (CI: -8.05, -0.4) and motile sperm count by 26.0% (CI: -46.2%, 1.7%). In contrast, in the H_1BH_2-arm (Table 1.3B & Figure 1.2), semen parameters did non-significantly change after crossover or crossback.

We found consistent results from the sensitivity analyses using empirical standard errors in LMEMs and after further adjustment for BMI and smoking (data not shown) and from FEMs which fully adjust for both observed and unobserved factors that do not change within a person across visits(97) (Supplementary Table 1.1).

Discussion

In our crossover-crossback study, men who were on non-DBP mesalamine at baseline (B_1HB_2-arm) and newly exposed for four months (crossover period) to high-DBP had lower semen parameters, primarily % total sperm motility, % progressive sperm motility and motile sperm count. This decrease was more pronounced and became statistically significant even after crossback for four months to their original non-DBP mesalamine. Thus, the effect of high-DBP on sperm motility parameters was not reversible after four months. Among men who were on high-DBP at baseline (H_1BH_2-arm), there was no statistically significant change in semen parameters after crossover to non-DBP mesalamine or crossback to their original high-DBP mesalamine. Therefore, in both arms, the four month wash-out period was *not* long enough to reverse the effect of high-DBP exposure.

In the cross-sectional analysis of baseline semen characteristics, unexpectedly, men who were on high-DBP mesalamine at baseline (H_1BH_2-arm) had non-significantly higher percent sperm motility and normal morphology than men who were on non-DBP mesalamine at baseline (B_1HB_2-arm). However, among men in the H_1BH_2-arm, there was a downward trend in all semen parameters associated with increased duration on high-DBP mesalamine. This negative association with years on high-DBP mesalamine among men in H_1BH_2-arm argues against a 'protective' effect of high-DBP and suggests chance sampling variability at baseline due to non-randomization at the baseline and cautions against cross-sectional analyses. These results highlight the power of the study that by design removes subject to subject variability from the comparison of interest, thus avoiding the purely cross-sectional analysis that is likely to suffer from this random variability.

There is an accumulating literature on the association of background low environmental exposure to phthalates with semen quality. A recent meta-analysis that included 14 publications on phthalates and semen quality concluded that higher urinary MBP concentrations were associated with reduced sperm

concentration and straight line velocity(99). All 14 publications were cross-sectional with 11 conducted in infertility clinics. Two recent US studies on fertile men not included in the meta-analysis(42,43) did not find significant associations. Our study differs in many respects from the earlier cross-sectional studies. We used a more powerful crossover-crossback prospective design and therefore had repeated semen samples from men during both high and background DBP exposure. This allowed for within-person comparisons and was by design adjusted for non-time varying covariates. Furthermore, our design allowed us to determine the carryover effect that was not observable in the earlier cross-sectional designs.

Experimental studies, primarily during postnatal exposure(87,100), have shown decreased serum testosterone concentration and increased follicle stimulating hormone (FSH) and luteinizing hormone (LH) concentrations in male rats exposed to BBzP which suggested tubular atrophy and loss of the germinal epithelium as a result of Sertoli cell injury.

Postnatal exposure to other anti-androgenic phthalates (di(2-ethylhexyl)phthalate) caused germ cell apoptosis with increased membrane localization of Fas(101,102) or germ cells detachment from the seminiferous epithelium probably due to cellular adhesion loss between Sertoli cells(103). Also, studies suggested that in older animals DBP preferentially targets Sertoli cells(104,105).

Studies during fetal development also have shown that DBP is anti-androgenic. It decreases Leydig cell testosterone production by interfering with steroidogenesis by down-regulating gene and/or protein expression important in the steroidogenic pathway, cholesterol transport and metabolism(106-112), while increasing oxidative stress along with reactive oxygen species(113).

Several studies have identified numerous medications as a source of phthalate exposure(23-27,35). Phthalate levels in medications may not be openly displayed, due to proprietary formulations(23). Medications with phthalates in the coating include mesalamine, didanosine, omeprazole, theophylline(24) and other medications including over-the-counter preparations(23,27,32). Our research and others have

shown that mesalamine medications with DBP contribute to high-DBP exposure as measured by urinary MBP concentrations(24-26).

The human daily DBP intake from the maximum recommended dose of Asacol® is 21 mg(34,114). Therefore, the estimated maximum daily DBP intake is 350 μg/kg/day based upon 60 kg body weight. Similarly, the human DBP daily intake from the maximum recommended dose of Asacol®HD is 48 mg(115) with the estimated maximum daily DBP intake of 800 μg/kg/day. These intakes are orders of magnitude higher than the estimated average DBP human intake (0.84-5.22 μg/kg/day)(116), several times higher than the US Environmental Protection Agency (EPA) reference dose (100 μg/kg/day)(26) and orders of magnitude higher than the European tolerable daily intake (10 μg/kg/day)(117).

We considered measuring urinary MBP concentrations in the urine sample collected at each study visit. However, due to the unique crossover-crossback design and the very high exposure from Asacol® (1000 fold higher than urinary levels when not taking Asacol®), we concluded that urinary MBP concentrations would not add significant information beyond using medication as an indicator of high and low DBP exposure. We offer three primary justifications for not measuring urinary MBP concentrations. First, clear documentation that Asacol® contributes to very high exposure to DBP(23-27,32,34,114,115). Second, the study is considered interventional in which we assigned participants to the different medications. Therefore, the data was appropriately analyzed based on the assigned medication (as an intention to treat analysis) for our interventional design. Third, from a statistical perspective, continuous urinary data would essentially dichotomize exposure given the very large differences in urinary MBP when taking Asacol® and we would use the same modeling approach. Specifically, the estimates of exposure response, even if we had the continuous urinary MBP concentrations, would have been completely dominated by the medication type (1000 times difference when taking Asacol®) with no overlap in urinary MBP concentrations between those visits when taking Asacol® and those visits when not taking Asacol®.

Our study had several potential limitations including non-randomization of mesalamine at baseline which may have resulted in chance sampling variability at the baseline. However, there is no reason to believe that the prescribing practices of physicians were associated with the presence or absence of DBP enteric coating. To assign high-DBP exposure, we relied on self-reported use of mesalamine medications as prescribed over the study period(118) rather than measuring urinary MBP concentrations which only represent recent exposure (i.e., past several hours) due to the short DBP half-life. Although the sample size for this study was not very large due to the innovative study and length of participation, the power of the study came from the unique design that removes subject to subject variability from the comparison of interest avoiding the purely cross-sectional analyses that likely to suffer from this random variability. We also conducted a post-hoc power analysis and the study had a sufficient number of participants to provide 80% power for detecting at least 23% decrease in sperm concentration and an absolute mean difference of at least 0.4 ml of ejaculate volume, 1% fewer morphologically normal sperm, 6% fewer motile sperm and 4% fewer progressive motile sperm, which is reasonable for a study of this design. Finally, although semen quality is an imperfect predictor of fertility(119), semen analysis is considered the cornerstone of the laboratory evaluation for infertile men(120).

Our study had several important strengths. We implemented a unique innovative study design that is rarely performed in environmental epidemiology. We were able to compare, within the same men, their semen parameters during periods of high-DBP to background-DBP exposure and vice versa accounting for confounding by measured and unmeasured non-time-varying characteristics (121). This is a major strength as compared to previous cross-sectional studies. We were able to explore whether there was a carryover effect of high-DBP exposure. Our study was not restricted to infertile men as most of the previous literature. The means of all semen parameters were comparable to those for fertile US adult men(10). Although there may be a concern of generalizing results from men with IBD, which has a significantly increasing temporal trend in incidence worldwide especially in industrialized countries (122), these results raise concern about high-DBP exposure and poor semen quality. In addition, there is

no evidence that IBD or mesalamine is linked to male infertility (123-127). Asacol® and its mesalamine alternatives for treating IBD have the same active ingredients, therefore confounding by indication was unlikely.

Compared to environmental background exposure, we were able to explore high-DBP exposure (1000 times background). A recent gastroenterology consensus report has recommended switching to non-DBP mesalamine in pregnant women or women who may contemplate pregnancy(28), without any recommendations for men of reproductive age. Therefore, our results will be informative for future recommendations of use of non-DBP mesalamine formulations among men. To our knowledge, no previous human studies investigated effects of such *high*-DBP exposure on semen parameters.

Conclusions

High-DBP exposure from Asacol® (mesalamine) had adverse effects on semen parameters in adult men, with the most robust effects on sperm motility. Most importantly, the effect of DBP carried-over even after removing the exposure suggesting that high-DBP had longer term effects on spermatogenesis. Further studies are needed to explore whether the carryover effect is potentially reversible after a wash-out period longer than four months. Finally, studies of the effect of high-DBP exposure on sperm epigenetics and reproductive and non-reproductive hormones would add additional insights. Attention should be given to such high-exposure sources and our study and further research will provide more guidance on further regulation of DBP containing coating of medications.

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Table 1.1: Demographics of 73 men contributing 332 visits in the MARS Study by arm.

	B_1HB_2-arm (26 men, 133 visits)	H_1BH_2-arm (47 men, 199 visits)	Total (73 men, 332 visits)	
	N(%)/Mean (SD),[Range]	N(%)/Mean (SD),[Range]	N(%)/Mean (SD),[Range]	P-value[b]
Baseline characteristics (at Visit 1), # men (N)[a]				
Age (Years)	33.9 (8.97), [20.2, 54.6]	34.9 (9.66), [19.6, 55.7]	34.6 (9.37), [19.6, 55.7]	0.82
Race				0.27
Caucasian	25 (96%)	38 (81%)	63 (86%)	-
Black/African American	1 (4%)	2 (4%)	3 (4%)	-
Asian	0	4 (9%)	4 (6%)	-
Other	0	3 (6%)	3 (4%)	-
BMI (Kg/m2)	25.3 (3.56), [19.4, 32.7]	26.4 (6.39), [19.5, 54.0]	26.0 (5.54), [19.4, 54.0]	0.88
BMI-categories				0.99
Normal weight (18.5 ≤BMI<25)	14 (54%)	24 (51%)	38 (52%)	-
Overweight (25≤BMI<30)	9 (35%)	16 (34%)	25 (34%)	-
Obese (BMI≥30)	3 (11%)	7 (15%)	10 (14%)	-
Education				0.82
Below college	3 (14%)	6 (14%)	9 (14%)	-
College graduate	10 (48%)	16 (38%)	26 (41%)	-
Graduate degree	8 (38%)	20 (48%)	28 (45%)	-
Smoking status				0.009
Never smoker	22 (85%)	36 (77%)	58 (80%)	-
Former smoker	1 (4%)	11 (23%)	12 (16%)	-
Current smoker	3 (11%)	0 (0%)	3 (4%)	-
Warm season at baseline[c]	5 (19%)	20 (43%)	25 (34%)	0.07
IBD diagnosis				0.61
Ulcerative colitis	16 (62%)	32 (68%)	48 (66%)	-
Crohn's disease	10 (38%)	15 (32%)	25 (34%)	-
IBD score[d]	1.65 (1.38), [0, 4]	1.19 (1.35), [0, 5]	1.36 (1.37), [0, 5]	0.13
Time-varying characteristics, #visits[a]				
Warm season [c]	61 (46%)	92 (46%)	153 (46%)	0.99
Ejaculation abstinence time				0.84
< 2 Days	10 (8%)	19 (10%)	29 (9%)	-
2≤Days<4	115 (86%)	169 (85%)	284 (85%)	-
≥4 Days	8 (6%)	11 (5%)	19 (6%)	-

B_1HB_2-arm, B_1 represents background-exposure at baseline, **H** represents high-DBP exposure after crossover and **B_2** represents background-exposure after crossback; included 26 men taking mesalamine that did not contain DBP at baseline; 16 men (62%) on Lialda®, 8 men (31%) on Pentasa®, one man (4%) on Apriso® and one man (4%) was not on mesalamine medication at the time of recruitment.

H_1BH_2-arm, H_1 represents high-DBP exposure at baseline, **B** represents background-exposure after crossover and **H_2** represents high-DBP exposure after crossback; included 47 men taking mesalamine-containing DBP at baseline; 24 men (51%) on Asacol® and 23 men (49%) on Asacol®HD.

[a]:N (%) for categorical/binary variables and mean (SD), [Range] for continuous variables

[b]: P-values are based on Fisher exact test for categorical variables and Kruskal Wallis test for continuous variables.

[c]: Season of sample collection, Warm: April Through September

10 men missing information on education; for 2 semen samples missing abstinence times, we imputed values for their categories based on the other samples given by the same man (both men had the same abstinence time category for all their other samples).

Table 1.1 (Continued): [d]**:** IBD score: simple clinical colitis activity index, includes bowel frequency and urgency, presence of blood in the stool and general wellbeing. Mild IBD score: 5 or less for UC and 4 or less for CD. **Abbreviations:** MARS, Mesalamine And Reproductive health Study; BMI, body mass index; Kg, Kilogram; m, meter; SD, standard deviation; IBD, Inflammatory Bowel Disease; UC, ulcerative colitis; CD, Crohn's disease; N, number of men; DBP, dibutyl phthalate; B_1HB_2, $Background_1$-High-$Background_2$ DBP exposure; H_1BH_2, $High_1$-Background-$High_2$ DBP exposure.

Table 1.2: Semen quality parameters for 73 men (332 semen samples) in the MARS study.

Semen quality parameters	Mean (SD)	Median [Range]	n (%) < WHO lower reference limits[a]
Ejaculate volume (mL)	2.71 (1.21)	2.5 [0.23, 7.10]	45 (14%)
Sperm concentration (million/mL)	74.4 (68.2)	59.8 [2.7, 670]	22 (7%)
Total sperm count (million)	192 (151)	168 [0.62, 828]	35 (11%)
%Total sperm motility	49.0 (20.6)	51.0 [0, 88]	111 (33%)
%Progressive sperm motility	29.4 (14.2)	30.0 [0, 77]	177 (53%)
Motile sperm count (million)[b]	111 (115)	74.3 [0, 621]	-
% Normal sperm morphology	7.01 (3.61)	7 [0, 23]	93 (28%)
Morphologically normal sperm count (million)[c]	15.6 (17.7)	9.63 [0, 131]	-

[a] Binary semen quality parameters less than WHO lower reference limits (2010)(1)**:** ejaculate volume<15 ml; sperm concentration <15 million/ml; total sperm count< 39 million; total motile sperm<40%; progressive motile sperm <32% and normal sperm morphology< 4% using "strict" Tygerberg method; **n:** # semen samples. There were 2 samples missing morphology **(n=330).**
[b] Motile count= Total Sperm Count x % Motile
[c] Morphologically normal count =Total Sperm Count x % normal morphology
Abbreviations: MARS, Mesalamine And Reproductive health Study; WHO: World health organization.

Table 1.3A: Adjusted effect (95%CI) of crossover, crossback and carryover on semen parameters among men starting on mesalamine medication not containing dibutyl phthalate (B1HB2-arm)[a]

B_1HB_2-arm [Background (B_1)- High (H)- Background (B_2) dibutyl phthalate exposure]: 26 men, 133 semen samples

Semen quality parameter	Fitted Means (95%CI)			Comparisons					
	Baseline (B_1)	Crossover (H)	Crossback (B_2)	Crossover effect (95%CI) H-B_1	P value	Crossback effect (95%CI) B_2-H	P value	Carryover effect (95%CI) B_2-B_1	P value
Ejaculate volume (mL)	2.88 (2.4, 3.37)	2.97 (2.46, 3.47)	2.70 (2.19, 3.22)	0.09 (-0.22, 0.39)	0.57	-0.27 (-0.58, 0.05)	0.10	-0.18 (-0.51, 0.15)	0.29
Sperm concentration (million/mL)[b]	55.5 (40.1, 76.8)	48.9 (34.9, 68.5)	50.1 (35.5, 70.6)	-11.9% (-27.6%, 7.23%)	0.21	2.36% (-16.6%, 25.6%)	0.82	-9.8% -27.0%, 11.5%)	0.34
Total sperm count (million)[b]	140 (94, 207)	127 (84.3, 192)	120 (79.1, 183)	-8.96% (-28.7%, 16.2%)	0.45	-5.45% (-26.7%, 22.0%)	0.67	-13.9% (-33.9%, 12.1%)	0.26
%Total sperm motility	43.8 (35.5, 52)	39.6 (31, 48.1)	36.2 (27.4, 44.9)	-4.19 (-9.25, 0.86)	0.10	-3.41 (-8.69, 1.86)	0.20	-7.61 (-13.1, -2.15)	0.007
%progressive sperm motility	25.2 (19.5, 31)	23.5 (17.5, 29.4)	21.0 (14.9, 27.1)	-1.76 (-5.29, 1.78)	0.33	-2.47 (-6.16, 1.22)	0.19	-4.23 (-8.05, -0.40)	0.03
Motile sperm count (million)[b]	52.1 (30.4, 88.8)	44.8 (25.6, 77.8)	38.3 (21.6, 67.1)	-13.7% (-35.7%, 15.8%)	0.32	-14.3% (-36.9%, 16.5%)	0.32	-26.0% (-46.2%, 1.70%)	0.06
%Normal sperm morphology	6.17 (4.77, 7.57)	6.31 (4.83, 7.79)	6.02 (4.47, 7.56)	0.13 (-1.06, 1.33)	0.82	-0.29 (-1.54, 0.96)	0.65	-0.16 (-1.45, 1.13)	0.81
Morphologically normal sperm count (million)[b]	8.25 (5.08, 13.1)	7.90 (4.76, 12.8)	7.08 (4.18, 11.6)	-3.78% (-25.9%, 24.9%)	0.77	-9.14% (-30.8%, 19.4%)	0.49	-12.6% (-34.2%, 16.1%)	0.35

Table 1.3B: Adjusted effect (95%CI) of crossover, crossback and carryover on semen parameters among men starting on mesalamine medication containing dibutyl phthalate (H1BH2-arm)[a]

H_1BH_2-arm[High (H_1)-Background (B) - High (H_2)] dibutyl phthalate exposure]: 47 men, 199 semen samples									
	Fitted Means (95%CI)			Comparisons					
Semen quality parameter	**Baseline (H_1)**	**Crossover (B)**	**Crossback (H_2)**	**Crossover Effect (95%CI) B-H_1**	**P value**	**Crossback Effect (95%CI) H_2-B**	**P value**	**Carryover Effect (95%CI) H_2-H_1**	**P value**
Ejaculate volume (mL)	2.47 (2.11, 2.84)	2.51 (2.11, 2.91)	2.44 (2.02, 2.86)	0.04 (-0.22, 0.29)	0.77	-0.07 (-0.35, 0.21)	0.62	-0.03 (-0.32, 0.25)	0.81
Sperm concentration (million/mL)[b]	57.5 (45, 73.5)	55.4 (42.5, 72.1)	60.6 (46, 79.9)	-3.70% (-18.4%, 13.6%)	0.65	9.49% (-8.77%, 31.4%)	0.33	5.43% (-12.1%, 26.5%)	0.57
Total sperm count (million)[b]	124 (92.1, 167)	124 (89.6, 171)	130 (92.6, 182)	-0.31% (-18.9%, 22.5%)	0.98	4.86% (-16.5%, 31.6%)	0.68	4.54% (-16.7%, 31.1%)	0.70
%Total sperm motility	52.8 (46.6, 59.1)	49.6 (42.8, 56.3)	51.6 (44.6, 58.6)	-3.28 (-7.54, 0.97)	0.13	2.02 (-2.68, 6.72)	0.40	-1.26 (-5.95, 3.42)	0.60
%progressive sperm motility	32.6 (28.2, 36.9)	31.1 (26.4, 35.8)	32.3 (27.4, 37.2)	-1.49 (-4.46, 1.49)	0.33	1.20 (-2.09, 4.49)	0.47	-0.29 (-3.56, 2.99)	0.86
Motile sperm count (million)[b]	62.7 (42, 93.4)	54.7 (35.5, 83.9)	63.3 (40.5, 98.6)	-12.6% (-31.8%, 12.1%)	0.29	15.4% (-12.2%, 51.8%)	0.30	0.91% (-23.2%, 32.7%)	0.95
%Normal sperm morphology	7.75 (6.67, 8.83)	6.74 (5.52, 7.96)	7.64 (6.33, 8.95)	-1.01 (-2.0, -0.02)	0.04	0.90 (-0.21, 2.01)	0.11	-0.11 (-1.19, 0.97)	0.84
Morphologically normal sperm count (million)[b]	10.3 (7.23, 14.5)	8.52 (5.76, 12.4)	10.6 (7.12, 15.6)	-15.6% (-32.2%, 5.05%)	0.13	22.0% (-4.23%, 55.3%)	0.11	2.95% (-19.1%, 31%)	0.81

[a]: Adjusted for abstinence time (categorical: < 2 days, 2≤days<4 and ≥ 4 days), age (continuous), season (warm (April through September) vs cold) and period (# years on DBP-containing mesalamine medication at baseline) in the mixed effect model.

[b]: Sperm concentration, total sperm count, motile sperm count and morphologically normal sperm count are presented as percentage change by exponentiating the log-transformed Beta coefficient. The sum of the percent changes for crossover and cross back do not equal the total carryover effect for the log-transformed variables because they are on the multiplicative scale. For the variables that are not log-transformed, the sum of the percent changes for crossover and cross back equal the total carryover effect as they represent the absolute difference on the additive scale

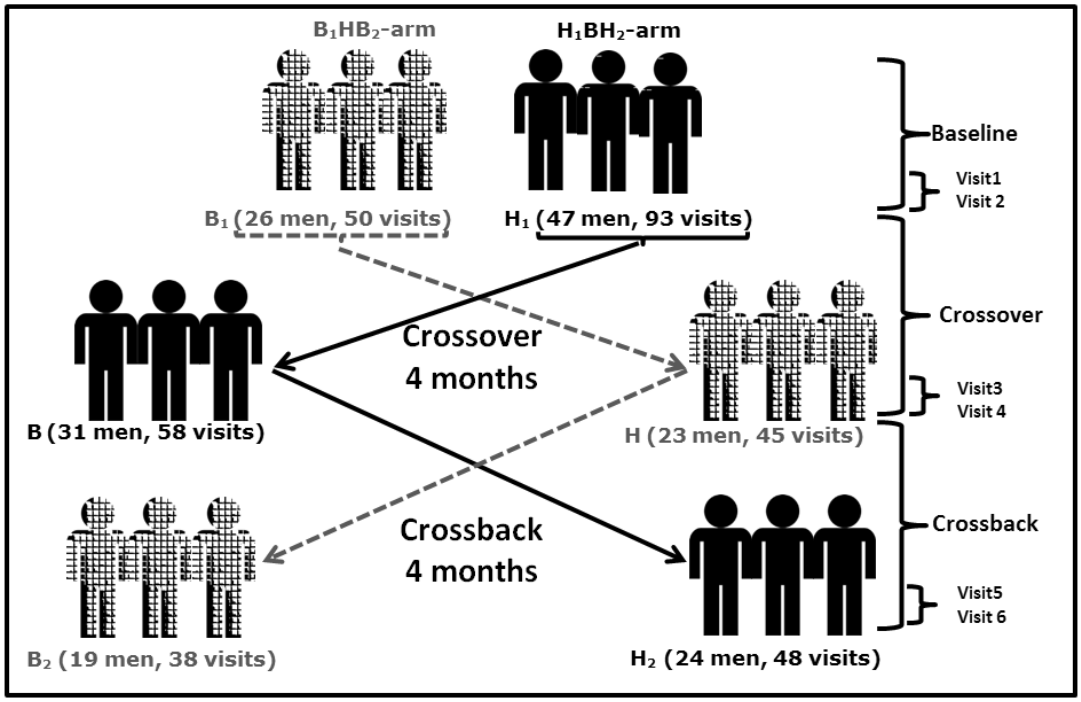
Motile sperm count (million) and morphologically normal sperm count (million) were log-transformed after adding 1 due to few zero values, they were back transformed and 1 was subtracted from the means.

B_1HB_2-arm: B_1 represents background low-DBP exposure at baseline, H represents high-DBP exposure after crossover and B_2 represents background low-exposure after crossback.

H_1BH_2-arm: H_1 represents high-DBP at baseline, B represents background low-DBP exposure after crossover and **H_2** represents high-DBP after crossback.

Note: 2 samples missing morphology; negative sign means a decrease or % decrease for the log-transformed variables compared to the measure in the previous period. **Abbreviations**: DBP, dibutyl phthalate; B_1HB_2: Background$_1$-High-Background$_2$ DBP exposure; H_1BH_2; High$_1$-Background-High$_2$ DBP exposure; 95%CI, 95% Confidence Interval.

Figure 1.1: Mesalamine And Reproductive health Study (MARS) Design.



B_1HB_2-arm: B_1 represents background-exposure at baseline, **H** represents high-DBP exposure after crossover and **B_2** represents background-exposure after crossback; included 26 men taking mesalamine that did not contain DBP at baseline. **H_1BH_2-arm: H_1** represents high-DBP exposure at baseline, **B** represents background-exposure after crossover and **H_2** represents high-DBP exposure after crossback; included 47 men taking mesalamine-containing DBP at baseline. **Abbreviations: MARS**, Mesalamine And Reproductive health Study; **DBP,** dibutyl phthalate.

Figure 1.2: Unadjusted and Adjusted means (95%CI) of semen parameters at baseline, crossover and crossback for the 2 arms-MARS.

Panel A **Panel B**

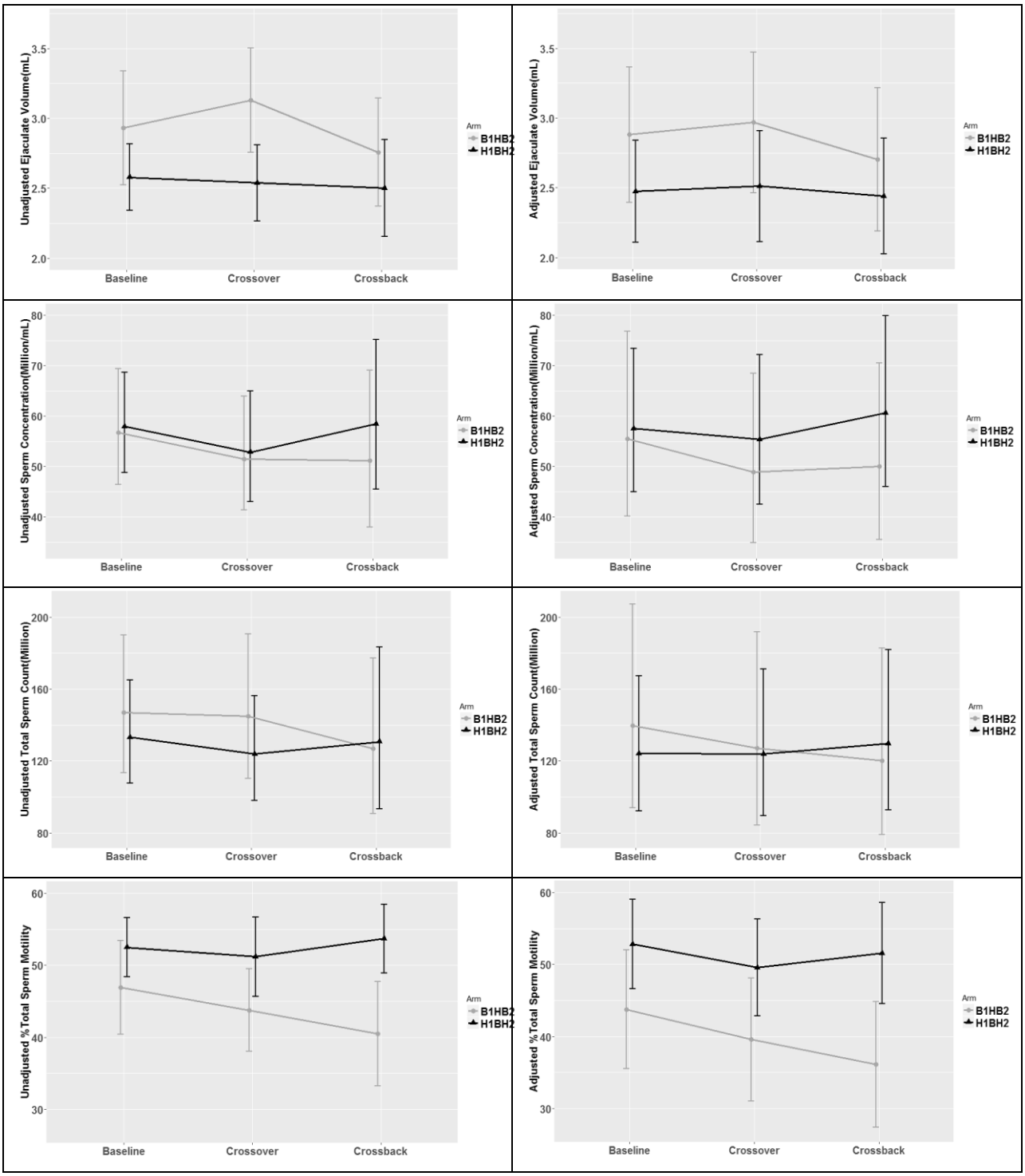


Figure 1.2 (Continued): Unadjusted and adjusted means (95%CI) of semen parameters at baseline, crossover and crossback for the 2 arms-MARS.

Panel A **Panel B**

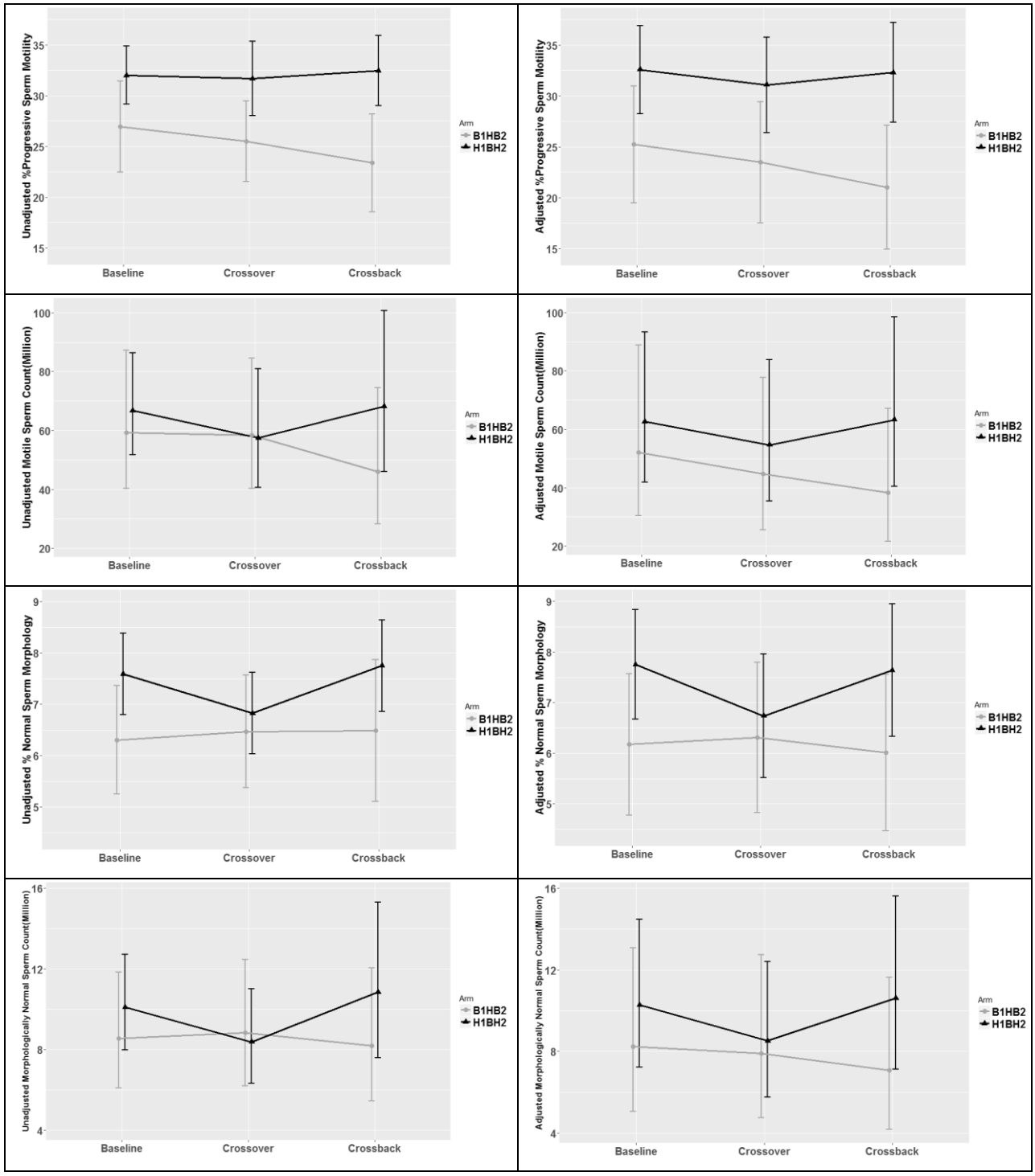
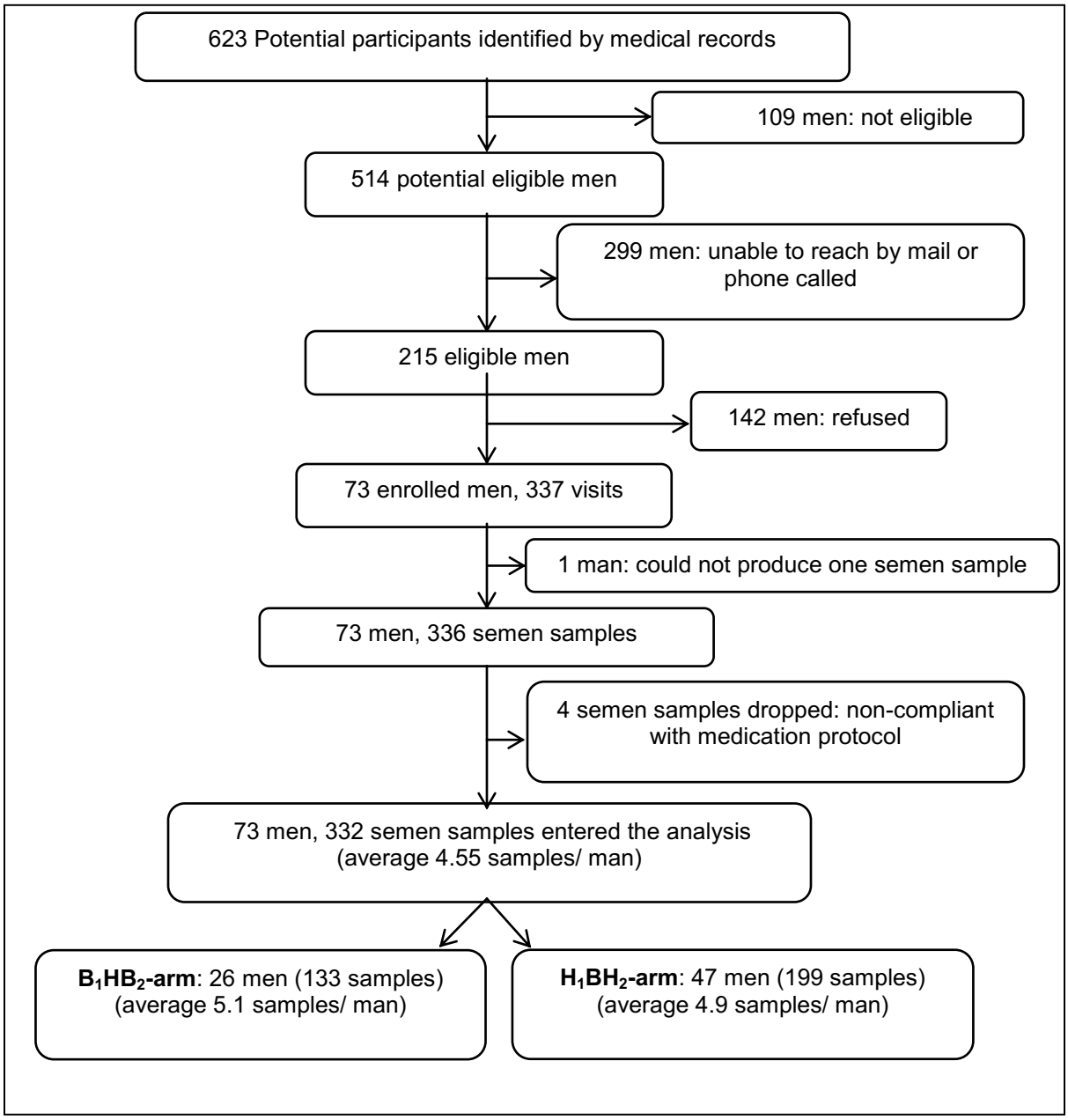


Figure 1.2: Panel A shows the unadjusted means and 95% CI and **Panel B** shows the adjusted means and 95% CI [adjusted for abstinence time (categorical), age (continuous), season (warm versus cold) and duration on DBP-containing mesalamine medication at baseline (in years)]. For the log-transformed outcomes (sperm concentration, total sperm count, motile sperm count and morphologically normal sperm count), the geometric means and 95% CI are presented. **B_1HB_2-arm, B_1** represents background-exposure at baseline, **H** represents high-DBP exposure after crossover and **B_2** represents background-exposure after crossback; included 26 men taking mesalamine that did not contain DBP at baseline. **H_1BH_2-arm, H_1** represents high-DBP exposure at baseline, **B represents** background-exposure after crossover and **H_2** represents high-DBP exposure after crossback; included 47 men taking mesalamine-containing DBP at baseline. **Abbreviations: MARS**, Mesalamine And Reproductive health Study; **95% CI**, 95% Confidence Interval; **DBP**, dibutyl phthalate.

Supplementary Materials

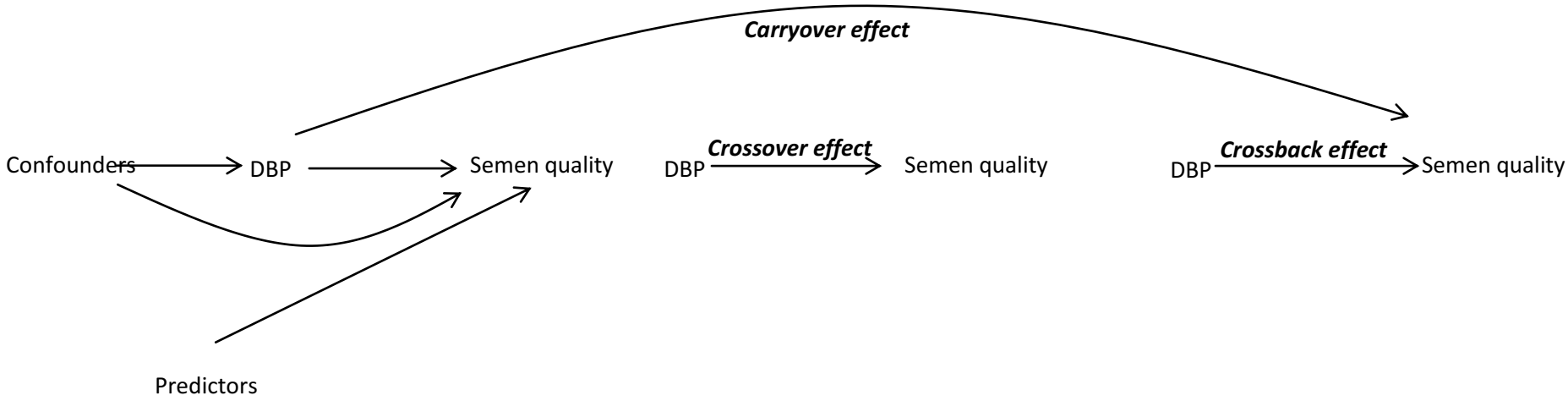
Supplementary Figure 1.1: Sample Size Flow Chart-Mesalamine And Reproductive health Study (MARS)



MARS Eligibility criteria: 18 to 55 years old males; diagnosed with mild IBD, visited gastroenterology clinics at Massachusetts General Hospital (MGH), Beth Israel Deaconess Medical Center (BIMC) or Brigham and Women's Hospital (BWH), Boston, MA, USA from October 2010 through October 2015. Participants must have had mild IBD score (5 or less for UC and 4 or less for CD) on the simple clinical colitis activity index, on oral mesalamine for at least the previous 3 months with at least 800 mg/3 times/ day. Men with history of infertility were not excluded. Men with history of vasectomy, steroid medication in the last 3 months, diabetes mellitus, hepatic or renal diseases were not eligible. **Potential eligible**: men who are eligible according to the medical records but we could not confirm eligibility due to failure to reach them. **Eligible**: eligibility confirmed after contacting the man.

Supplementary Figure 1.1: B_1HB_2-arm, B_1 represents background-exposure at baseline, **H** represents high-DBP exposure after crossover and **B_2** represents background-exposure after crossback included 26 men taking mesalamine that did not contain DBP at baseline.
H_1BH_2-arm, H_1 represents high-DBP exposure at baseline, **B** represents background-exposure after crossover and **H_2** represents high-DBP exposure after crossback included 47 men taking mesalamine containing DBP at baseline.
Abbreviations: MARS, Mesalamine And Reproductive health Study; IBD, Inflammatory Bowel Disease; UC, ulcerative colitis; CD, Crohn's disease; DBP, dibutyl phthalate; B_1HB_2: $Background_1$-High-$Background_2$ DBP exposure; H_1BH_2; $High_1$-Background-$High_2$ DBP exposure.

Supplementary Figure 1.2: Directed Acyclic Graph (DAG) for Mesalamine And Reproductive health Study (MARS).



Confounders e.g., period under High-DBP before enrollment
Predictors e.g., abstinence time, age and season
DBP exposure could be background as non-DBP mesalamine or high as high-DBP mesalamine
Abbreviations: DBP, dibutyl phthalate

Supplementary Table 1.1.A: Adjusted effect (95%CI) of crossover, crossback and carryover on semen parameters among men starting on mesalamine medication not containing dibutyl phthalate (B1HB2-arm)[a]

B_1HB_2-arm [Background (B_1) -High (H)-Background (B_2) dibutyl phthalate exposure]: (26 men, 133 semen samples)

	Means (SE)	Comparisons					
Semen quality parameter	**Baseline (B_1)**	**Crossover Effect (95%CI) H-B_1**	**P value**	**Crossback Effect (95%CI) B_2-H**	**P value**	**Carryover Effect (95%CI) B_2-B_1**	**P value**
Ejaculate volume (mL)	2.93 (2.52, 3.34)	0.07 (-0.24, 0.37)	0.67	-0.26 (-0.58, 0.06)	0.11	-0.2 (-0.53, 0.14)	0.24
Sperm concentration (million/mL)[b]	56.8 (46.5, 69.4)	-11.5% (-27.4%, 7.88%)	0.23	2.87% (-16.23%, 26.32%)	0.79	-8.95% (-26.5%, 12.8%)	0.39
Total sperm count (million)[b]	147 (113, 190)	-9.34% (-29.2%, 16.0%)	0.43	-4.79% (-26.3%, 23.0%)	0.71	-13.7% (-33.9%, 12.8%)	0.28
%Total sperm motility	46.9 (40.4, 53.5)	-4.38 (-9.47, 0.71)	0.09	-3.39 (-8.68, 1.89)	0.21	-7.78 (-13.3, -2.26)	0.006
%progressive sperm motility	27.0 (22.5, 31.5)	-1.83 (-5.39, 1.74)	0.31	-2.53 (-6.23, 1.17)	0.18	-4.35 (-8.21, -0.49)	0.03
Motile sperm count (million)[b]	59.3 (40.2, 87.1)	-14.8% (-36.6%, 14.7%)	0.29	-13.7% (-36.6%, 17.4%)	0.35	-26.5% (-46.7%, 1.42%)	0.06
% Normal sperm morphology	6.31 (5.25, 7.36)	0.11 (-1.1, 1.32)	0.86	-0.40 (-1.66, 0.85)	0.53	-0.29 (-1.61, 1.03)	0.66
Morphologically normal sperm count (million)[b]	8.55 (6.11, 11.8)	-4.94% (-26.9%, 23.7%)	0.71	-9.49% (-31.2%, 19.0%)	0.47	-14.0% (-35.4%, 14.6%)	0.30

Supplementary Table 1.1.B: Adjusted effect (95%CI) of crossover, crossback and carryover on semen parameters among men starting on mesalamine medication containing dibutyl phthalate (H_1BH_2-arm)[a]

H_1BH_2-arm[High (H_1)-Background (B) – High (H_2) dibutyl phthalate exposure]: (47 men, 199 semen samples)							
	Means (SE)	Comparisons					
Semen quality parameter	Baseline (H_1)	Crossover Effect (95%CI) B-H_1	P value	Crossback Effect (95%CI) H_2-B	P value	carryover Effect (95%CI) H_2-H_1	P value
Ejaculate volume (mL)	2.58 (2.34, 2.82)	0.04, -0.22, 0.3)	0.78	-0.08 (-0.36, 0.21)	0.59	-0.04 (-0.33, 0.25)	0.77
Sperm concentration (million/mL)[b]	57.9 (48.8, 68.7)	-2.4% (-17.5%, 15.5%)	0.78	9.66% (-8.71%, 31.7%)	0.32	7.02% (-11.1%, 28.9%)	0.47
Total sperm count (million)[b]	133 (108, 165)	0.72% (-18.4%, 24.3%)	0.95	4.96% (-16.5%, 31.9%)	0.68	5.73% (-16.1%, 33.3%)	0.64
%Total sperm motility	52.51 (48.4, 56.6)	-3.84 (-8.17, 0.49)	0.08	2.04 (-2.67, 6.76)	0.39	-1.8 (-6.57, 2.98)	0.46
%progressive sperm motility	32 (29.1, 34.9)	-1.83 (-4.86, 1.2)	0.24	1.28 (-2.02, 4.58)	0.45	-0.55 (-3.89, 2.79)	0.75
Motile sperm count (million)[b]	66.8 (51.7, 86.3)	-13.0% (-32.4%, 12%)	0.28	15.4% (-12.3%, 51.9%)	0.31	0.41% (-24.0%, 32.63%)	0.98
% Normal sperm morphology	7.59 (6.8, 8.38)	-1.17 (-2.2, -0.15)	0.03	0.91 (-0.21, 2.03)	0.11	-0.27 (-1.4, 0.87)	0.65
Morphologically normal sperm count (million)[b]	10.1 (7.99, 12.7)	-16.1% (-32.9%, 4.89%)	0.12	21.6% (-4.61%, 55.0%)	0.11	2.05% (-20.2%, 30.5%)	0.87

[a]: Adjusted for abstinence time (categorical: < 2 days, 2≤days<4 and ≥ 4 days), age (continuous), season (warm (April through September) vs cold) and period (# years on DBP-containing mesalamine medication at baseline) in the fixed effect model.

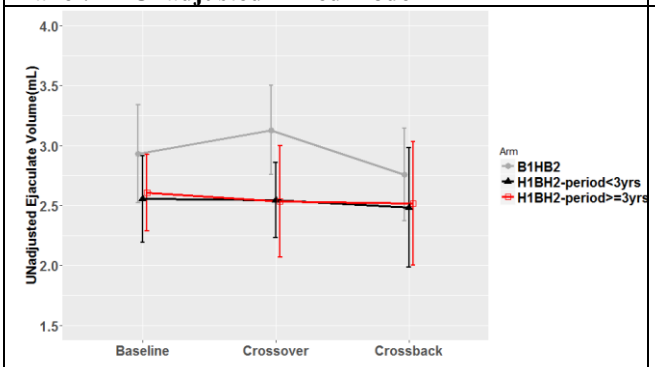
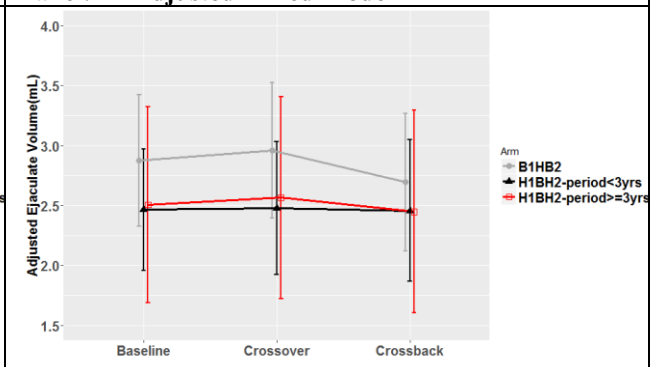
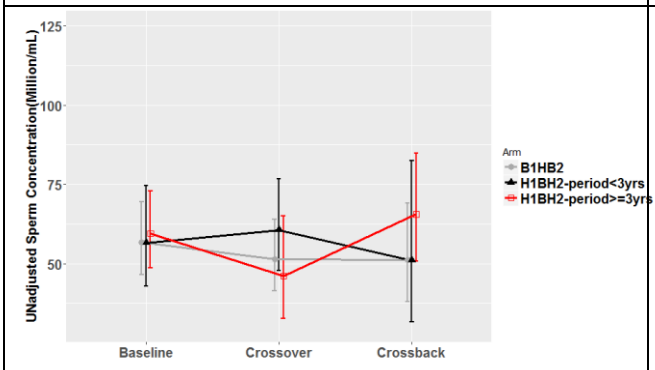
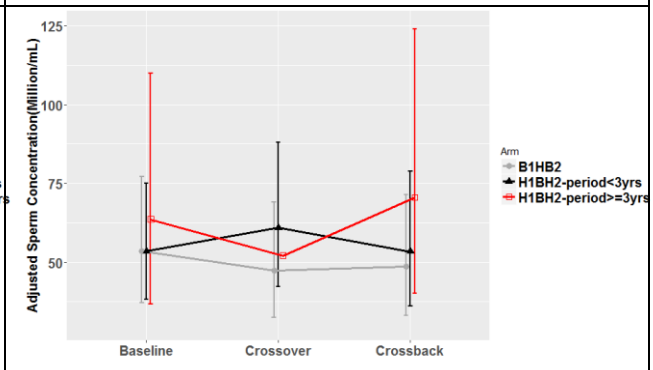
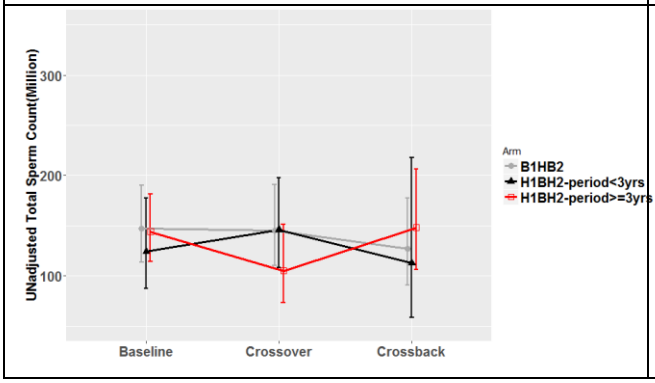
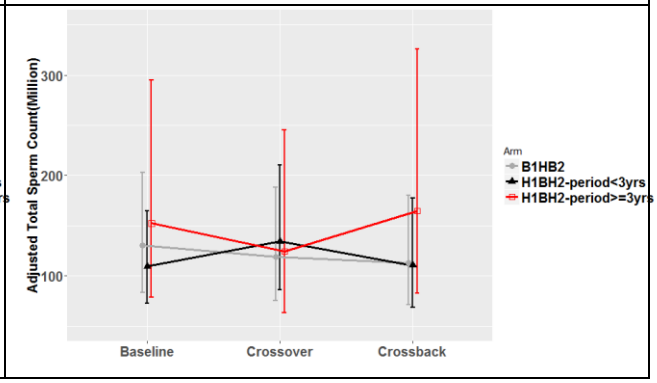
[b]: Sperm concentration, total sperm count, motile sperm count and morphologically normal sperm count are presented as percentage change by exponentiating the log transformed Beta coefficient. The sum of the percent changes for crossover and cross back do not equal the total carryover effect for the log transformed variables because they are on the multiplicative scale). For the variables that are not log transformed, the sum of the percent changes for crossover and cross back equal the total carryover effect as they represent the absolute difference on the additive scale.

Motile sperm count (million) and morphologically normal sperm count (million) were log transformed after adding 1 due to few zero values, they were back transformed and 1 was subtracted from the means.

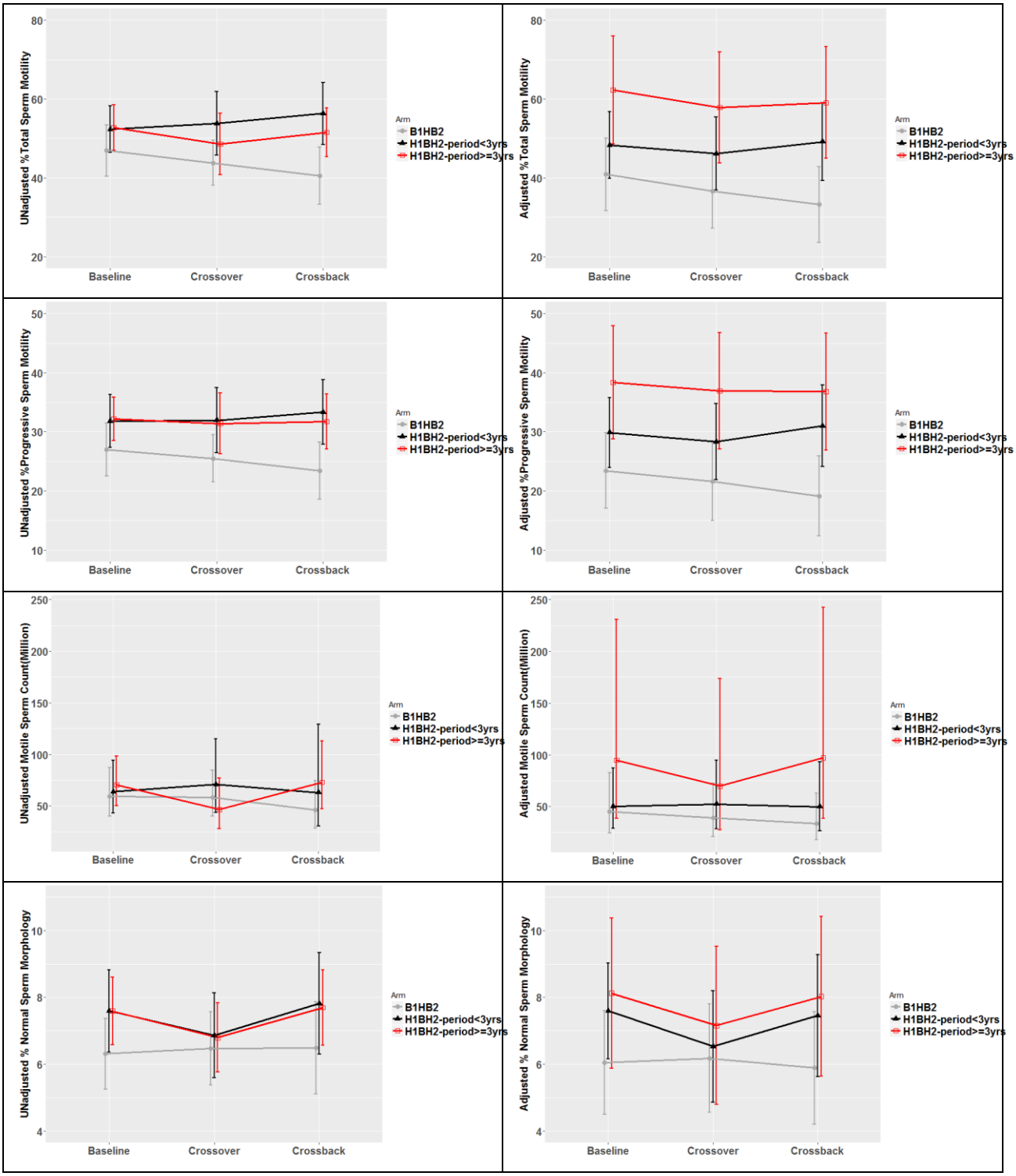
Supplementary Table 1.1. (Continued): B_1HB_2-arm: B_1 represents background-DBP exposure at baseline, H represents high-DBP exposure after crossover and B_2 represents background-exposure after crossback.
H_1BH_2-arm: H_1 represents high-DBP at baseline, B represents background-DBP exposure after crossover and H_2 represents high-DBP after crossback.
Note: 2 samples missing morphology
Note: Negative sign means a decrease or % decrease for the log transformed variables compared to the measure in the previous period
Abbreviations: DBP, dibutyl phthalate; B_1HB_2: $Background_1$-High-$Background_2$DBP exposure; H_1BH_2; $High_1$-Background-$High_2$ DBP exposure; 95%CI, 95% Confidence Interval.

Chapter 1 Appendix

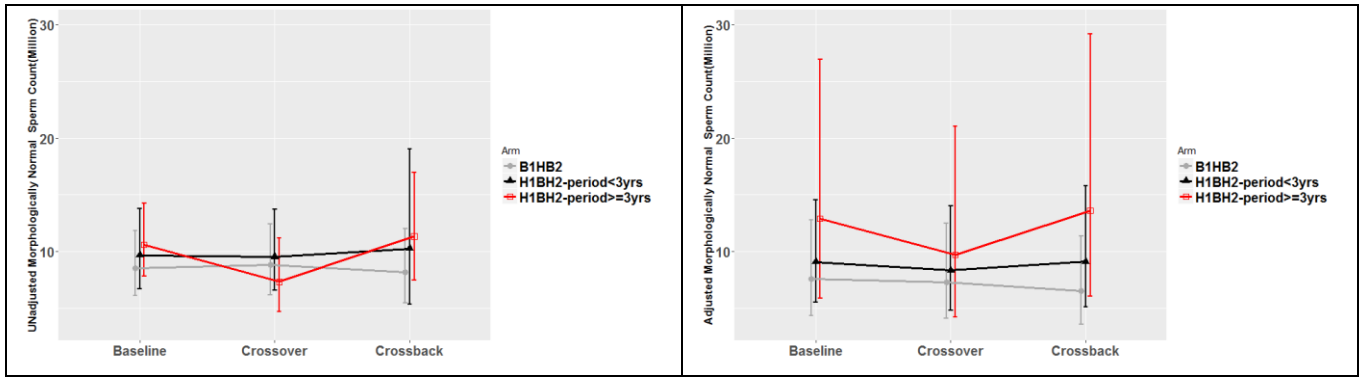
Supplementary Figure 1.3. Means (95%CI) of semen parameters at baseline, crossover and crossback for the 3-arms (B1HB2--arm, H1BH2-arm with duration on DBP-containing mesalamine medication at baseline (period) <3 years and H1BH2-arm with period ≥ 3 years) -MARS

Panel: A- Unadjusted Mixed model	Panel: B- Adjusted Mixed Model
	
	
	

Supplementary Figure 1.3. (Continued)



Supplementary Figure 1.3. (Continued)



Supplementary Figure 1.3. : Panel A shows the unadjusted means and 95%CI and **Panel B** shows the adjusted means and 95%CI (adjusted for abstinence time (categorical), age (continuous), season (warm versus cold) and duration on DBP-containing mesalamine medication at baseline (in years).

For the log transformed outcomes (sperm concentration, total sperm count, motile sperm count and morphologically normal sperm count), the geometric means and 95% CI are presented.

B_1HB_2-arm (26 men, 133 semen samples): included men taking mesalamine that did not contain DBP at baseline. **H_1BH_2-arm (47 men, 199 semen samples): included** men taking mesalamine containing DBP at baseline. **H_1BH_2-arm, <3ys**: 25 men, 100 semen samples and **H_1BH_2-arm, ≥3ys**: 22 men and 99 semen samples.

In B_1HB_2-arm, Background$_1$ represents background-DBP exposure at baseline, **H** represents high-DBP exposure after crossover and **Background$_2$** represents background-DBP exposure after crossback.

In H_1BH_2-arm, H_1 represents high-DBP exposure at baseline, **B** represents background-DBP exposure after crossover and **H_2** represents high-DBP exposure after crossback.

Abbreviations: MARS, Mesalamine And Reproductive health Study; **95% CI**, 95% Confidence Interval; **DBP**, dibutyl phthalate.

Supplementary Table 1.2.A: Unadjusted effect (95% CI) of crossover, crossback and carryover on semen parameters among men starting on mesalamine medication not containing dibutyl phthalate (B1HB2-arm: B1HB2) -Mixed Model[a].

B_1HB_2-arm [Background$_1$(B_1)-High(H)-Background$_2$(B_2) dibutyl phthalate exposure]: (26 men, 133 semen samples)

	Means (95%CI)			Comparisons					
Semen quality parameter	**Baseline (B_1)**	**Crossover (H)**	**Crossback (B_2)**	**Crossover Effect (95%CI) H-B_1**	**P value**	**Crossback Effect (95%CI) B_2-H**	**P value**	**Carryover Effect (95%CI) B_2-B_1**	**P value**
Ejaculate volume (mL)	2.95 (2.52, 3.38)	3.01 (2.57, 3.45)	2.70 (2.24, 3.15)	0.06 (-0.24, 0.35)	0.71	-0.31 (-0.63, 0)	0.05	-0.26 (-0.57, 0.06)	0.11
Sperm concentration (million/mL)b	57.2 (43.1, 75.9)	51.5 (38.5, 68.8)	52.1 (38.7, 70.3)	-9.98% (-25.6%, 8.92%)	0.28	1.29% (-17.4%, 24.1%)	0.90	-8.82% (-25.6%, 11.8%)	0.37
Total sperm count (million)b	149 (106, 210)	138 (96.7, 196)	126 (87.5, 181)	-7.66% (-27.2%, 17.2%)	0.51	-8.53% (-29.1%, 18.0%)	0.49	-15.5% (-34.5%, 8.95%)	0.19
%Total sperm motility	47.1 (39.8, 54.3)	42.6 (35.2, 50.0)	39.0 (31.3, 46.6)	-4.44 (-9.34, 0.45)	0.07	-3.65 (-8.87, 1.57)	0.17	-8.10 (-13.3, -2.87)	0.003
%progressive sperm motility	26.9 (21.9, 32.0)	24.7 (19.6, 29.9)	22.0 (16.8, 27.3)	-2.2 (-5.63, 1.23)	0.21	-2.71 (-6.36, 0.95)	0.15	-4.91 (-8.57, -1.24)	0.009
Motile sperm count (million)b	60.5 (37.8, 96.5)	52.9 (32.7, 85.2)	43.6 (26.5, 71.2)	-12.4% (-34.2%, 16.7%)	0.37	-17.3% (-39.1%, 12.2%)	0.22	-27.5% (-46.6%, -1.60%)	0.04
% Normal sperm morphology	6.23 (5.05, 7.41)	6.31 (5.09, 7.54)	6.05 (4.75, 7.35)	0.09 (-1.07, 1.24)	0.89	-0.27 (-1.51, 0.98)	0.67	-0.18 (-1.42, 1.06)	0.78
Morphologically normal sperm count (million)b	8.41 (5.54, 12.5)	7.99 (5.2, 12.0)	7.05 (4.49, 10.8)	-4.48% (-25.8%, 23.0%)	0.72	-10.4% (-31.6%, 17.4%)	0.42	-14.4% (-34.8%, 12.4%)	0.26

Supplementary Table 1.2.B: Unadjusted effect (95% CI) of crossover, crossback and carryover on semen parameters among men starting on mesalamine medication containing dibutyl phthalate (H_1BH_2-arm) -Mixed Model[a.]

H_1BH_2-arm [High$_1$(H_1)-Background(B)–High$_2$(H_2)] dibutyl phthalate exposure]: (47 men, 199 semen samples)									
	Means (95%CI)			Comparisons					
	Baseline (B_1)	**Crossover (H)**	**Crossback (B_2)**	**Crossover Effect (95%CI) B-H_1**	**P value**	**Crossback Effect (95%CI) H2-B**	**P value**	**Carryover Effect (95%CI) H_2-H_1**	**P value**
Ejaculate volume (mL)	2.58 (2.26, 2.9)	2.59 (2.23, 2.94)	2.48 (2.12, 2.85)	0.00 (-0.25, 0.26)	0.97	-0.10 (-0.38, 0.18)	0.48	-0.10 (-0.37, 0.18)	0.49
Sperm concentration (million/mL)[b]	57.8 (46.9, 71.4)	55.5 (44.1, 69.9)	60.2 (47.3, 76.5)	-4.06% (-18.6%, 13.1%)	0.62	8.44% (-9.56%, 30.0%)	0.38	4.03% (-12.9%, 24.3%)	0.66
Total sperm count (million)[b]	133 (103, 172)	130 (98.4, 173)	134 (99.5, 179)	-2.21% (-20.4%, 20.1%)	0.83	2.42% (-18.4%, 28.5%)	0.84	0.16% (-19.8%, 25.1%)	0.99
%Total sperm motility	52.6 (47.2, 58.0)	49.1 (43.2, 55.0)	51.1 (44.9, 57.2)	-3.45 (-7.66, 0.76)	0.11	1.94 (-2.72, 6.6)	0.41	-1.51 (-6.07, 3.05)	0.52
%progressive sperm motility	32.0 (28.3, 35.7)	30.4 (26.3, 34.4)	31.4 (27.1, 35.7)	-1.68 (-4.63, 1.27)	0.26	1.05 (-2.21, 4.32)	0.53	-0.63 (-3.82, 2.57)	0.70
Motile sperm count (million)[b]	67.0 (47.3, 94.6)	57.2 (39.2, 83.3)	64.6 (43.7, 95.4)	-14.3% (-33.1%, 9.68%)	0.22	12.7% (-14.2%, 48.0%)	0.39	-3.48% (-26.2%, 26.2%)	0.79
% Normal sperm morphology	7.6 (6.73, 8.47)	6.61 (5.59, 7.64)	7.54 (6.44, 8.64)	-0.98 (-1.96, -0.01)	0.05	0.93 (-0.17, 2.03)	0.10	-0.05 (-1.11, 1)	0.92
Morphologically normal sperm count (million)[b]	10.1 (7.51, 13.5)	8.27 (5.9, 11.5)	10.2 (7.18, 14.2)	-16.6% (-32.9%, 3.51%)	0.10	20.3% (-5.36%, 52.8%)	0.13	0.25% (-20.7%, 26.7%)	0.98

[a] Adjusted for abstinence time (categorical: < 2 days, 2≤days<4 and ≥ 4 days), age (continuous), season (warm (April through September) vs cold) and period (# years on DBP containing mesalamine medication at baseline).

[b] Sperm concentration, total sperm count, motile sperm count and morphologically normal sperm count are presented as percentage change by exponentiating the log transformed Beta coefficient. The sum of the percent changes for crossover and cross back do not equal the total carryover effect for the log transformed variables because they are on the multiplicative scale). For the variables that are not log transformed, the sum of the percent changes for crossover and cross back equal the total carryover effect as they represent the absolute difference on the additive scale

Motile sperm count (million) and morphologically normal sperm count (million) were log transformed after adding 1 due to few zero values, they were back transformed and 1 was subtracted from the means. **B_1HB_2-arm: Background$_1$** represents low exposure to DBP at baseline, **H** represents high DBP exposure after crossover and Background2 represents low DBP after crossback. **H_1BH_2-arm: H_1** represents high DBP at baseline, **B** represents Background exposure to DBP after crossover and **H_2** represents high DBP after crossback. **Note:** 2 samples missing morphology **Note:** Negative sign means a decrease or% decrease for the log transformed variables compared to the measure in the previous period**. Abbreviations: DBP,** dibutyl phthalate; **B_1HB_2:** Background-High-Background DBP exposure; **H_1BH_2;** High-Background-High DBP exposure**; 95% CI**, 95% Confidence Interval.

Supplementary Table 1.3: Unadjusted effect (95% CI) of crossover, crossback and carryover on semen parameters among men starting on mesalamine medication not containing dibutyl phthalate (B_1HB_2-arm: B_1HB_2) and men starting on mesalamine medication containing dibutyl phthalate (H_1BH_2-arm) -Fixed Model[a].

B_1HB_2-arm [Background$_1$(B_1)-High(H)-Background$_2$(B_2) dibutyl phthalate exposure]: (26 men, 133 semen samples)							
	Means (95%CI)	Comparisons					
Semen Quality Parameter	**Baseline (Background$_1$)**	**Crossover Effect (95%CI) H- Background$_1$**	**P value**	**Crossback Effect (95%CI) B_2-H**	**P value**	**Carryover Effect (95%CI) Background$_2$-Background$_1$**	**P value**
Ejaculate volume (mL)	2.93 (2.52, 3.34)	0.02 (-0.28, 0.32)	0.90	-0.3 (-0.62, 0.01)	0.06	-0.28 (-0.6, 0.04)	0.08
Sperm concentration (million/mL)[b]	56.8 (46.5, 69.4)	-9.91% (-25.6%, 9.14%)	0.29	1.47% (-17.2%, 24.4%)	0.89	-8.58% (-25.6%, 12.3%)	0.39
Total sperm count (million)[b]	147 (113, 190)	-9.01% (-28.4%, 15.7%)	0.44	-8% (-28.7%, 18.8%)	0.52	-16.3% (-35.3%, 8.26%)	0.17
%Total sperm motility	46.9 (40.4, 53.5)	-4.74 (-9.66, 0.17)	0.06	-3.72 (-8.95, 1.5)	0.16	-8.47 (-13.7, -3.2)	0.002
%progressive sperm motility	27.0 (22.5, 31.5)	-2.39 (-5.84, 1.05)	0.17	-2.79 (-6.46, 0.87)	0.13	-5.19 (-8.88, -1.5)	0.006
Motile sperm count (million)[b]	59.3 (40.2, 87.1)	-14.2% (-35.7%, 14.5%)	0.30	-17.0% (-38.9%, 12.8%)	0.23	-28.8% (-47.7%, 3.03%)	0.03
% Normal sperm morphology	6.31 (5.25, 7.36)	-0.01 (-1.18, 1.16)	0.98	-0.37 (-1.62, 0.87)	0.56	-0.38 (-1.65, 0.88)	0.55
Morphologically normal sperm count (million)[b]	8.60 (6.11, 11.8)	-6.72% (-27.7%, 20.3%)	0.59	-10.9% (-32.0%, 16.9%)	0.40	-16.9% (-36.8%, 9.46%)	0.19

Supplementary Table 1.3 (Continued):

H_1BH_2-arm [$High_1(H_1)$-Background(B)–$High_2(H_2)$] dibutyl phthalate exposure]: (47 men, 199 semen samples)

	Means (95%CI)	Comparisons					
Semen quality parameter	**Baseline (A_1)**	**Crossover Effect (95%CI) B-H_1**	**P value**	**Crossback Effect (95%CI) H2-B**	**P value**	**Carryover Effect (95%CI) H_2-H_1**	**P value**
Ejaculate volume (mL)	2.58 (2.34, 2.82)	0.00 (-0.25, 0.26)	0.97	-0.11 (-0.39, 0.18)	0.46	-0.10 (-0.38, 0.18)	0.47
Sperm concentration (million/mL)b	57.9 (48.8, 68.7)	-3.1% (-18.0%, 14.5%)	0.71	8.23% (-9.8%, 29.9%)	0.39	4.88% (-12.5%, 25.7%)	0.61
Total sperm count (million)b	133 (108, 165)	-1.48% (-20.1%, 21.5%)	0.89	2.14% (-18.7%, 28.3%)	0.86	0.63% (-19.8%, 26.3%)	0.96
%Total sperm motility	52.5 (48.4, 56.6)	-4.07 (-8.35, 0.22)	0.06	1.89 (-2.78, 6.56)	0.43	-2.18 (-6.83, 2.47)	0.36
%progressive sperm motility	32.0 (29.1, 34.9)	-2.08 (-5.08, 0.93)	0.17	1.11 (-2.17, 4.38)	0.51	-0.97 (-4.23, 2.28)	0.56
Motile sperm count (million)b	66.8 (51.7, 86.3)	-15.0% (-33.9%, 9.3%)	0.20	12.3% (-14.6%, 47.6%)	0.41	-4.56% (-27.3%, 25.3%)	0.74
% Normal sperm morphology	7.59 (6.80, 8.38)	-1.17 (-2.18, -0.15)	0.02	0.94 (-0.17, 2.04)	0.10	-0.23 (-1.33, 0.87)	0.68
Morphologically normal sperm count (million)b	10.1 (7.99, 12.7)	-17.4% (-33.8%, 2.95%)	0.09	19.6% (-5.96%, 52.1%)	0.14	-1.24% (-22.2%, 25.4%)	0.92

[a] **Unadjusted fixed effect model.**

[b] Sperm concentration, total sperm count, motile sperm count and morphologically normal sperm count are presented as percentage change by exponentiating the log transformed Beta coefficient. The sum of the percent changes for crossover and cross back do not equal the total carryover effect for the log transformed variables because they are on the multiplicative scale). For the variables that are not log transformed, the sum of the percent changes for crossover and cross back equal the total carryover effect as they represent the absolute difference on the additive scale

Motile sperm count (million) and morphologically normal sperm count (million) were log transformed after adding 1 due to few zero values, they were back transformed and 1 was subtracted from the means.

B_1HB_2-arm: Background$_1$ represents low exposure to DBP at baseline, **H** represents high DBP exposure after crossover and Background2 represents low DBP after crossback.

H_1BH_2-arm: H_1 represents high DBP at baseline, **B** represents Background exposure to DBP after crossover and **H_2** represents high DBP after crossback.

Note: 2 samples missing morphology

Note: Negative sign means a decrease or% decrease for the log transformed variables compared to the measure in the previous period

Abbreviations: DBP, dibutyl phthalate; **B_1HB_2:** Background-High-Background DBP exposure; **H_1BH_2;** High-Background-High DBP exposure**;95% CI**, 95% Confidence Interval.

Supplementary Table 1.4: Adjusted[a] effect (95% CI) of crossover, crossback and carryover on semen parameters among men starting on mesalamine medication not containing dibutyl phthalate (B1HB2-arm) and men starting on mesalamine medication containing dibutyl phthalate (H1BH2-arm with duration on DBP-containing mesalamine medication at baseline (period) <3 years and H1BH2-arm with period ≥ 3 years)-Mixed model-3-arms[a]

B_1HB_2-arm [Background$_1$(B_1)-High(H)- Background$_2$(B_2) dibutyl phthalate exposure]: (26 men, 133 semen samples)

Semen Quality Parameter	Mean (95%CI)			Comparisons				
	Baseline (B1)	Crossover (H)	Crossback (B2)	Crossover effect (95%CI) H- B1	P value	Crossback effect (95%CI) B2-H	P value	Carryover effect (95%CI) B2- B1	P value
Ejaculate Volume(mL)	2.87 (2.33,3.42)	2.96 (2.39,3.53)	2.69 (2.12,3.27)	0.09 (-0.22, 0.39)	0.58	-0.27 (-0.58, 0.05)	0.10	-0.18 (-0.51, 0.15	0.28
Sperm Concentration(Million/mL)[b]	53.5 (37.1,77.1)	47.3 (32.4,69)	48.6 (33.1,71.3)	-11.5% (-27.2%, 7.47%)	0.22	2.80% (-16.1%, 25.9%)	0.79	-9.05% (-26.3%, 12.2%)	0.38
Total Sperm Count(Million)[b]	130 (83.6,203)	119 (75.3,188)	113 (71.1,180)	-8.63% (-28.3%, 16.5%)	0.47	-4.99% (-26.3%, 22.5%)	0.69	-13.2% (-33.3%, 12.9%)	0.29
%Total Sperm Motility	40.9 (31.7,50.1)	36.6 (27.1,46.1)	33.2 (23.6,42.8)	-4.24 (-9.31, 0.82)	0.10	-3.42 (-8.71, 1.87)	0.20	-7.66 (-13.1, -2.18)	**0.006**
%Progressive Sperm Motility	23.4 (17,29.9)	21.6 (15,28.3)	19.1 (12.4,25.9)	-1.80 (-5.35, 1.74)	0.32	-2.49 (-6.19, 1.21)	0.19	-4.29 (-8.12, -0.46)	**0.03**
Motile Sperm Count(Million)[b]	45.2 (24.6,82.4)	39.0 (20.8,72.3)	33.4 (17.6,62.6)	-13.5% (-35.6%, 16.1)	0.33	-14.0 (-36.7%, 17.0%)	0.34	-25.6% (-45.9%, 2.33%)	**0.07**
% Normal Sperm Morphology	6.05 (4.5,7.59)	6.18 (4.55,7.8)	5.88 (4.2,7.57)	0.13 (-1.06, 1.33)	0.83	-0.29 (-1.55, 0.96)	0.65	-0.16 (-1.46, 1.13)	0.81
Morphologically Normal Sperm Count(Million)[b]	7.60 (4.36,12.8)	7.28 (4.09,12.5)	6.54 (3.59,11.4)	-3.66% (-25.8%, 25.1%)	0.78	-8.97% (-30.8%, 19.7%)	0.50	-12.3% (-34.0%, 16.5%)	0.36

Supplementary Table 1.4 (Continued):

H_1BH_2,<3ys-arm[High (H_1)-Background (B) - High (H_2)] dibutyl phthalate exposure-period<3ys]: 25 men, 100 semen samples

	Mean (95%CI)			Comparisons					
Semen Quality Parameter	**Baseline (H1)**	**Crossover (B)**	**Crossback (H2)**	**Crossover Effect (95%CI) B-H1**	**P value**	**Crossback Effect (95%CI) H2-B**	**P value**	**Carryover Effect (95%CI) H2-H1**	**P value**
Ejaculate Volume(mL)	2.46 (1.96,2.97)	2.48 (1.92,3.03)	2.46 (1.86,3.05)	0.01 (-0.35, 0.38)	0.94	-0.02 (-0.43, 0.39)	0.92	-0.01 (-0.42, 0.41)	0.97
Sperm Concentration(Million/mL)[b]	53.5 (38.2,75)	61 (42.2,88.1)	53.4 (36.1,78.8)	13.9% (-9.97%, 44.1%)	0.28	-12.5% (-32.7%, 13.9%)	0.32	-0.29% (-23.5%, 30.0%)	0.98
Total Sperm Count(Million)[b]	109 (72.8,165)	134 (85.8,210)	110 (68.6,178)	22.6% (-8.57%, 64.5%)	0.17	-17.8% (-40.8%, 14.2%)	0.24	0.83% (-27.6%, 40.4%)	0.96
%Total Sperm Motility	48.3 (39.8,56.7)	46.1 (36.9,55.4)	49.1 (39.3,59)	-2.12 (-8.23, 4.00)	0.50	2.99 (-3.86, 9.84)	0.39	0.87 (-6.02, 7.76)	0.80
%Progressive Sperm Motility	29.9 (24,35.8)	28.3 (21.8,34.8)	31 (24.1,37.9)	-1.55 (-5.83, 2.73)	0.48	2.67 (-2.12, 7.47)	0.27	1.12 (-3.70, 5.95)	0.65
Motile Sperm Count(Million)[b]	50.3 (28.8,87.2)	52.3 (28.6,94.9)	49.8 (26.4,93.4)	4.00% (-27.2%, 48.6%)	0.83	-4.69% (-36.0%, 41.9%)	0.81	-0.88% (-33.7%, 48.1%)	0.97
% Normal Sperm Morphology	7.59 (6.16,9.03)	6.53 (4.86,8.19)	7.45 (5.62,9.28)	-1.07 (-2.48, 0.35)	0.14	0.92 (-0.69, 2.54)	0.26	-0.14 (-1.73, 1.45)	0.86
Morphologically Normal Sperm Count(Million)[b]	9.10 (5.54,14.6)	8.34 (4.8,14.0)	9.12 (5.1,15.8)	-7.52% (-32.5%, 26.6%)	0.62	8.38% (-23.8%, 54.1%)	0.65	0.22% (-29.7%, 42.9%)	0.99

Supplementary Table 1.4 (Continued):

H_1BH_2,≥3ys-arm[High (H_1)-Background (B) - High (H_2)] dibutyl phthalate exposure-period≥3ys]: 22 men, 99 semen samples

	Mean (95%CI)			Comparisons					
Semen Quality Parameter	**Baseline (H1)**	**Crossover (B)**	**Crossback (H2)**	**Crossover Effect (95%CI) B-H1**	**P value**	**Crossback Effect (95%CI) H2-B**	**P value**	**Carryover Effect (95%CI) H2-H1**	**P value**
Ejaculate Volume (mL)	2.50 (1.69,3.32)	2.56 (1.72,3.41)	2.45 (1.6,3.29)	0.06 (-0.3, 0.42)	0.74	-0.12 (-0.51,0.28)	0.56	-0.06 (-0.44,0.33)	0.77
Sperm Concentration (Million/mL)[b]	63.6 (36.8,110)	52.1 (29.7,91.4)	70.5 (40.1,124)	-18.0% (-34.8%,3.04%)	0.09	35.3% (5.26%,74.0%)	0.02	10.9% (-13.1, 41.7%)	0.40
Total Sperm Count (Million)[b]	152 (78.5,295)	124 (63.0,245)	164 (83.0,326)	-18.4% (-38.6%,8.65%)	0.16	32.3% (-3.36%,81.1%)	0.08	8.02% (-20.4%, 46.6%)	0.62
%Total Sperm Motility	62.3 (48.6,76)	57.8 (43.7,71.9)	59.1 (44.9,73.3)	-4.44 (-10.4,1.51)	0.14	1.26 (-5.28,7.81)	0.70	-3.18 (-9.54,3.18)	0.33
%Progressive Sperm Motility	38.4 (28.8,47.9)	36.9 (27.1,46.8)	36.8 (26.9,46.7)	-1.46 (-5.62,2.71)	0.49	-0.11 (-4.69,4.47)	0.96	-1.57 (-6.02,2.88)	0.49
Motile Sperm Count (Million)[b]	94.8 (38.6,231)	69.8 (27.6,174)	97.1 (38.5,242)	-26.2% (-47.8%,4.41%)	0.09	38.7% (-5.18%,103%)	0.09	2.4% (-29.3%,48.3%)	0.90
Sperm Morphology (% Normal)	8.12 (5.88,10.4)	7.16 (4.79,9.52)	8.03 (5.63,10.4)	-0.97 (-2.36,0.42)	0.17	0.87 (-0.67,2.42)	0.27	-0.10 (-1.58,1.38)	0.90
Morphologically Normal Sperm Count (Million)[b]	12.9 (5.90,27.0)	9.72 (4.22,21.0)	13.6 (6.09,29.2)	-22.8% (-43.2%,4.81%)	0.10	36.4% (-2.61%,90.9%)	0.07	5.25% (-24.1%,45.9%)	0.76

[a] Adjusted for abstinence time (categorical: < 2 days, 2≤days<4 and ≥ 4 days), age (continuous), season (warm (April through September) vs cold) and period (# years on DBP containing mesalamine medication at baseline).

[b] Sperm concentration, total sperm count, motile sperm count and morphologically normal sperm count are presented as percentage change by exponentiating the log transformed Beta coefficient. The sum of the percent changes for crossover and cross back do not equal the total carryover effect for the log transformed variables because they are on the multiplicative scale. For the variables that are not log transformed, the sum of the percent changes for crossover and cross back equal the total carryover effect as they represent the absolute difference on the additive scale

Motile sperm count (million) and morphologically normal sperm count (million) were log transformed after adding 1 due to few zero values, they were back transformed and 1 was subtracted from the means. **B_1HB_2-arm: Background$_1$** represents low exposure to DBP at baseline, **H** represents high DBP exposure after crossover and Background2 represents low DBP after crossback. 26 men, 133 semen samples.**H_1BH_2-arm: H_1** represents high DBP at baseline, **B** represents Background exposure to DBP after crossover and **H_2** represents high DBP after crossback. **H_1BH_2-arm, <3ys**: 25 men, 100 semen samples and **H_1BH_2-arm, ≥3ys**: 22 men and 99 semen samples. **Note:** 2 samples missing morphology **Note:** Negative sign means a decrease or% decrease for the log transformed variables compared to the measure in the previous period **Abbreviations: DBP,** dibutyl phthalate; **B_1HB_2:** Background-High-Background DBP exposure; **H_1BH_2;** High-Background-High DBP exposure; **95% CI**, 95% Confidence Interval**.**

Supplementary Table 1.5: Adjusted effect (95% CI) of crossover, crossback and carryover on semen parameters among men starting on mesalamine medication not containing dibutyl phthalate (B1HB2-arm) and men starting on mesalamine medication containing dibutyl phthalate (H1BH2-arm with duration on DBP-containing mesalamine medication at baseline (period) <3 years and H1BH2-arm with period ≥ 3 years)-Fixed model-3arms[a]

B_1HB_2-arm [Background$_1$(B_1)-High(H)- Background$_2$(B_2) dibutyl phthalate exposure]: (26 men, 133 semen samples)

Semen quality Parameter	Mean (95%CI)	Comparisons					
	Baseline (B1)	Crossover effect (95%CI) H- B1	P value	Crossback effect (95%CI) B2-H	P value	Carryover effect (95%CI) B2- B1	P value
Ejaculate Volume (mL)	2.93 (2.52, 3.34)	0.06 (-0.24,0.37	0.68	-0.26 (-0.58,0.06)	0.10	-0.20 (-0.53, 0.13)	0.24
Sperm Concentration (Million/mL)[b]	56.8 (46.5,69.4)	-11.1% (-27.0%,8.13%)	0.24	3.33% (-15.7%,26.7%)	0.75	-8.17% (-25.8%,13.6%)	0.43
Total Sperm Count (Million)[b]	147 (113,190)	-8.98% (-28.8%,16.3%)	0.45	-4.30% (-25.8%,23.5%)	0.73	-12.9% (-33.2%,13.7%)	0.31
%Total Sperm Motility	46.9 (40.4, 53.5)	-4.41 (-9.52,0.69)	0.09	-3.39 (-8.69,1.91)	0.21	-7.80 (-13.3,-2.27)	0.006
%Progressive Sperm Motility	27.0 (22.5, 31.5)	-1.86 (-5.43,1.71)	0.31	-2.55 (-6.25,1.16)	0.18	-4.41 (-8.28,-0.54)	0.03
Motile Sperm Count (Million)[b]	59.3 (40.2,87.1)	-14.5% (-36.4%,15.0%)	0.30	-13.4% (-36.3%,17.8%)	0.36	-25.9% (-46.3%,2.15%)	0.07
Sperm Morphology (% Normal)	6.31 (5.25, 7.36)	0.11 (-1.10,1.33)	0.85	-0.41 (-1.67,0.86)	0.53	-0.29 (-1.62,1.03)	0.66
Morphologically Normal Sperm Count (Million)[b]	8.60 (6.11,11.8)	-4.76% (-26.9%,24%)	0.72	-9.32% (-31.1%,19.3%)	0.48	-13.6% (-35.2%,15.1%)	0.32

Supplementary Table 1.5 (Continued):

H_1BH_2,<3ys-arm[High (H_1)-Background (B) - High (H_2)] dibutyl phthalate exposure-period<3ys]: 25 men, 100 semen samples

Semen quality Parameter	Mean (95%CI) Baseline (H1)	Comparisons Crossover Effect (95%CI) B-H1	P value	Crossback Effect (95%CI) H2-B	P value	Carryover Effect (95%CI) H2-H1	P value
Ejaculate Volume (mL)	2.55 (2.19, 2.91)	0.00 (-0.38,0.38)	0.99	0.00 (-0.42,0.41)	0.99	0.00 (-0.43,0.42)	0.99
Sperm Concentration (Million/mL)[b]	56.6 (42.9,74.5)	15.9% (-8.91%,47.4%)	0.23	-12.1% (-32.5%,14.5%)	0.34	1.86% (-22.4%,33.6%)	0.89
Total Sperm Count (Million)[b]	124 (87.1,177)	23.5% (-8.59%,67.0%)	0.17	-16.7% (-40.1%,16%)	0.28	2.95% (-26.7%,44.6%)	0.87
%Total Sperm Motility	52.3 (46.4, 58.2)	-2.89 (-9.16,3.37)	0.36	3.14 (-3.74,10.0)	0.37	0.25 (-6.82,7.31)	0.95
%Progressive Sperm Motility	31.8 (27.4, 36.3)	-1.98 (-6.36,2.40)	0.37	2.87 (-1.94,7.68)	0.24	0.89 (-4.05,5.83)	0.72
Motile Sperm Count (Million)[b]	63.7 (42.9,94.5)	2.65% (-28.7%,47.7%)	0.89	-3.53% (-35.3%,43.8%)	0.86	-0.98% (-34.3%,49.3%)	0.96
Sperm Morphology (% Normal)	7.59 (6.36, 8.83)	-1.29 (-2.77,0.20)	0.09	0.92 (-0.71,2.55)	0.27	-0.37 (-2.05,1.31)	0.66
Morphologically Normal Sperm Count (Million)[b]	9.70 (6.71,13.8)	-9.26% (-34.3%,25.3%)	0.55	8.69% (-23.7%, 54.8%)	0.64	-1.37% (-31.5%,41.9%)	0.94

Supplementary Table 1.5 (Continued):

H_1BH_2,≥3ys-arm[High (H_1)-Background (B) - High (H_2)] dibutyl phthalate exposure-period≥3ys]: 22 men, 99 semen samples

Semen quality Parameter	Mean (95%CI)	Comparisons					
	Baseline (H1)	Crossover Effect (95%CI) B-H1	P value	Crossback Effect (95%CI) H2-B	P value	Carryover Effect (95%CI) H2-H1	P value
Ejaculate Volume (mL)	2.61 (2.29, 2.93)	0.07 (-0.29,0.43)	0.70	-0.15 (-0.54,0.25)	0.46	-0.08 (-0.47,0.31)	0.70
Sperm Concentration (Million/mL)[b]	59.5 (48.6,72.8)	-17.1% (-34.2%,4.61%)	0.11	35.3% (5.13%,74.2%)	0.02	12.3% (-12.4%,43.9%)	0.36
Total Sperm Count (Million)[b]	144 (114,181)	-16.9% (-37.8%,11.1%)	0.21	31.1% (-4.43%,79.7%)	0.09	8.88% (-20.2%,48.5%)	0.59
%Total Sperm Motility	52.8 (47.0, 58.6)	-4.68 (-10.7,1.36)	0.13	1.18 (-5.39,7.75)	0.72	-3.50 (-9.96,2.96)	0.29
%Progressive Sperm Motility	32.1 (28.5, 35.8)	-1.65 (-5.88,2.57)	0.44	-0.12 (-4.72,4.47)	0.96	-1.78 (-6.3,2.74)	0.44
Motile Sperm Count (Million)[b]	70.3 (50.3,98.1)	-25.5% (-47.6%,5.74%)	0.10	37.2% (-6.32%,101%)	0.10	2.17% (-29.8%,48.7%)	0.91
Sperm Morphology (% Normal)	7.59 (6.58, 8.60)	-1.07 (-2.50,0.36)	0.14	0.89 (-0.67,2.45)	0.26	-0.18 (-1.71,1.35)	0.82
Morphologically Normal Sperm Count (Million)[b]	10.6 (7.83,14.2)	-22.1% (-42.9,6.3)	0.12	35.1% (-3.60%,89.5%)	0.08	5.34% (-24.4%,46.9%)	0.76

[a] Adjusted for abstinence time (categorical: < 2 days, 2≤days<4 and ≥ 4 days), age (continuous), season (warm (April through September) vs cold) and period (# years on DBP containing mesalamine medication at baseline).

[b] Sperm concentration, total sperm count, motile sperm count and morphologically normal sperm count are presented as percentage change by exponentiating the log transformed Beta coefficient. The sum of the percent changes for crossover and cross back do not equal the total carryover effect for the log transformed variables because they are on the multiplicative scale. For the variables that are not log transformed, the sum of the percent changes for crossover and cross back equal the total carryover effect as they represent the absolute difference on the additive scale

Motile sperm count (million) and morphologically normal sperm count (million) were log transformed after adding 1 due to few zero values, they were back transformed and 1 was subtracted from the means.

B_1HB_2-arm: Background$_1$ represents low exposure to DBP at baseline, **H** represents high DBP exposure after crossover and Background2 represents low DBP after crossback. 26 men, 133 semen samples.

H_1BH_2-arm: H_1 represents high DBP at baseline, **B** represents Background exposure to DBP after crossover and **H_2** represents high DBP after crossback.

H_1BH_2-arm, <3ys: 25 men, 100 semen samples and **H_1BH_2-arm, ≥3ys**: 22 men and 99 semen samples.

Note: 2 samples missing morphology **Note:** Negative sign means a decrease or% decrease for the log transformed variables compared to the measure in the previous period**. Abbreviations: DBP,** dibutyl phthalate; **B_1HB_2:** Background-High-Background DBP exposure; **H_1BH_2;** High-Background-High DBP exposure; **95% CI**, 95% Confidence Interval

Chapter 2

A crossover–crossback prospective study of dibutyl-phthalate exposure from mesalamine medications and serum reproductive hormones in men with inflammatory bowel disease

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Abbreviated title: DBP from mesalamine medications and reproductive hormones in men

Key terms: Phthalate, endocrine disruptor, crossover study, hormones, men

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Abbreviations

DBP, dibutyl phthalate; FDA, Food and Drug Administration; 5-ASA, 5-aminosalicylic acid; IBD, Inflammatory Bowel Disease; UC, ulcerative colitis; CD, Crohn's disease; MBP, monobutyl phthalate;

NHANES, National Health and Nutrition Examination Survey; MARS, Mesalamine And Reproductive health Study; BIMC, Beth Israel Deaconess Medical Center; BWH, Brigham and Women's Hospital; MGH, Massachusetts General Hospital; IRB, institutional review board; B_1HB_2, $Background_1$-High-$Background_2$ DBP exposure; H_1BH_2, $High_1$-Background-$High_2$ DBP exposure; BMI, body mass index; Kg, Kilogram; m, meter; SD, standard deviation; LMEM, linear mixed effects models; FEM, fixed effect models; N, number of men; EPA, Environmental Protection Agency; TT, total testosterone; FT, free testosterone; LH: luteinizing hormone; FSH, follicle-stimulating hormone.

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Abstract

Phthalates, such as dibutyl phthalate (DBP), are endocrine disruptors used in some medication coatings e.g., mesalamine to treat inflammatory bowel disease (IBD). Taking advantage of different mesalamine formulations with/without DBP, we assessed whether DBP from mesalamine (>1000x background) altered serum hormones. 73 men with IBD participated in a crossover-crossback prospective study and provided up to 6 serum samples/man (2: baseline, 2: crossover, 2: crossback). Men on non-DBP mesalamine (**b**ackground) at baseline crossed-over for 4 months to DBP mesalamine (**h**igh) and then crossed-back for 4 months to non-DBP mesalamine (B_1HB_2-arm) and vice versa for men on DBP mesalamine at baseline (H_1BH_2-arm). Because duration on DBP mesalamine at baseline had a wide range (0.3-24 years),we divided H_1BH_2-arm at the median (H_1<3yrs or $H_1\geq$3yrs). We estimated crossover (crossover versus baseline) and crossback (crossback versus crossover) % changes using multivariable linear mixed effect models. When B_1HB_2-arm (26 men,134 samples) crossed-over, luteinizing hormone decreased 13.9% (95%CI:-23.6,-3.0) and testosterone, inhibin-B, and follicle-stimulating hormone (FSH) marginally decreased; after crossback all increased 8-14%. H_1BH_2-arm, $H_1\geq$3yrs (25 men,107samples) had no changes at crossover or crossback whereas in H_1BH_2-arm,H_1<3yrs (22 men,100samples) after crossover, inhibin-B increased 13.2% (95%CI: 4.2,22.9), FSH decreased 9.9% (95%CI:-17.9,-1.1) and after crossback, inhibin-B further increased 11.3%, and FSH marginally increased. High-DBP exposure disrupted serum levels of pituitary-gonadal hormones that largely reversed after exposure removal, but only in men with no history of or a short duration (<3yrs) of previous high-exposure. Paradoxically, among men with longer duration of high-DBP exposure, exposure removal did not change hormone levels, suggesting that long-term high-DBP exposure may alter the pituitary-gonadal axis and make it insensitive to changes in exposures.

Introduction

Ortho-phthalates (hereto referred to as phthalates) are high production volume chemicals with global production of 11 billion pounds per year(3) and more than 470 million pounds produced or imported into the United States (US) annually(4). Because of their widespread use in consumer and personal care products, use as plasticizers for polyvinyl chloride plastic products, and use in medical supplies and pharmaceuticals(32,128), phthalate exposure is ubiquitous in the general population(2).

In experimental studies, several phthalates, including dibutyl phthalate (DBP), have adverse impacts on male reproductive health(37-39). Primarily for this reason, DBP was banned in toys and child care products in the European Union (EU) in 2005(20) and in the US in 2008(21) . In the EU, DBP was also banned in cosmetics in 2004(22). However, despite these bans and restrictions, DBP is still used in the enteric coatings of some medications including specific mesalamine formulations and this represents a source of very high exposure to DBP(23,24,26-32). Recently, the U.S. Food and Drug Administration (FDA) recommended that certain phthalates including DBP should not be used as excipients in medications(33). Mesalamine, the active ingredient in Asacol®, Asacol®HD, Lialda®, Pentasa®, Apriso®, and Delzicol®, is a commonly prescribed for inflammatory bowel diseases (IBD), including ulcerative colitis (UC) and Crohn's disease (CD). The enteric coating of Asacol®, and Asacol®HD contains DBP as an excipient(34), whereas it is not used in Lialda®, Pentasa®, Apriso®, or Delzicol®(23,35). Asacol® and Asacol®HD are prescribed as six to eight tablets per day leading to high-DBP exposure as measured by urinary monobutyl phthalate (MBP) concentrations, the primary DBP metabolite(24-26), with urinary concentrations approximately1000 times higher than the median concentrations reported for men in the U.S. general population (National Health and Nutrition Examination Survey (NHANES))(36).

Experimental studies have shown that DBP was anti-androgenic and a reproductive toxicant, however, most studies focused on *in utero* exposure(37-39) rather than postnatal or adult exposures(40,41). There is

limited evidence from epidemiologic studies on the association of general population DBP exposure with serum reproductive hormones(44-48). However, there are no studies that we are aware of on very high-DBP exposures from medications and their potential effects. Therefore, we took advantage of the difference in DBP-exposure from different mesalamine formulations and conducted a crossover-crossback prospective cohort study to examine the associations between the high-DBP exposure from mesalamine medications and serum reproductive hormones in adult men.

Methods

Participants

As previously described(29), 73 men were enrolled in the Mesalamine And Reproductive health Study (MARS) from gastroenterology clinics at three Boston hospitals, Beth Israel Deaconess Medical Center (BIMC), Brigham and Women's Hospital (BWH) and Massachusetts General Hospital (MGH) between October 2010 and March 2016. Eligible men were 18 to 55 years old, mild severity IBD, five or less for UC (the simple clinical colitis activity index(92)) or four or less for CD (Harvey-Bradshaw index(93)), and on mesalamine medication for at least three months at enrollment. There were no exclusions based on history of infertility. Men with history of steroid medication use in the last three months, vasectomy, diabetes mellitus, hepatic, or renal diseases were not eligible. MARS was approved by the institutional review boards (IRBs) of Harvard T.H. Chan School of Public Health, BIMC, BWH and MGH. All men signed informed consents.

Study design

Men participated in up to six visits (v) (baseline: v1&v2, crossover: v3&v4 and crossback: v5&v6). Men who started on non-DBP mesalamine (background-DBP exposure) crossed-over to high-DBP mesalamine (high-DBP exposure) then crossed-back to non-DBP mesalamine (background) (B_1HB_2-arm;$Background_1$-$High$-$Background_2$) and vice versa in men who started on DBP mesalamine (H_1BH_2-arm;$High_1$-$Background$-$High_2$) (Figure 2.1). Crossover and crossback periods (between v2&v3 and

v4&v5) were designed to be four months and durations between v1&v2, v3&v4 and v5&v6 were designed to be two weeks. We *a priori* chose the four month periods to be longer than the spermatogenesis cycle (around 70 days)(94), as one of our aims was to study semen parameters(29). At baseline, men reported demographic, lifestyle and health information on questionnaires and our research nurse measured their height and weight. At each visit, men reported time varying characteristics such as fever and illness in the previous three months and gave blood, semen and urine samples.

13 out of the 47 men in the H_1BH_2-arm participated only in a short protocol consisting of up to four visits that required only crossover without crossback. At enrollment, they chose not to change medications, but because the manufacturer was reformulating Asacol® to remove DBP, we anticipated that their physicians would prescribe the new formulated mesalamine without DBP. Warner Chilcott discontinued Asacol® in 2013 and introduced Delzicol® (non-DBP mesalamine) to the market, but Asacol®HD (containing DBP) remained on the market. Only three men crossed-over to Delzicol® and the remainder never crossed-over but did contribute data at baseline.

Exposure assessment

High-DBP exposure was defined by medication type i.e., DBP containing mesalamine versus non-DBP mesalamine.

Serum hormone analysis

Nurses collected non-fasting blood samples in red top tubes (Coviden) from men at each visit between 7 am and 2 pm and reported the exact time. Blood was allowed to clot, centrifuged, and the serum separated, frozen, and stored at -80°C until analyses were performed. All samples were shipped in a single batch for analysis to the laboratories at Ansh (inhibin-B and sex hormone-binding globulin (SHBG)), National Institute of Environmental Health Sciences (NIEHS) (follicle-stimulating hormone (FSH) and luteinizing hormone (LH)), and Mayo Clinic (total testosterone (TT) and albumin).

SHBG was measured by enzyme linked immunosorbent assay (ELISA) (quantitative two-step sandwich type immunoassay). Specificity of the SHBG ELISA was studied by measuring SHBG response caused by high levels of thyroxine binding globulin (TBG) and cortisol binding globulin (CBG). No cross-reactions were found when testing up to 500 mg/L of TBG and 500 mg/L of CBG. The analytical measurable range of the assay was between 4 – 260 nmol/L and was sensitive to approximately 0.77 nmol/L. The intra- and inter-assay coefficients of variation (CV) were <9.0% and <8.0%, respectively.

Testosterone was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Agilent Technologies, Santa Clara, CA 95051). Intra- and inter-assay CV were ≤9.0% and ≤8.9%, respectively. Albumin, used to calculate the free testosterone concentration, was measured by an enzymatic colorimetric assay on the Roche Cobas c311 chemistry analyzer (Roche Diagnostics, Indianapolis, IN 46250). Intra- and inter-assay CV were ≤1.4% and ≤2.1%, respectively. The lower limit of detection for albumin was 0.2 g/dL. Inhibin-B was measured by ELISA (quantitative three-step sandwich type immunoassay). Ansh laboratory has developed highly specific antibodies for inhibin-B with no detectable cross-reactivity to inhibin-A or activin-A and AB, and negligible cross-reactivity to activin-B (0.04% at 50 ng/ml). The assay has an analytical measurable range of 14-1300 pg/ml and is sensitive to approximately 6.5 pg/ml. The intra- and inter-assay CV were <4.38% and <7.42%, respectively.
LH and FSH concentrations were measured by chemiluminescent microparticle immunoassay (CMIA) technology (Architect i100SR Immunoassay (Architect i100SR Immunoassay Analyzer, Abbott Diagnostics, Abbott Park, IL). The intra-assay CV for LH and FSH were 2.8% and 1.8%, respectively, with inter-assay CV were 5.7% and 12.7%, respectively.

All laboratories were blinded to all participants' information. We calculated the free testosterone (FT) from the measured concentrations of TT, SHBG, and albumin using the method of Vermeulen et al.(129). We also calculated TT/LH, FT/LH, and Inhibin-B/FSH ratios.

Statistical Analysis

In primary analyses, to define exposure we used a six-level indicator variable cross-classifying each observation based on the medication type (high versus non-DBP) at each period (baseline, crossover and crossback) for the two study arms (H_1BH_2 and B_1HB_2). We natural log-transformed all the hormones due to right skewness of their distributions and modeled them as continuous outcomes.

We performed descriptive statistics on men's baseline and time-varying characteristics. We also tested for any differences between men in the different arms using Fisher's exact test for categorical variables and Kruskal-Wallis test for continuous variables. We calculated intraclass correlation coefficients (ICC) using random intercept mixed effect models to estimate within and between men variability across the repeated measures of the log-transformed hormone concentrations.

We selected the covariates based on directed acyclic graphs and statistical considerations (>10% change in the effect estimate). The final model included age (continuous), body mass index (BMI) (continuous), sample drawing time (continuous, time varying), season of the sample collection (warm and cold, time varying), race (Caucasian or not), current smoking (binary), and duration on high-DBP mesalamine at baseline (continuous).

In preliminary models, we considered adjustment for IBD severity score, IBD diagnosis (UC/CD), duration since diagnosis, history of reproductive diseases or surgeries, education, alcohol consumption, fever and illness in the previous three months but they were not confounders and thus not retained in the final models.

We estimated the DBP crossover (crossover compared to baseline), crossback (crossback compared to crossover), and carryover (crossback compared to baseline) percent changes in the hormone parameters within each arm, as well as the cross-sectional differences at baseline between arms. We used linear mixed effect models (LMEM) with a random intercept to account for within-man correlation among

longitudinal measures of a given hormone parameter arising from man-to-man heterogeneity across the study participants.

Because men in H_1BH_2-arm were on their high-DBP mesalamine at baseline for a wide range of duration (4 months to 24 years), in post-hoc analyses we explored whether the length of time the participants were on high-DBP mesalamine at baseline modified the associations of interest by adding an interaction term. Additionally, we subdivided men in the H_1BH_2-arm based on the median duration of three years on high-DBP mesalamine at baseline i.e., (H_1BH_2-arm, H_1<3yrs) and (H_1BH_2-arm, $H_1\geq$3yrs) and ran all analyses based on the resulting three arms using the same method as the two-arm analyses above.

As a sensitivity analysis, we applied fixed effect models (FEM) adjusting for the covariates above. A FEM isolates the longitudinal within-man exposure effect by fully adjusting for both observed and unobserved covariates that are not time-varying within a man across visits(97). We also assessed LMEM model sensitivity to the covariance structure implied by the random intercept model, using the robust empirical standard errors(97). We repeated all sensitivity analyses considering both two and three arms. We considered two-sided alpha <0.05 as statistically significant. We conducted all statistical analyses using SAS version 9.4 (SAS Institute Inc., Cary, NC) and used the R package ggplot for generation of graphical output.

Results

Of the 215 eligible men who were contacted, 73 men (mean age 34.6 years) agreed to participate and provided 341 blood samples (average: five samples/ man) (Supplementary Figure 2.1). B_1HB_2-arm (26 men, 134 samples) were on their non-DBP mesalamine medication at baseline for a median of 1 year. H_1BH_2-arm (47 men, 207 serum samples) were on their high-DBP mesalamine medication at baseline (H_1) for a median duration of 3 years. Men in the two arms had comparable characteristics (Table 2.1).

Among the 60 men who participated in the full-protocol, 51 men (85%) crossed-over and 47 (78%) crossed-back; 89% and 77%, respectively, in B_1HB_2-arm, 82% and 79%, respectively, in H_1BH_2-arm. During the follow-up, nine men were lost follow-up, three in B_1HB_2-arm and six in H_1BH_2-arm (p-value= 0.64). The median crossover duration (between v2 & v3), and crossback duration (between v4 & v5) was 121 days. The median duration between visits on the same medication (v1 & v2), (v3 & 4) and (v5 & v6) was 16 days.

All serum samples had detectable concentrations for all hormones (Table 2.2) and there was a moderate to high ICC ranging from 0.5 for FT to 0.9 for FSH (data not shown), indicating good reliability within-man across the repeated measures.

In the adjusted two-arm analyses (Table 2.3 & Supplementary Figure 2.3), on average, when men in B_1HB_2-arm were newly-exposed to high-DBP mesalamine, LH decreased by 13.9% (95% confidence interval (CI): –23.6, –3.0) and TT, FT, SHBG, inhibin-B, and FSH decreased though not significantly. After crossback to their original non-DBP mesalamine, apart from SHBG which continued to decrease non-significantly, LH increased by 13.8% (CI: 0.5, 28.9), TT by 10.4% (CI: 0.4, 21.4), FT by 10.4% (CI: 0.2, 21.6), and inhibin-B and FSH marginally significantly increased. There was no carryover effect in B_1HB_2-arm. When men in H_1BH_2-arm crossed-over to non-DBP mesalamine, on average, inhibin-B increased by 8.5% (CI: 2.4, 15.1) and continued to increase by an additional 7.7% (CI: 1.3, 14.5) after crossback to their original high-DBP mesalamine. LH and FSH decreased by 10.1% and 8.4%, respectively, after crossover and increased by 9 to 10% after crossback (significantly for FSH). TT/LH, FT/LH and inhibin-B/FSH ratios increased significantly by 11 to 18% after crossover and decreased non-significantly after crossback despite non-significant changes in TT and FT.

In the cross-sectional analysis of values at baseline, on average, men in H_1BH_2-arm had lower TT, FT, LH, and FSH than men in the B_1HB_2-arm. Because of the wide range in duration on high-DBP

mesalamaine and to further explore the effect of duration on high-DBP mesalamine at baseline we divided the men in the H_1BH_2-arm at the median. At baseline, men on high-DBP mesalamine for less than 3 years (H_1<3 yrs) had higher TT, FT, and LH by 15- 20% and lower by 18% for FSH (although none were statistically significant) than men in H_1BH_2-arm on high-DBP mesalamine for 3 years or more ($H_1\geq3$ yrs). There was effect modification of the DBP exposure effect by the duration of high-DBP mesalamine at baseline for TT (p-value= 0.06), LH (p-value= 0.002) and FSH (p-value= 0.08) (Figure 2.2).

Based on these results, we conducted a post-hoc three-arm analysis: H_1BH_2-arm, H_1<3yrs (25 men, 107 serum samples) had an average H_1 duration of 1.2 years, H_1BH_2-arm, $H_1\geq3$yrs (22 men, 100 serum samples) had an average H_1 duration of 11.1 years (Supplementary Table 2.1& Supplementary Figure 2.2) and B_1HB_2-arm (Figure 2.3 & Supplementary table 2.2). We found heterogeneity of the exposure responses among the three arms. Although on average, men in H_1BH_2-arm, $H_1\geq3$ yrs had no significant changes in hormones in crossover or crossback, men in H_1BH_2-arm, H_1<3 yrs had significant changes similar to men in the B_1HB_2-arm. When men in H_1BH_2-arm, H_1<3 years crossed-over, on average, inhibin-B increased by 13.2% (CI: 4.2, 22.9), and FSH decreased by 9.9% (CI: -17.9, -1.1), while TT/LH, FT/LH and inhibin-B/FSH ratios significantly increased by 18 to 26%. After crossback, inhibin-B had an additional increase of 11.3% (CI: 2.2, 21.3) and FSH marginally significantly increased by 9.7% (CI: -0.3, 20.8). Although not significant, there was a pattern after crossover that TT, FT, and SHBG increased and LH decreased. After crossback, SHBG decreased, TT, and FT had smaller additional increases, and LH increased. There was a significant carryover effect for FT, inhibin-B and inhibin-B/FSH ratio. Both the unadjusted and the adjusted LMEM and FEM analyses and the two-arm and three-arm analyses gave consistent results across the primary and sensitivity analyses, indicating the robustness of the results (Supplementary Tables 2.3 to 2.9).

Discussion

In our crossover-crossback study, when men who were on non-DBP mesalamine at baseline (B_1HB_2-arm) were newly-exposed to high-DBP for four months, serum levels of testicular hormones (testosterone and inhibin-B) and gonadotropins (LH and FSH) decreased. After crossback for four months to their original non-DBP mesalamine, both testicular hormone and gonadotropin concentrations increased and exceeded their baseline concentrations. In our post-hoc three-arm analysis, in the H_1BH_2-arm the duration on high-DBP mesalamine at baseline modified the hormone response. The hormone changes were only found in H_1BH_2-arm, < 3yrs and not in the H_1BH_2-arm, ≥3 yrs.When men in the H_1BH_2-arm, <3 yrs crossed-over to non-DBP mesalamine, testicular hormones increased and gonadotropins decreased, suggesting a functioning negative feedback response. When they crossed-back to their original high-DBP mesalamine, their testicular hormones continued to increase but at slower rates while gonadotropins increased. TT/LH, FT/LH and inhibin-B/FSH ratios increased after crossover to non-DBP mesalamine, suggesting improved Leydig and Sertoli cell functions.

Observed decreases in testosterone, inhibin-B and FSH in B_1HB_2-arm, essential hormones for spermatogenesis and testicular function, may explain our previously observed decreasing trend in the semen quality parameters, primarily sperm motility in the same cohort of men(29). However, after exposure removal for four months, in contrast to the observed reversal of hormonal changes, the semen quality decline was not reversed and instead further declined(29). This could be explained by the long process of spermatogenesis(94) which potentially may result in a lagged response to removal of high-DBP exposure. Interestingly among the very long exposed men(H_1BH_2-arm, H_1≥3 yrs), the four month wash-out period during the crossover to non-DBP mesalamine was not long enough to 'reverse' the apparent effect of high-DBP exposure on hormones. Similarly, semen quality did not change in H_1BH_2-arm after four months of high-DBP exposure removal(29). We also found in a cross-sectional analysis that men exposed to high-DBP for a longer duration (H_1BH_2-arm, H_1≥3 yrs) had lower TT, FT, and LH

concentrations than men exposed for a shorter duration (H_1BH_2-arm, <3 yrs) or men who were not exposed at all (B_1HB_2-arm).

In experimental studies, peri-pubertal DBP-exposure altered differential expression of proteins involved in spermatogenesis and changed number and function of Sertoli and Leydig cells(40,41). Other studies in older animals showed that DBP preferentially targeted Sertoli cells(104). Studies during fetal development showed that DBP was anti-androgenic resulting in decreased testosterone production by Leydig cells due to interference with steroidogenesis, down-regulating gene and/or protein expression essential for the steroidogenic pathway, and cholesterol transport and metabolism(107,110,111). Suppression of both gonadotropin and testosterone production as well as decreased semen quality were observed in di(2-ethylhexyl)phthalate (DEHP) newly-exposed pre-pubertal and mature animals(130,131), similar to our results on men newly exposed to high-DBP mesalamine. In adult DEHP-exposed animals, pituitary LH and FSH protein expression decreased significantly(132,133). Decreased testosterone may indicate suppression of Leydig cell function and decreased inhibin-B may indicate suppression of Sertoli cell function, as well as germ cell production in adult testis(134).

In humans, both inhibin-B and FSH decreased with higher-DEHP exposure(135) suggesting that the normal endocrine communication between the pituitary and the testis might be disrupted(41,135) hence disruption of the hypothalamo-pituitary-testis (HPT) axis. Similarly, based on our results of the suppression of both testicular hormones and gonadotropins, we speculate that high-DBP exposure from mesalamine was associated with HPT axis disruption targeting the gonads and/or the pituitary in the newly exposed men. In the H_1BH_2-arm, H_1<3 yrs, gonadotropin negative feedback was partially regained after a relatively short removal of high-DBP exposure and partially compensated the testicular suppression after re-exposure. However, after longer durations of high-DBP exposure, as we observed in

men in H_1BH_2-arm, $H_1 \geq 3$ yrs, the reproductive hormones were suppressed and the hormonal responses may be desensitized to DBP exposure removal. Therefore, our results from the three different arms suggest that there may be different effects of the hormone responses modified by the duration of high-DBP mesalamine history. There is a caveat that there are limited comparable animal data to explain modes of action of high-DBP exposure in adult life, especially with such unique crossover-crossback design, and no data on the potentially modified hormone responses by the duration of exposure. In addition, many of the experimental animal studies only measured testosterone and not gonadotropins.

In epidemiological studies on the association between environmental (background) DBP-exposures and reproductive hormones in adult men, the results were inconsistent with the main focus on testosterone. Urinary MBP was negatively correlated with FT among occupationally exposed Chinese men(136). Studies in infertility clinics observed that urinary MBP was associated with a significant testosterone decrease in men from Slovenia(49), China(57), and India (only in the unadjusted analysis)(50) but not in men from Poland(58). Both pooled and non-pooled analyses of infertile and fertile men in the US failed to find statistically significant associations between urinary MBP and any of the reproductive hormones(44-46). Only a few studies included men from the general population and found that urinary MBP was inversely associated with testosterone only in men aged 40-60 years from NHANES(47), but no evidence from studies in Denmark (only a significant increase in LH)(48), Sweden(137), or China(138). All the studies described above were cross-sectional and used a single serum sample to measure the hormone concentrations at the same time when urinary MBP was measured. In addition, most of the studies were based in infertility clinics. To our knowledge, Janjua et al(139) conducted the only human study with some similarity to our design examining the association between DBP-exposure (dermally) and short term effects on reproductive hormones. It was a placebo controlled crossover study in which men were assigned to daily whole-body topical DBP-exposure for a week after a week of placebo exposure. They did not find associations of topical DBP-exposure with reproductive hormones. These results must be interpreted cautiously due to a small sample size (n=26), non-randomization, likely too short washout

period (one week), only assessing the short term effects, and considering only topical exposures with much lower anticipated exposure levels compared to our study.

Several studies have characterized DBP and other phthalates in coatings of many licensed medications including over-the-counter medications in the US(23-26), Canada(28,35), United Kingdom(30), Germany(27,32) and China(31). Phthalate levels may not be openly displayed, due to proprietary formulations of medications(23). The estimated maximum daily DBP intake for a 60 kg person who is taking high-DBP mesalamine (Asacol® or Asacol®HD) is 350- 800 μg/kg/day(29) compared to the estimated average DBP human intake of 0.84 - 5.22 μg/kg/day(116), the US Environmental Protection Agency (EPA) reference dose of 100 μg/kg/day(26), and the European tolerable daily intake of 10 μg/kg/day(117). The urinary MBP in patients taking Asacol® was approximately 1000 times higher than the median levels reported for men in the US general population (NHANES)(36). Therefore, we used *a priori* medication type as a proxy for high-DBP exposure(29) rather than measuring urinary MBP concentrations which represent only exposure over the past several hours due to the short DBP half-life(139). Due to the unique crossover-crossback design and the well-documented very high-DBP exposure from Asacol®(23-27,32,34,114,115), measuring urinary MBP concentrations would have not added significant information beyond using the assigned medication (as an intention to treat analysis). Also due to the expected non-overlapping large differences in urinary MBP, statistically, we would essentially dichotomize exposure according to the different mesalamine formulations (DBP versus non-DBP containing) and use the same modeling approach we used even if we had the continuous urinary data. Thus, the effect estimates would have been completely dominated by the medication type(29).

Our study had several potential limitations including that we did not randomize the men to high-DBP or non-DBP mesalamine at baseline. However the cross-sectional results were consistent with the

longitudinal associations. In addition, physicians do not prescribe mesalamine based on DBP in the enteric coating or in relation to hormonal concentrations or semen quality, arguing against potential confounding by medication at baseline. Although our sample size was not very large due to the innovative study and length of participation, the power of the study is derived from the unique design and the intra-person comparison rather than the inter-person variability avoiding the purely cross-sectional analysis that may be confounded and lacks temporality. We also conducted a post-hoc power analysis and the study had a sufficient number of participants to provide 80% power for detecting at least a 7% change in TT or FT, 8% in SHBG, 6% in inhibin-B and FSH and 9% in LH which were reasonable for a study of this design. Although there may be a concern of generalizing results from men with IBD, these results raise concerns about high-DBP exposure and reproductive hormones. In addition, there is no evidence that IBD (with significantly increasing incidence worldwide(140)) or mesalamine is linked to male infertility(123,124).

Our study had several important strengths including a unique innovative design rarely conducted in environmental epidemiology. We were able to compare, within the same men, their hormone parameters during periods of high-DBP to background-DBP exposure and vice versa accounting for confounding by measured and unmeasured non-time-varying characteristics(121). This is a major strength compared to cross-sectional studies. We had repeated measures up to six time points per man which increased the reliability of the measures. The different orders of exposure and non-exposure in the different arms gave us a unique opportunity to observe the reversibility and carryover effect of the changes under the study period. Our study was not restricted to infertile men compared to most of the previous literature. Confounding by indication was unlikely because mesalamine medications (with or without DBP coating) prescribed for treating IBD have the same active ingredient. We used the gold standard methods for hormone analyses especially for testosterone, which was accurately and precisely measured using LC-MS/MS methodology(141). We did not use the free androgen index (FAI) as a surrogate for FT as most of the studies did because the FAI correlates poorly with FT in men(141). Instead, we used the actual

measured SHBG, albumin and TT applying Vermeulen et al.'s method which is considered the most appropriate estimate of FT in blood(129,141). We had the opportunity to examine high-DBP exposure (1000 times background) compared to environmental background exposure. Therefore, we had more power to detect significant changes.

Our results are of a concern because testosterone deficiency in adult men is a risk factor not only for decreased semen quality and infertility but also for decreased libido, energy, intellectual capacity, bone mass, and muscle strength, and associated with erectile dysfunction, osteoporosis, abdominal obesity, type-2 diabetes, metabolic syndrome, and, cardiovascular disease, and hence increased mortality(142-145). Recently, there was a consensus that women with IBD who are pregnant or might become pregnant may need to avoid high-DBP mesalamine(28) leaving men of reproductive age with no recommendations and no guidance about any other medications that contain phthalate coating. Therefore, our results suggest the need to reconsider the use of DBP containing mesalamine and other DBP containing medications for men.

Conclusions

Although, some medications are a source of high-DBP exposure especially for certain subpopulations such as IBD patients, this source is relatively overlooked and the focus has been more on background environmental exposure. To our knowledge, MARS is the only and the first study that examined the association between such high-DBP exposure and health outcomes. Our results present evidence that high-DBP exposure disrupted serum levels of pituitary-gonadal hormones that largely reversed after exposure removal, but only in men with no history of or a short duration (<3yrs) of previous high-exposure. Paradoxically, among men with longer duration of high-DBP exposure, exposure removal did not change hormone levels, suggesting that long-term high-DBP exposure may alter the pituitary-gonadal axis and make it insensitive to changes in exposures.

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Table 2.1. Demographics of 73 men contributing 341 blood samples in the MARS Study by arm

	B_1HB_2-arm (26 men, 134 visits)	H_1BH_2-arm (47 men, 207 visits)	Total (73 men, 341 visits)	
	N(%)/Mean (SD),[Range]	N(%)/Mean (SD),[Range]	N(%)/Mean (SD),[Range]	P-value[b]
Baseline characteristics (at Visit 1), # men (N)[a]				
Duration on High-DBP mesalamine at baseline (H_1, years)	0	5.86 (6.16), [0.33, 24.1]	3.77 (5.68), [0, 24.1]	-
Age (Years)	33.9 (8.97), [20.2, 54.6]	34.9 (9.66), [19.6, 55.7]	34.6 (9.37), [19.6, 55.7]	0.82
Race				0.27
Caucasian	25 (96%)	38 (81%)	63 (86%)	-
Black/African American	1 (4%)	2 (4%)	3 (4%)	-
Asian	0	4 (9%)	4 (6%)	-
Other	0	3 (6%)	3 (4%)	-
BMI (Kg/m2)	25.3 (3.56), [19.4, 32.7]	26.4 (6.39), [19.5, 54.0]	26.0 (5.54), [19.4, 54.0]	0.88
BMI-categories				0.99
Normal weight (18.5 ≤BMI<25)	14 (54%)	24 (51%)	38 (52%)	-
Overweight (25≤BMI<30)	9 (35%)	16 (34%)	25 (34%)	-
Obese (BMI≥30)	3 (11%)	7 (15%)	10 (14%)	-
Education				0.82
Below college	3 (14%)	6 (14%)	9 (14%)	-
College graduate	10 (48%)	16 (38%)	26 (41%)	-
Graduate degree	8 (38%)	20 (48%)	28 (45%)	-
Smoking status				0.009
Never smoker	22 (85%)	36 (77%)	58 (80%)	-
Former smoker	1 (4%)	11 (23%)	12 (16%)	-
Current smoker	3 (11%)	0	3 (4%)	-
Warm season at baseline[c]	5 (19%)	20 (43%)	25 (34%)	0.07
IBD diagnosis				0.61
Ulcerative colitis	16 (62%)	32 (68%)	48 (66%)	-
Crohn's disease	10 (38%)	15 (32%)	25 (34%)	-
IBD score[d]	1.65 (1.38), [0, 4]	1.19 (1.35), [0, 5]	1.36 (1.37), [0, 5]	0.13
Duration since IBD diagnosis (years)	9.00 (7.84), [0.28, 29.5]	10.8 (9.24), [0.7, 39.0]	10.1 (8.76), [0.28, 39.0]	0.41
Time-varying characteristics , # visits[a]				
Warm season of blood draw [c]	60 (45%)	94 (45%)	154 (45%)	0.91
Hour of the blood draw	9.68 (1.62), [7.25, 13.9]	9.72 (1.61), [7.17, 13.5]	9.71 (1.61), [7.17, 13.9]	0.80
Hour of the blood draw-categories				0.74

7 am to 10 am	74 (55%)	110 (53%)	184 (54%)	-
After 10 am to 2 pm	60 (45%)	97 (47%)	157 (46%)	-

Table 2.1.: B_1HB_2-arm, B_1 represents background-exposure at baseline, H represents high-DBP exposure after crossover and B_2 represents background-exposure after crossback; included 26 men taking mesalamine that did not contain DBP at baseline; 16 men (62%) on Lialda®, 8 men (31%) on Pentasa®, one man (4%) on Apriso® and one man (4%) was not on mesalamine medication at the time of recruitment.

H_1BH_2-arm, H_1 represents high-DBP exposure at baseline, B represents background-exposure after crossover and H_2 represents high-DBP exposure after crossback; included 47 men taking mesalamine-containing DBP at baseline; 24 men (51%) on Asacol® and 23 men (49%) on Asacol®HD.

[a] N (%) for categorical/binary variables and mean (SD), [Range] for continuous variables

[b] P-values are based on Fisher exact test for categorical variables and Kruskal Wallis test for continuous variables.

[c] Season of sample collection, Warm: April Through September

[d] IBD score: included bowel frequency and urgency, presence of blood in the stool and general wellbeing. Mild IBD score: 5 or less on the simple clinical colitis activity index for UC and 4 or less on the Harvey-Bradshaw index for CD.

Abbreviations: MARS, Mesalamine And Reproductive health Study; BMI, body mass index; Kg, Kilogram; m, meter; SD, standard deviation; IBD, Inflammatory Bowel Disease; UC, ulcerative colitis; CD, Crohn's disease; N, number of men; DBP, dibutyl phthalate; B_1HB_2, $Background_1$-High-$Background_2$ DBP exposure; H_1BH_2, $High_1$-Background-$High_2$ DBP exposure.

Table 2.2. Hormone concentrations for 73 men (341 blood samples) in the MARS study

Hormone Parameters	Mean	SD	Geometric Mean	Minimum	5th Perc	25th Perc	50th Perc	75th Perc	95th Perc	Maximum	QR
Total Testosterone (nmol/L)	17.2	6.18	16.1	4.41	8.36	13.3	16.3	21.1	28.4	40.5	7.88
Free Testosterone (pmol/L)	345	105	329	44.3	198	275	333	400	541	730	125
SHBG (nmol/L)	34.3	16.6	30.2	3.89	11.2	22.8	31.5	44.5	64.7	98.7	21.7
Inhibin-B (pg/mL)	191	77.6	176	52.0	81.8	134	171	241	334	432	107
LH (IU/L)	3.23	1.37	2.96	0.90	1.42	2.22	3.04	3.94	5.64	10.8	1.72
FSH (IU/L)	3.20	1.74	2.77	0.62	1.13	1.85	2.97	4.04	6.73	9.99	2.19
Total Testosterone/LH Ratio	6.10	2.94	5.45	1.78	2.46	3.93	5.40	7.92	11.7	17.7	3.99
Free Testosterone/LH Ratio	123	56.5	111	23.8	51.6	81.6	111	155	223	360	73.0
Inhibin-B/FSH Ratio	90.1	78.4	63.4	7.59	15.6	37.5	61.9	120	265	391	82.7

Abbreviations: MARS, Mesalamine And Reproductive health Study; **SD**, standard deviation; **Perc**, percentile; **QR**: quartile range; **SHBG**, sex hormone-binding globulin; **LH**, luteinizing hormone; **FSH**, follicle-stimulating hormone

Table 2.3A. Adjusted percent changes (95%CI) of crossover, crossback and carryover on hormone concentrations among men starting on mesalamine medication not containing dibutyl phthalate (B_1HB_2-arm)[a]

Table 3A. Adjusted B_1HB_2-arm [Background (B_1)- High (H)- Background (B_2) dibutyl phthalate exposure]: 26 men, 134 blood samples

Hormone serum concentrations	Fitted Geometric Means (95%CI)			Comparisons					
	Baseline (B_1)	Crossover (H)	Crossback (B_2)	Crossover % Change (95%CI) H-B_1	P value	Crossback % Change (95%CI) B_2-H	P value	Carryover % Change (95%CI) B_2-B_1	P value
Total Testosterone (nmol/L)	16.3 (14.2, 18.8)	15.6 (13.6, 18.0)	17.2 (14.9, 19.9)	-4.28 (-12.7, 4.88)	0.35	10.4 (0.37, 21.4)	0.04	5.64 (-4.29, 16.6)	0.27
Free Testosterone (pmol/L)	330 (292, 373)	322 (284, 365)	355 (312, 404)	-2.36 (-11.1, 7.19)	0.61	10.4 (0.16, 21.6)	0.05	7.77 (-2.53, 19.2)	0.14
SHBG (nmol/L)	29.6 (24.5, 35.7)	29.4 (24.3, 35.6)	28.8 (23.7, 34.9)	-0.52 (-10.3, 10.3)	0.92	-2.14 (-12.1, 8.91)	0.69	-2.65 (-12.9, 8.85)	0.64
Inhibin-B (pg/mL)	162 (137, 192)	161 (136, 190)	173 (146, 205)	-0.89 -7.57, 6.27)	0.80	7.28 (-0.21, 15.3)	0.06	6.32 (-1.44, 14.7)	0.11
LH (IU/L)	3.29 (2.81, 3.85)	2.83 (2.42, 3.32)	3.22 (2.73, 3.80)	-13.9 (-23.6, -3.02)	0.01	13.8 (0.53, 28.9)	0.04	-2.04 (-13.8, 11.4)	0.75
FSH (IU/L)	3.23 (2.57, 4.07)	3.05 (2.42, 3.84)	3.3 (2.62, 4.17)	-5.79 (-12.8, 1.82)	0.13	8.35 (-0.04, 17.4)	0.05	2.08 (-6.19, 11.1)	0.63
Total Testosterone/LH Ratio	4.95 (4.13, 5.92)	5.5 (4.59, 6.6)	5.33 (4.42, 6.42)	11.1 (-1.11, 24.9)	0.08	-3.17 (-14.2, 9.33)	0.60	7.62 (-5.13, 22.1)	0.25
Free Testosterone/LH Ratio	100 (84.7, 119)	114 (95.9, 135)	110 (92.4, 132)	13.4 (-0.71, 29.6)	0.06	-3.04 (-15.6, 11.4)	0.66	9.98 (-4.68, 26.9)	0.19
Inhibin-B/FSH Ratio	50.0 (35.0, 71.5)	52.7 (36.8, 75.3)	52.3 (36.5, 75.0)	5.32 (-4.45, 16.1)	0.30	-0.68 (-10.2, 9.88)	0.90	4.61 (-5.93, 16.3)	0.40

Table 2.3B. Adjusted percent changes (95%CI) of crossover, crossback and carryover on hormone concentrations among men starting on mesalamine medication containing dibutyl phthalate (H_1BH_2-arm)[a]

H_1BH_2-arm[High (H_1)-Background (B) - High (H_2)] dibutyl phthalate exposure]: 47 men, 207 blood samples									
Hormone serum concentrations	**Fitted Geometric Means (95%CI)**			**Comparisons**					
	Baseline (H_1)	**Crossover (B)**	**Crossback (H_2)**	**Crossover % Change (95%CI) B-H_1**	**P value**	**Crossback % Change (95%CI) H_2-B**	**P value**	**Carryover % Change (95%CI) H_2-H_1**	**P value**
Total Testosterone (nmol/L)	16.0 (14.5, 17.7)	15.9 (14.3, 17.7)	17.0 (15.3, 19.0)	-0.58 (-7.86, 7.27)	0.88	7.13 (-1.18, 16.1)	0.09	6.50 (-1.59, 15.3)	0.12
Free Testosterone (pmol/L)	316 (290, 345)	324 (294, 358)	343 (311, 380)	2.52 (-5.1, 10.8)	0.53	5.90 (-2.49, 15.0)	0.17	8.57 (0.18, 17.7)	0.05
SHBG (nmol/L)	31.7 (27.7, 36.3)	33.2 (28.8, 38.3)	31.7 (27.4, 36.6)	4.65 (-3.97, 14.1)	0.30	-4.50 (-12.8, 4.58)	0.32	-0.05 (-8.60, 9.30)	0.99
Inhibin-B (pg/mL)	171 (152, 193)	185 (164, 210)	200 (176, 226)	8.52 (2.37, 15.1)	0.006	7.71 (1.31, 14.5)	0.02	16.9 (10.0, 24.2)	<0.0001
LH (IU/L)	2.96 (2.65, 3.31)	2.66 (2.35, 3.01)	2.90 (2.55, 3.29)	-10.1 (-18.6, -0.78)	0.03	8.93 (-1.98, 21.1)	0.11	-2.09 (-11.7, 8.51)	0.69
FSH (IU/L)	2.64 (2.24, 3.11)	2.42 (2.05, 2.87)	2.65 (2.24, 3.14)	-8.39 (-14.2, -2.23)	0.009	9.63 (2.40, 17.4)	0.008	0.43 (-6.14, 7.46)	0.9
Total Testosterone/LH Ratio	5.41 (4.76, 6.15)	6.01 (5.23, 6.90)	5.90 (5.12, 6.80)	11.0 (0.73, 22.3)	0.04	-1.80 (-11.4, 8.86)	0.73	9.01 (-1.47, 20.6)	0.09
Free Testosterone/LH Ratio	107 (94.9, 120)	122 (107, 139)	118 (103, 136)	14.0 (2.07, 27.2)	0.02	-2.74 (-13.6, 9.42)	0.64	10.8 (-1.16, 24.3)	0.08
Inhibin-B/FSH Ratio	64.7 (50.1, 83.4)	76.6 (59.1, 99.2)	75.2 (58.0, 97.5)	18.4 (9.12, 28.5)	<0.0001	-1.80 (-9.84, 6.96)	0.68	16.3 (6.82, 26.6)	0.001

Table 2.3: [a] Adjusted for race (Caucasian or not), age (continuous), BMI (continuous), hour of sample draw (continuous), season (warm or cold), current smoking, age (continuous), and duration on DBP-containing mesalamine medication at baseline (in years) in the mixed effect model.

[b] Percentage changes are presented by exponentiating the beta coefficient. Negative sign means % decrease for the corresponding outcome compared to the measure in the previous period.

[c] **B_1HB_2-arm:** B_1 represents background low-DBP exposure at baseline, H represents high-DBP exposure after crossover and B_2 represents background low-exposure after crossback.

[d] **H_1BH_2-arm: H_1** represents high-DBP at baseline, **B** represents background-DBP exposure after crossover and **H_2** represents high-DBP after crossback.

Abbreviations: MARS, Mesalamine And Reproductive health Study; N, number of men; B_1HB_2, Background$_1$-High-Background$_2$ DBP exposure; H_1BH_2, High$_1$-Background-High$_2$ DBP exposure; **95% CI**, 95% Confidence Interval; **DBP**, dibutyl phthalate; **SHBG**, sex hormone-binding globulin; **LH**, luteinizing hormone; **FSH**, follicle-stimulating hormone.

Figure 2.1. Mesalamine And Reproductive health Study (MARS) Design.

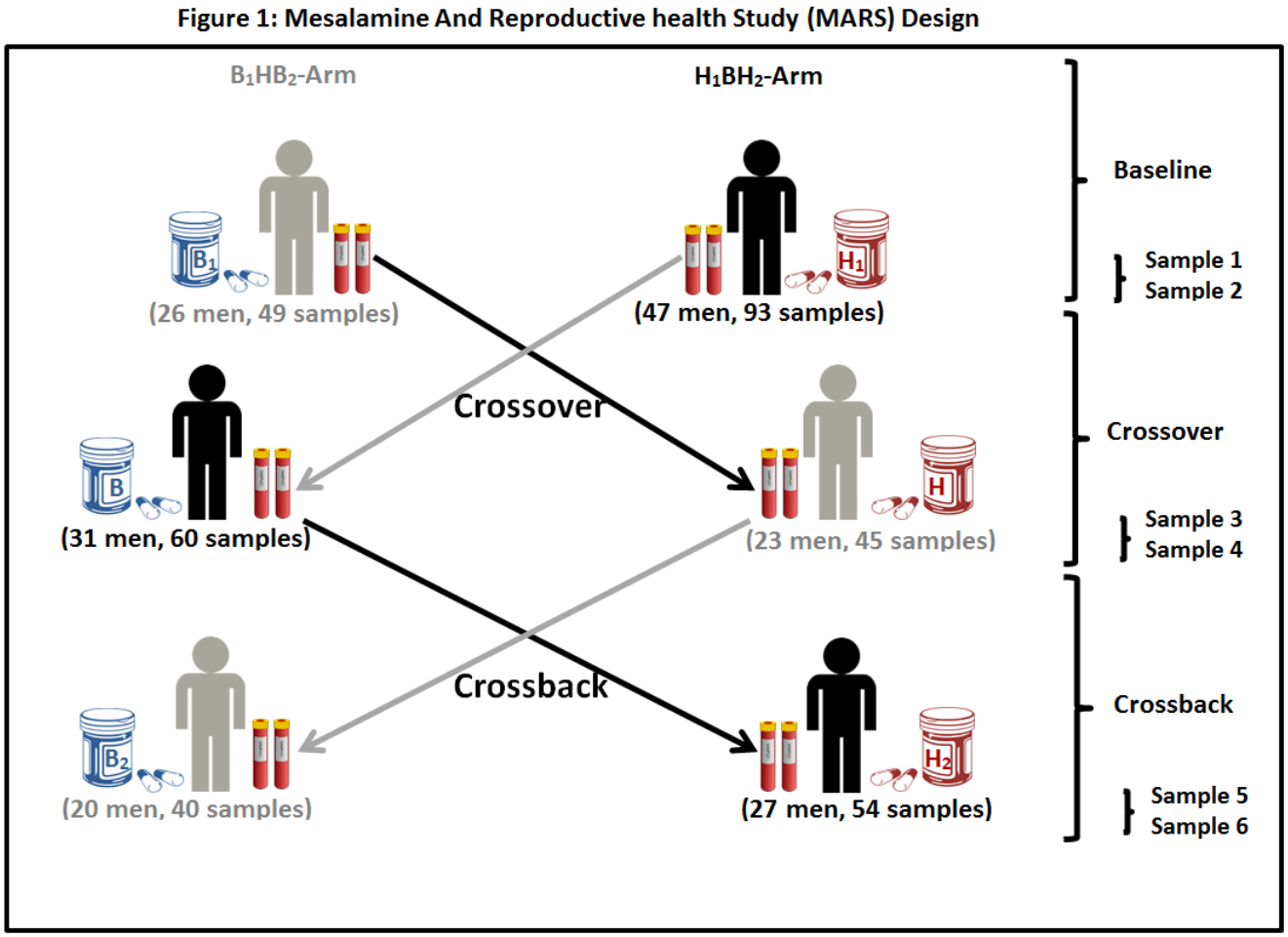


Figure 2.1: B_1HB_2-arm: B_1 represents background-exposure at baseline, **H** represents high-DBP exposure after crossover and **B_2** represents background-exposure after crossback; included 26 men taking mesalamine that did not contain DBP at baseline. **H_1BH_2-arm: H_1** represents high-DBP exposure at baseline, **B** represents background-exposure after crossover and **H_2** represents high-DBP exposure after crossback; included 47 men taking mesalamine containing DBP at baseline (25 men for less than 3 years and 22 men with 3 years or more). **Abbreviations: MARS**, Mesalamine And Reproductive health Study; **DBP,** dibutyl phthalate.

Figure 2.2 Geometric means (95%CI) of reproductive hormone parameters at baseline, crossover and crossback for the 3 arms (B1HB2- arm, H1BH2-arm with duration on DBP-containing mesalamine medication at baseline (period) <3 years and H1BH2-arm with period ≥ 3 years) –MARS.

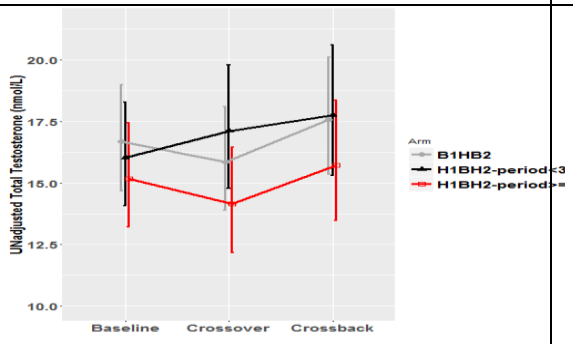
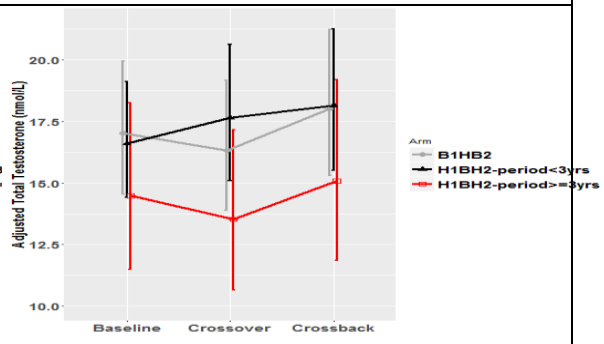
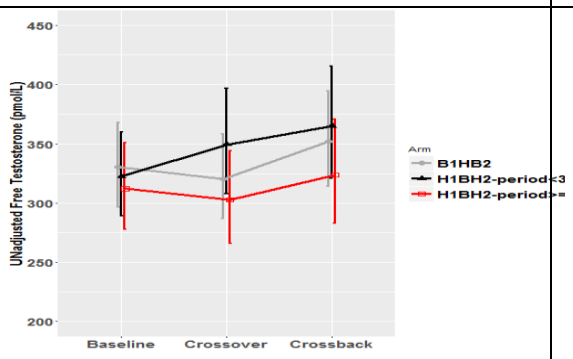
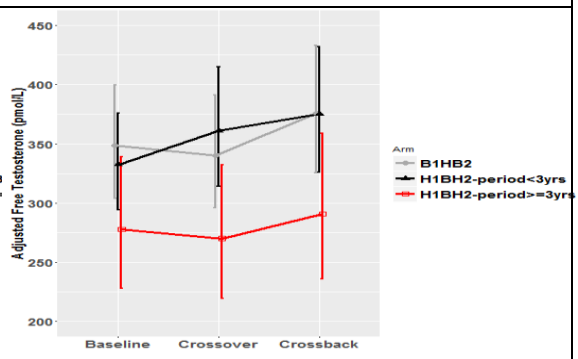
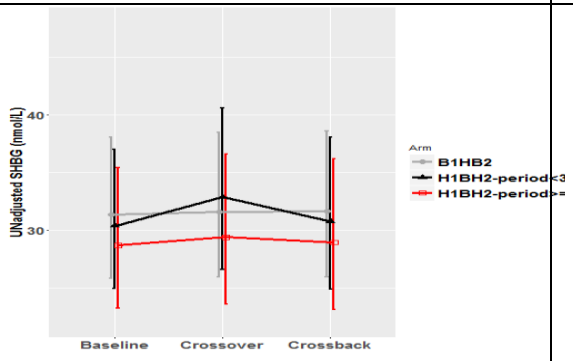
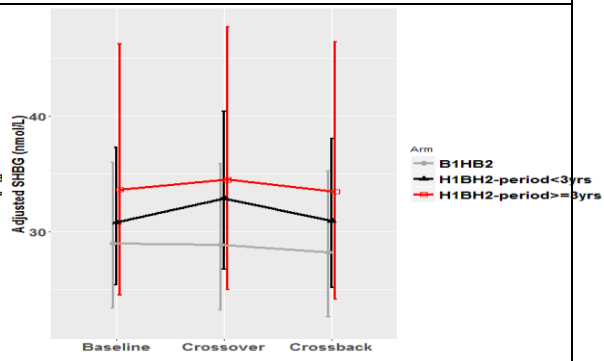
Panel 2.2A. Unadjusted geometric means (95%CI)	Panel 2.2B. Adjusted geometric means (95%CI)
	
	
	

Figure 2.2. (Continued)

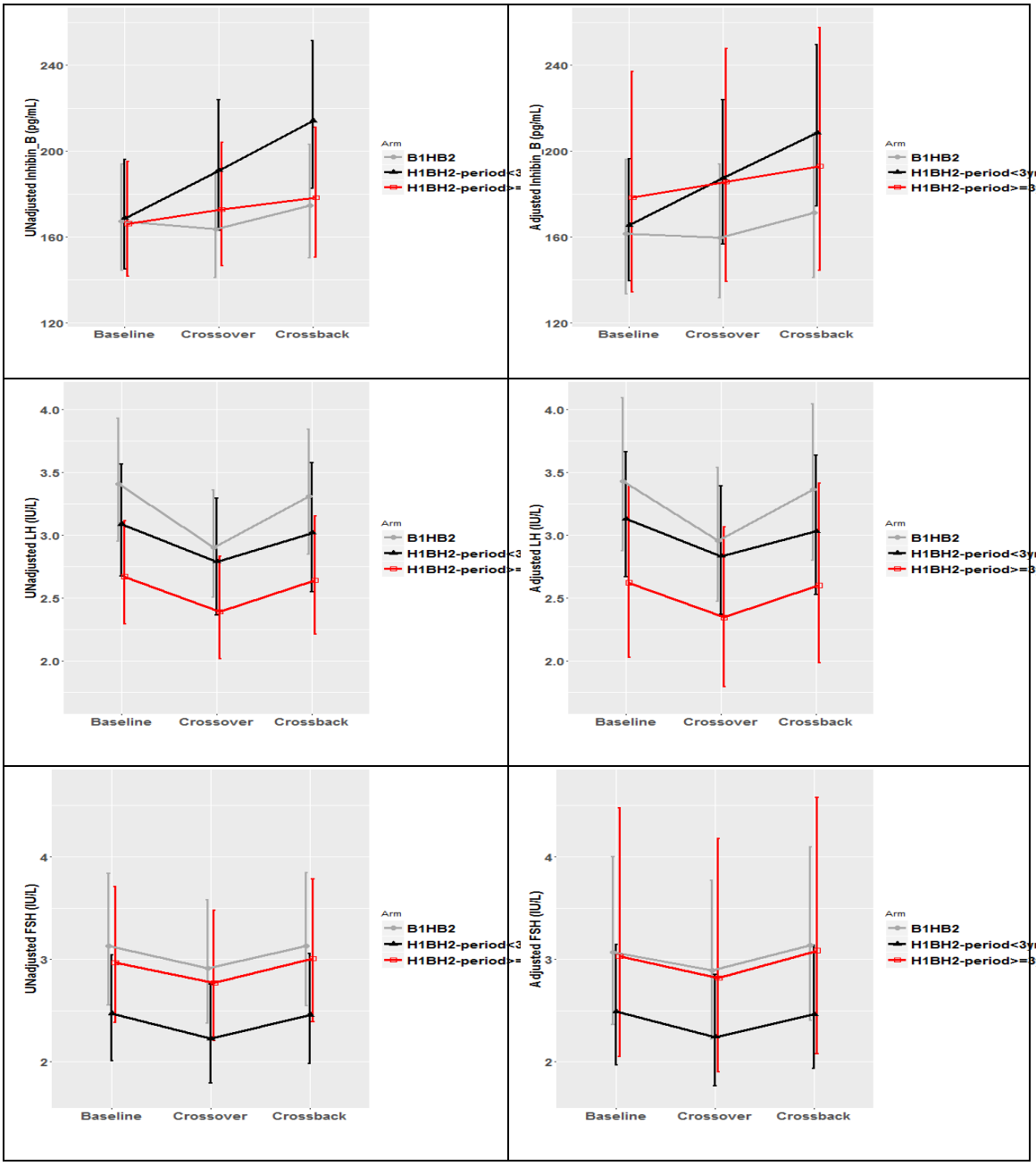


Figure 2.2. (Continued)

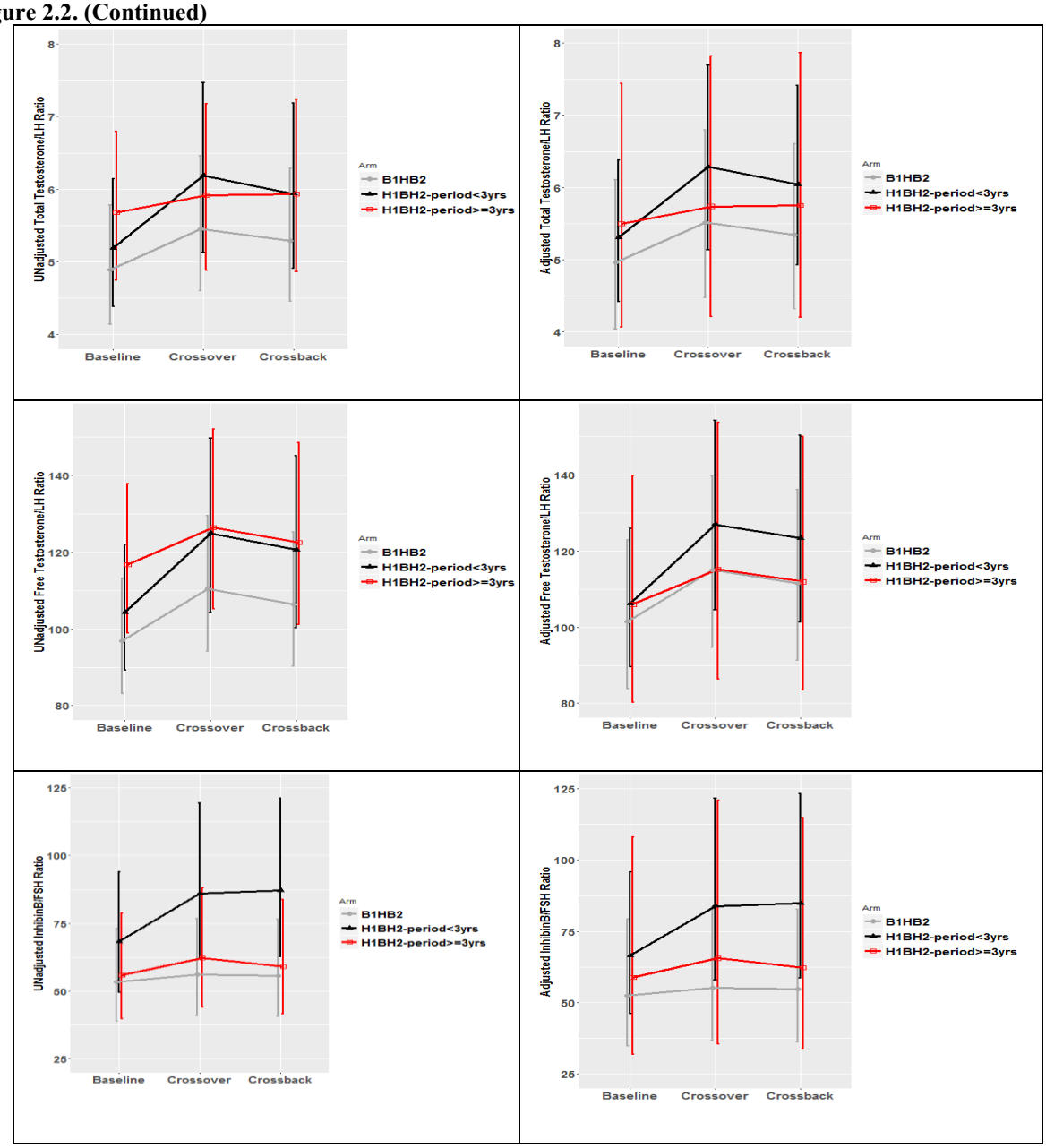
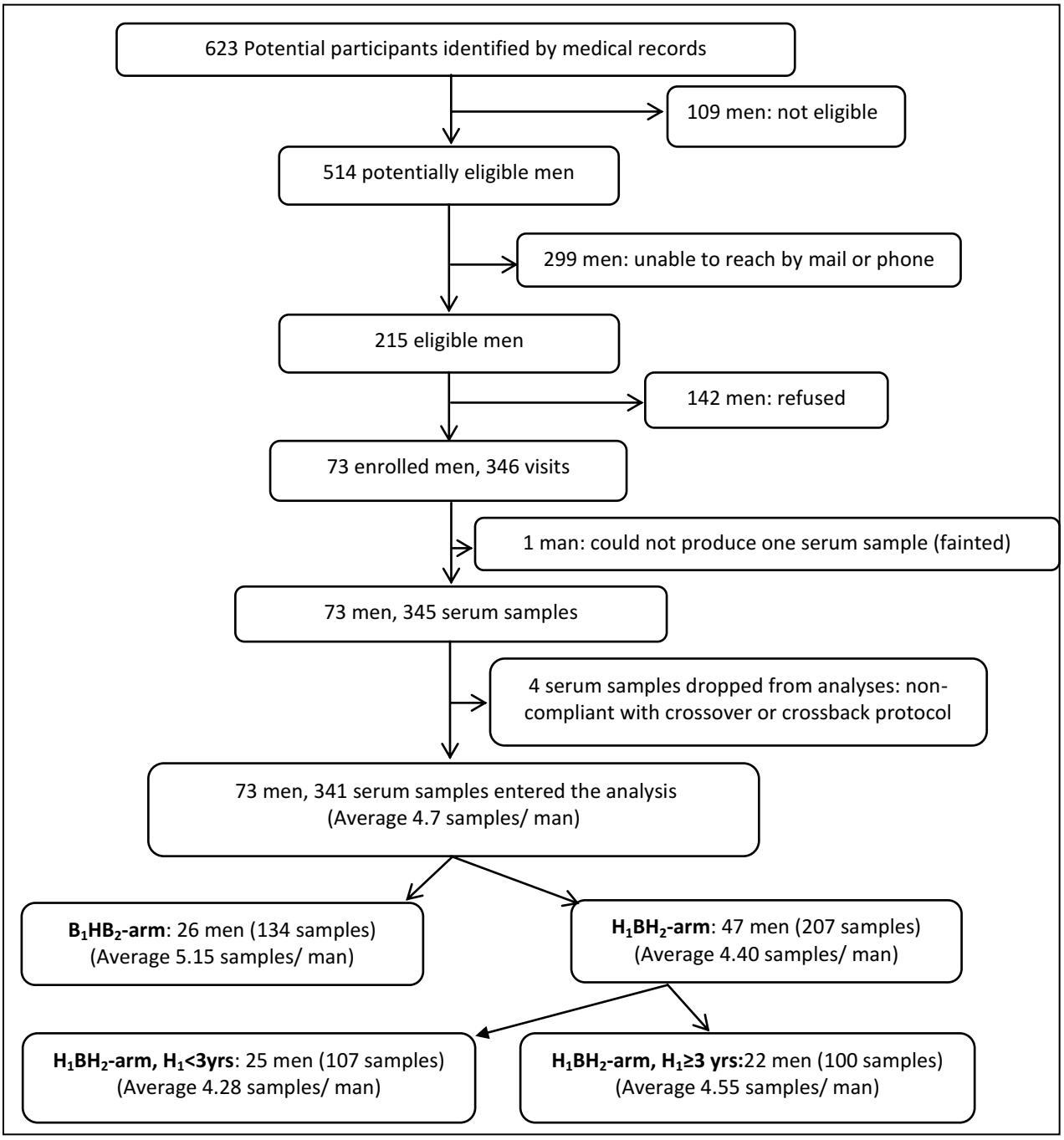


Figure 2.2. shows the adjusted geometric means and 95% CI [adjusted for race (Caucasian or not), age (continuous), BMI (continuous), hour of sample draw (continuous), season (warm or cold), current smoking, age (continuous), and duration on DBP-containing mesalamine medication at baseline (in years)]. **B_1HB_2-arm, B_1** represents background-exposure at baseline, **H** represents high-DBP exposure after crossover and **B_2** represents background-exposure after crossback; included 26 men taking mesalamine that did not contain DBP at baseline. **H_1BH_2-arm, H_1** represents high-DBP exposure at baseline, **B represents** background-exposure after crossover and **H_2** represents high-DBP exposure after crossback; included 47 men taking mesalamine-containing DBP at baseline. H_1BH_2-arm is subdivided into two arms based on the median duration on DBP-containing mesalamine medication at baseline **(period) i.e., <3 years and >= 3 years.**

Abbreviations: MARS, Mesalamine And Reproductive health Study; **95% CI**, 95% Confidence Interval; **DBP**, dibutyl phthalate; **SHBG**, sex hormone-binding globulin; **LH**, luteinizing hormone; **FSH**, follicle-stimulating hormone.

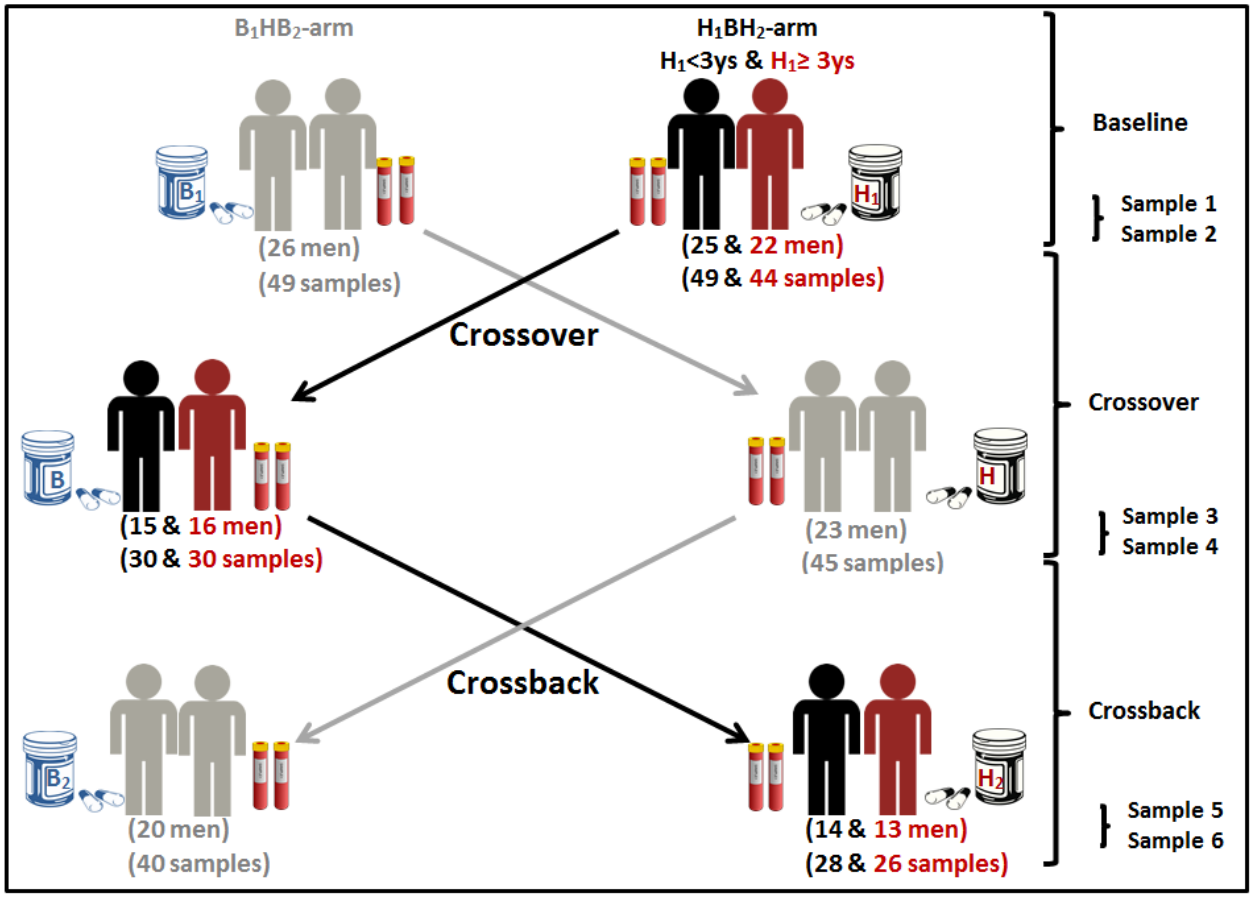
Supplementary Materials

Supplementary Figure 2.1: Sample Size Flow Chart-Mesalamine And Reproductive health Study (MARS)



Supplementary Figure 2.1. (Continued): MARS Eligibility criteria: 18 to 55 years old males; diagnosed with mild IBD, visited gastroenterology clinics at Massachusetts General Hospital (MGH), Beth Israel Deaconess Medical Center (BIMC) or Brigham and Women's Hospital (BWH), Boston, MA, USA from October 2010 through March 2016. Participants must have had mild IBD severity, on oral mesalamine for at least the previous 3 months. Men with history of infertility were not excluded. Men with history of vasectomy, steroid medication in the previous 3 months, diabetes mellitus, hepatic or renal diseases were not eligible. **Potentially eligible**: men who were eligible according to the medical records but we could not confirm eligibility due to failure to reach them. **Eligible**: eligibility confirmed after contacting the men.
B_1HB_2-arm, B_1 represents background-exposure at baseline, **H** represents high-DBP exposure after crossover and **B_2** represents background-exposure after crossback included 26 men taking mesalamine that did not contain DBP at baseline.
H_1BH_2-arm, H_1 represents high-DBP exposure at baseline, **B** represents background-exposure after crossover and **H_2** represents high-DBP exposure after crossback included 47 men taking mesalamine containing DBP at baseline.
H_1< 3 Yrs: represents high-DBP exposure at baseline for less than 3 years. **$H_1 \geq$ 3 Yrs:** represents high-DBP exposure at baseline for 3 years or more.
Note: 3 years was the median duration for men in the H_1BH_2-arm on high-DBP exposure at baseline (H_1).
Abbreviations: MARS, Mesalamine And Reproductive health Study; **IBD**, Inflammatory Bowel Disease; **UC**, ulcerative colitis; **CD**, Crohn's disease; **DBP**, dibutyl phthalate; **B_1HB_2**: $Background_1$-High-$Background_2$ DBP exposure; **H_1BH_2**; $High_1$-Background-$High_2$ DBP exposure.

Supplementary Figure 2.2: Mesalamine And Reproductive health Study (MARS) Design.



Supplementary Figure 2.2: B_1HB_2-arm: B_1 represents background-exposure at baseline, **H** represents high-DBP exposure after crossover and **B_2** represents background-exposure after crossback; included 26 men taking mesalamine that did not contain DBP at baseline. **H_1BH_2-arm: H_1** represents high-DBP exposure at baseline, **B** represents background-exposure after crossover and **H_2** represents high-DBP exposure after crossback; included 47 men taking mesalamine containing DBP at baseline (25 men for less than 3 years and 22 men with 3 years or more). **H_1< 3 Yrs:** represents high-DBP exposure at baseline for less than 3 years. **H_1≥ 3 Yrs:** represents high-DBP exposure at baseline for 3 years or more.
Note: 3 years was the median duration for men in the H_1BH_2-arm on high-DBP exposure at baseline (H_1).
Abbreviations: MARS, Mesalamine And Reproductive health Study; **DBP,** dibutyl phthalate.

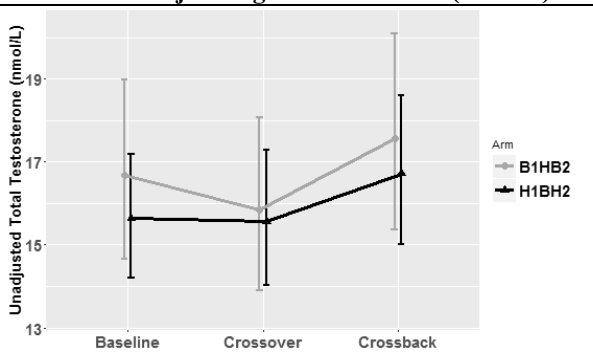
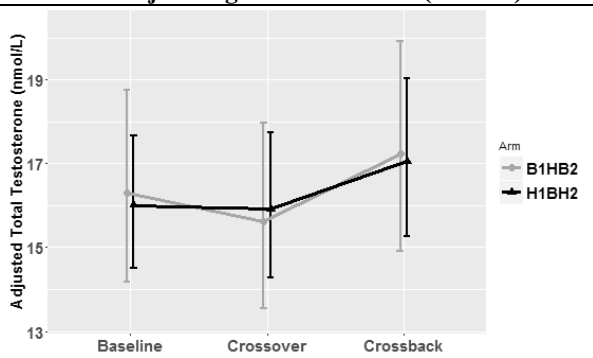
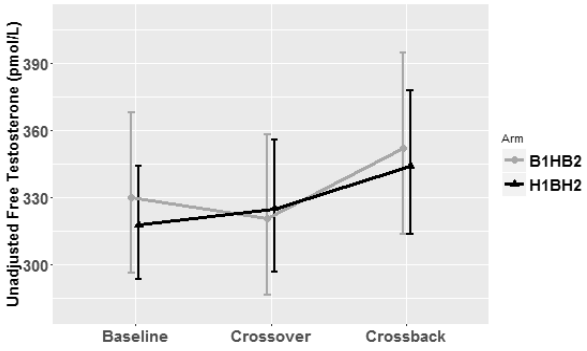
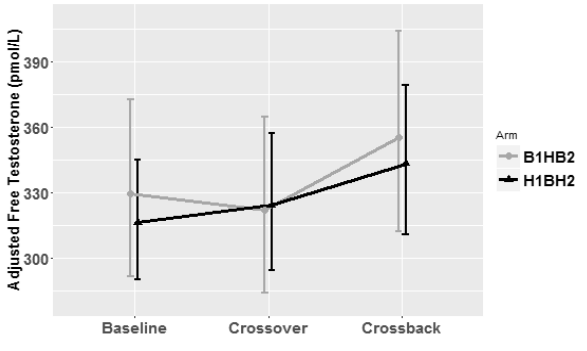
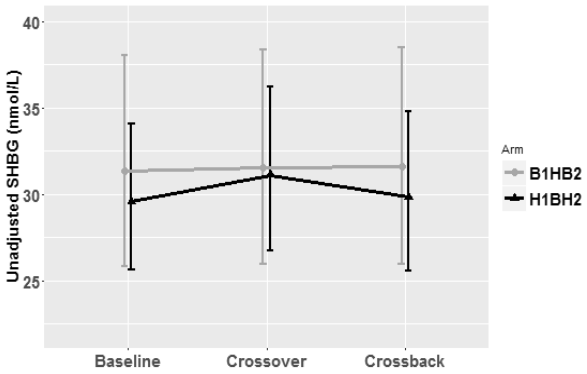
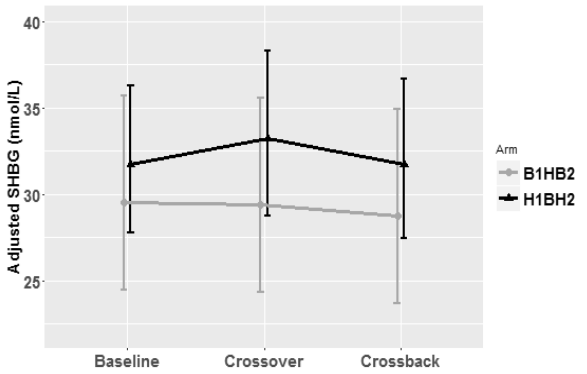
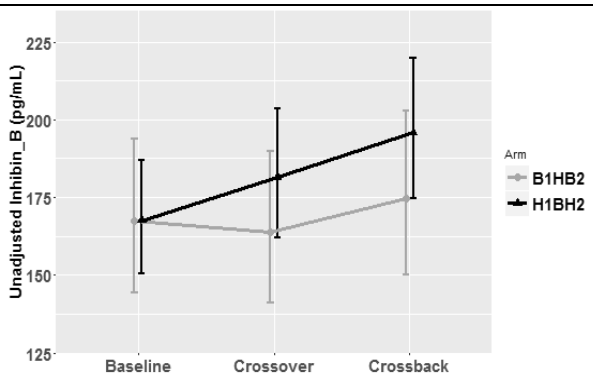
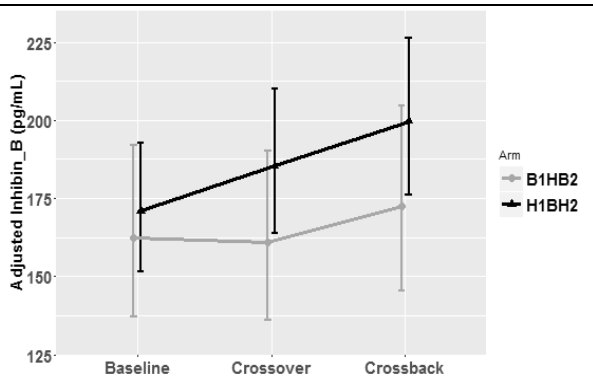
Supplementary Table 2.1. Demographics of 73 men contributing 341 blood samples in the MARS Study by arm.

Baseline characteristics (at Visit 1),# men (N)[a]	B_1HB_2-arm (26 men, 134 visits)	H_1BH_2-arm, H_1< 3 Yrs (25 men, 107 visits)	H_1BH_2-arm, $H_1\geq$ 3 Yrs (22 men, 100 visits)	Total (73 men, 341 visits)	P-value[b]
			N(%)/Mean (SD),[Range]		
Duration on High-DBP mesalamine at baseline (H_1), years	0	1.21 (0.77), [0.33, 2.97]	11.1 (5.26), [4.07, 24.1]	3.77 (5.68), [0, 24.1]	-
Age (Years)	33.9 (8.97), [20.2, 54.6]	32.0 (9.80), [19.6, 55.7]	38.2 (8.57),[24.2, 55.6]	34.6 (9.37), [19.6, 55.7]	0.04
Race					0.44
Caucasian	25 (96%)	21 (84%)	17 (77%)	63 (86%)	-
Black/African American	1 (4%)	1 (4%)	1 (5%)	3 (4%)	-
Asian	0	2 (8%)	2 (9%)	4 (6%)	-
Other	0	1 (4%)	2 (9%)	3 (4%)	-
BMI (Kg/m2)	25.3 (3.56), [19.4, 32.7]	25.3 (6.79), [19.5, 54.0]	27.5 (5.84), [19.7, 47.4]	26.0 (5.54), [19.4, 54.0]	0.09
BMI-categories					0.38
Normal weight (18.5 ≤BMI<25)	14 (54%)	16 (64%)	8 (37%)	38 (52%)	-
Overweight (25≤BMI<30)	9 (35%)	7 (28%)	9 (41%)	25 (34%)	-
Obese (BMI≥30)	3 (11%)	2 (8%)	5 (23%)	10 (14%)	-
Education					0.92
Below college	3 (14%)	3 (13%)	3 (17%)	9 (14%)	-
College graduate	10 (48%)	10 (42%)	6 (33%)	26 (41%)	-
Graduate degree	8 (38%)	11 (46%)	9 (50%)	28 (45%)	-
Smoking status					0.04
Never smoker	22 (85%)	20 (80%)	16 (73%)	58 (80%)	-
Former smoker	1 (4%)	5 (20%)	6 (28%)	12 (16%)	-
Current smoker	3 (11%)	0	0	3 (4%)	-
Warm season at baseline[c]	5 (19%)	13 (52%)	7 (32%)	25 (34%)	0.05
IBD diagnosis					0.75
Ulcerative colitis	16 (62%)	18 (72%)	14 (64%)	48 (66%)	-
Crohn's disease	10 (38%)	7 (28%)	8 (36%)	25 (34%)	-
IBD score[d]	1.65 (1.38), [0, 4]	1.28 (1.21), [0, 4]	1.09 (1.51), [0, 5]	1.36 (1.37), [0, 5]	0.22
Duration since IBD diagnosis (years)	9.00 (7.84), [0.28, 29.5]	7.24 (8.47), [0.70, 39.0]	14.8 (8.59), [4.47, 38.0]	10.1 (8.76), [0.28, 39.0]	0.003
Time-varying characteristics, #visits[a]					
Warm season of blood draw [c]	60 (45%)	51 (48%)	43 (43%)	154 (45%)	0.79
Hour of the blood draw	9.68 (1.62), [7.25, 13.9]	9.82 (1.65), [7.33, 13.5]	9.62 (1.56), [7.17, 13.3]	9.71 (1.61), [7.17, 13.9]	0.57
Hour of blood draw-categories					0.53
7 am to 10 am	74 (55%)	53 (50%)	57 (57%)	184 (54%)	-
After 10 am to 2 pm	60 (45%)	54 (50%)	43 (43%)	157 (46%)	-
Fever in previous 3 months	2 (2%)	1 (1%)	2 (2%)	5 (1%)	0.87
Illness in previous 3mo	27 (25%)	14 (15%)	15 (17%)	56 (19%)	0.21

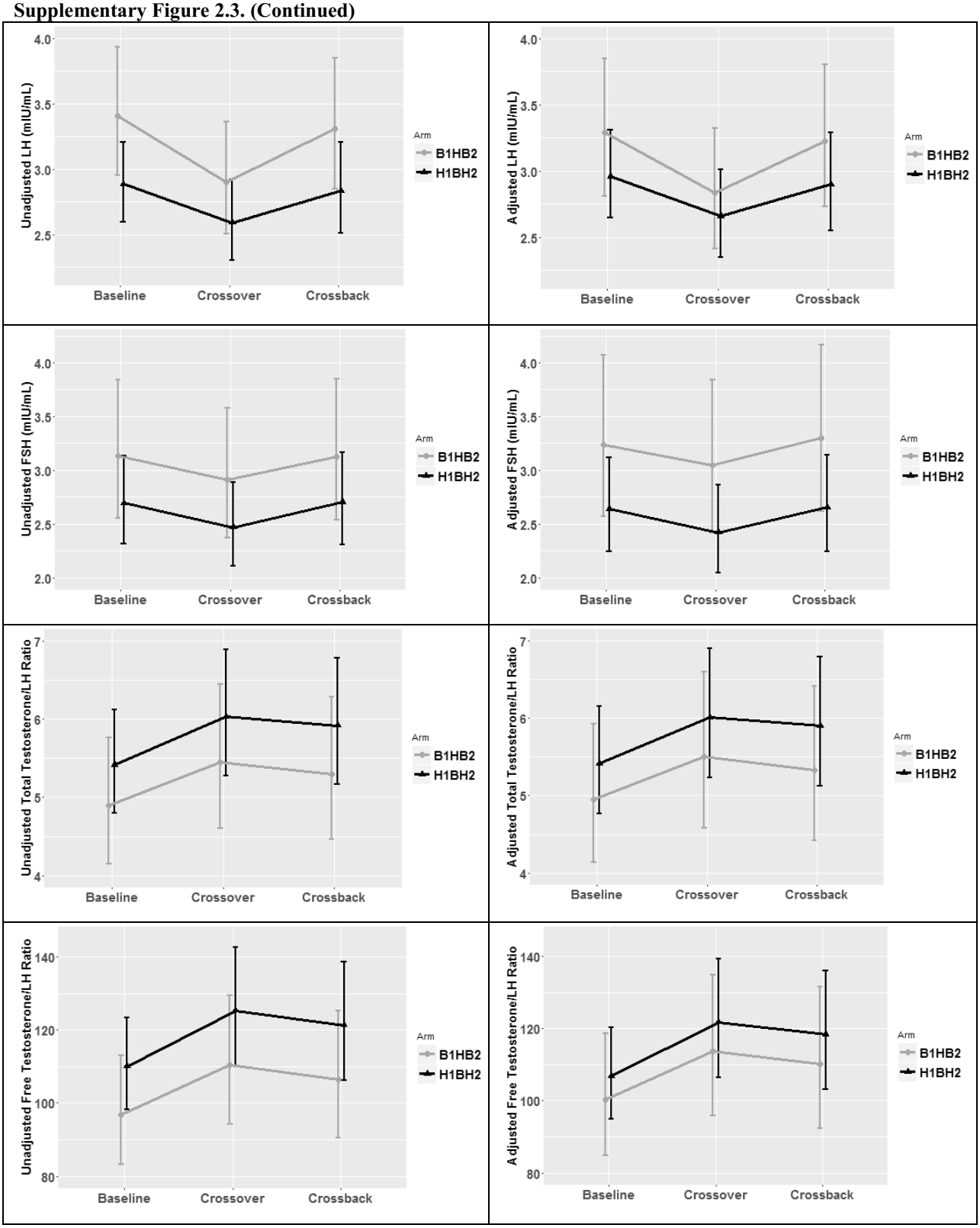
Supplementary Table 2.1.: B_1HB_2-arm, B_1 represents background-exposure at baseline, H represents high-DBP exposure after crossover and B_2 represents background-exposure after crossback; included 26 men taking mesalamine that did not contain DBP at baseline; 16 men (62%) on Lialda®, 8 men (31%) on Pentasa®, one man (4%) on Apriso® and one man (4%) was not on mesalamine medication at the time of recruitment.
H_1BH_2-arm, H_1 represents high-DBP exposure at baseline, B represents background-exposure after crossover and H_2 represents high-DBP exposure after crossback; included 47 men taking mesalamine-containing DBP at baseline; 24 men (51%) on Asacol® and 23 men (49%) on Asacol®HD.
H_1< 3 Yrs: represents high-DBP exposure at baseline for less than 3 years. **H_1≥ 3 Yrs:** represents high-DBP exposure at baseline for 3 years or more.
Note: 3 years was the median duration for men in the H_1BH_2-arm on high-DBP exposure at baseline (H_1).

[a] N (%) for categorical/binary variables and mean (SD), [Range] for continuous variables
[b] P-values are based on Fisher exact test for categorical variables and Kruskal Wallis test for continuous variables.
[c] Season of sample collection, Warm: April Through September
[d] IBD score: included bowel frequency and urgency, presence of blood in the stool and general wellbeing. Mild IBD score: 5 or less on the simple clinical colitis activity index for UC and 4 or less on the Harvey-Bradshaw index for CD.
[e] Alcohol: the average number of days per week the men drank alcoholic beverages, over the previous 3 months.
Abbreviations: MARS, Mesalamine And Reproductive health Study; BMI, body mass index; Kg, Kilogram; m, meter; SD, standard deviation; IBD, Inflammatory Bowel Disease; UC, ulcerative colitis; CD, Crohn's disease; N, number of men; DBP, dibutyl phthalate; B_1HB_2, Background$_1$-High-Background$_2$ DBP exposure; H_1BH_2, High$_1$-Background-High$_2$ DBP exposure.

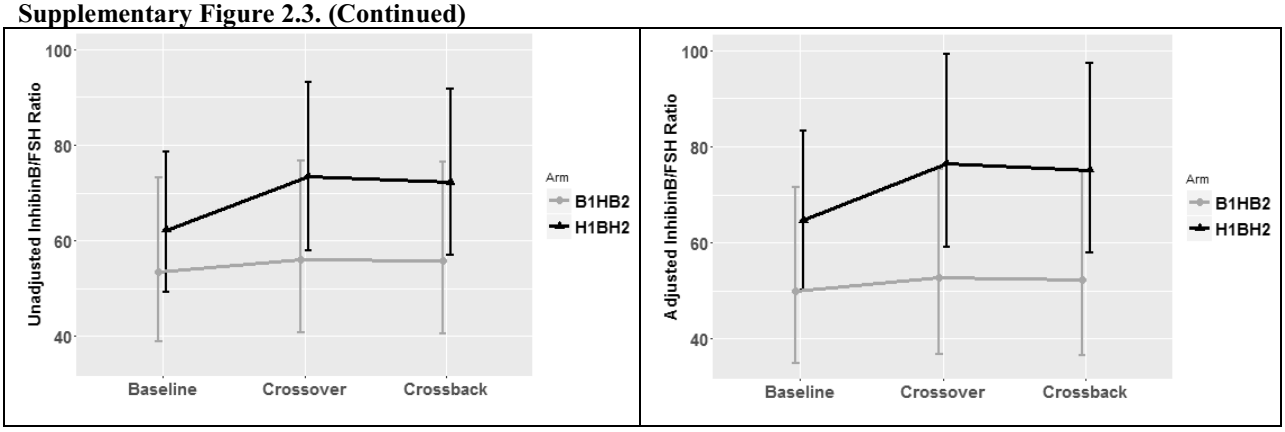
Supplementary Figure 2.3. based on mixed model: 2 arms
Geometric means (95%CI) of reproductive hormone parameters at baseline, crossover and crossback for the 2 arms (B_1HB_2- arm and H_1BH_2-arm -MARS

Panel: A- Unadjusted geometric means (95%CI)	Panel: B- Adjusted geometric means (95%CI)
	
	
	
	

Supplementary Figure 2.3. (Continued)



Supplementary Figure 2.3. (Continued)



Supplementary Figure 2.3. shows the adjusted geometric means and 95% CI [adjusted for race (Caucasian or not), age (continuous), BMI (continuous), hour of sample draw (continuous), season (warm or cold), current smoking, age (continuous), and duration on DBP-containing mesalamine medication at baseline (in years)]. **B_1HB_2-arm, B_1** represents background-exposure at baseline, **H** represents high-DBP exposure after crossover and **B_2** represents background-exposure after crossback; included 26 men taking mesalamine that did not contain DBP at baseline. **H_1BH_2-arm, H_1** represents high-DBP exposure at baseline, **B represents** background-exposure after crossover and **H_2** represents high-DBP exposure after crossback; included 47 men taking mesalamine-containing DBP at baseline. **Abbreviations: MARS**, Mesalamine And Reproductive health Study; **95% CI**, 95% Confidence Interval; **DBP**, dibutyl phthalate; **SHBG**, sex hormone-binding globulin; **LH**, luteinizing hormone; **FSH**, follicle-stimulating hormone.

Adjusted Mixed model: 3 arms:

Supplementary Table 2.2. Adjusted[a] percent change[b] (95%CI) of crossover, crossback and carryover on reproductive hormone parameters among men starting on mesalamine medication not containing dibutyl phthalate (B_1HB_2-arm)[c]

Table 2.2.A. Adjusted B_1HB_2-arm [Background (B_1)- High (H)- Background (B_2) dibutyl phthalate exposure]: 26 men, 134 blood samples

Hormone serum concentrations	Fitted Geometric Means (95%CI)			Comparisons					
	Baseline (B_1)	Crossover (H)	Crossback (B_2)	Crossover % Change (95%CI) H-B_1	P value	Crossback % Change (95%CI) B_2-H	P value	Carryover % Change (95%CI) B_2-B_1	P value
Total Testosterone (nmol/L)	17.0 (14.5, 19.9)	16.3 (13.9, 19.5)	18.0 (15.3, 21.2)	-4.18 (-12.6, 4.98)	0.36	10.4 (0.43, 21.4)	0.04	5.80 (-4.13, 16.8)	0.26
Free Testosterone (pmol/L)	348 (304, 400)	340 (296, 391)	376 (326, 433)	-2.33 (-11.1, 7.23)	0.62	10.4 (0.15, 21.6)	0.05	7.79 (-2.52, 19.2)	0.14
SHBG (nmol/L)	29.0 (23.4, 36.0	28.9 (23.2, 35.9)	28.2 (22.6, 35.2)	-0.49 (-10.3, 10.4)	0.93	-2.12 (-12.1, 8.98)	0.70	-2.60 (-12.92, 8.96)	0.64
Inhibin-B (pg/mL)	162 (133, 196)	160 (132, 194)	171 (141, 208)	-1.19 (-7.80, 5.90)	0.73	7.23 (-0.20, 15.2)	0.06	5.95 (-1.74, 14.2)	0.13
LH (IU/L)	3.43 (2.87, 4.10)	2.96 (2.47, 3.54)	3.37 (2.80, 4.05)	-13.8 (-23.6, -2.83)	0.02	13.8 (0.49, 28.9)	0.04	-1.9 (-13.77, 11.6)	0.77
FSH (IU/L)	3.07 (2.36, 4.00)	2.89 (2.22, 3.77)	3.14 (2.40, 4.09)	-5.76 (-12.83, 1.88)	0.14	8.36 (-0.05, 17.5)	0.05	2.11 (-6.20, 11.2)	0.63
Total Testosterone/LH Ratio	4.97 (4.04, 6.10)	5.52 (4.48, 6.79)	5.34 (4.32, 6.61)	11.1 (-1.15, 24.9)	0.08	-3.14 (-14.2, 9.35)	0.61	7.61 (-5.15, 22.1)	0.25
Free Testosterone/LH Ratio	102 (83.8, 123)	115 (94.7, 140)	112 (91.3, 136)	13.3 (-0.84, 29.5)	0.07	-3.04 (-15.6, 11.4)	0.66	9.87 (-4.81, 26.8)	0.20
Inhibin-B/FSH Ratio	52.5 (34.8, 79.2)	55.2 (36.5, 83.2)	54.7 (36.2, 82.7)	4.96 (-4.74, 15.6)	0.33	-0.74 (-10.2, 9.75)	0.88	4.18 (-6.27, 15.8)	0.45

Supplementary Table 2.2.B. Adjusted H1BH2-arm[High (H1)-Background (B) - High (H2)] dibutyl phthalate exposure-period< 3 Yrs]: 25 men, 107 blood samples

Hormone serum concentrations	Fitted Geometric Means (95%CI)			Comparisons					
	Baseline (H_1)	Crossover (B)	Crossback (H_2)	Crossover % Change (95%CI) B-H_1	P value	Crossback % Change (95%CI) H_2-B	P value	Carryover % Change (95%CI) H_2-H_1	P value
Total Testosterone (nmol/L)	16.6 (14.4, 19.1)	17.6 (15.1, 20.6)	18.2 (15.5, 21.2)	6.24 (-4.61, 18.3)	0.27	2.89 (-8.13, 15.2)	0.62	9.32 (-2.18, 22.2)	0.12
Free Testosterone (pmol/L)	332 (294, 376)	361 (314, 415)	375 (326, 432)	8.53 (-2.73, 21.1)	0.14	3.96 (-7.45, 16.8)	0.51	12.8 (0.78, 26.3)	0.04
SHBG (nmol/L)	30.8 (25.4, 37.3)	32.9 (26.8, 40.4)	30.9 (25.1, 38.1)	6.86 (-5.47, 20.8)	0.29	-5.94 (-17.3, 6.91)	0.35	0.51 (-11.4, 14.1)	0.94
Inhibin-B (pg/mL)	166 (140, 196)	187 (157, 224)	209 (174, 250)	13.2 (4.23, 22.9)	0.003	11.3 (2.19, 21.3)	0.01	26.0 (15.7, 37.2)	<0.0001
LH (IU/L)	3.13 (2.67, 3.67)	2.84 (2.37, 3.39)	3.03 (2.53, 3.64)	-9.42 (-21.3, 4.27)	0.17	7.00 (-7.83, 24.2)	0.37	-3.08 (-16.2, 12.0)	0.67
FSH (IU/L)	2.49 (1.97, 3.15)	2.24 (1.76, 2.85)	2.46 (1.93, 3.13)	-9.90 (-17.9, -1.12)	0.03	9.74 (-0.32, 20.8)	0.06	-1.12 (-10.2, 8.82)	0.82
Total Testosterone/LH Ratio	5.31 (4.42, 6.38)	6.28 (5.14, 7.69)	6.04 (4.93, 7.41)	18.4 (3.11, 35.9)	0.02	-3.84 (-16.8, 11.2)	0.60	13.8 (-1.27, 31.2)	0.07
Free Testosterone/LH Ratio	106 (89.5, 126)	127 (105, 154)	123 (101, 150)	19.6 (2.31, 39.9)	0.02	-2.83 (-17.7, 14.7)	0.73	16.2 (-1.07, 36.6)	0.07
Inhibin-B/FSH Ratio	66.6 (46.3, 95.8)	83.9 (57.9, 122)	84.9 (58.5, 123)	26.0 (12.2, 41.5)	0.0001	1.21 (-10.2, 14.1)	0.84	27.5 (13.2, 43.7)	<0.0001

Supplementary Table 2.2.C: Adjusted H1BH2-arm[High (H1)-Background (B) - High (H2)] dibutyl phthalate exposure-period>= 3 Yrs]: 22 men, 100 blood samples

Hormone serum concentrations	Fitted Geometric Means (95%CI)			Comparisons					
	Baseline (H_1)	Crossover (B)	Crossback (H_2)	Crossover % Change (95%CI) $B-H_1$	P value	Crossback % Change (95%CI) H_2-B	P value	Carryover % Change (95%CI) H_2-H_1	P value
Testosterone_nmol_L	14.5 (11.5, 18.3)	13.5 (10.7, 17.2)	15.1 (11.9, 19.2)	-6.64 (-16.1, 3.85)	0.20	11.5 (-0.70, 25.3)	0.07	4.13 (-7.09, 16.7)	0.49
FT_pmol_L	278 (228, 339)	270 (220, 332)	291 (236, 359)	-2.78 (-12.8, 8.37)	0.61	7.69 (-4.40, 21.3)	0.22	4.69 (-6.80, 17.6)	0.44
SHBG_nmol_L	33.6 (24.5, 46.2)	34.5 (24.95, 47.7)	33.5 (24.1, 46.4)	2.53 (-9.14, 15.7)	0.68	-2.97 (-14.9, 10.7)	0.65	-0.52 (-12.6, 13.3)	0.94
Inhibin-B_pg_mL	178 (134, 237)	186 (139, 248)	193 (144, 258)	4.12 (-3.99, 12.9)	0.33	3.85 (-4.88, 13.4)	0.40	8.13 (-0.87, 18.0)	0.08
LH (IU/L)	2.62 (2.03, 3.39)	2.35 (1.8, 3.07)	2.60 (1.99, 3.41)	-10.6 (-22.2, 2.83)	0.12	10.9 (-4.83, 29.2)	0.18	-0.80 (-14.6, 15.2)	0.92
FSH (IU/L)	3.03 (2.05, 4.47)	2.82 (1.90, 4.18)	3.09 (2.08, 4.58)	-6.96 (-15.1, 1.93)	0.12	9.58 (-0.73, 21.0)	0.07	1.95 (-7.56, 12.5)	0.7
Total Testosterone/LH Ratio	5.50 (4.07, 7.44)	5.74 (4.21, 7.82)	5.75 (4.20, 7.87)	4.34 (-8.95, 19.6)	0.54	0.21 (-13.6, 16.3)	0.98	4.56 (-9.64, 21.0)	0.55
Free Testosterone/LH Ratio	106 (80.3, 140)	115 (86.3, 154)	112 (83.5, 150)	8.70 (-6.90, 26.9)	0.29	-2.84 (-18.1, 15.2)	0.74	5.62 (-10.5, 24.7)	0.52
Inhibin-B/FSH Ratio	58.8 (32.0, 108)	65.5 (35.5, 121)	62.2 (33.7, 115)	11.5 (-0.46, 24.9)	0.06	-5.11 (-16.1, 7.28)	0.40	5.80 (-6.34, 19.5)	0.36

Supplementary Table 2.2.: [a] Adjusted for race (Caucasian or not), age (continuous), BMI (continuous), hour of sample draw (continuous), season (warm or cold), current smoking, age (continuous), and duration on DBP-containing mesalamine medication at baseline (in years) in the mixed effect model.

[b] Percentage changes are presented by exponentiating the Beta coefficient. Negative sign means % decrease for the corresponding outcome compared to the measure in the previous period.

[c] **B_1HB_2-arm:** B_1 represents background low-DBP exposure at baseline, H represents high-DBP exposure after crossover and B_2 represents background low-exposure after crossback. [d] **H_1BH_2-arm, period<3 yrs: H_1** represents high-DBP at baseline for duration less than 3 years, **B** represents background low-DBP exposure after crossover and **H_2** represents high-DBP after crossback.

[e] **H_1BH_2-arm, period≥3 yrs: H_1** represents high-DBP at baseline for duration 3 years or more, **B** represents background low-DBP exposure after crossover and **H_2** represents high-DBP after crossback.**Abbreviations:** MARS, Mesalamine And Reproductive health Study; BMI, body mass index; Kg, Kilogram; m, meter; SD, standard deviation; IBD, Inflammatory Bowel Disease; UC, ulcerative colitis; CD, Crohn's disease; N, number of men; DBP, dibutyl phthalate; B_1HB_2, $Background_1$-High-$Background_2$ DBP exposure; H_1BH_2, $High_1$-Background-$High_2$ DBP exposure.

Sensitivity analyses

Supplementary Table 2.3. Unadjusted Mixed model: 2 arms

Supplementary Table 2.3.A. Unadjusted percent change (95%CI) of crossover, crossback and carryover on hormone concentrations among men starting on mesalamine medication not containing dibutyl phthalate (B_1HB_2-arm)[a]

Hormone serum concentrations	Fitted Geometric Means (95%CI)			Comparisons					
	Baseline (B_1)	Crossover (H)	Crossback (B_2)	Crossover % Change (95%CI) H-B_1	P value	Crossback % Change (95%CI) B_2-H	P value	Carryover % Change (95%CI) B_2-B_1	P value
Total Testosterone (nmol/L)	16.7 (14.7, 19.0)	15.9 (13.9, 18.1)	17.6 (15.4, 20.1)	-4.98 (-13.1, 3.90)	0.26	10.8 (0.92, 21.6)	0.03	5.27 (-4.12, 15.6)	0.28
Free Testosterone (pmol/L)	330 (296, 368)	320 (287, 358)	352 (314, 395)	-2.97 (-11.43, 6.30)	0.52	9.82 (-0.19, 20.8)	0.05	6.56 (-3.14, 17.2)	0.19
SHBG (nmol/L)	31.3 (25.8, 38.0)	31.6 (26.0, 38.4)	31.6 (25.9, 38.5)	0.76 (-8.90, 11.4)	0.88	0.11 (-9.89, 11.2)	0.98	0.87 (-9.24, 12.1)	0.87
Inhibin-B (pg/mL)	167 (144, 194)	164 (141, 190)	175 (150, 203)	-2.14 (-8.61, 4.78)	0.53	6.66 (-0.68, 14.5)	0.08	4.37 (-2.84, 12.1)	0.24
LH (IU/L)	3.41 (2.95, 3.93)	2.90 (2.51, 3.36)	3.31 (2.84, 3.85)	-14.8 (-24.2, -4.29)	0.01	14.0 (0.93, 28.9)	0.04	-2.86 (-14.0, 9.74)	0.64
FSH (IU/L)	3.13 (2.55, 3.84)	2.91 (2.37, 3.58)	3.13 (2.54, 3.85)	-6.93 (-13.7, 0.39)	0.06	7.32 (-0.82, 16.1)	0.08	-0.11 (-7.73, 8.13)	0.98
Total Testosterone/LH Ratio	4.89 (4.15, 5.77)	5.45 (4.61, 6.45)	5.29 (4.46, 6.28)	11.4 (-0.56, 24.8)	0.06	-2.88 (-13.8, 9.37)	0.63	8.2 (-3.93, 21.9)	0.19
Free Testosterone/LH Ratio	97 (83.1, 113)	110 (94.3, 129)	106 (90.4, 125)	14.0 (0.07, 29.8)	0.05	-3.70 (-16, 10.4)	0.59	9.76 (-4.2, 25.7)	0.18
Inhibin-B/FSH Ratio	53.4 (39.0, 73.1)	56.05 (40.9, 76.8)	55.76 (40.6, 76.5)	4.95 (-4.54, 15.4)	0.32	-0.51 (-9.87, 9.80)	0.92	4.41 (-5.46, 15.3)	0.39

Supplementary Table 2.3.B. Unadjusted percent change (95%CI) of crossover, crossback and carryover on hormone concentrations among men starting on mesalamine medication containing dibutyl phthalate (H_1BH_2-arm)[a]

Hormone serum concentrations	Fitted Geometric Means (95%CI)			Comparisons					
	Baseline (H_1)	Crossover (B)	Crossback (H_2)	Crossover % Change (95%CI) $B-H_1$	P value	Crossback % Change (95%CI) H_2-B	P value	Carryover % Change (95%CI) H_2-H_1	P value
Total Testosterone (nmol/L)	15.6 (14.2, 17.2)	15.6 (14.0, 17.3)	16.7 (15.0, 18.6)	-0.45 (-7.69, 7.36)	0.91	7.41 (-0.88, 16.4)	0.08	6.93 (-1.19, 15.7)	0.10
Free Testosterone (pmol/L)	318 (293, 344)	325 (297, 356)	344 (313, 378)	2.33 (-5.23, 10.5)	0.56	5.86 (-2.50, 15.0)	0.17	8.33 (-0.01, 17.4)	0.05
SHBG (nmol/L)	29.6 (25.6, 34.1)	31.1 (26.7, 36.2)	29.8 (25.6, 34.8)	5.23 (-3.42, 14.7)	0.24	-4.07 (-12.4, 5.02)	0.37	0.95 (-7.71, 10.4)	0.84
Inhibin-B (pg/mL)	167.62 (150, 187)	181.6 (162, 204)	196 (174, 220)	8.33 (2.20, 14.8)	0.007	7.85 (1.44, 14.7)	0.02	16.8 (9.94, 24.2)	<0.0001
LH (IU/L)	2.89 (2.60, 3.21)	2.59 (2.3, 2.92)	2.83 (2.51, 3.20)	-10.2 (-18.6, -0.95)	0.03	9.38 (-1.54, 21.5)	0.09	-1.80 (-11.4, 8.81)	0.73
FSH (IU/L)	2.69 (2.31, 3.14)	2.47 (2.11, 2.89)	2.71 (2.31, 3.17)	-8.33 (-14.1, -2.20)	0.009	9.60 (2.4, 17.3)	0.008	0.47 (-6.1, 7.49)	0.89
Total Testosterone/LH Ratio	5.42 (4.80, 6.12)	6.03 (5.28, 6.89)	5.92 (5.16, 6.78)	11.3 (1.12, 22.6)	0.03	-1.88 (-11.4, 8.68)	0.72	9.24 (-1.2, 20.8)	0.09
Free Testosterone/LH Ratio	110 (98.3, 123)	125 (110, 142)	121 (106, 139)	13.8 (2.04, 27.0)	0.02	-3.19 (-13.9, 8.85)	0.59	10.2 (-1.69, 23.5)	0.1
Inhibin-B/FSH Ratio	62.3 (49.3, 78.6)	73.5 (57.9, 93.3)	72.3 (56.9, 91.8)	18.1 (8.89, 28.0)	<0.0001	-1.64 (-9.65, 7.08)	0.70	16.1 (6.72, 26.4)	0.001

Supplementary Table 2.4. Fixed model: 2 arms:

Supplementary Table 2.4.A. B_1HB_2 Arm

B_1HB_2-arm [Background (B_1) -High (H)-Background (B_2) dibutyl phthalate exposure]: (26 men, 134 blood samples)												
	Unadjusted Comparisons						Adjusted Comparisons					
Hormone serum concentrations	Crossover % Change (95%CI) $H-B_1$	P value	Crossback % Change (95%CI) B_2-H	P value	Carryover % Change (95%CI) B_2-B_1	P value	Crossover % Change (95%CI) $H-B_1$	P value	Crossback % Change (95%CI) B_2-H	P value	Carryover % Change (95%CI) B_2-B_1	P value
Total Testosterone (nmol/L)	-5.00 (-13.2, 3.95)	0.26	10.6 (0.70, 21.5)	0.04	5.06 (-4.40, 15.5)	0.30	-3.87 (-12.4, 5.44)	0.40	11.2 (1.02, 22.4)	0.03	6.88 (-3.39, 18.2)	0.20
Free Testosterone (pmol/L)	-3.22 (-11.7, 6.13)	0.49	10.0 (-0.06, 21.1)	0.05	6.47 (-3.34, 17.3)	0.20	-1.96 (-10.8, 7.77)	0.68	11.4 (0.95, 22.8)	0.03	9.17 (-1.55, 21.1)	0.10
SHBG (nmol/L)	1.08 (-8.65, 11.9)	0.83	-0.10 (-10.1, 11.0)	0.99	0.98 (-9.18, 12.3)	0.86	-0.12 (-9.96, 10.8)	0.98	-1.78 (-11.8, 9.37)	0.74	-1.9 (-12.4, 9.87)	0.74
Inhibin-B (pg/mL)	-2.25 (-8.73, 4.69)	0.51	6.79 (-0.57, 14.7)	0.07	4.39 (-2.86, 12.2)	0.24	-0.59 (-7.31, 6.63)	0.87	7.80 (0.25, 15.9)	0.04	7.17 (-0.73, 15.7)	0.08
LH (IU/L)	-14.4 (-23.9, -3.67)	0.01	14.14 (0.95, 29.1)	0.03	-2.29 (-13.7, 10.6)	0.71	-13.6 (-23.5, -2.46)	0.02	14.5 (0.96, 29.9)	0.04	-1.09 (-13.4, 12.9)	0.87
FSH (IU/L)	-6.59 (-13.4, 0.77)	0.08	7.23 (-0.91, 16.0)	0.08	0.16 (-7.49, 8.46)	0.97	-5.63 (-12.7, 2.01)	0.14	8.23 (-0.17, 17.3)	0.05	2.14 (-6.19, 11.2)	0.62
Total Testosterone/LH Ratio	11.0 (-1.05, 24.5)	0.08	-3.12 (-14.0, 9.18)	0.60	7.52 (-4.67, 21.3)	0.24	11.3 (-1.12, 25.2)	0.08	-2.90 (-14.1, 9.76)	0.64	8.05 (-5.03, 22.9)	0.24
Free Testosterone/LH Ratio	13.1 (-0.89, 29.0)	0.07	-3.62 (-16.0, 10.5)	0.60	8.96 (-5.09, 25.1)	0.22	13.5 (-0.90, 30.0)	0.07	-2.74 (-15.5, 11.9)	0.70	10.38 (-4.81, 28.0)	0.19
Inhibin-B/FSH Ratio	4.64 (-4.83, 15.1)	0.35	-0.41 (-9.78, 9.93)	0.94	4.22 (-5.65, 15.1)	0.41	5.34 (-4.45, 16.1)	0.29	-0.39 (-9.98, 10.2)	0.94	4.93 (-5.68, 16.7)	0.38

Supplementary Table 2.4.B. Fixed: H_1BH_2 Arm

H_1BH_2-arm[High (H_1)-Background (B) – High (H_2) dibutyl phthalate exposure]: (47 men, 207 blood samples)												
	Unadjusted Comparisons						Adjusted Comparisons					
Hormone serum concentrations	Crossover % Change (95%CI) $B-H_1$	P value	Crossback % Change (95%CI) H_2-B	P value	Carryover % Change (95%CI) H_2-H_1	P value	Crossover % Change (95%CI) $B-H_1$	P value	Crossback % Change (95%CI) H_2-B	P value	Carryover % Change (95%CI) H_2-H_1	P value
Total Testosterone (nmol/L)	-0.49 (-7.88, 7.49)	0.90	7.55 (-0.78, 16.6)	0.08	7.02 (-1.27, 16.0)	0.10	-0.24 (-7.70, 7.83)	0.95	7.41 (-0.95, 16.5)	0.08	7.15 (-1.16, 16.2)	0.09
Free Testosterone (pmol/L)	2.30 (-5.47, 10.7)	0.57	5.95 (-2.45, 15.1)	0.17	8.39 (-0.21, 17.7)	0.06	2.27 (-5.56, 10.7)	0.58	6.07 (-2.37, 15.2)	0.16	8.48 (-0.13, 17.8)	0.05
SHBG (nmol/L)	5.43 (-3.33, 15.0)	0.23	-3.98 (-12.3, 5.13)	0.38	1.24 (-7.53, 10.8)	0.79	5.76 (-3.07, 15.4)	0.21	-4.31 (-12.6, 4.80)	0.34	1.21 (-7.56, 10.8)	0.79
Inhibin-B (pg/mL)	8.16 (1.98, 14.7)	0.009	7.75 (1.32, 14.6)	0.02	16.5 (9.59, 23.9)	<0.0001	8.46 (2.25, 15.0)	0.007	7.62 (1.21, 14.4)	0.02	16.7 (9.80, 24.1)	<0.0001
LH (IU/L)	-11.5 (-20.0, -2.07)	0.02	9.65 (-1.35, 21.9)	0.09	-2.95 (-12.7, 7.87)	0.58	-11.3 (-19.9, -1.74)	0.02	9.5 (-1.55, 21.8)	0.09	-2.85 (-12.6, 8.02)	0.59
FSH (IU/L)	-8.36 (-14.1, -2.21)	0.009	9.57 (2.37, 17.3)	0.009	0.40 (-6.19, 7.46)	0.91	-8.37 (-14.2, -2.17)	0.009	9.65 (2.42, 17.4)	0.008	0.47 (-6.14, 7.54)	0.89
Total Testosterone/ LH Ratio	12.4 (1.89, 24.1)	0.02	-1.92 (-11.5, 8.70)	0.71	10.3 (-0.50, 22.2)	0.06	12.4 (1.80, 24.2)	0.02	-1.91 (-11.6, 8.80)	0.71	10.3 (-0.52, 22.3)	0.06
Free Testosterone/ LH Ratio	15.6 (3.24, 29.4)	0.01	-3.38 (-14.1, 8.72)	0.57	11.7 (-0.75, 25.7)	0.07	15.3 (2.85, 29.2)	0.01	-3.13 (-14.0, 9.09)	0.6	11.7 (-0.81, 25.7)	0.07
Inhibin-B/FSH Ratio	18.0 (8.81, 28.0)	<0.0001	-1.66 (-9.67, 7.06)	0.7	16.1 (6.61, 26.4)	0.0006	18.4 (9.04, 28.5)	<0.0001	-1.85 (-9.89, 6.92)	0.67	16.2 (6.68, 26.5)	0.0006

Supplementary Table 2.5. Sensitivity: Mixed adjusted model with empirical standard error (sandwich estimator)

B_1HB_2-arm [Background (B_1)- High (H)- Background (B_2) dibutyl phthalate exposure]: 26 men, 134 blood samples-empirical----- Mixed model sensitivity:

Hormone serum concentrations	Fitted Geometric Means (95%CI)			Comparisons					
	Baseline (B_1)	Crossover (H)	Crossback (B_2)	Crossover % Change (95%CI) H-B_1	P value	Crossback % Change (95%CI) B_2-H	P value	Carryover % Change (95%CI) B_2-B_1	P value
Total Testosterone (nmol/L)	16.3 (14.4, 18.5)	15.6 (13.9, 17.6)	17.2 (15.3, 19.4)	-4.28 (-13.3, 5.63)	0.38	10.4 (1.94, 19.5)	0.02	5.64 (-1.99, 13.9)	0.15
Free Testosterone (pmol/L)	330 (296, 367)	322 (285, 364)	355 (318, 397)	-2.36 (-11.6, 7.87)	0.64	10.4 (-0.12, 22)	0.05	7.77 (-1.29, 17.7)	0.09
SHBG (nmol/L)	29.6 (24.2, 36.1)	29.4 (24.3, 35.6)	28.8 (23.88, 34.7)	-0.52 (-9.10, 8.80)	0.91	-2.14 (-11, 7.65)	0.66	-2.65 (-11.3, 6.88)	0.57
Inhibin-B (pg/mL)	162 (135, 195)	161 (135, 192)	173 (142, 209)	-0.89 (-6.13, 4.65)	0.75	7.28 (2.13, 12.7)	0.01	6.32 (0.57, 12.4)	0.03
LH (IU/L)	3.29 (2.86, 3.79)	2.83 (2.40, 3.34)	3.22 (2.78, 3.73)	-13.9 (-23.6, -3.03)	0.01	13.8 (3.11, 25.6)	0.01	-2.04 (-12, 9.00)	0.70
FSH (IU/L)	3.23 (2.57, 4.08)	3.05 (2.41, 3.85)	3.30 (2.57, 4.24)	-5.79 (-11.4, 0.13)	0.06	8.35 (1.12, 16.1)	0.02	2.08 (-5.55, 10.3)	0.60
Total Testosterone/LH Ratio	4.95 (4.09, 5.99)	5.50 (4.60, 6.58)	5.33 (4.39, 6.47)	11.1 (0.09, 23.4)	0.05	-3.17 (-12.4, 7.01)	0.53	7.62 (-5.35, 22.4)	0.26
Free Testosterone/LH Ratio	100 (84.1, 120)	114 (97.2, 133)	110 (91.9, 132)	13.4 (1.50, 26.8)	0.03	-3.04 (-13.5, 8.70)	0.59	9.98 (-3.17, 24.9)	0.14
Inhibin-B/FSH Ratio	50.0 (34.2, 73.2)	52.7 (36.3, 76.5)	52.3 (34.8, 78.7)	5.32 (-1.61, 12.7)	0.14	-0.68 (-8.3, 7.58)	0.87	4.61 (-5.39, 15.7)	0.38

Supplementary Table 2.5. (Continued)

H_1BH_2-arm[High (H_1)-Background (B) - High (H_2)] dibutyl phthalate exposure]: 47 men, 207 blood samples-empirical----- Mixed model sensitivity:									
Hormone serum concentrations	**Fitted Geometric Means (95%CI)**			**Comparisons**					
	Baseline (H_1)	**Crossover (B)**	**Crossback (H_2)**	**Crossover % Change (95%CI) B-H_1**	**P value**	**Crossback % Change (95%CI) H_2-B**	**P value**	**Carryover % Change (95%CI) H_2-H_1**	**P value**
Total Testosterone (nmol/L)	16.0 (14.4, 17.8)	15.9 (14.1, 17.9)	17.0 (15.6, 18.7)0	-0.58 (-7.63, 7.01)	0.88	7.13 (-1.39, 16.4)	0.10	6.5 (-2.70, 16.6)	0.17
Free Testosterone (pmol/L)	316 (285, 351)	324 (292, 360)	343 (317, 373)	2.52 (-5.17, 10.8)	0.53	5.90 (-1.74, 14.1)	0.13	8.57 (-1.69, 19.9)	0.10
SHBG (nmol/L)	31.7 (27.9, 36.1)	33.2 (29.3, 37.7)	31.7 (27.5, 36.5)	4.65 (-4.37, 14.5)	0.32	-4.50 (-10.7, 2.19)	0.18	-0.05 (-10.41, 11.5)	0.99
Inhibin-B (pg/mL)	171 (153, 191)	185 (169, 203)	200 (179, 222)	8.52 (2.13, 15.3)	0.009	7.71 (-0.11, 16.1)	0.05	16.9 (6.70, 28.0)	0.0009
LH (IU/L)	2.96 (2.64, 3.32)	2.66 (2.37, 2.99)	2.90 (2.59, 3.24)	-10.1 (-17.9, -1.55)	0.02	8.93 (0.08, 18.6)	0.05	-2.09 (-11.1, 7.80)	0.67
FSH (IU/L)	2.64 (2.28, 3.06)	2.42 (2.08, 2.81)	2.65 (2.29, 3.08)	-8.39 (-14.5, -1.88)	0.01	9.63 (3.85, 15.7)	0.001	0.43 (-5.43, 6.64)	0.89
Total Testosterone/LH Ratio	5.41 (4.80, 6.10)	6.01 (5.19, 6.95)	5.90 (5.21, 6.68)	11.0 (1.24, 21.7)	0.03	-1.80 (-11.6, 9.09)	0.73	9.01 (0.28, 18.5)	0.04
Free Testosterone/LH Ratio	107 (95.2, 120)	122 (107, 139)	118 (105, 134)	14.0 (2.85, 26.3)	0.01	-2.74 (-12.6, 8.17)	0.61	10.8 (1.45, 21.1)	0.02
Inhibin-B/FSH Ratio	64.7 (51.4, 81.4)	76.6 (62.3, 94.1)	75.2 (60.4, 93.6)	18.4 (8.60, 29.1)	0.0002	-1.80 (-11.19, 8.58)	0.72	16.3 (3.72, 30.4)	0.01

Supplementary Table 2.6. Unadjusted Mixed model: 3 arms

Supplementary Table 2.6.A: Unadjusted B_1HB_2-arm [Background (B_1)- High (H)- Background (B_2) dibutyl phthalate exposure]: 26 men, 134 blood samples

Hormone serum concentrations	Fitted Geometric Means (95%CI)			Comparisons					
	Baseline (B_1)	Crossover (H)	Crossback (B_2)	Crossover % Change (95%CI) H-B_1	P value	Crossback % Change (95%CI) B_2-H	P value	Carryover % Change (95%CI) B_2-B_1	P value
Total Testosterone (nmol/L)	16.7 (14.7, 19.0)	15.9 (13.9, 18.1)	17.6 (15.4, 20.1)	-4.98 (-13.1, 3.86)	0.26	10.8 (0.95, 21.6)	0.03	5.27 (-4.09, 15.6)	0.28
Free Testosterone (pmol/L)	330 (296, 368)	320 (287, 358)	352 (314, 395)	-2.97 (-11.4, 0)	0.52	9.8 (-0.19, 20.8)	0.05	6.56 (-3.14, 17.2)	0.19
SHBG (nmol/L)	31.3 (25.8, 38.1)	31.6 (25.9, 38.4)	31.6 (25.9, 38.6)	0.76 (-8.92, 11.5)	0.88	0.10 (-9.91, 11.2)	0.98	0.87 (-9.26, 12.1)	0.87
Inhibin-B (pg/mL)	167 (144, 194)	164 (141, 190)	175 (150, 203)	-2.15 (-8.54, 4.69)	0.53	6.66 (-0.60, 14.5)	0.07	4.37 (-2.76, 12.0)	0.24
LH (IU/L)	3.41 (2.96, 3.93)	2.9 (2.51, 3.36)	3.31 (2.85, 3.85)	-14.8 (-24.25, -4.25)	0.007	14.04 (0.88, 28.9)	0.04	-2.88 (-14.1, 9.78)	0.64
FSH (IU/L)	3.13 (2.55, 3.84)	2.91 (2.37, 3.58)	3.13 (2.54, 3.85)	-6.93 (-13.7, 0.41)	0.06	7.32 (-0.84, 16.2)	0.08	-0.12 (-7.76, 8.16)	0.98
Total Testosterone/LH Ratio	4.89 (4.14, 5.78)	5.45 (4.60, 6.46)	5.29 (4.45, 6.29)	11.4 (-0.55, 24.8)	0.06	-2.89 (-13.8, 9.36)	0.63	8.19 (-3.94, 21.9)	0.19
Free Testosterone/LH Ratio	97.0 (83.1, 113)	110 (94.3, 129)	106 (90.3, 125)	14.0 (0.04, 29.8)	0.05	-3.7 (-16.0, 10.4)	0.59	9.75 (-4.22, 25.8)	0.18
Inhibin-B/FSH Ratio	53.4 (39.0, 73.1)	56.0 (40.9, 76.8)	55.76 (40.6, 76.5)	4.94 (-4.48, 15.3)	0.31	-0.51 (-9.81, 9.73)	0.92	4.4 (-5.40, 15.2)	0.39

Supplementary Table 2.6.B: Unadjusted H1BH2-arm [High (H1)-Background (B) - High (H2)] dibutyl phthalate exposure-period< 3 Yrs]: 25 men, 107 blood samples

Hormone serum concentrations	Fitted Geometric Means (95%CI)			Comparisons					
	Baseline (H_1)	Crossover (B)	Crossback (H_2)	Crossover % Change (95%CI) $B-H_1$	P value	Crossback % Change (95%CI) H_2-B	P value	Carryover % Change (95%CI) H_2-H_1	P value
Total Testosterone (nmol/L)	16.0 (14.1, 18.3)	17.1 (14.8, 19.8)	17.8 (15.3, 20.6)	6.70 (-4.16, 18.8)	0.24	3.76 (-7.18, 16.0)	0.52	10.7 (-0.84, 23.6)	0.07
Free Testosterone (pmol/L)	322 (289, 360)	349 (308, 397)	365 (321, 416)	8.37 (-2.84, 20.9)	0.15	4.48 (-6.84, 17.2)	0.45	13.2 (1.22, 26.6)	0.03
SHBG (nmol/L)	30.4 (24.9, 37.0)	32.9 (26.6, 40.6)	30.7 (24.8, 38.1)	8.22 (-4.29, 22.4)	0.21	-6.41 (-17.5, 6.18)	0.3	1.28 (-10.7, 14.9)	0.84
Inhibin-B (pg/mL)	169 (145, 196)	191 (163, 224)	214 (183, 251)	13.2 (4.26, 22.9)	0.003	12.1 (3.06, 22.0)	0.008	27.0 (16.7, 38.1)	<0.0001
LH (IU/L)	3.09 (2.67, 3.57)	2.79 (2.36, 3.30)	3.02 (2.55, 3.58)	-9.62 (-21.5, 3.98)	0.16	8.20 (-6.6, 25.3)	0.29	-2.22 (-15.3, 12.9)	0.76
FSH (IU/L)	2.47 (2.01, 3.04)	2.22 (1.79, 2.76)	2.46 (1.98, 3.05)	-10.0 (-18.0, -1.27)	0.03	10.6 (0.58, 21.6)	0.04	-0.50 (-9.53, 9.43)	0.92
Total Testosterone/LH Ratio	5.19 (4.39, 6.14)	6.19 (5.13, 7.46)	5.94 (4.91, 7.18)	19.2 (3.91, 36.7)	0.01	-4.04 (-16.8, 10.6)	0.57	14.4 (-0.65, 31.6)	0.06
Free Testosterone/LH Ratio	104 (89.3, 122)	125 (104, 150)	121 (100, 145)	19.7 (2.45, 39.9)	0.02	-3.45 (-18.0, 13.7)	0.67	15.6 (-1.49, 35.6)	0.08
Inhibin-B/FSH Ratio	68.3 (49.6, 94.0)	86.0 (62.0, 119)	87.2 (62.8, 121)	26.0 (12.3, 41.4)	<0.0001	1.39 (-9.83, 14.0)	0.82	27.8 (13.52, 43.8)	<0.0001

Supplementary Table 2.6.C: Unadjusted H1BH2-arm[High (H1)-Background (B) - High (H2)] dibutyl phthalate exposure-period>= 3 Yrs]: 22 men, 100 blood samples

Hormone serum concentrations	Fitted Geometric Means (95%CI)			Comparisons					
	Baseline (H_1)	Crossover (B)	Crossback (H_2)	Crossover % Change (95%CI) $B-H_1$	P value	Crossback % Change (95%CI) H_2-B	P value	Carryover % Change (95%CI) H_2-H_1	P value
Testosterone_nmol_L	15.2 (13.2, 17.4)	14.2 (12.2, 16.4)	15.7 (13.5, 18.4)	-6.80 (-16.16, 3.61)	0.19	11.1 (-0.97, 24.7)	0.07	3.56 (-7.46, 15.9)	0.54
FT_pmol_L	312 (278, 351)	303 (266, 344)	324 (283, 371)	-3.08 (-13.0, 7.99)	0.57	7.04 (-4.90, 20.5)	0.26	3.75 (-7.50, 16.4)	0.53
SHBG_nmol_L	28.7 (23.2, 35.4)	29.4 (23.6, 36.6)	28.9 (23.1, 36.2)	2.46 (-9.18, 15.6)	0.69	-1.59 (-13.6, 12.1)	0.81	0.83 (-11.3, 14.6)	0.9
Inhibin-B_pg_mL	166 (142, 195)	173 (146, 204)	178 (151, 211)	4.02 (-4.06, 12.8)	0.34	3.14 (-5.48, 12.6)	0.49	7.28 (-1.55, 16.9)	0.11
LH (IU/L)	2.67 (2.29, 3.11)	2.39 (2.02, 2.83)	2.64 (2.22, 3.15)	-10.5 (-22.1, 2.85)	0.12	10.5 (-5.04, 28.6)	0.20	-1.07 (-14.6, 14.6)	0.89
FSH (IU/L)	2.97 (2.38, 3.71)	2.77 (2.21, 3.47)	3.01 (2.39, 3.78)	-6.76 (-14.86, 2.11)	0.13	8.67 (-1.48, 19.9)	0.10	1.32 (-8.01, 11.6)	0.79
Total Testosterone/LH Ratio	5.68 (4.75, 6.79)	5.92 (4.88, 7.18)	5.93 (4.86, 7.24)	4.21 (-8.96, 19.3)	0.55	0.24 (-13.5, 16.1)	0.97	4.46 (-9.51, 20.6)	0.55
Free Testosterone/LH Ratio	117 (99.0, 138)	126 (105, 152)	123 (101, 149)	8.26 (-7.22, 26.3)	0.31	-3.09 (-18.1, 14.7)	0.71	4.91 (-10.9, 23.6)	0.57
Inhibin-B/FSH Ratio	56.0 (39.8, 78.7)	62.3 (44.0, 88.0)	59.1 (41.7, 83.7)	11.2 (-0.64, 24.5)	0.06	-5.14 (-16.0, 7.10)	0.39	5.50 (-6.42, 18.9)	0.38

Supplementary Table 2.7. Adjusted Fixed model- 3 arms

Supplementary Table 2.7.A. Adjusted percent change (95%CI) of crossover, crossback and carryover on reproductive hormone parameters among men starting on mesalamine medication not containing dibutyl phthalate (B1HB2-arm)[a]

Adjusted B_1HB_2-arm [Background (B_1) -High (H)-Background (B_2) dibutyl phthalate exposure]: (26 men, 134 blood samples)							
	Means (95% CI)	Comparisons					
Hormone serum concentrations	Baseline (B_1)	Crossover % Change (95%CI) $H-B_1$	P value	Crossback % Change (95%CI) B_2-H	P value	Carryover % Change (95%CI) B_2-B_1	P value
Total Testosterone (nmol/L)	17.0 (14.5, 19.9)	-3.79 (-12.3, 5.51)	0.41	11.3 (1.11, 22.4)	0.03	7.03 (-3.24, 18.4)	0.19
Free Testosterone (pmol/L)	348.42 (304, 400)	-1.99 (-10.9, 7.75)	0.68	11.4 (0.97, 22.9)	0.03	9.17 (-1.57, 21.1)	0.10
SHBG (nmol/L)	29.0 (23.4, 36.0)	-0.07 (-9.95, 10.9)	0.99	-1.75 (-11.8, 9.44)	0.75	-1.82 (-12.38, 10.0)	0.75
Inhibin-B (pg/mL)	162 (133, 196)	-0.90 (-7.56, 6.24)	0.80	7.73 (0.24, 15.8)	0.04	6.76 (-1.06, 15.2)	0.09
LH (IU/L)	3.43 (2.87, 4.10)	-13.47 (-23.4, -2.25)	0.02	14.6 (0.96, 30.0)	0.04	-0.88 (-13.24, 13.3)	0.9
FSH (IU/L)	3.07 (2.36, 4.00)	-5.59 (-12.7, 2.09)	0.15	8.23 (-0.19, 17.4)	0.06	2.18 (-6.19, 11.3)	0.62
Total Testosterone/LH Ratio	4.97 (4.04, 6.10)	11.2 (-1.2, 25.1)	0.08	-2.88 (-14.07, 9.76)	0.64	7.98 (-5.10, 22.9)	0.24
Free Testosterone/LH Ratio	102 (83.8, 123)	13.3 (-1.11, 29.7)	0.07	-2.76 (-15.52, 11.9)	0.70	10.1 (-5.05, 27.8)	0.20
Inhibin-B/FSH Ratio	52.5 (34.8, 79.2)	4.97 (-4.75, 15.7)	0.33	-0.46 (-9.99, 10.1)	0.93	1.04 (-6.04, 16.2)	0.42

Supplementary Table 2.7.B. Adjusted percent change (95%CI) of crossover, crossback and carryover on reproductive hormone parameters among men starting on mesalamine medication containing dibutyl phthalate (H_1BH_2-arm)[a]

Adjusted H1BH2-arm[High (H1)-Background (B) - High (H2)] dibutyl phthalate exposure-period< 3 Yrs]: (25 men, 107 blood samples)							
	Means (95% CI)	Comparisons					
Hormone serum concentrations	Baseline (H_1)	Crossover % Change (95%CI) B-H_1	P value	Crossback % Change (95%CI) H_2-B	P value	Carryover % Change (95%CI) H_2-H_1	P value
Total Testosterone (nmol/L)	16.6 (14.4, 19.1)	7.37 (-3.86, 19.9)	0.21	2.56 (-8.45, 14.9)	0.66	10.1 (-1.73, 23.4)	0.1
Free Testosterone (pmol/L)	332 (294, 376)	8.27 (-3.34, 21.3)	0.17	3.86 (-7.58, 16.7)	0.52	12.4 (0.04, 26.4)	0.05
SHBG (nmol/L)	30.8 (25.4, 37.3)	9.32 (-3.49, 23.8)	0.161	-6.42 (-17.7, 6.39)	0.31	2.30 (-10.03, 16.3)	0.73
Inhibin-B (pg/mL)	166 (140, 197)	13.5 (4.46, 23.4)	0.00	11.0 (1.88, 20.9)	0.02	26.01 (15.64, 37.3)	<0.0001
LH (IU/L)	3.13 (2.67, 3.67)	-12.01 (-24.0, 1.82)	0.086	6.96 (-7.95, 24.3)	0.38	-5.88 (-19.03, 9.39)	0.43
FSH (IU/L)	2.49 (1.97, 3.15)	-10.2 (-18.3, -1.43)	0.024	9.91 (-0.18, 21.0)	0.05	-1.34 (-10.41, 8.65)	0.783
Total Testosterone/LH Ratio	5.31 (4.42, 6.38)	22.0 (5.93, 40.6)	0.01	-4.11 (-17.09, 10.9)	0.57	17.0 (1.14, 35.4)	0.035
Free Testosterone/LH Ratio	106 (89.523, 126)	23.0 (4.59, 44.7)	0.01	-2.9 (-17.8, 14.8)	0.73	19.5 (1.05, 41.3)	0.037
Inhibin-B/FSH Ratio	66.6 (46.3, 95.8)	26.5 (12.6, 42.1)	<0.0001	0.99 (-10.4, 13.8)	0.87	27.7 (13.3, 44.0)	<0.0001

Supplementary Table 2.7.C. Adjusted percent change (95%CI) of crossover, crossback and carryover on reproductive hormone parameters among men starting on mesalamine medication containing dibutyl phthalate (H_1BH_2-arm)[a]

Adjusted H1BH2-arm[High (H1)-Background (B) - High (H2)] dibutyl phthalate exposure-period>= 3 Yrs]:22 men, 100 blood samples							
Hormone serum concentrations	**Means (95% CI)**	**Comparisons**					
	Baseline (H_1)	**Crossover % Change (95%CI) B-H_1**	**P value**	**Crossback % Change (95%CI) H_2-B**	**P value**	**Carryover % Change (95%CI) H_2-H_1**	**P value**
Testosterone_nmol_L	14.5 (11.49, 18.3)	-7.03 (-16.6, 3.60)	0.19	12.6 (0.18, 26.5)	0.047	4.68 (-6.8, 17.6)	0.44
FT_pmol_L	278 (228, 339)	-3.19 (-13.4, 8.19)	0.57	8.3 (-3.93, 22.1)	0.19	4.85 (-6.93, 18.1)	0.43
SHBG_nmol_L	33.6 (24.48, 46.2)	2.46 (-9.32, 15.8)	0.695	-2.1 (-14.2, 11.7)	0.752	0.31 (-12.0, 14.4)	0.96
Inhibin-B_pg_mL	178 (134, 237)	3.76 (-4.38, 12.6)	0.37	3.99 (-4.78, 13.6)	0.38	7.89 (-1.15, 17.8)	0.09
LH (IU/L)	2.62 (2.03, 3.39)	-10.54 (-22.5, 3.21)	0.126	12.3 (-3.75, 31.0)	0.14	0.46 (-13.83, 17.1)	0.95
FSH (IU/L)	3.03 (2.05, 4.47)	-6.54 (-14.7, 2.44)	0.15	9.44 (-0.87, 20.8)	0.07	2.29 (-7.30, 12.9)	0.65
Total Testosterone/LH Ratio	5.50 (4.07, 7.44)	3.93 (-9.52, 19.4)	0.58	0.257 (-13.7, 16.4)	0.97	4.20 (-10.2, 20.9)	0.59
Free Testosterone/LH Ratio	106 (80.3, 140)	8.23 (-7.70, 26.9)	0.33	-3.56 (-18.8, 14.5)	0.68	4.37 (-12.0, 23.8)	0.62
Inhibin-B/FSH Ratio	58.8 (32.0, 108)	11.0 (-0.94, 24.4)	0.07	-4.98 (-16.0, 7.44)	0.41	5.48 (-6.66, 19.2)	0.39

Supplementary Table 2.8. Unadjusted Fixed model- 3 arms

Supplementary Table 2.8.A. Unadjusted percent change (95%CI) of crossover, crossback and carryover on reproductive hormone parameters among men starting on mesalamine medication not containing dibutyl phthalate (B1HB2-arm)[a]

B_1HB_2-arm [Background (B_1) -High (H)-Background (B_2) dibutyl phthalate exposure]: (26 men, 134 blood samples)							
Hormone serum concentrations	**Means (95% CI)**	**Comparisons**					
	Baseline (B_1)	**Crossover % Change (95%CI)**	**P value**	**Crossback % Change (95%CI)**	**P value**	**Carryover % Change (95%CI)**	**P value**
		H-B_1		**B_2-H**		**B_2-B_1**	
Total Testosterone (nmol/L)	16.7 (14.7, 19.0)	-5.00 (-13.2, 3.93)	0.26	10.6 (0.72, 21.4)	0.03	5.06 (-4.37, 15.4)	0.30
Free Testosterone (pmol/L)	330 (296, 368)	-3.22 (-11.74, 6.13)	0.49	10.0 (-0.06, 21.1)	0.05	6.47 (-3.34, 17.3)	0.20
SHBG (nmol/L)	31.3 (25.8, 38.1)	1.08 (-8.67, 11.9)	0.83	-0.10 (-10.1, 11.0)	0.99	0.98 (-9.20, 12.3)	0.86
Inhibin-B (pg/mL)	167 (144, 194)	-2.25 (-8.67, 4.62)	0.51	6.79 (-0.50, 14.6)	0.07	4.39 (-2.78, 12.1)	0.24
LH (IU/L)	3.41 (2.96, 3.93)	-14.4 (-24.0, -3.63)	0.01	14.1 (0.91, 29.1)	0.04	-2.29 (-13.7, 10.6)	0.71
FSH (IU/L)	3.13 (2.55, 3.84)	-6.59 (-13.4, 0.80)	0.08	7.23 (-0.94, 16.1)	0.08	0.16 (-7.52, 8.48)	0.97
Total Testosterone/LH Ratio	4.89 (4.14, 5.78)	11.0 (-1.04, 24.5)	0.07	-3.12 (-14.0, 9.16)	0.60	7.52 (-4.65, 21.3)	0.24
Free Testosterone/LH Ratio	97.0 (83.1, 113)	13.1 (-0.9, 29.0)	0.07	-3.62 (-16.0, 10.6)	0.60	8.96 (-5.10, 25.1)	0.22
Inhibin-B/FSH Ratio	53.4 (39.0, 73.1)	4.64 (-4.77, 15.0)	0.34	-0.41 (-9.72, 9.86)	0.93	1.04 (-5.59, 15.0)	0.41

Supplementary Table 2.8.B. Unadjusted percent change (95%CI) of crossover, crossback and carryover on reproductive hormone parameters among men starting on mesalamine medication containing dibutyl phthalate (H1BH2-arm)[a]

H1BH2-arm[High (H1)-Background (B) - High (H2)] dibutyl phthalate exposure-period< 3 Yrs]: (25 men, 107 blood samples)							
Hormone serum concentrations	**Means (95% CI)**	**Comparisons**					
	Baseline (H_1)	**Crossover % Change (95%CI) B-H_1**	**P value**	**Crossback % Change (95%CI) H_2-B**	**P value**	**Carryover % Change (95%CI) H_2-H_1**	**P value**
Total Testosterone (nmol/L)	16.0 (14.1, 18.3)	7.06 (-4.10, 19.5)	0.22	3.63 (-7.33, 15.9)	0.53	10.95 (-0.91, 24.2)	**0.07**
Free Testosterone (pmol/L)	322 (289, 360)	8.22 (-3.35, 21.2)	0.17	4.50 (-6.84, 17.2)	0.45	13.09 (0.69, 27.0)	0.04
SHBG (nmol/L)	30.4 (24.9, 37,0)	9.14 (-3.63, 23.6)	0.17	-6.62 (-17.7, 5.96)	0.29	1.92 (-10.3, 15.8)	0.77
Inhibin-B (pg/mL)	169 (145, 196)	13.3 (4.22, 23.1)	0.004	12.1 (3.00, 22.0)	0.008	27.0 (16.6, 38.3)	<0.0001
LH (IU/L)	3.09 (2.67, 3.57)	-12.3 (-24.1, 1.44)	0.08	8.01 (-6.80, 25.2)	0.31	-5.24 (-18.4, 9.99)	0.48
FSH (IU/L)	2.47 (2.01, 3.04)	-10.3 (-18.3, -1.54)	0.02	10.6 (0.57, 21.6)	0.04	-0.83 (-9.89, 9.14)	0.86
Total Testosterone/LH Ratio	5.19 (4.39, 6.14)	22.0 (6.04, 40.5)	0.006	-4.06 (-16.8, 10.7)	0.57	17.1 (1.36, 35.3)	0.03
Free Testosterone/LH Ratio	104 (89.3, 122)	23.4 (4.95, 45.0)	0.01	-3.25 (-17.9, 14.0)	0.69	19.4 (1.10, 40.9)	0.04
Inhibin-B/FSH Ratio	68.3 (49.6, 94.0)	26.3 (12.5, 41.8)	<0.0001	1.37 (-9.86, 14.0)	0.82	28.0 (13.7, 44.2)	<0.0001

Supplementary Table 2.8.C. Unadjusted percent change (95%CI) of crossover, crossback and carryover on reproductive hormone parameters among men starting on mesalamine medication containing dibutyl phthalate (H1BH2-arm)[a]

H1BH2-arm[High (H1)-Background (B) - High (H2)] dibutyl phthalate exposure-period>= 3 Yrs]: (22 men, 100 blood samples)							
Hormone serum concentrations	**Means (95% CI)**	**Comparisons**					
	Baseline (H_1)	**Crossover % Change (95%CI) B-H_1**	**P value**	**Crossback % Change (95%CI) H_2-B**	**P value**	**Carryover % Change (95%CI) H_2-H_1**	**P value**
Testosterone_nmol_L	15.2 (13.2, 17.4)	-7.22 (-16.7, 3.33)	0.17	11.6 (-0.57, 25.3)	0.06	3.57 (-7.64, 16.1)	0.55
FT_pmol_L	312 (278, 351)	-3.02 (-13.2, 8.33)	0.59	7.28 (-4.75, 20.8)	0.25	4.04 (-7.51, 17.0)	0.51
SHBG_nmol_L	28.7 (23.2, 35.4)	1.97 (-9.71, 15.2)	0.75	-1.16 (-13.28, 12.7)	0.86	0.79 (-11.5, 14.7)	0.91
Inhibin-B_pg_mL	166 (142, 195)	3.66 (-4.44, 12.5)	0.39	2.99 (-5.64, 12.4)	0.51	6.76 (-2.10, 16.4)	0.14
LH (IU/L)	2.67 (2.29, 3.11)	-10.8 (-22.6, 2.83)	0.12	11.5 (-4.27, 29.9)	0.16	-0.51 (-14.5, 15.7)	0.95
FSH (IU/L)	2.97 (2.38, 3.71)	-6.46 (-14.6, 2.47)	0.15	8.58 (-1.56, 19.8)	0.10	1.56 (-7.84, 11.9)	0.75
Total Testosterone/LH Ratio	5.68 (4.75, 6.79)	3.99 (-9.36, 19.3)	0.58	0.11 (-13.6, 16.1)	0.99	4.11 (-10.1, 20.5)	0.59
Free Testosterone/LH Ratio	117 (99.0, 138)	8.71 (-7.19, 27.3)	0.30	-3.80 (-18.8, 14.0)	0.65	4.58 (-11.6, 23.7)	0.60
Inhibin-B/FSH Ratio	56.0 (39.8, 78.7)	10.8 (-1.02, 24.1)	0.08	-5.15 (-160, 7.10)	0.39	5.12 (-6.79, 18.6)	0.41

Supplementary Table 2.9. Empirical Adjusted 3 arms analyses

Adjusted B_1HB_2-arm [Background (B_1)- High (H)- Background (B_2) dibutyl phthalate exposure]: 26 men, 132 blood samples

Hormone serum concentrations	Fitted Means (95%CI)			Comparisons					
	Baseline (B_1)	Crossover (H)	Crossback (B_2)	Crossover effect (95%CI) $H-B_1$	P value	Crossback effect (95%CI) B_2-H	P value	Carryover effect (95%CI) B_2-B_1	P value
Total Testosterone (nmol/L)	17.0 (14.7, 19.8)	16.3 (14.1, 18.8)	18.0 (15.6, 20.8)	-4.18 (-13.2, 5.77)	0.4	10.4 (2.02, 19.5)	0.01	5.80 (-1.92, 14.1)	0.14
Free Testosterone (pmol/L)	348 (309, 393)	340 (298, 389)	376 (332, 425)	-2.33 (-11.6, 0)	0.64	10.4 (-0.12, 22.0)	0.05	7.79 (-1.31, 17.7)	0.10
LNSHBG_nmol_L	29.0 (22.8, 36.8)	28.9 (22.8, 36.5)	28.2 (22.5, 35.5)	-0.49 (-9.1, 8.93)	0.91	-2.12 (-11.0, 7.68)	0.66	-2.6 (-11.4, 7.07)	0.58
Inhibin-B (pg/mL)	162 (133, 197)	160 (132, 194)	171 (139, 210)	-1.19 (-6.37, 4.28)	0.66	7.23 (2.15, 12.6)	0.005	5.95 (0.26, 12.0)	0.04
LH (IU/L)	3.43 (2.92, 4.03)	2.96 (2.47, 3.54)	3.37 (2.85, 3.98)	-13.8 (-23.5, -2.90)	0.01	13.8 (3.16, 25.6)	0.01	-1.9 (-11.8, 9.12)	0.72
FSH (IU/L)	3.07 (2.32, 4.07)	2.89 (2.17, 3.85)	3.14 (2.32, 4.24)	-5.76 (-11.4, 0.18)	0.06	8.36 (1.13, 16.1)	0.02	2.11 (-5.55, 10.4)	0.6
Total Testosterone/LH Ratio	4.97 (4.08, 6.04)	5.52 (4.59, 6.63)	5.34 (4.38, 6.52)	11.1 (0.04, 23.4)	0.05	-3.14 (-12.4, 7.05)	0.53	7.61 (-5.35, 22.3)	0.26
Free Testosterone/LH Ratio	102 (84.7, 122)	115 (97.7, 135)	112 (92.5, 135)	13.3 (1.38, 26.7)	0.03	-3.04 (-13.5, 8.70)	0.59	9.87 (-3.25, 24.8)	0.15
Inhibin-B/FSH Ratio	52.5 (34, 81.2)	55.2 (35.8, 85.0)	54.74 (34.4, 87.2)	4.96 (-2.01, 12.4)	0.17	-0.74 (-8.31, 7.46)	0.85	4.18 (-5.79, 15.2)	0.42

Supplementary Table 2.9. (Continued)

Adjusted H1BH2-arm[High (H1)-Background (B) - High (H2)] dibutyl phthalate exposure-period<3yrs]: 25 men, 107 blood samples									
Hormone serum concentrations	Fitted Means (95%CI)			Comparisons					
	Baseline (H_1)	Crossover (B)	Crossback (H_2)	Crossover Effect (95%CI) $B-H_1$	P value	Crossback Effect (95%CI) H_2-B	P value	Carryover Effect (95%CI) H_2-H_1	P value
Total Testosterone (nmol/L)	16.6 (14.3, 19.2)	17.6 (15.1, 20.6)	18.2 (15.9, 20.7)	6.24 (-5.2, 19.1)	0.3	2.89 (-10.7, 18.5)	0.69	9.32 (-7.05, 28.6)	0.28
Free Testosterone (pmol/L)	332 (285, 388)	361 (313, 415)	375 (333, 423)	8.53 (-4.24, 23.0)	0.20	3.96 (-7.51, 16.9)	0.51	12.8 (-5.06, 34.1)	0.17
LNSHBG_nmol_L	30.8 (25.3, 37.4)	32.9 (27.0, 40.0)	30.9 (25.0, 38.2)	6.86 (-8.80, 25.2)	0.41	-5.94 (-15.1, 4.18)	0.24	0.51 (-17.0, 21.7)	0.96
Inhibin-B (pg/mL)	166 (142, 193)	187 (164, 215)	209 (176, 247)	13.2 (3.24, 24.1)	0.009	11.3 (-3.06, 27.8)	0.13	26.0 (8.01, 47.0)	0.003
LH (IU/L)	3.13 (2.67, 3.67)	2.84 (2.39, 3.36)	3.03 (2.59, 3.56)	-9.42 (-21.1, 3.93)	0.16	7.00 (-5.06, 20.6)	0.27	-3.08 (-17.6, 14.1)	0.71
FSH (IU/L)	2.49 (2.01, 3.08)	2.24 (1.78, 2.82)	2.46 (1.96, 3.09)	-9.90 (-20.5, 2.14)	0.10	9.74 (0.27, 20.1)	0.04	-1.12 (-11.6, 10.6)	0.84
Total Testosterone/LH Ratio	5.31 (4.47, 6.30)	6.28 (5.00, 7.89)	6.04 (5.02, 7.27)	18.4 (2.83, 36.2)	0.02	-3.84 (-18.3, 13.2)	0.64	13.81 (-0.44, 30.1)	0.06
Free Testosterone/LH Ratio	106 (90.2, 125)	127 (104, 156)	123 (105, 145)	19.6 (2.41, 39.7)	0.02	-2.83 (-16.5, 13.1)	0.71	16.2 (2.84, 31.4)	0.02
Inhibin-B/FSH Ratio	66.6 (47.9, 92.4)	83.9 (61.0, 115)	84.9 (60.6, 119)	26.0 (10.1, 44.2)	0.0008	1.21 (-15.8, 21.6)	0.90	27.5 (4.92, 55.0)	0.01

Supplementary Table 2.9. (Continued)

Adjusted H1BH2-arm[High (H1)-Background (B) - High (H2)] dibutyl phthalate exposure-period>=3yrs]: 22 men, 102 blood samples									
Hormone serum concentrations	Fitted Means (95%CI)			Comparisons					
	Baseline (H_1)	Crossover (B)	Crossback (H_2)	Crossover Effect (95%CI) B-H_1	P value	Crossback Effect (95%CI) H_2-B	P value	Carryover Effect (95%CI) H_2-H_1	P value
Testosterone_nmol_L	14.5 (11.0, 19.1)	13.5 (10.0, 18.2)	15.1 (11.6, 19.7)	-6.64 (-13.4, 0.60)	0.07	11.5 (1.67, 22.4)	0.02	4.13 (-3.89, 12.8)	0.32
FT_pmol_L	278 (231, 334)	270 (217, 336)	291 (244, 347)	-2.78 (-10.3, 5.34)	0.49	7.69 (-2.49, 18.9)	0.14	4.69 (-5.28, 15.7)	0.37
SHBG_nmol_L	33.6 (23.7, 47.7)	34.5 (24.1, 49.4)	33.5 (22.6, 49.5)	2.53 (-6.19, 12.1)	0.58	-2.97 (-11.8, 6.74)	0.53	-0.52 (-11.4, 11.7)	0.93
InhibinB_pg_mL	178 (140, 227)	186 (149, 231)	193 (154, 242)	4.12 (-3.25, 12.1)	0.28	3.85 (-0.09, 7.96)	0.06	8.13 (1.54, 15.2)	0.02
LH (IU/L)	2.62 (1.94, 3.56)	2.35 (1.77, 3.12)	2.60 (1.93, 3.51)	-10.6 (-20.9, 1.14)	0.08	10.9 (-1.6, 25.0)	0.09	-0.80 (-10.6, 10.1)	0.88
FSH (IU/L)	3.03 (1.90, 4.81)	2.82 (1.80, 4.40)	3.09 (1.97, 4.83)	-6.96 (-12.3, -1.32)	0.02	9.58 (2.79, 16.8)	0.01	1.95 (-3.04, 7.20)	0.45
Total Testosterone/LH Ratio	5.50 (4.40, 6.88)	5.74 (4.59, 7.17)	5.75 (4.65, 7.11)	4.34 (-5.86, 15.6)	0.42	0.21 (-12.3, 14.5)	0.97	4.56 (-4.53, 14.5)	0.34
Free Testosterone/LH Ratio	106 (84.7, 133)	115 (92.8, 143)	112 (87.7, 143)	8.70 (-4.05, 23.2)	0.19	-2.84 (-16.3, 12.8)	0.71	5.62 (-6.57, 19.4)	0.38
Inhibin-B/FSH Ratio	58.8 (30.9, 112)	65.5 (36.6, 117)	62.2 (34.2, 113)	11.5 (1.00, 23.1)	0.03	-5.11 (-12.0, 2.31)	0.17	5.80 (-3.54, 16.1)	0.23

Chapter 3

Personal care product use in men predicts urinary concentrations of select phthalate metabolites and parabens: recommendations to improve exposure assessment

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Short running title: Personal care product use in men, phthalates and parabens

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Abstract

Background: Personal care products (PCPs) are exposure sources to phthalates and parabens, however their contribution to men's exposure is understudied.

Objectives: We examined the association between PCP-use and urinary concentrations of phthalate metabolites and parabens in men.

Methods: In a prospective cohort, at multiple study visits, men self-reported their use of 14 PCPs and provided a urine sample (2004-2015, Boston, MA). We measured urinary concentrations of nine phthalate metabolites and methylparaben, propylparaben, and butylparaben. We estimated the covariate-adjusted percent change in urinary concentrations associated with PCP-use using linear mixed and Tobit mixed regressions. We also estimated weights for each PCP in a weighted binary score regression and modeled the resulting composite weighted PCP-use.

Results: 400 men contributed 1,037 urine samples (mean of 3 /man). The largest percent increase in monoethyl phthalate (MEP) was associated with use of cologne/perfume (83%, p-value<0.01) and deodorant (74%, p-value<0.01). In contrast, the largest percent increase for parabens was associated with the use of suntan/sunblock lotion (66-156%) and hand/body lotion (79-147%). Increases in MEP and parabens were generally greater with PCP-use within six hours of urine collection. A subset of ten PCPs that were used within six hours of urine collection contributed to at least 70% of the weighted score and predicted a 254% to 1,333% increase in MEP and parabens concentrations. Associations between PCP-use and concentrations of the other phthalate metabolites were not statistically significant.

Conclusions: We identified ten PCPs of relevance and demonstrated that their use within six hours of urine collection strongly predicted MEP and paraben urinary concentrations.

Introduction

There is ubiquitous general population exposure to ortho-phthalates (hereto referred to as phthalates) and several parabens (2). Phthalates are a family of chemicals commonly used as plasticizers in polyvinyl chloride plastics and in consumer products, including personal care products (PCPs), medications, and food processing and packaging materials (2). Diethyl phthalate (DEP) is the most commonly used phthalate in PCPs (67). Parabens are a family of chemicals with antimicrobial preservative properties that are also widely used in PCPs, pharmaceuticals, food, and beverages to increase the shelf life of the product (59-61,146). Methylparaben, propylparaben and butylparaben are most commonly used in PCPs (59,68,69).

In multiple urine samples collected from two men and three women, Koch et al. (147) found that concentrations of the metabolites of low molecular weight phthalates such as DEP, di-n-butyl phthalate (DnBP), and di-isobutyl phthalate (DiBP) had a cyclical pattern of rise and decline suggestive of ongoing repeated non-food exposures. Koch et al (147) also found that monoethyl phthalate (MEP), metabolite of DEP, concentrations increased following showers which suggested PCPs as a major source of DEP exposure. Koch et al (148) also found that urinary concentrations of the parabens were lower when the participants were given products without parabens.

Exposure to phthalates and parabens from PCP-use occurs through direct dermal application (62,63), inhalation, oral ingestion or even transdermal exposure from air (64). Phthalates and parabens have short biological half-lives (6-24 hours) (62,65,149), and urinary concentrations of phthalate metabolites and parabens are the preferred exposure biomarkers (2,66).

Certain phthalates and parabens are endocrine disruptors and have been linked to adverse health outcomes (150-155). Identifying the most important sources of phthalate and paraben exposure is of paramount importance given the widespread use and potential health effects of these chemicals. Prior

epidemiological studies on the contribution of PCP-use to exposure to phthalates and parabens focused primarily on women and children (68,69,76-82). However, there have been limited studies in men (68,83). Given the differences in type and frequency of PCP-use across genders, better understanding of the specific PCPs contributing to urinary phthalate metabolite and paraben concentrations in men is necessary.

Given this gap in knowledge on the contribution of PCP-use to exposure to phthalates and parabens in men, we explored the association between self-reported use of 14 PCPs and urinary concentrations of nine urinary phthalate metabolites and three parabens in men recruited as part of a prospective cohort study. Because of the known use of DEP, DiBP and parabens in PCPs (32,59), we focused on MEP, mono-isobutyl phthalate (MiBP), a metabolite of DiBP, methylparaben, propylparaben and butylparaben. We considered mono-n-butyl phthalate (MnBP), a metabolite of DnBP, but because DnBP is infrequently used in PCPs (67) it was not a focus of our analysis.

Methods

Participants

The Environment And Reproductive Health (EARTH) Study (2004-present), a prospective cohort, enrolled couples seeking fertility treatment at the Massachusetts General Hospital (MGH) Fertility Center to identify determinants of fertility (69,156). Among male partners who were 18–55 years of age, approximately 50% agreed to participate. Male participants were followed from study enrollment until their partner had a live birth or the couple discontinued treatment at MGH. Approximately 30% of EARTH men had a primary diagnosis of male factor infertility (156). In the current analysis, men were eligible if they provided at least one urine sample and completed the PCP questionnaire (Supplementary material) between December 2004 and June 2015. All men provided informed consent. The EARTH

study was approved by institutional review boards at MGH, the Harvard T.H. Chan School of Public Health, and the Centers for Disease Control and Prevention (CDC).

Personal care product (PCP) use questionnaire

Upon enrollment, men completed questionnaires that collected information on demographics, lifestyle and health information and a research nurse measured their height and weight. At recruitment and each subsequent visit, men completed a questionnaire on PCP-use within the past 24-hours and at what time they last used each PCP prior to the collection of each urine sample. There were questions for the use of 16 PCPs but in the analysis we excluded toothpaste (used in 98% of men) and nail polish (<1% use). Therefore, we included deodorants, shampoo, conditioner/crème rinse, hairspray/hair gel, combined other hair care products (including mousse, hair bleach, relaxer, perm and straightener), shaving cream, aftershave, cologne/perfume, mouthwash, bar soap, liquid soap/body wash, hand sanitizer, hand/body lotion and suntan/sunblock lotion.

Based on our previous publication that showed higher urinary concentrations of MEP following PCP-use in men from the same fertility clinic population (83) and another study that showed higher urine concentrations following dermal exposure through lotion application (139),we *a priori* decided to explore both a six hour and a 24 hour time window for PCP-use before the urine collection. Furthermore, in women from the same fertility clinic population, we reported higher urinary biomarker concentrations when the product was used within six hours prior to urine sample collection (69).

Urinary measurements of phthalate metabolite and paraben concentrations

At each visit, men collected urine in a sterile polypropylene cup using standard procedures. Study staff recorded time of collection and measured specific gravity (SG) using a handheld refractometer (National

Instrument Co. Inc., Baltimore, MD). Urine samples were divided into aliquots, frozen, and stored at –80°C before shipment on dry ice to the CDC. Briefly, the analytical technique for quantification of the urinary biomarkers involved enzymatic deconjugation of the urinary metabolites, followed by solid-phase extraction, separation by high-performance liquid chromatography, and detection by isotope-dilution tandem mass spectrometry.

As previously described (157,158), the CDC used solid phase extraction-high performance liquid chromatography-isotope dilution tandem mass spectrometry to quantify total (free plus conjugated) urinary concentrations (μg/L) of three parabens (methylparaben, propylparaben and butylparaben) and nine phthalate metabolites: MEP, MnBP, MiBP, monobenzyl phthalate (MBzP) metabolite of butylbenzyl phthalate (BBzP), mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP) metabolites of di(2-ethylhexyl) phthalate (DEHP), and mono(3-carboxypropyl) phthalate (MCPP), non-specific metabolite of DnBP, di-n-octyl phthalate (DOP) and other high molecular weight phthalates. We also calculated the molar sum of DEHP metabolites (∑DEHP) (69). The limits of detection (LOD) ranged from 0.10 (propylparaben, butylparaben) to 1.20 μg/L (MEHP). Concentrations below the LOD were replaced by LOD/√2 for all biomarkers detected in at least 90% of the samples (159,160).

Statistical analysis

We calculated descriptive statistics for participants' baseline characteristics, e.g., age, race, education, BMI, and smoking and time-varying characteristics, e.g., time of the day, season, and calendar year of the sample collection and personal care product use patterns. Because of the relatively short half-lives of phthalates and parabens (62,65,149) and previous publications, we defined PCP-use within the last 24 and 6 hours before urine collection. We used two approaches for assessing men's use of PCPs. The first

approach (single-PCP analyses) defined use of each PCP as a binary variable (yes/no use within 24 hours preceding urine collection). Because multiple PCPs contributed to the overall body burden of phthalates and parabens, in our second approach (multi-PCP analyses) we used a composite weighted PCP-score (continuous variable) as explained below.

The nine urinary concentrations of phthalate metabolites, ∑DEHP, and three parabens were modeled as continuous variables. We examined distributions for the urinary concentrations, and due to observed skewness we used a natural log-transformation to satisfy regression model assumptions. We examined the Spearman correlations among the concentrations of phthalate metabolites and parabens.

Covariate adjustment

Selection of covariates was based a priori on directed acyclic graphs and previous literature (69,83,161). The final model included urine specific gravity (continuous) (162) to account for urine dilution, race (Caucasian or not), age (continuous), body mass index (BMI) (continuous), calendar year (continuous) as it has been shown that urinary concentrations of several phthalate metabolites have changed over time in the United States (U.S.) (163), three categories for time of urine sample collection (early morning (between 5am to 9am), late morning (between 9:01am to 12pm) or afternoon (after 12:01pm)), current smoking (yes/no) and warm season (April through September) (yes/no) to account for seasonality in Boston with higher use of certain products, specifically sunscreen, in the summer.

Multiple imputations of the data

Some men had incomplete PCP-use data across their repeated visits and two men were missing BMI. The missingness for each PCP question ranged from 5 (for shampoo) to 339 (for hand/body lotion) (Table 3.2). Distributions of the urinary concentrations of phthalate metabolites and parabens were not

statistically different when comparing the complete and incomplete PCP-use data suggesting that missingness was not related to our outcomes and would not likely impact our findings.. We investigated missingness patterns for PCPs-use and the concordance of PCP-use within the same man over repeated visits. We used multiple imputation to account for the missing data, using chained equations (MICE) (164,165) for imputing missing PCP-use data and covariates (two men missing BMI). For any given PCP at any time point, the predictors were the same PCP used at other time points, urine specific gravity (162), race, age, BMI, calendar year, time of sample collection, current smoking and season. We generated 10 imputed datasets for each analysis and used these imputed datasets in the main analysis using both of the analytical approaches described below using proc mianalyze.

Regression models analysis

(1) Single-PCP Analyses

We first regressed each biomarker (and ∑DEHP) concentration on a single-PCP and covariates in separate linear mixed effects models (LMEM) with a random intercept to account for within-person correlation among multiple longitudinal measures of a given biomarker. Because MEHP and butylparaben had >10% non-detectable concentrations, we used mixed Tobit regression (166) models with random intercepts for left-censored data (160). For MEHP and butylparaben, we took into account LODs from different analytic batches over the study period by specifying a unique LOD for each sample collection within the Tobit regression. In addition, we regressed all biomarkers on a continuous variable for total sum (0 to 14) of the PCPs used by each man at each visit.

We created heatmaps for better visualization of the estimated percent change in the urinary concentrations associated with each PCP-use based on LMEM (or Tobit models for MEHP and butylparaben). We adjusted for the covariates mentioned above in all models. We considered two-sided alpha <0.05 as statistically significant and indicated significance as stars in heatmaps.

We further explored the association between the PCP-use within six hours before urine collection (yes/no) for five of the urinary concentrations that were most likely to have PCP-use as sources of their parent compound i.e., MEP (for DEP), MiBP (for DiBP), methylparaben, propylparaben and butylparaben (32,59,77,79).

(2) Multi-PCP Analyses

To analyze the simultaneous impact of the 14 PCPs on a given biomarker concentration, we modified the weighted quantile score (WQS) regression model previously used for quantifying the impact of environmental mixtures on an outcome (167-171). Due to the high dimensionality and inherent correlations among the use of different PCPs as well as correlations among phthalate metabolites and parabens urinary concentrations, traditional regression is unsuitable (169). In addition, in previous simulation analyses, the WQS showed improvements over ordinary regression and LASSO (169). The WQS approach assumes that a given biomarker urinary concentration is associated with a PCP-use composite score, where this score encompasses a linear combination of PCP (0/1) use indicators. For a given urinary concentration, the approach estimates simultaneously PCP weights and an effect estimate for the resulting composite score within a regression model framework. Within each imputed dataset, we used bootstrapping to empirically estimate the weights, and the data set was split (50/50) into a training and a test set to provide valid inferences (e.g., p-values) for the estimated slope of the composite score. For biomarkers with a small percent of non-detectable concentrations (up-to 10% below LOD), we constructed a weighted binary score (WBS) within the linear regression framework. For biomarkers with a moderate percent of concentrations below the LOD (>10% and <70% below LOD), we performed WBS estimation within the Tobit regression model.

We present results as weighted percentage change in urinary concentrations of MEP, MiBP and the three parabens associated with an increase in the weighted score of the 14 PCPs as well as the corresponding

weight for each PCP (sum to 100%). Similarly, we a priori chose to present the number of PCPs (could be different depending on the chemical) that summed to at least 70% of the weighted score. The 70% of the weighted score should provide a reasonable prediction of exposure as compared to 100% of the weighted score, and may therefore be applicable for future research on exposure from PCPs.We repeated these analyses for PCPs-use with six hours of urine collection for the five urinary concentrations of higher interest.

Sensitivity analyses

Given the low detection frequency for butylparaben (31%), we performed a secondary analysis using generalized estimating equations (GEE) with logit link after we dichotomized butylparaben urinary concentrations into detectable versus non-detectable (156). We adjusted for the same covariates as in the primary analyses. We report adjusted odds ratios of detection associated with PCP-use along with p-values. We repeated all analyses using only the complete case analyses (with no imputation). Analyses were also repeated after imputing the missing PCP data as "0", assuming missing was no use.

We conducted statistical analyses using SAS version 9.4 (SAS Institute Inc., Cary, NC) and used the (heatmap.2) R package for heatmap generation.

Results

In the final analysis, 400 men contributed 1,037 urine samples with an average of three samples per man (upto 12 samples); 29% of the men provided one urine sample, 31% provided two, and 40% provided at least three. The median time between any two consecutive urine collections per man was 77 days (interquartile range: 34 to126 days).

The men were mostly Caucasian (86%), non-smokers (93%), had a median age of 36 years, median BMI of 27 kg/m^2, and a college or graduate education (84%) at time of enrollment. Urine collection time ranged from 6 am to 6 pm with 42% collected between 9 am and noon, and 46% of the samples collected in the warm season (Table 3.1).

Deodorants and shampoos were the most frequently (> 80%) used PCPs. Suntan/sunblock lotion and other hair care products were the least frequently (< 10%) used PCPs (Table 3.2). The concordance between specific PCP-use at different study visits within the same men was relatively high, ranging from 47% for hand sanitizer to 93% for suntan/sunblock lotion.

The percentage of samples (2004 to 2015) with detectable urinary concentrations of phthalates and parabens biomarkers ranged from 90% to 100% except for MEHP (78%) and butylparaben (31%) (Table 3.3). The men's median urinary MEP and paraben concentrations were substantially lower than the medians for women from the same cohort (69) but comparable to male urinary concentrations from NHANES (2) (Table 3.3). Spearman correlations were generally weak between phthalate metabolites and parabens (range: 0.09 to 0.32), weak to strong among different phthalate metabolites (range: 0.22 to >0.99) with the strongest correlation occurring between metabolites from the same parent compound (e.g., DEHP), and moderate to strong among different parabens (range: 0.41 to 0.80) (Figure. S1). Strong correlations indicate similar sources and weak correlations indicate different sources of exposure. These correlations are in accordance with results shown in Figure 3.1.

The adjusted percent changes in urinary concentrations (by the single-PCP approach) were highest for MEP (e.g., 83% increase with cologne/perfume use and 74% increase with deodorant use) (Figure 3.1).

Hairspray/hair gel, aftershave, mouthwash, shaving cream and other hair care products predicted MEP concentrations (18 to 31% increase). For the three parabens, the strongest predictors were use of suntan/sunblock lotion (66% to 156% increase) and of hand/body lotion (79% to147% increase). Hairspray/hair gel, shaving cream, aftershave, mouthwash and deodorant were moderate predictors for parabens. Liquid soap/body wash use was a strong predictor only for butylparaben (86%). Apart from hand sanitizer and bar soap, the rest of the PCPs were moderate to weak predictors of the three parabens. Each additional type of PCP used, regardless of which PCP, was associated with a 12% increase in MEP and a 14% to 29% increase in parabens urinary concentrations. While shampoo use was significantly associated with a 33% increase in the urinary concentrations of MEHP, it was not significantly associated with the other DEHP metabolites.The associations between PCP-use and urinary concentrations of other phthalate metabolites were weak to null, and sometimes even negative, but none were statistically significant. The percent increase in the urinary concentrations associated with PCP-use within six hours of urine collection was generally larger and more statistically significant than for use within 24 hours (Figure 3.2).

In the multi-PCP approach, we presented the different weights for each PCP depending on the association with each biomarker so that the 14 PCPs sum to 100%. When regressing these composite scores, we found that a one unit increase in the weighted score of the use of all 14 PCPs was associated with an increase in MEP and paraben urinary concentrations ranging from 700% to 1,398% for samples collected within 24 hours of PCP-use (Figure 3.3-Panel A). When we limited PCP-use to the last six hours, one unit increase in the weighted score was associated with a 458% to 3,626% increase in urinary concentrations of MEP and the parabens (Figure 3.3-Panel B).

We also present in Figure 3.3 (Panels C&D) the adjusted weighted % change in the urinary concentrations associated with the PCPs making up at least 70% of the weighted score as contributed by

the highest weighted PCPs for each chemical use within 24 and 6 hours of urine collection, along with the weights for each PCP (sum to at least 70%). The adjusted weighted % change in the urinary concentrations of MEP and the three parabens was associated with six or fewer PCPs (depending on the biomarker) and ranged from a 111% to 591% increase within 24 hours of urine collection and up-to 1,333% increase within six hours of urine collection. MiBP urinary concentration was not strongly predicted by the weighted PCP-score.

For MEP and the three parabens individually, seven or fewer PCPs used within 24 or 6 hours explained at least 70% of the weighted PCP-score. The adjusted weighted % changes within six hours were generally higher than within 24 hours of urine collection. Overall, use of ten PCPs within six hours prior to collection explained at least 70% of the weighted score. These included: cologne/perfume, deodorant, suntan/sunblock lotion, hand/body lotion, aftershave, other hair care products, mouthwash, conditioner/crème rinse, hairspray/hair gel and liquid soap/body wash which explained at least a 254% and up-to a 1,333% increase in MEP and the three parabens urinary concentrations (Figure 3.3 Panel C and D).

Secondary analyses performed by modeling butylparaben as a dichotomous outcome (detected versus non-detected) provided odds ratios that were consistent with the direction of the % changes from Tobit regression (Table 3S.2). Sensitivity analyses using the complete case analysis (with no imputation) and after imputing the missing PCP-use as no use gave consistent results with the main analysis (Figure. 3S.2 to 3S.5 and Tables 3S.3 to 3S.6).

Discussion

We found that self-reported PCP-use among men was associated with higher urinary concentrations of MEP and three parabens (methyparaben, propylparaben and butylparaben). As expected and consistent with a prior study of women in the same cohort (69), due to the short half-lives of phthalates and parabens (62,65,149) and the episodic use of PCPs, observed associations were stronger for PCP-use within six hours of urine collection as compared with use 24 hours prior to collection. PCP-use did not predict MiBP or the other phthalate metabolite urinary concentrations, consistent with infrequent use of these phthalates in PCPs (59).

To our knowledge only two large scale studies investigated the association between PCP-use and urinary phthalate metabolites and parabens in men. Duty et al.(83) explored associations of five PCPs used within 48 hours of a single spot urine sample collection in 406 men recruited from the MGH Andrology Laboratory (2000-2003); cologne and aftershave were predictors of MEP urinary concentrations (83). More recently, using data from the National Health and Nutrition Examination Survey (NHANES), Ferguson et al.(68) reported that mouthwash was a more important source of paraben exposure in men than in women. Our results were consistent with the findings from these previous studies even though both relied on only one spot urine sample per participant and had limited information about the time of PCP-use relevant to the urine collection. Duty et al.(83) assessed PCP-use within 48 hours before urine collection, likely to be too long a time window that could contribute to attenuation of associations. Ferguson et al.(68) had no information on last time of use relative to urine sample collection. Instead, PCP-use was recorded as always, sometimes or never, introducing exposure misclassification that is likely non-differential. Our study, with repeated urine samples from the same man and more detailed information on time of PCP-use extends the literature by identifying specific predictors of phthalates and parabens exposure.

In our study, men's self-reported information on PCP-use was predictive of select phthalate (mainly DEP) and paraben exposures. Based on our results, the exposure assessment of phthalates and parabens can be improved by asking only about the most "important" PCPs used within six hours before urine collection. We showed that ten PCPs contributed to at least 70% of the weighted score and explained more than a 250% increase in the urinary concentrations of MEP and the three parabens. Our recommendation for refining the questionnaire includes focusing on the following ten PCPs: cologne/perfume, deodorant, suntan/sunblock lotion, hand/body lotion, aftershave, other hair care products, mouthwash, conditioner/crème rinse, hairspray/hair gel, and liquid soap/body wash. Shortening the questionnaire would likely decrease missingness and improve participant's response rate. In addition, restricting the period of inquiry to the six hours prior to urine collection would provide a more precise estimate of exposure to phthalates and parabens since the urinary concentrations of the biomarkers are a better reflection of exposure in the last 6 hours than in 24 hours. It is also likely that participants will better recall their PCP-use within a shorter time frame.

Our study had several potential limitations. First, our single-PCP approach may be susceptible to multiple testing statistical issues. However, we also used a multi-PCP approach using WBS regression to account for multiple testing and the resulting estimated % changes were even larger, making it unlikely that any false positives in the single-PCP analysis accounted for our findings. Butylparaben had a low detection frequency which was consistent with findings from NHANES (2,68,172) and a German study (173). We addressed this in two ways. First, to explicitly model non-detectable concentrations, we used Tobit regression which accounts for missingness better than replacing those concentrations with LOD/$\sqrt{2}$ and linear regression (68). Second, we applied a sensitivity analysis using GEE (detected versus non-detected) as performed before (156), and these sensitivity analyses produced comparable results to the Tobit regression.

We had no information about frequency of PCP-use, amount of product used, whether it was used with hot or cold water, phthalates and parabens product content or brand names of the PCPs. Nevertheless, the lack of this information would have likely introduced non-differential measurement error and thus attenuated our results toward the null. In addition, knowing the brand name may not remedy this potential limitation as even the same brand might change product formulations over time and chemicals are not always listed on product labels. Because of short half-lives and episodic use, urinary concentrations of the biomarkers will depend on the frequency of voids between PCP-use and urine collection and we did not collect this information. We only focused on exposures from PCP-use, not accounting for other exposure sources. However, this is less likely to affect our results for MEP and the parabens because the PCPs are considered a major source of exposure to these compounds (or their precursors) (62,69,81,139) whereas for other phthalates such as DEHP, food is a major source of exposure (174). In addition, it is unlikely that other sources of exposure such as diet would meet the confounder definition as they are unlikely to be associated with PCP-use, hence not affecting the study validity. Finally, most men in our study were Caucasian, overweight or obese, highly educated, and non-smokers. Therefore, our results may be only generalized to men with similar characteristics.

Our study had several strengths including the use of repeated collection of self-reported PCP-use and urinary concentrations of phthalate metabolites and parabens for a large number of men over a ten year period; these repeated urine samples likely increased the precision of the measurements compared to a single sample. We included in the analysis 14 PCPs, the largest number of PCPs investigated in men upto date. We also included a large number of phthalate metabolites and three parabens. We assessed PCP-use within 24 and 6 hours before urine collection, more relevant exposure windows compared to the last 48 hours (81,83).

Another novel aspect of our study compared to previous research was the use of a weighted PCP-score to predict urinary concentrations. The WBS analyses yielded different weights for each PCP depending on the association with each biomarker. Using the single-PCP analyses for modeling, the crude sum of PCPs likely attenuated the effect due to non-differential misclassification by giving equal weights to each PCP in association with each biomarker. In addition, the multi-PCP approach was able to adjust for the other correlated PCPs and estimate the joint effect of multiple PCP-use, rather than simply using the crude sum of the PCPs or modeling each PCP separately. This approach has been used in linear regression settings (167-171), and we extended the basic weighted quantile strategy in earlier work to the repeated measures data and in Tobit regression framework.

Conclusions

Among a large cohort of men, we identified PCP predictors of urinary concentrations of MEP and three parabens. The results will be useful in future exposure assessment studies on PCP-use as an important source of exposure to phthalates (mainly DEP) and parabens. Collecting concise PCP-use information can be achieved by only asking specific questions about the use of the most relevant PCPs within a narrow exposure window (six hours) to potentially decrease missingness and improve recall decreasing misclassification while also optimizing research cost and time.

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Table 3.1. Demographics for 400 men who contributed 1,037 urine samples in the Environment and Reproductive Health (EARTH) Study

Baseline characteristics	N(%) or Mean± SD[Range]
Age (years)	36.5± 5.50 [23.9, 66.6]
Race	
Caucasian	343 (86%)
Black/ African American	13 (3%)
Asian	29 (7%)
Other	15 (4%)
BMI (Kg/m^2)[a]	27.5± 4.45 [18.6, 50.0]
BMI Categories	
Underweight: <18.5	2 (1%)
Normal weight: 18.5 <=bmi<25	114(28%)
Overweight: 25<=bmi<30	192 (48%)
Obese: bmi>=30	92 (23%)
Education Categories	
Less than college graduate	50 (16%)
College graduate	109 (34%)
Graduate degree	158 (50%)
Current Smoking Status	
Yes	26 (7%)
No	374 (93%)
Time varying Characteristics for urine sample collection	**n(%)**
Warm season (April through September)	477 (46%)
Calendar year	
2004 through 2009	522 (50%)
2010 through 2015	515 (50%)
Time of the day	
Early morning: 5am< and <=9am	385 (37%)
Late morning until noon: 9am< and <=12pm	432 (42%)
Afternoon: >12pm	220 (21%)

Table 3.1. :Abbreviations: N, men's number; n, urine samples' number; SD, standard deviation; BMI, body mass index; Kg, Kilogram; m, meter.
N or n (%) for categorical/binary variables and mean± SD [Range] for continuous variables**.**
[a] 2 men missing information on BMI and 83 missing education.

Table 3.2. Self-reported use of 14 personal care products (PCPs) within 24 and 6 hours of collection of urine sample among 400 men in the EARTH Study

Personal care products (PCPs)	n (answered yes/no)	n (answered yes/no and reported time since last used)	n (%) yes within 24 hours	n (%) yes within 6 hours	% concordant[a] over time
Deodorant	1029	1023	879 (85%)	723 (71%)	82%
Shampoo	1032	1024	832 (81%)	584 (57%)	74%
Conditioner/Creme Rinse	946	941	220 (23%)	149 (16%)	76%
Hairspray/Hair Gel	952	948	291 (31%)	222 (23%)	83%
Other hair care products[b]	914	910	66 (7%)	46 (5%)	90%
Shaving Cream	993	993	390 (39%)	263 (26%)	62%
Aftershave	800	799	79 (10%)	53 (7%)	87%
Cologne/Perfume	955	952	202 (21%)	145 (15%)	79%
Mouthwash	975	969	325 (33%)	199 (21%)	72%
Bar Soap	1003	1001	755 (75%)	567 (57%)	81%
Liquid Soap/Body Wash	1016	990	732 (72%)	508 (51%)	55%
Hand Sanitizer	726	714	219 (30%)	128 (18%)	47%
Hand/Body lotion	698	695	162 (23%)	102 (15%)	81%
Suntan/Sunblock Lotion	863	862	25 (3%)	12 (1%)	93%

Table 3.2.: Abbreviations: EARTH, Environment and Reproductive Health Study; n, number of visits/ urine samples; PCPs, personal care products.
[a] Concordant: same answer for product use within 24 hours over different visits for the same man, only includes observations for men who completed at least 2 visits.
[b] Combined other hair care products included mousse, hair bleach, relaxer, perm and straightener.

Table 3.3. Distributions of urinary concentrations of phthalate metabolites and parabens measured in 400 men (n=1,037 urine samples)[a] in the Environment and Reproductive Health (EARTH) Study

Urinary concentrations[b] (µg/L)[c]	% (>LOD)[d]	GM	10th perc	25th perc	50th perc	75th perc	90th perc	GM (NHANES)[e] 2005-2006	GM (NHANES)[e] 2009-2010	GM (NHANES)[e] 2011-2012
Monoethyl phthalate (MEP)	100%	48.8	6.86	16.6	42.0	145	458	107	61.0	38.1
Mono-n-butyl phthalate (MnBP)	98%	10.6	2.00	4.90	12.4	24.7	43.6	19.8	14.5	8.14
Mono-isobutyl phthalate (MiBP)	97%	6.44	1.20	3.20	7.40	14.9	27.2	5.65	7.80	6.54
Monobenzyl phthalate (MBzP)	95%	3.25	0.50	1.37	3.60	8.10	16.2	9.47	6.93	4.80
Mono(2-ethylhexyl) phthalate (MEHP)	78%	3.03	<LOD	0.85	2.80	7.80	24.9	3.40	1.83	1.51
Mono(2-ethyl-5-oxohexyl) phthalate (MEOHP)	99%	10.3	1.40	3.80	9.80	24.8	84.1	18.3	9.14	5.50
Mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)	100%	17.3	2.40	6.00	16.2	45.3	148	29.6	15.2	8.71
Mono(2-ethyl-5-carboxypentyl) phthalate (MECPP)	100%	27.7	4.30	10.5	26.1	65.5	209	43.6	23.4	14.3
∑DEHP (Di(2-ethylhexyl) phthalate) metabolites (µmol/L)[f]	-	0.20	0.03	0.07	0.19	0.49	1.57	-	-	-
Mono(3-carboxypropyl) phthalate (MCPP)	97%	3.85	0.60	1.60	3.70	9.70	23.2	2.32	3.37	3.52
Methylparaben	99%	28.0	4.70	9.40	23.2	80.4	226	29.8	31.7	23.2
Propylparaben	90%	2.86	0.14	0.60	2.30	12.1	58.6	2.96	2.77	2.44
Butylparaben	31%	0.26	<LOD	<LOD	<LOD	0.30	2.20	< LOD	< LOD	< LOD

Table 3.3. Abbreviations: SD, standard deviation; LOD, limit of detection; GM, geometric mean; NHANES, the National Health and Nutrition Examination Survey; Perc, percentile.

[a] Phthalate metabolites were measured in 1037 urine samples from 400 men and parabens in 965 samples from 383 men.

[b] Urinary concentrations were ordered according to the molecular weights within phthalates and within parabens.

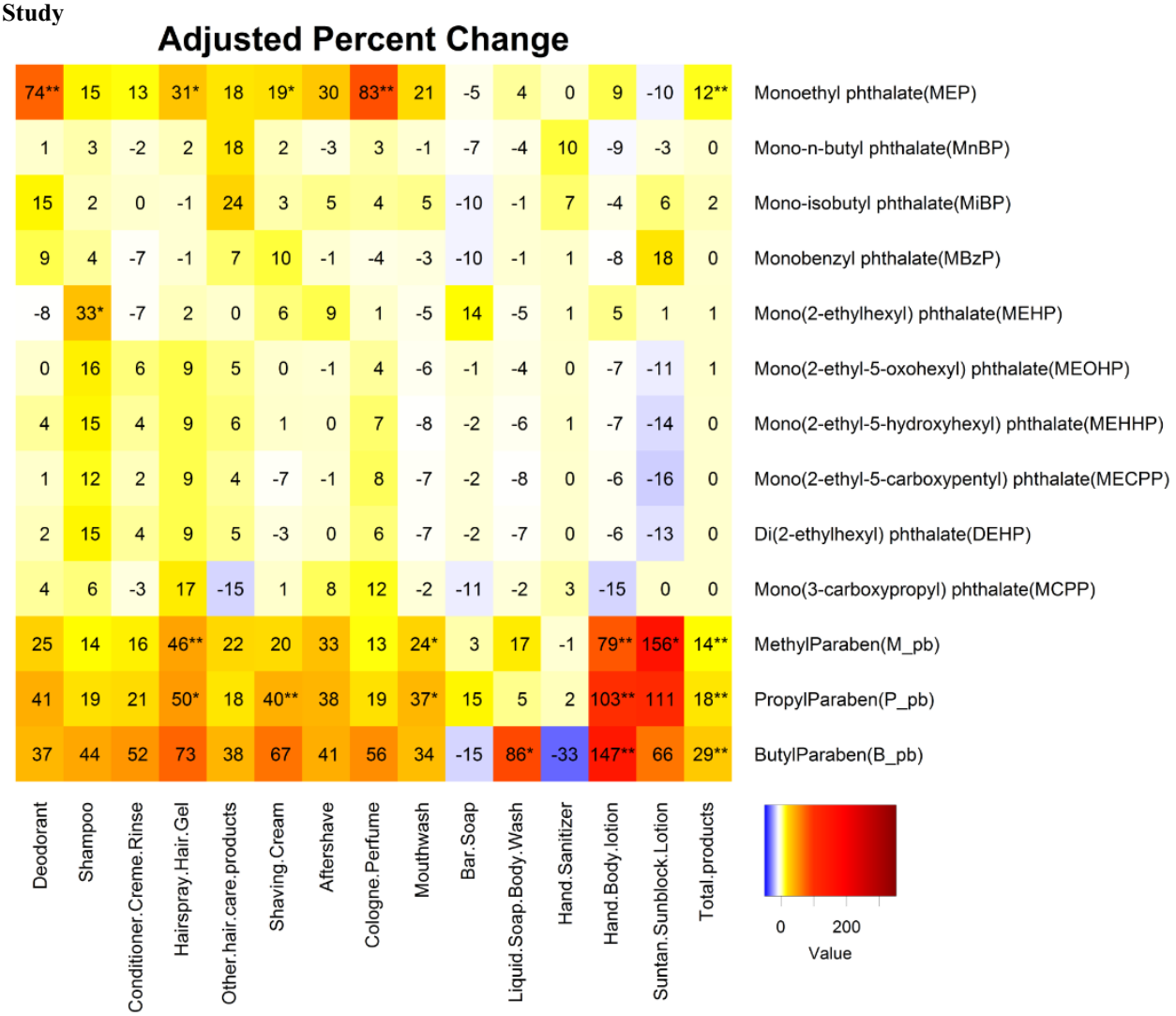
[c] Concentrations in µg/L (except for ∑DEHP) not corrected for urine dilution; concentrations < LOD were replaced by LOD/√2.

[d] LOD range for [MnBP: 0.4, 0.6], [MCPP: 0.18, 0.2],[MEHHP: 0.2, 0.7], [MEOHP: 0.2, 0.7], [MEHP: 0.5, 1.2], [MiBP: 0.2, 0.3], [MBzP: 0.2, 0.3], (M_pb: 1),[B_pb: 0.1, 0.2], [B_pb: 0.1, 0.2]µg/L.

[e] Concentrations from NHANES (2005-2006), (2009-2010), and (2011-2012) because our data encompassed the years 2004-2015.

[f] ∑DEHP metabolites (µmol/L)= µmol/L sum of MEHP + MEOHP + MEHHP + MECP.

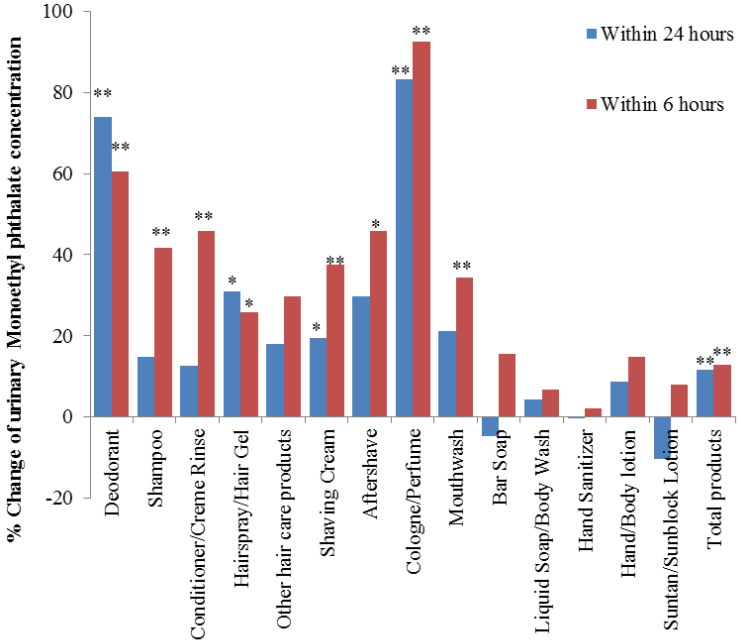
Figure 3.1. Heatmap for adjusted % change in urinary phthalate metabolite and parabens concentrations associated with self-reported use of personal care products (PCPs) within 24 hours of urine sample collection among 400 men who contributed 1037 urine samples in the Environment and Reproductive Health (EARTH) Study



Statistical significance indicated as *: if p-value < 0.05, **: if p-value < 0.01.
DEHP means: ∑DEHP metabolites (µmol/L)= sum of µmol/L of MEHP+ MEOHP+ MEHHP+ MECPP
Analysis was based on 10 imputed data using chained equations method.
Multiple imputing (MI) missing based on concordance of product use within persons. For any given PCP at any time point, the imputation model included PCP-use at other time points, urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no) and warm season (April through September) (yes/no).
Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon (>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).Total products: the crude sum of PCP used within 24 hours. The last column for the total products represents percent changes associated with each additional type of PCP used, regardless of which PCP.
Urinary concentrations were ordered according to the molecular weights within phthalates and within parabens.
Combined other hair care products included mousse, hair bleach, relaxer, perm and straightener.

Figure 3.2. (Panels A to E): Adjusted % change in urinary concentrations of parabens and phthalate metabolites associated with 14 personal care products used within 24 and 6 hours of urine collection.

Panel 3.2A. Monoethyl phthalate (MEP) urinary concentration



Panel 3.2B. Mono-isobutyl phthalate (MiBP) urinary concentration

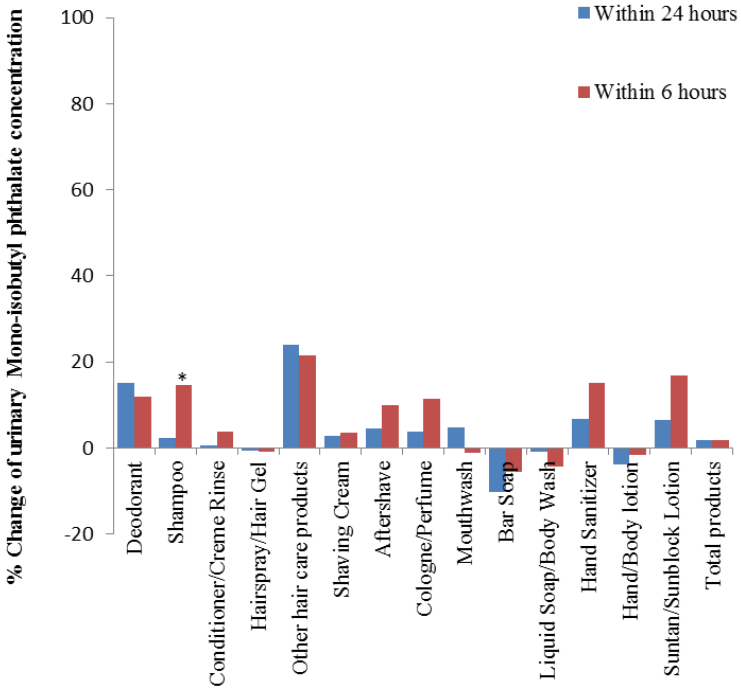
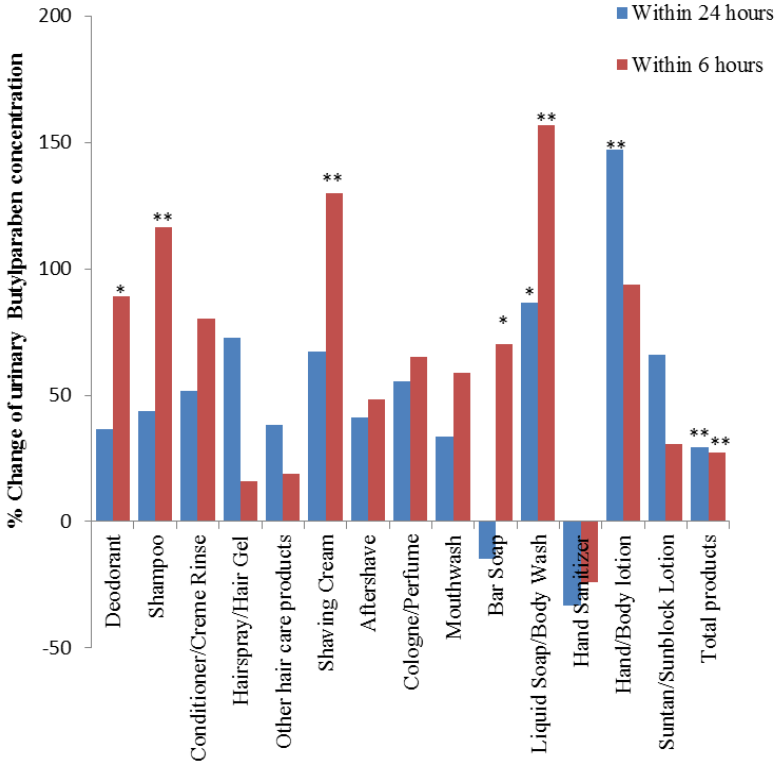


Figure 3.2.(Continued)

Panel 3.2C. Butylparaben urinary concentration



Panel 3.2D. Methylparaben urinary concentration

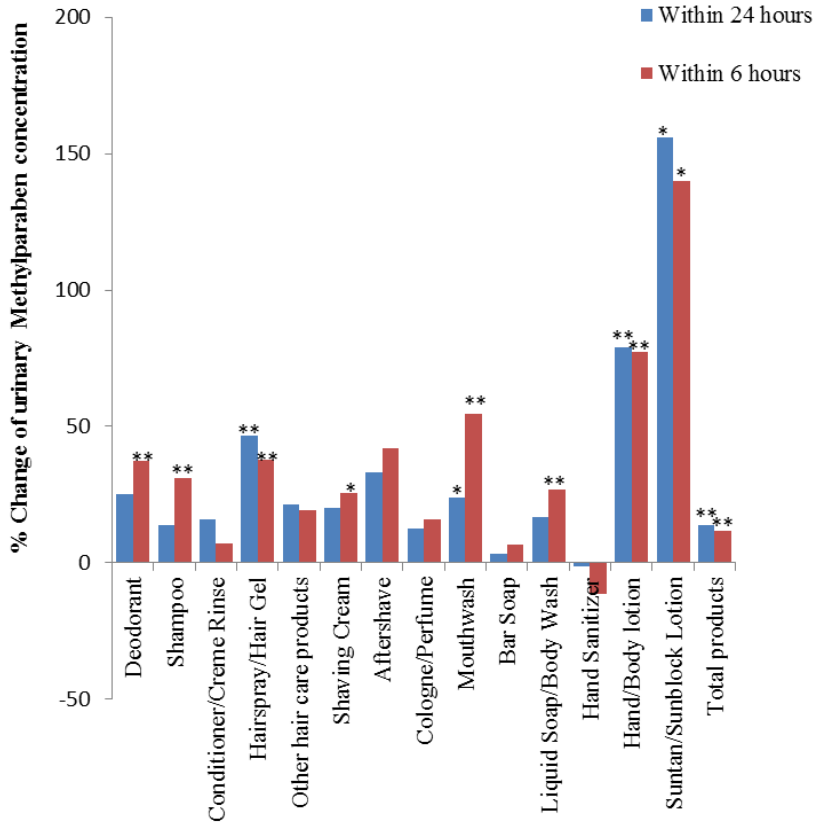


Figure 3.2.(Continued)

Panel 3.2E. Propylparaben urinary concentration

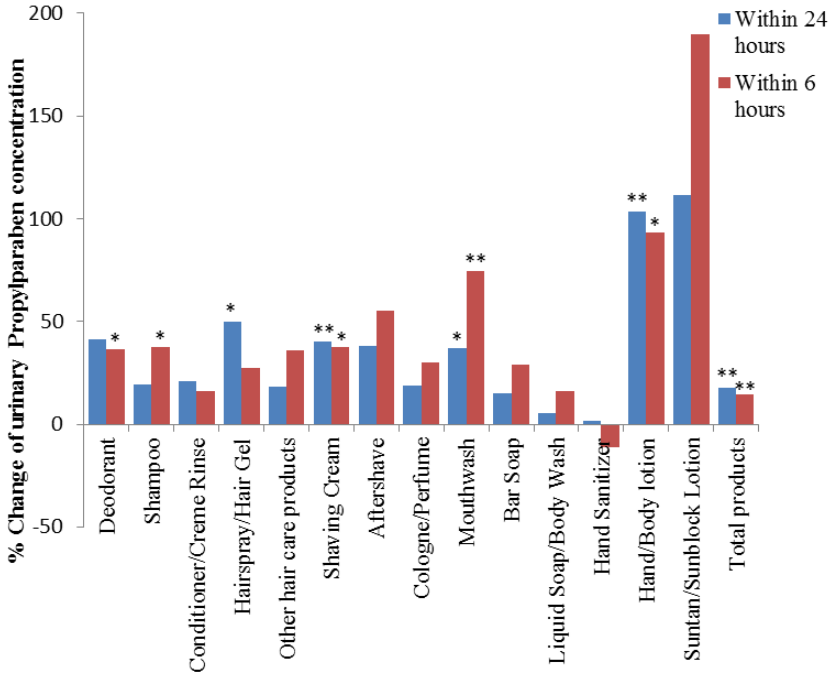
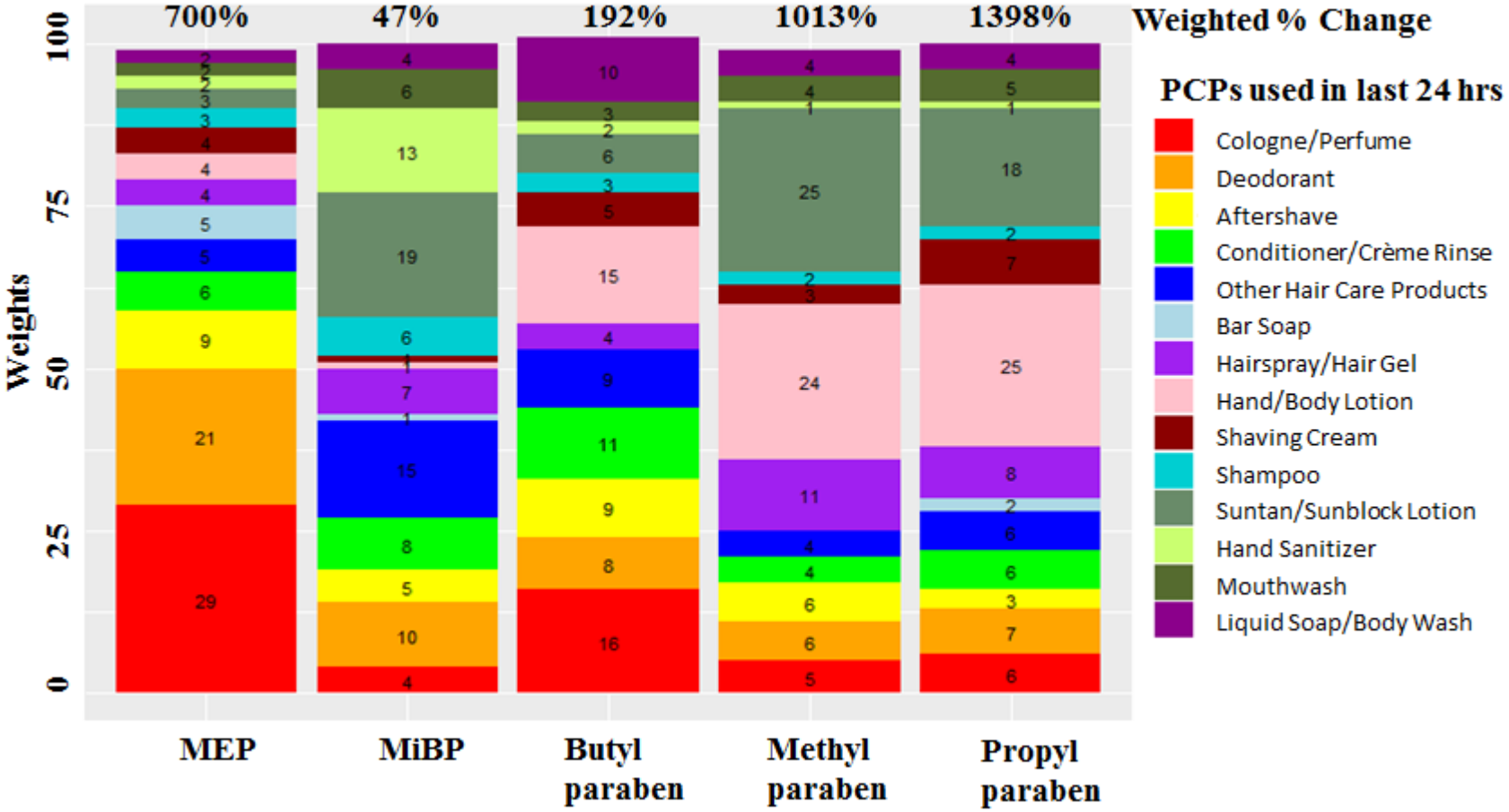


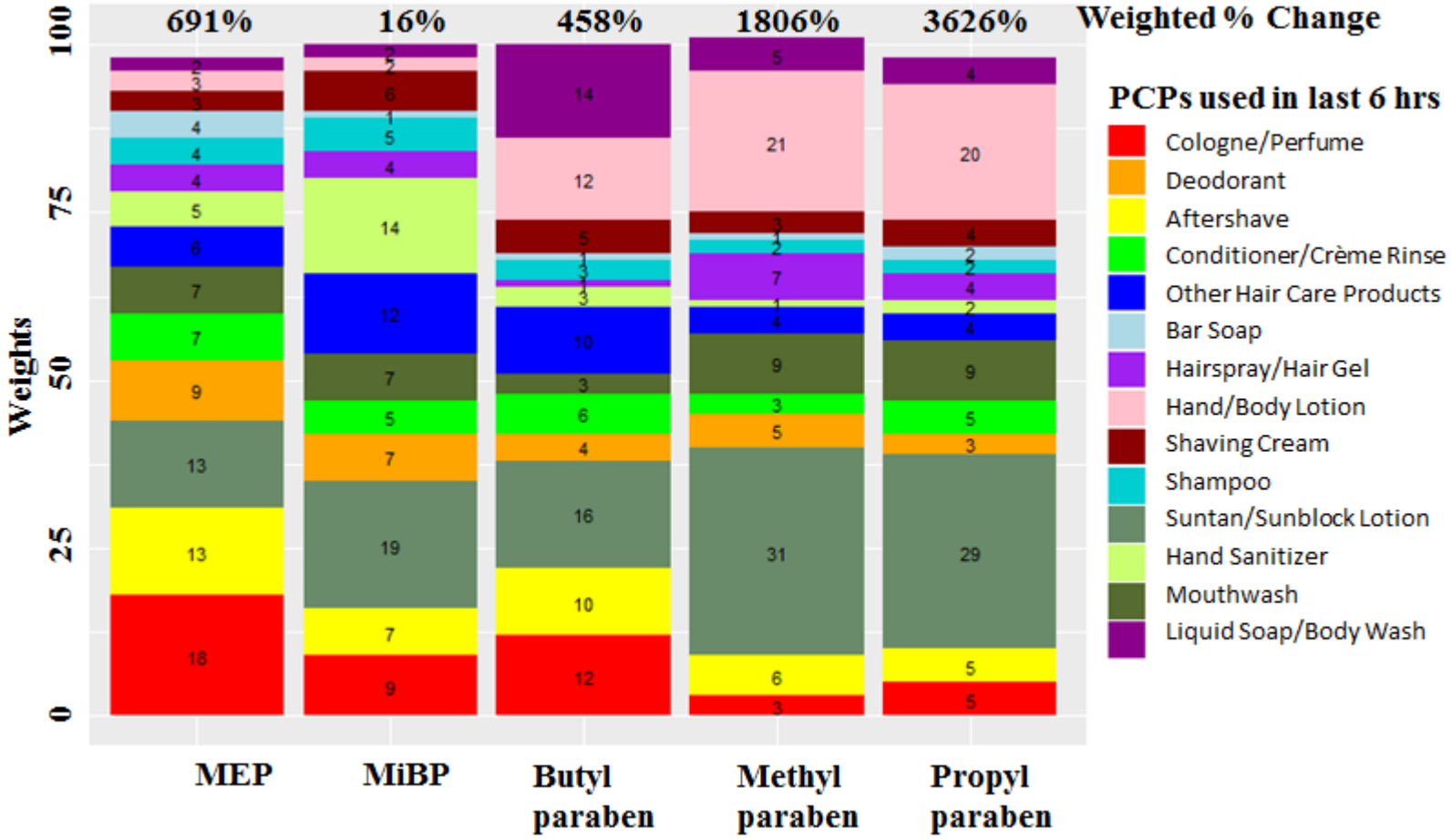
Figure 3.2. Statistical significance indicated as *: if p-value < 0.05, **: if p-value < 0.01.
Analysis was based on 10 imputed data using chained equations method.
Multiple imputing missing based on concordance of product use within persons. Imputation model included urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no) and warm season (April through September) (yes/no).
Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).
Total products: the crude sum of PCP used within 24 hours.The total products' bars represent percent changes associated with each additional type of PCP used, regardless of which PCP. Combined other hair care products included mousse, hair bleach, relaxer, perm and straightener.

Figure 3.3. Weights of personal care products (PCPs) that contribute 100% and at least 70% to the overall urinary concentrations of biomarkers associated with PCPs-use within 24 hours and 6 hours of sample collection

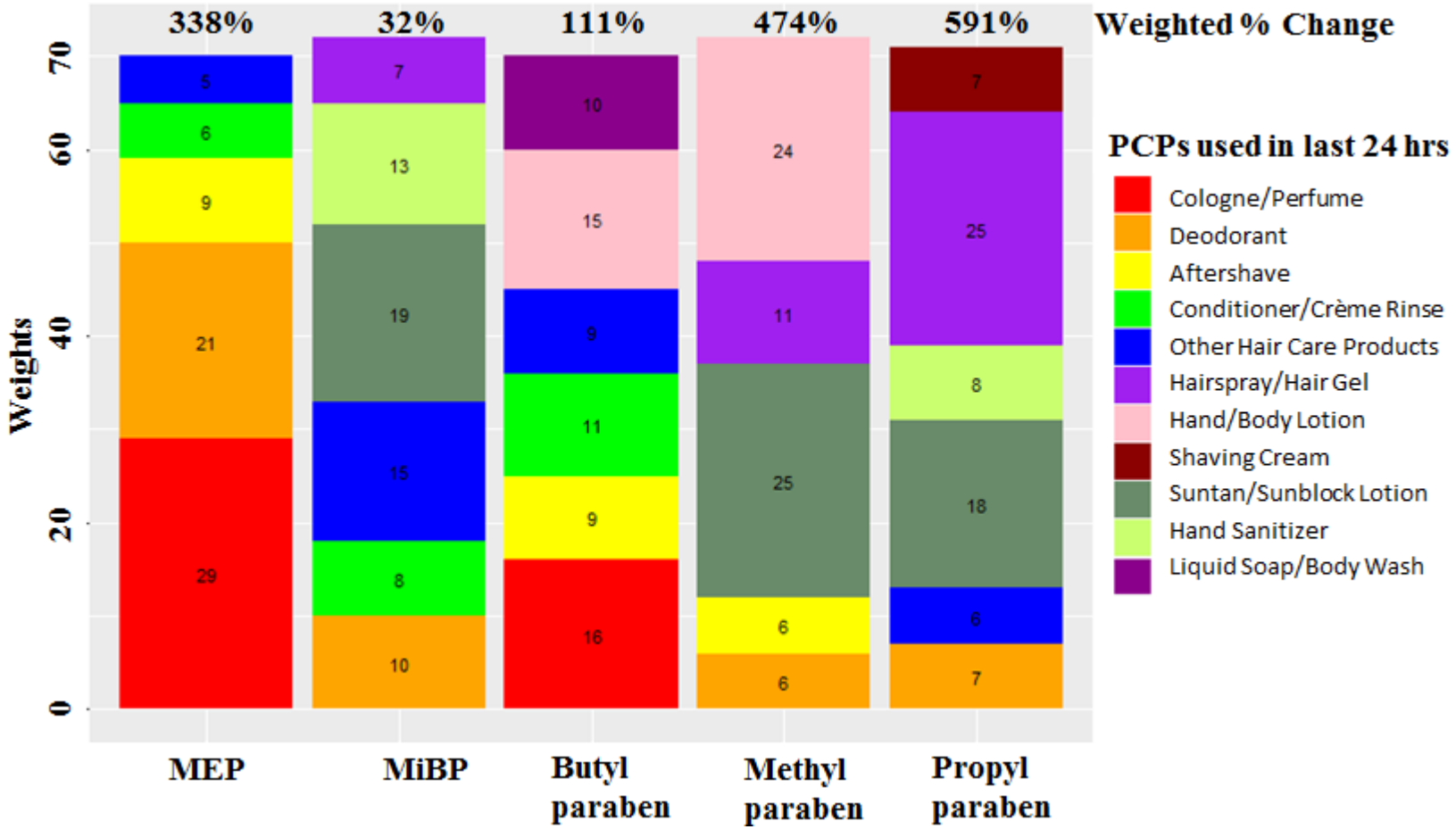
Panel 3.3.A. Weights of personal care products (PCPs) that contribute 100% to the overall urinary concentrations associated with PCPs-use within 24 hours and their overall weighted percent change



Panel 3.3.B. Weights of personal care products (PCPs) that contribute 100% to the overall urinary concentrations associated with PCPs-use within 6 hours and their overall weighted percent change



Panel 3.3.C. Weights of personal care products (PCPs) that contribute at least 70% to the overall urinary concentrations associated with PCPs-use within 24 hours and their overall weighted percent change



Panel 3.3.D. Weights of personal care products (PCPs) that contribute at least 70% to the overall urinary concentrations associated with PCPs-use within 6 hours and their overall weighted percent change

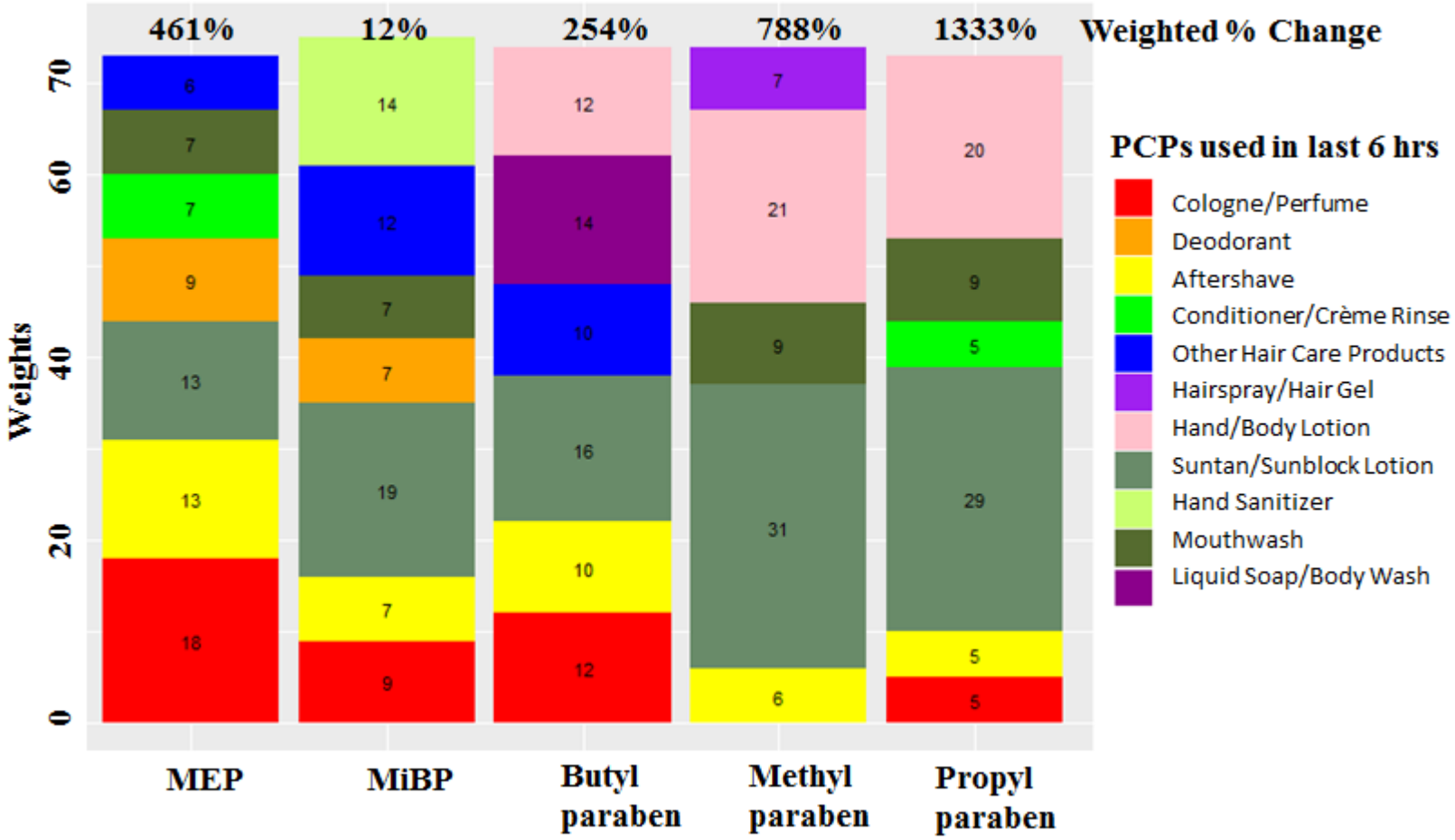


Figure 3.3. (Continued): Numbers presented inside the stack bars represent the weights associated with PCP-use. The weights of the 14 PCPs are summed to ~100 due to approximation.
The percent presented above the stacked bars represent the percent change associated with PCPs.
The weights presented are NOT summed to the percent change, instead summed to 100% for the 14 PCPs and 70 % or more for the PCPs presented above.
The percent changes are calculated by exponentiation of the weighted beta coefficient based on the PCPs used given the weights.
Analysis was based on 10 imputed data using chained equations method.
Multiple imputing missing based on concordance of product use within persons. For any given PCP at any time point, the imputation model included PCP-use at other time points, urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no) and warm season (April through September) (yes/no).
Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).
Combined other hair care products included mousse, hair bleach, relaxer, perm and straightener.

Supplemental Material

Product Use Questionnaire in the Environment and Reproductive Health (EARTH) Study.

DATE: ___ / ___ / ___ TIME: ___ : ___ AM / PM

PRODUCT USE QUESTIONNAIRE
(Complete on same day as urine collection)

EARTH Study

1. Please indicate use of any of these products in the PAST 24 HOURS.	Used in past 24 hours? No	Used in past 24 hours? Yes	Time last used? (Please circle AM or PM)	Yesterday or today? Yesterday	Yesterday or today? Today	Scented? Scented	Scented? Not Scented	Scented? Don't know	Comments
Deodorant	◯	◯	__ __ : __ __ AM / PM	◯	◯	◯	◯	◯	
Shampoo	◯	◯	__ __ : __ __ AM / PM	◯	◯	◯	◯	◯	
Conditioner/Creme Rinse	◯	◯	__ __ : __ __ AM / PM	◯	◯	◯	◯	◯	
Hairspray/Hair Gel	◯	◯	__ __ : __ __ AM / PM	◯	◯	◯	◯	◯	
Other hair care products *(e.g., mousse, hair bleach, relaxer, Perm, straightener)*	◯	◯	__ __ : __ __ AM / PM	◯	◯	◯	◯	◯	
Toothpaste	◯	◯	__ __ : __ __ AM / PM	◯	◯				
Mouthwash	◯	◯	__ __ : __ __ AM / PM	◯	◯				
Shaving Cream	◯	◯	__ __ : __ __ AM / PM	◯	◯	◯	◯	◯	
Cologne/Perfume	◯	◯	__ __ : __ __ AM / PM	◯	◯	◯	◯	◯	
Aftershave	◯	◯	__ __ : __ __ AM / PM	◯	◯	◯	◯	◯	
Bar Soap	◯	◯	__ __ : __ __ AM / PM	◯	◯	◯	◯	◯	
Liquid Soap/Body Wash	◯	◯	__ __ : __ __ AM / PM	◯	◯	◯	◯	◯	
Hand Sanitizer	◯	◯	__ __ : __ __ AM / PM	◯	◯	◯	◯	◯	
Hand/Body lotion	◯	◯	__ __ : __ __ AM / PM	◯	◯	◯	◯	◯	Contains sunscreen? ◯ Yes ◯ No
Face moisturizer/lotion	◯	◯	__ __ : __ __ AM / PM	◯	◯	◯	◯	◯	Contains sunscreen? ◯ Yes ◯ No
Colored Cosmetics *(e.g., hair dye, makeup)*	◯	◯	__ __ : __ __ AM / PM	◯	◯	◯	◯	◯	Contains sunscreen? ◯ Yes ◯ No
Suntan/Sunblock Lotion	◯	◯	__ __ : __ __ AM / PM	◯	◯	◯	◯	◯	
Nail Polish	◯	◯	__ __ : __ __ AM / PM	◯	◯	◯	◯	◯	
Nail Polish Remover	◯	◯	__ __ : __ __ AM / PM	◯	◯	◯	◯	◯	
Other toiletries *(e.g., wax, vaseline, lip balm)*	◯	◯	AM / PM	◯	◯	◯	◯	◯	Contains sunscreen? ◯ Yes ◯ No

Figure 3.S1. Spearman correlation between urinary concentrations of phthalate metabolites and parabens among 965 samples provided by men in the Environment and Reproductive Health (EARTH) Study.

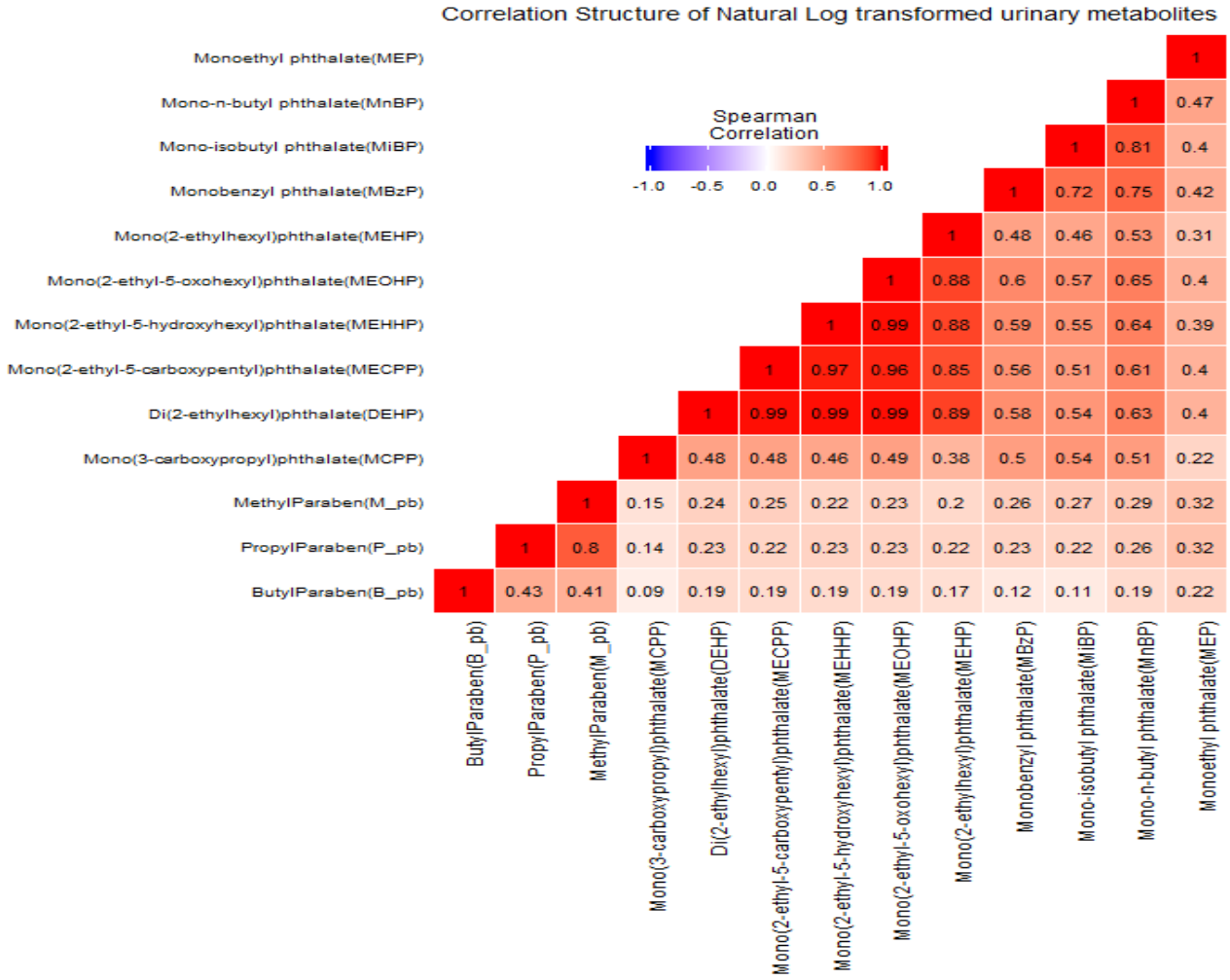


Figure 3.S1.: [a] 1037 urine samples were analyzed for phthalate metabolites and 965 for parabens.
[b] For Spearman correlations, data was restricted to 965 urine samples for which both phthalate metabolites and parabens were measured.

Table 3.S1. Adjusted [a] percent change associated with the weighted score: (based on binary use up-to 24 hours use and 6 hours) using the weighted binary score (WBS) in the 10 imputed data.

	Percent change associated with the weighted score for the 14 PCPs: (based on binary use up-to 24 hours use)		Percent change associated with the weighted with the weighted score: (based on binary use up-to 6 hours use)	
Urinary biomarker	**% Change (95% CI)**	**p-value**	**% Change (95% CI)**	**p-value**
MEP	700 (258, 1686)	2.42E-06	961 (485, 1823)	1.80E-14
MnBP	39.0 (-29.5, 174)	0.33	13.7 (-18.5, 58.6)	0.45
MiBP	47.0 (-23.1, 181)	0.24	16.1 (-19.6, 67.6)	0.43
MBzP	20.6 (-41.3, 148)	0.60	-1.79 (-40.1, 61.1)	0.94
MEHP	-2.20 (-58.6, 131)	0.96	-21.4 (-56.7, 42.9)	0.43
MEOHP	9.14 (-46.5, 123)	0.81	-25.3 (-58.4, 34.2)	0.32
MEHHP	6.20 (-50.1, 126)	0.87	-20.6 (-55.5, 41.9)	0.43
MECPP	3.60 (-48.3, 107)	0.92	-19.6 (-51.8, 34.1)	0.40
∑DEHP [b]	5.46 (-47.6, 112)	0.88	-21.4 (-53.7, 33.5)	0.37
MCPP	3.48 (-59.5, 165)	0.94	-33.9 (-60.3, 9.99)	0.11
Methylparaben	1013 (-90.8, 135010)	0.29	1806 (417, 6925)	0.00008
Propylparaben	1398 (-96.1, 575248)	0.35	3626 (535, 21763)	0.0002
Butylparaben	192 (-77.1, 3617)	0.40	458 (58.3, 1868)	0.009

Abbreviations**:** PCP; personal care product, 95% CI; 95% confidence intervals, MEP; Monoethyl phthalate, MnBP; Mono-n-butyl phthalate, MiBP; Mono-isobutyl phthalate, MBzP; Monobenzyl phthalate, MEHP; Mono(2-ethylhexyl) phthalate, MEOHP; Mono(2-ethyl-5-oxohexyl) phthalate, MEHHP; Mono(2-ethyl-5-hydroxyhexyl) phthalate, MECPP; Mono(2-ethyl-5-carboxypentyl) phthalate, DEHP; (Di(2-ethylhexyl) phthalate) metabolites, MCPP; Mono(3-carboxypropyl) phthalate.

[a] Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).

[b] ∑DEHP metabolites= MEHP+ MEOHP+ MEHHP+ MECPP weighted by the molecular weight.

Table 3.S2. Adjusted [a] odds ratio for detected versus undetected urinary butylparaben associated with last 24 hours personal care product use in the 10 imputed data.

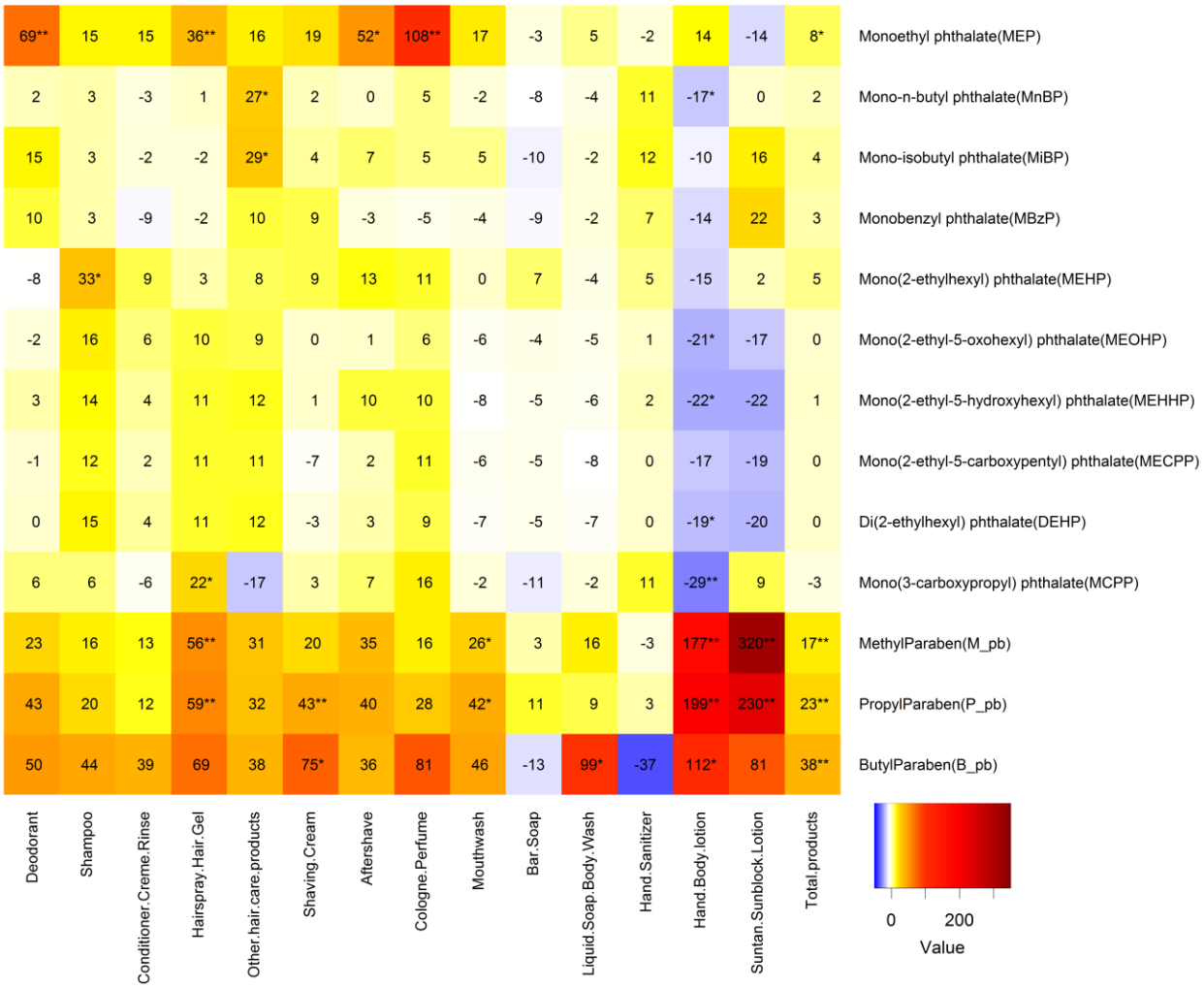
Personal care prodcut	Odds ratio (OR) (95% CI)	p-value
Total_poduct	1.14 (1.06, 1.23)	0.0004
Deodorant	1.08 (0.72, 1.63)	0.70
Shampoo	1.21 (0.86, 1.70)	0.26
Conditioner/Creme Rinse	1.24 (0.84, 1.83)	0.28
Hairspray/Hair Gel	1.34 (0.92, 1.96)	0.13
Other hair care products (e.g., mousse, hair bleach, relaxer, Perm, straightener)	1.62 (0.93, 2.84)	0.09
Shaving Cream	1.18 (0.90, 1.56)	0.23
Aftershave	1.08 (0.69, 1.68)	0.75
Cologne/Perfume	1.16 (0.81, 1.68)	0.41
Mouthwash	1.38 (1.00, 1.90)	0.049
Bar Soap	0.92 (0.65, 1.32)	0.66
Liquid Soap/Body Wash	1.39 (1.01, 1.93)	0.046
Hand Sanitizer	0.92 (0.63, 1.33)	0.65
Hand/Body lotion	1.39 (0.98, 1.96)	0.06
Suntan/Sunblock Lotion	2.27 (1.00, 5.18)	0.05

Abbreviations: OR; odds ratio, 95% CI; 95% confidence intervals, MEP; Monoethyl phthalate, MnBP; Mono-n-butyl phthalate, MiBP; Mono-isobutyl phthalate, MBzP; Monobenzyl phthalate, MEHP; Mono(2-ethylhexyl) phthalate, MEOHP; Mono(2-ethyl-5-oxohexyl) phthalate, MEHHP; Mono(2-ethyl-5-hydroxyhexyl) phthalate, MECPP; Mono(2-ethyl-5-carboxypentyl) phthalate, DEHP; (Di(2-ethylhexyl) phthalate) metabolites, MCPP; Mono(3-carboxypropyl) phthalate.

[a] Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).

[b] Generalized estimating equations (GEE) was used for modeling butylparaben as binary variable: detected vs undetected in the 10 imputed data.

Figure 3.S2. Heatmap for adjusted % change in urinary concentrations of phthalate metabolites and parabens associated with self-report use of personal care products (PCPs) within 24 hours of urine sample collection among 400 men who contributed 1037 urine samples in the Environment and Reproductive Health (EARTH) Study in the complete case data.



[a] Significance indicated as *: if p-value < 0.05, **: if p-value < 0.01
[b] DEHP means: ∑DEHP metabolites= MEHP+ MEOHP+ MEHHP+ MECPP weighted by the molecular weight
[c] Analysis was based on complete case analyses.
[d] Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).
[e] Total products: the crude sum of PCP used within 24 hours
[f] The last column for the total products represents percent changes associated with each additional type of PCP used, regardless of which PCP.
[g] Urinary concentrations were ordered according to the molecular weights within phthalates and within parabens.
[h] Combined other hair care products included mousse, hair bleach, relaxer, perm and straightener.

Table 3.S3. Adjusted [a] odds ratio for detected versus undetected urinary butylparaben associated with last 24 hours personal care product use in the complete case data.

Personal care prodcut	OR (95% CI)	p-value
Total_poduct	1.16 (1.04, 1.29)	0.0055
Deodorant	1.10 (0.72, 1.66)	0.6691
Shampoo	1.23 (0.87, 1.72)	0.2444
Conditioner/Creme Rinse	1.18 (0.80, 1.74)	0.4109
Hairspray/Hair Gel	1.36 (0.93, 1.97)	0.1087
Other hair care products (e.g., mousse, hair bleach, relaxer, Perm, straightener)	1.81 (0.98, 3.35)	0.0572
Shaving Cream	1.20 (0.91, 1.60)	0.1967
Aftershave	0.99 (0.61, 1.61)	0.978
Cologne/Perfume	1.29 (0.87, 1.90)	0.201
Mouthwash	1.43 (1.03, 1.98)	0.0331
Bar Soap	0.94 (0.65, 1.36)	0.7558
Liquid Soap/Body Wash	1.41 (1.01, 1.96)	0.0407
Hand Sanitizer	0.93 (0.66, 1.31)	0.6812
Hand/Body lotion	1.36 (0.91, 2.05)	0.1355
Suntan/Sunblock Lotion	3.44 (1.60, 7.40)	0.0016

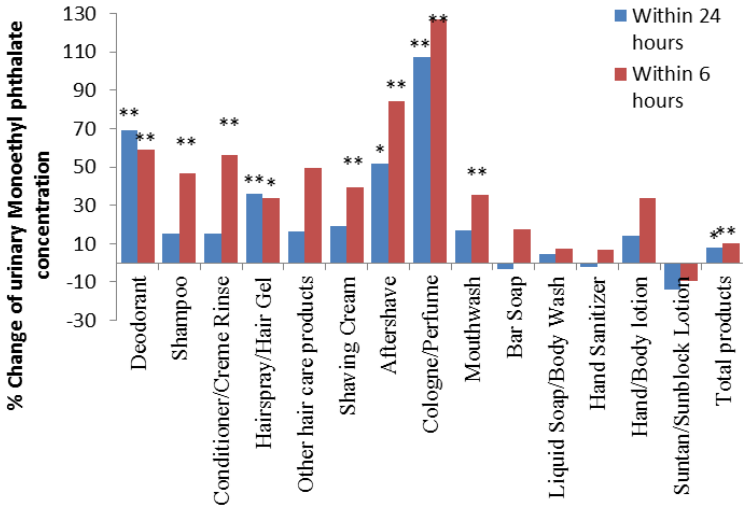
Abbreviations: OR; odds ratio, 95% CI; 95% confidence intervals, MEP; Monoethyl phthalate, MnBP; Mono-n-butyl phthalate, MiBP; Mono-isobutyl phthalate, MBzP; Monobenzyl phthalate, MEHP; Mono(2-ethylhexyl) phthalate, MEOHP; Mono(2-ethyl-5-oxohexyl) phthalate, MEHHP; Mono(2-ethyl-5-hydroxyhexyl) phthalate, MECPP; Mono(2-ethyl-5-carboxypentyl) phthalate, DEHP; (Di(2-ethylhexyl) phthalate) metabolites, MCPP; Mono(3-carboxypropyl) phthalate.

[a] Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).

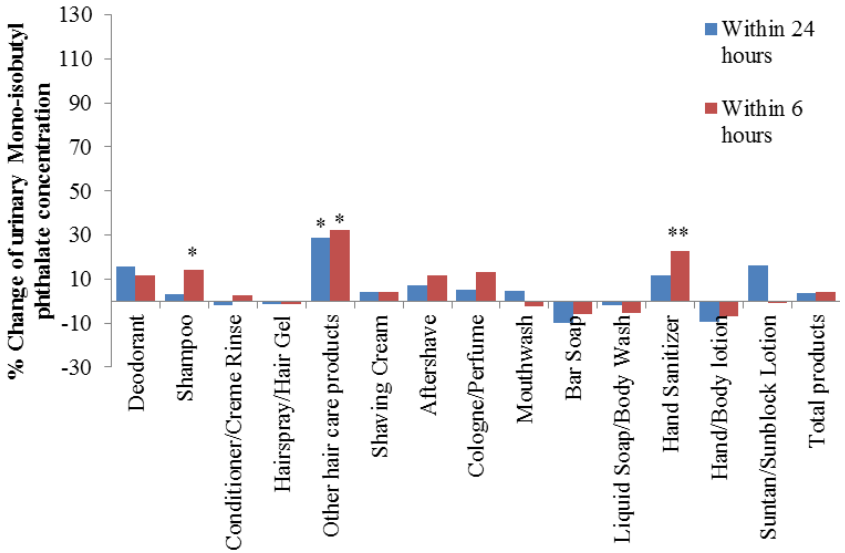
[b] Generalized estimating equations (GEE) was used for modeling butylparaben as binary variable: detected vs undetected in the complete case data.

Figure 3.S3. (Panels A to E): Adjusted % change in urinary concentrations of parabens and phthalate metabolites associated with use of 14 personal care products within 24 and 6 hours of urine collection in the complete case analysis.

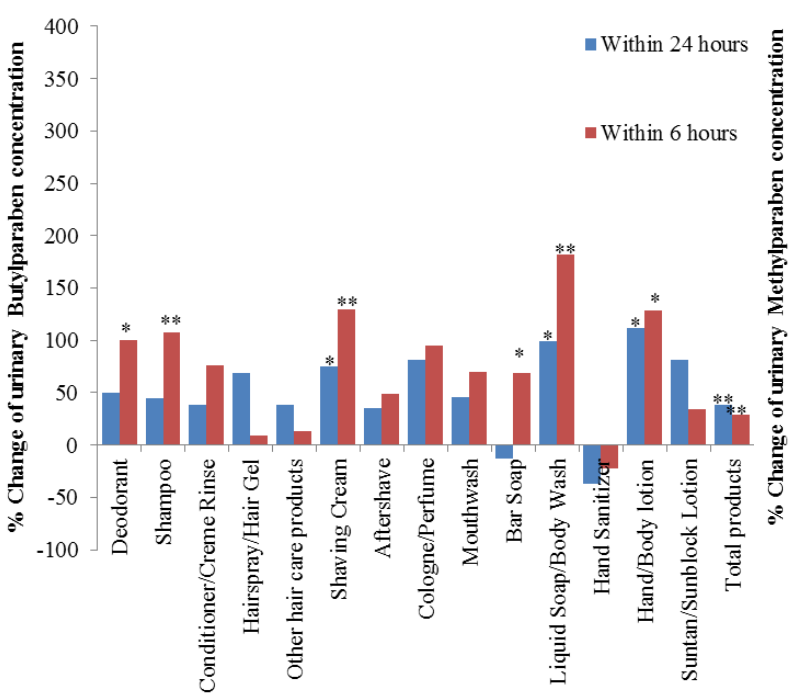
Panel 3.S3.A. Monoethyl phthalate (MEP) urinary concentration



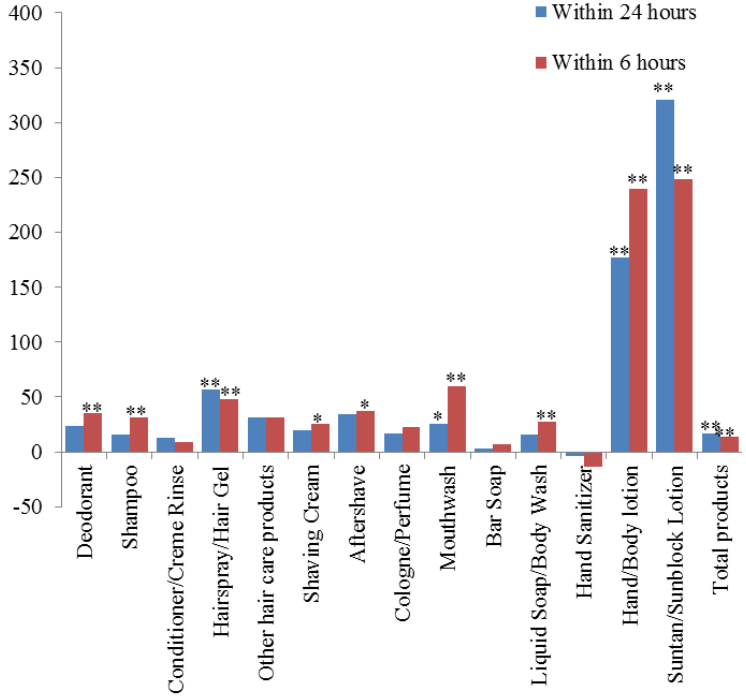
Panel 3.S3.B. Mono-isobutyl phthalate (MiBP) urinary concentration



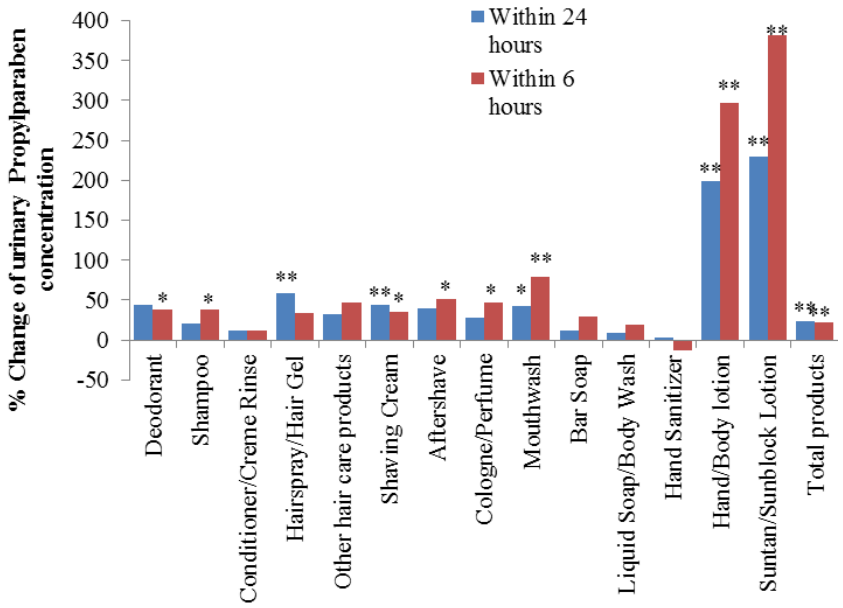
Panel 3.S3.C. Butylparaben urinary concentration



Panel 3.S3.D. Methylparaben urinary concentration



Panel 3.S3.E. Propylparaben urinary concentration



[a] Statistical significance indicated as *: if p-value < 0.05, **: if p-value < 0.01

[b] Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).

[c] Total products: the crude sum of PCP used within 24 hours

[d] The total products' bars represent percent changes associated with each additional type of PCP used, regardless of which PCP.

[e] Combined other hair care products included mousse, hair bleach, relaxer, perm and straightener

Table 3.S4. Adjusted [a] percent change associated with the weighted score: (based on binary use up-to 24 hours use and 6 hours) using the weighted binary score (WBS) in the complete case data.

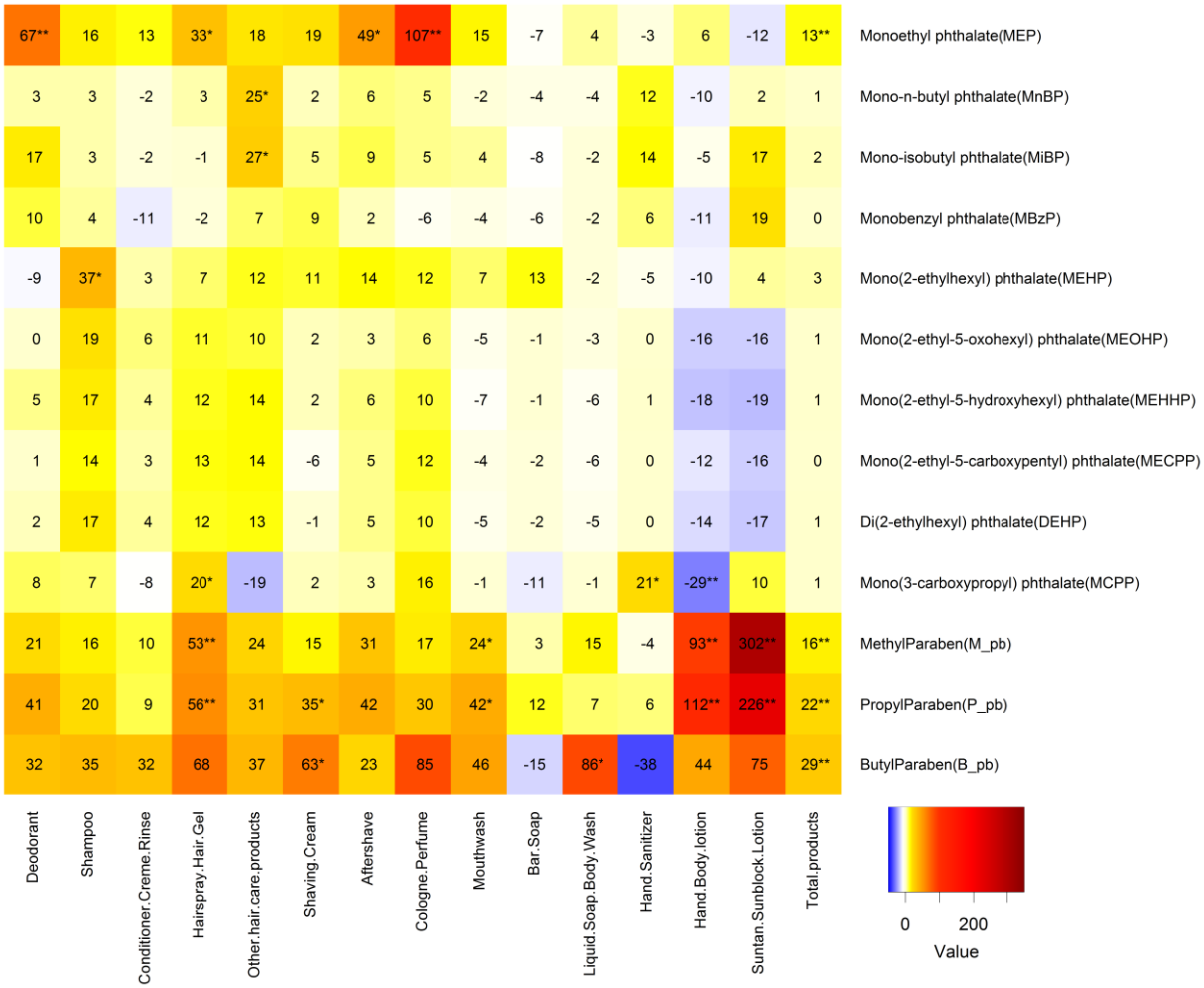
	Percent change associated with the weighted score: (based on binary use up-to 24 hours use)		Percent change associated with the weighted score: (based on binary use up-to 6 hours use)	
Urinary biomarker	**% Change (95% CI)**	**p-value**	**% Change(95% CI)**	**p-value**
MEP	281 (23, 1081)	0.02	429 (31, 2027)	0.02
MnBP	1 (-51, 107)	0.98	107 (4, 313)	0.04
MiBP	-16 (-59, 70)	0.62	62 (-31, 281)	0.26
MBzP	52 (-13, 164)	0.14	22 (-40, 147)	0.58
MEHP	-28 (-63, 39)	0.33	-41 (-74, 30)	0.19
MEOHP	-17 (-60, 74)	0.62	-34 (-70, 43)	0.29
MEHHP	-7 (-56, 96)	0.84	-28 (-67, 58)	0.41
MECPP	-26 (-64, 56)	0.43	-45 (-73, 12)	0.10
∑DEHP [b]	-15 (-57, 72)	0.66	-38 (-70, 26)	0.19
MCPP	-45 (-79, 46)	0.23	-69 (-88, -15)	0.02
Methylparaben	7597 (2087, 26997)	<.0001	9957 (2812, 34641)	<.0001
Propylparaben	4949 (721, 30966)	<.0001	23225 (3536, 149522)	<.0001
Butylparaben	577 (133, 1864)	0.0004	198 (-14, 928)	0.08

Abbreviations: 95% CI; 95% confidence intervals, MEP; Monoethyl phthalate, MnBP; Mono-n-butyl phthalate, MiBP; Mono-isobutyl phthalate, MBzP; Monobenzyl phthalate, MEHP; Mono(2-ethylhexyl) phthalate, MEOHP; Mono(2-ethyl-5-oxohexyl) phthalate, MEHHP; Mono(2-ethyl-5-hydroxyhexyl) phthalate, MECPP; Mono(2-ethyl-5-carboxypentyl) phthalate, DEHP; (Di(2-ethylhexyl) phthalate) metabolites, MCPP; Mono(3-carboxypropyl) phthalate.

[a] Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).

[b] ∑DEHP metabolites= MEHP+ MEOHP+ MEHHP+ MECPP weighted by the molecular weight.

Figure 3.S4. Heatmap for adjusted % change in urinary concentrations of phthalate metabolites and parabens associated with self-report use of personal care products (PCPs) within 24 hours of urine sample collection among 400 men who contributed 1037 urine samples in the Environment and Reproductive Health (EARTH) Study in the imputed missing as zero.



[a] Significance indicated as *: if p-value < 0.05, **: if p-value < 0.01
[b] Imputation for the missing values of PCP-use was assumed to be "0" i.e. no use.
[c] DEHP means: ∑DEHP metabolites= MEHP+ MEOHP+ MEHHP+ MECPP weighted by the molecular weight
[d] Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).
[e] Total products: the crude sum of PCP used within 24 hours
[f] The last column for the total products represents percent changes associated with each additional type of PCP used, regardless of which PCP.
[g] Combined other hair care products included mousse, hair bleach, relaxer, perm and straightener

Table 3.S5. Adjusted [a] odds ratio for detected versus undetected urinary butylparaben associated with last 24 hours personal care product use in the imputed missing as zero.

Personal care prodcut	OR (95% CI)	P-value
Total_poduct	1.14 (1.05, 1.23)	0.001
Deodorant	1.05 (0.70, 1.57)	0.83
Shampoo	1.23 (0.87, 1.73)	0.24
Conditioner/Creme Rinse	1.13 (0.78, 1.64)	0.52
Hairspray/Hair Gel	1.35 (0.94, 1.93)	0.10
Other hair care products (e.g., mousse, hair bleach, relaxer, Perm, straightener)	1.63 (0.91, 2.90)	0.10
Shaving Cream	1.19 (0.90, 1.57)	0.22
Aftershave	0.95 (0.61, 1.49)	0.82
Cologne/Perfume	1.28 (0.87, 1.88)	0.21
Mouthwash	1.40 (1.02, 1.93)	0.04
Bar Soap	0.93 (0.65, 1.33)	0.68
Liquid Soap/Body Wash	1.33 (0.97, 1.84)	0.08
Hand Sanitizer	0.88 (0.62, 1.24)	0.45
Hand/Body lotion	1.11 (0.78, 1.57)	0.56
Suntan/Sunblock Lotion	3.25 (1.53, 6.93)	0.002

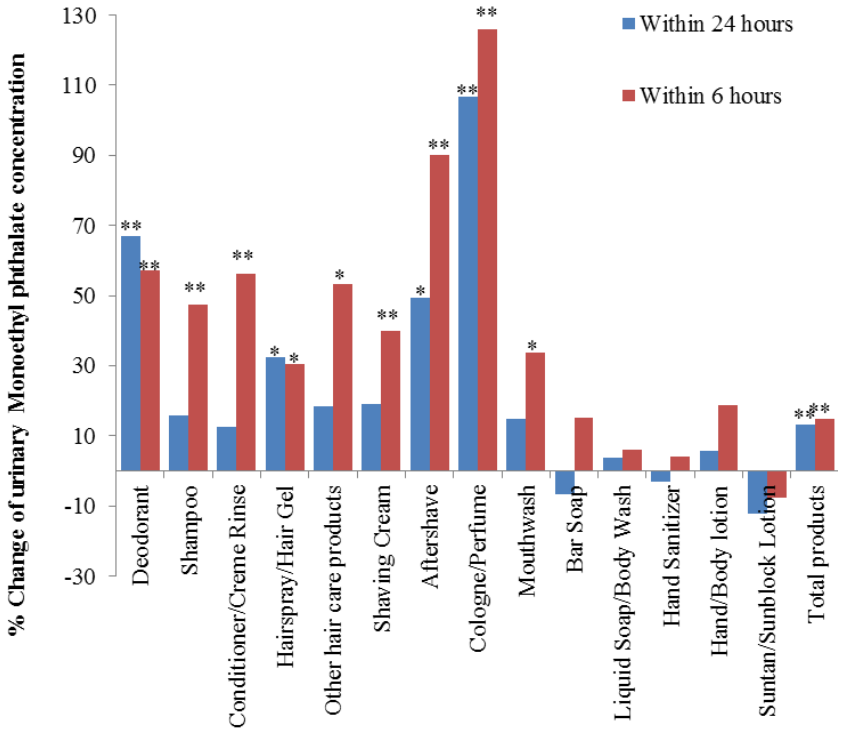
Abbreviations: OR; odds ratio, 95% CI; 95% confidence intervals, MEP; Monoethyl phthalate, MnBP; Mono-n-butyl phthalate, MiBP; Mono-isobutyl phthalate, MBzP; Monobenzyl phthalate, MEHP; Mono(2-ethylhexyl) phthalate, MEOHP; Mono(2-ethyl-5-oxohexyl) phthalate, MEHHP; Mono(2-ethyl-5-hydroxyhexyl) phthalate, MECPP; Mono(2-ethyl-5-carboxypentyl) phthalate, DEHP; (Di(2-ethylhexyl) phthalate) metabolites, MCPP; Mono(3-carboxypropyl) phthalate.

[a] Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).

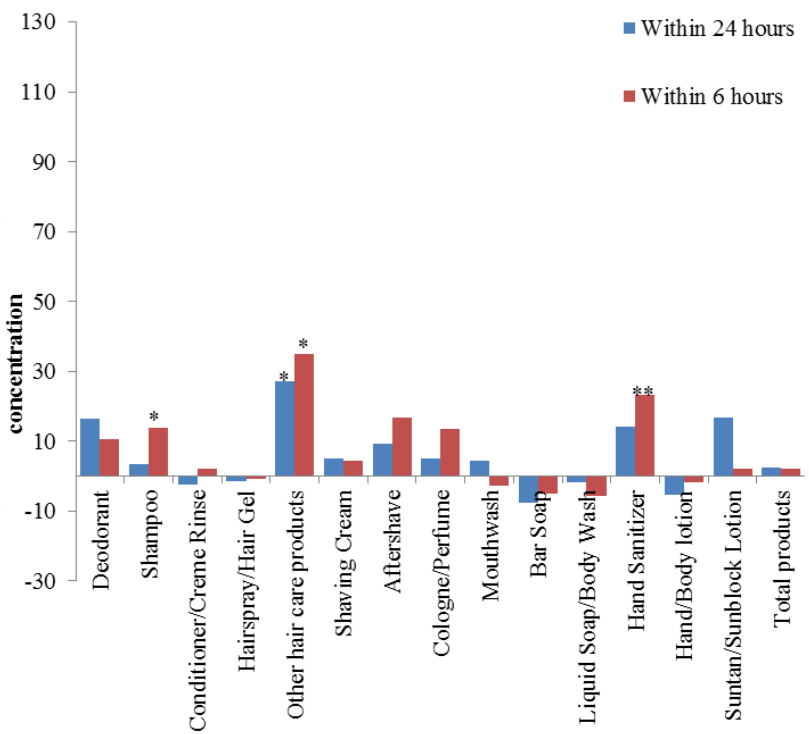
[b] Generalized estimating equations (GEE) was used for modeling butylparaben as binary variable: detected vs undetected in the imputed missing as zero.0.

Figure 3.S5. (Panels A to E): Adjusted % change in urinary concentrations of parabens and phthalate metabolites associated with use of 14 personal care products within 24 and 6 hours of urine collection in the imputed missing as zero.

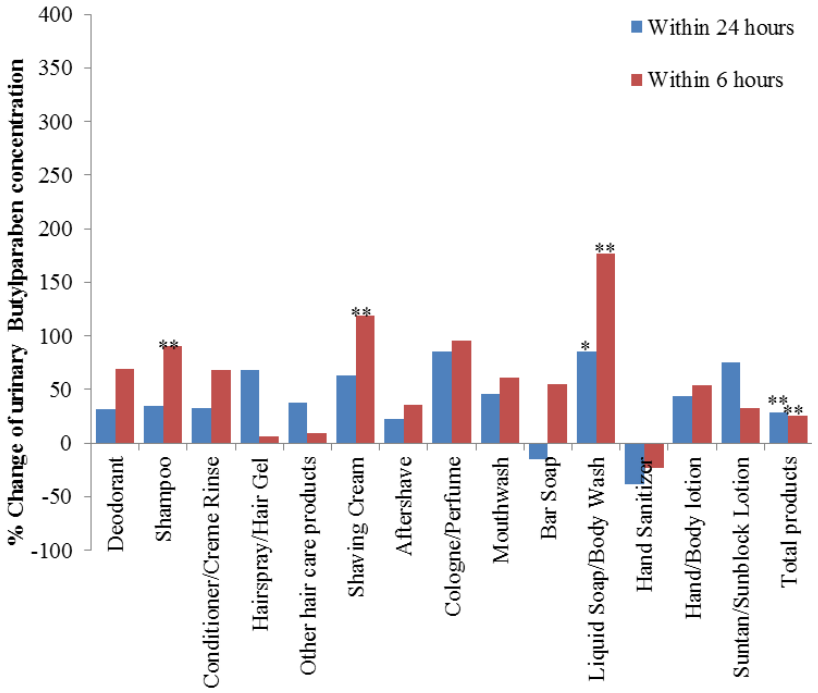
Panel 3.S5.A. Monoethyl phthalate (MEP) urinary concentration concentration



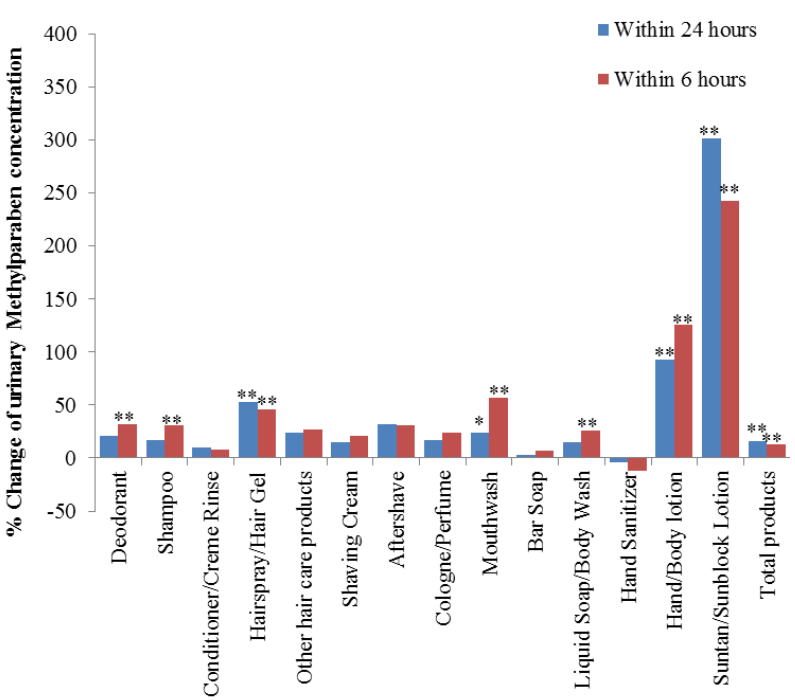
Panel 3.S5.B. Mono-isobutyl phthalate (MiBP) urinary



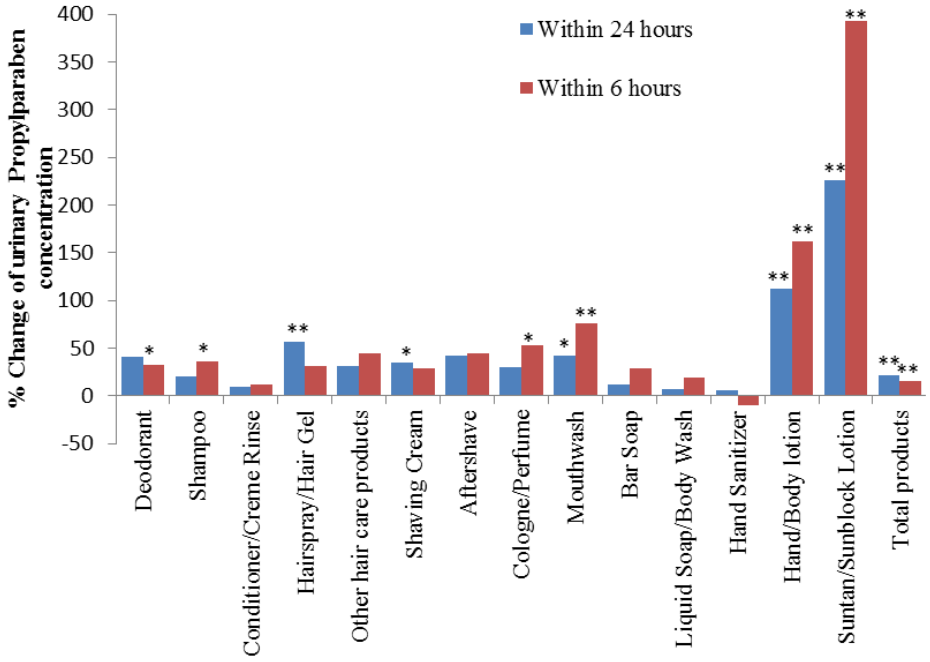
Panel 3.S5.C. Butylparaben urinary concentration



Panel 3.S5.D. Methylparaben urinary concentration



Panel 3.S5.E. Propylparaben urinary concentration



[a] Statistical significance indicated as *: if p-value < 0.05, **: if p-value < 0.01
[b] Imputation for the missing values of PCP-use was assumed to be "0" i.e. no use.
[c] Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).
[d] Total products: the crude sum of PCP used within 24 hours
[e] The total products' bars represent percent changes associated with each additional type of PCP used, regardless of which PCP.
[f] Combined other hair care products included mousse, hair bleach, relaxer, perm and straightener.

Table 3.S6. Adjusted [a] percent change associated with the weighted score: (based on binary use up-to 24 hours use and 6 hours) using the weighted binary score (WBS) in the imputed missing as zero.

	Percent change associated with the weighted score: (based on binary use up-to 24 hours use)		Percent change associated with the weighted score: (based on binary use up-to 6 hours use)	
Urinary biomarker	**% Change (95% CI)**	**p-value**	**% Change (95% CI)**	**p-value**
MEP	980 (496,1858)	<.0001	1483 (729,2924)	<.0001
MnBP	68 (11,155)	0.02	115 (34,246)	0.002
MiBP	89 (12,220)	0.02	102 (18,246)	0.01
MBzP	6 (-36,76)	0.81	10 (-37,94)	0.74
MEHP	3 (-38,71)	0.91	-4 (-47,75)	0.91
MEOHP	3 (-35,61)	0.91	13 (-28,76)	0.60
MEHHP	-1 (-38,60)	0.98	19 (-27,94)	0.49
MECPP	0 (-33,50)	0.99	12 (-21,59)	0.51
∑DEHP [b]	1 (-34,53)	0.98	12 (-24,65)	0.57
MCPP	47 (-19,167)	0.21	-9 (-51,72)	0.78
Methylparaben	6202 (2785,13668)	<.0001	9350 (3876,22362)	<.0001
Propylparaben	6512 (2099,19778)	<.0001	17283 (4909,60235)	<.0001
Butylparaben	199 (54,479)	0.001	487 (199,1055)	<.0001

Abbreviations: 95% CI; 95% confidence intervals, MEP; Monoethyl phthalate, MnBP; Mono-n-butyl phthalate, MiBP; Mono-isobutyl phthalate, MBzP; Monobenzyl phthalate, MEHP; Mono(2-ethylhexyl) phthalate, MEOHP; Mono(2-ethyl-5-oxohexyl) phthalate, MEHHP; Mono(2-ethyl-5-hydroxyhexyl) phthalate, MECPP; Mono(2-ethyl-5-carboxypentyl) phthalate, DEHP; (Di(2-ethylhexyl) phthalate) metabolites, MCPP; Mono(3-carboxypropyl) phthalate.

[a] Analysis adjusted for urine specific gravity (continuous), race (Caucasian or not), age (continuous), BMI (continuous), calendar year (continuous), time of sample collection (early morning (5am< and <=9am), late morning (9am< and <=12pm) or afternoon(>12pm)), current smoking (yes/no), warm season (April through September) (yes/no) and the product use within 24 hours (yes/no).

[b] ∑DEHP metabolites= MEHP+ MEOHP+ MEHHP+ MECPP weighted by the molecular weight.

Discussion and Conclusions

Phthalates and parabens are endocrine disrupting chemicals with ubiquitous human exposure. Medication coatings and personal care products (PCPs) are important exposure sources, however, their contribution to men's exposure as well as health effects are understudied.

Discussion of Chapters 1 and 2

In Chapters 1 and 2, we examined the association between very high exposure to dibutyl phthalate (DBP) from mesalamine medication with semen quality, and serum reproductive hormones in adult men. We used an innovative crossover-crossback study (Mesalamine And Reproductive health Study (MARS)). We found that on average when men who were on non-DBP mesalamine (background exposure) at baseline (B_1HB_2-arm; $Background_1$-High-$Background_2$) were newly-exposed to high-DBP mesalamine (crossover) for four months, their serum concentrations of both testicular hormones (testosterone and inhibin-B) and gonadotropins (luteinizing hormone (LH) and follicle-stimulating hormone (FSH)) decreased. As these hormones play critical roles in normal testicular function and spermatogenesis, there were also decrements in most of their semen parameters, primarily sperm motility (% total sperm motility, % progressive sperm motility and motile sperm count)(29). When the same men crossed-back to their original non-DBP mesalamine (background exposure) for four months, concentrations of both testicular hormones and gonadotropins increased and for some they even exceeded their baseline concentrations whereas their semen parameters continued to decrease despite removing high-DBP exposure(29). One potential explanation for the continued decline of semen parameters may relate to the longer duration for spermatogenesis (average of 70 days)(94) which potentially may result in a lagged spermatogenesis response to removal of high-DBP exposure. Also, the effect might be mainly on Sertoli cells as some studies suggested that DBP preferentially targets Sertoli cells in older animals(104,105).

Because Sertoli cell envelops and supports the germ cell undergoing progressive differentiation and development into mature spermatozoa(175), thus spermatogenesis response may persist longer.

These results differed as compared to men in the H_1BH_2-arm ($High_1$-Background-$High_2$). We found that the duration of high-DBP exposure history modified the responses of reproductive hormones and semen parameters to high-DBP exposure from mesalamine. The hormone changes were only found in men exposed to high-DBP for a shorter duration of less than three years (H_1BH_2-arm, <3 yrs) with a similar pattern of response as the newly exposed men (B_1HB_2-arm). This was in contrast to the response among men exposed to high-DBP for a long duration of three years or more (H_1BH_2-arm, $H_1 \geq 3$ yrs) who had non-significant hormonal changes either after crossover or crossback.

In general there was no change in semen parameters in the H_1BH_2-arm during either crossover or crossback as we found in Chapter 1. When we conducted the three-arm analysis for the semen parameters (Chapter 1 Appendix) as we did in Chapter 2 for the reproductive hormones, we found that, similar to the hormonal changes, the duration of high-DBP exposure at baseline modified the crossover and crossback effects on semen parameters primarily sperm concentration and count. Specifically, after removal of high-DBP exposure for four months (crossover), only men in (H_1BH_2-arm, < 3yrs) had an increase in their testosterone (total (TT) and free (FT)) concentrations along with corresponding increases in their sperm counts and sperm concentrations. In addition, TT/LH, FT/LH and inhibin-B/FSH ratios increased suggesting improved Leydig and Sertoli cell functions that was reflected primarily on sperm count and concentrations. After crossback to their high-DBP mesalamine, their testosterone concentrations increased but of smaller magnitudes along with decreased sperm counts and concentrations following similar patterns suggesting decreased functions of Leydig and Sertoli cell functions.

In contrast, among men with long duration of exposure to high-DBP mesalamine at baseline (H_1BH_2-arm, $H_1 \geq 3$ yrs), the four month wash-out period during the crossover to non-DBP mesalamine was not long

enough to 'reverse' the effects of high-DBP exposure on reproductive hormones or on semen parameters(29). One potential explanation is that the response following removal of high-DBP exposure differs based on duration of high-DBP exposure. These results may suggest potential different modes of actions for the endocrine and spermatogenesis responses to high-DBP based on the duration of DBP-exposure history which need further research.

In cross-sectional analysis of baseline values only, men exposed to high-DBP for a long duration of three years or more (H_1BH_2-arm, $H_1 \geq 3$ yrs) had the lowest TT, FT, and LH concentrations (non-significant though) than men exposed for a shorter duration of less than three years (H_1BH_2-arm, <3 yrs) which in turn had even lower baseline levels than men who were not exposed at all (B_1HB_2-arm). However, at baseline, unexpectedly, men exposed to high-DBP for a longer duration (H_1BH_2-arm, $H_1 \geq 3$ yrs) had non-significant slightly higher sperm parameters (except semen volume) than men exposed for a shorter duration (H_1BH_2-arm, <3 yrs). However, among men in the H_1BH_2-arm, there was a downward trend in all semen parameters at baseline associated with increased duration on high-DBP mesalamine. This negative association with the duration on high-DBP mesalamine among men in H_1BH_2-arm argues against a 'protective' effect on semen quality of high-DBP. The overall difference between H_1BH_2 and B_1HB_2arm at baseline suggests chance sampling variability due to non-randomization at the baseline and cautions against cross-sectional analyses. These results highlight the power of our crossover-crossback study that by design removes person to person variability from the comparison of interest, thus avoiding the purely cross-sectional analysis that is likely to suffer from this random variability and lack temporality.

Adequate testosterone production and stimulation by FSH are prerequisites for normal human spermatogenesis(176). Also, inhibin-B is a marker of testicular function and positively correlated with sperm count and testicular volume(177). Studies suggest that the regulation of inhibin-B production is mediated by a complex interaction between FSH, Sertoli cells, Leydig cells, and germ cells(178). Inhibin-

B and FSH are considered spermatogenesis markers and even a possible surrogate for measuring semen quality or fecundability in epidemiologic studies(179,180).

We speculate that effect on spermatogenesis may be through hormonal changes whose effects continued beyond one spermatogenesis cycle of 70 days but also perhaps through other independent or synergistic modes of actions. There is a caveat that there are limited comparable animal data to explain modes of action of high-DBP exposure in adult life especially with such unique crossover-crossback design, and no data on the potentially modified hormone responses by the duration of exposure. In addition, many of the experimental animal studies only measured testosterone and not gonadotropins.

Experimental studies have focused on the more vulnerable fetal exposure to DBP rather than adult exposure. Studies during fetal development showed that DBP was anti-androgenic resulting in decreased testosterone production by Leydig cell due to interference with steroidogenesis, down-regulating gene and/or protein expression essential for the steroidogenic pathway, and cholesterol transport and metabolism(107,110,111), while increasing oxidative stress along with reactive oxygen species(113). Experimental studies, primarily during postnatal exposure(87,100), showed that decreased serum testosterone concentration and FSH and LH concentrations in male rats exposed to butyl benzyl phthalate (BBzP), other anti-androgenic phthalate, which suggested tubular atrophy and loss of the germinal epithelium as a result of Sertoli cell injury. Postnatal exposure to other di(2-ethylhexyl)phthalate (DEHP), another anti-androgenic phthalate, caused germ cell apoptosis with increased membrane localization of Fas(101,102) or germ cells detachment from the seminiferous epithelium probably due to cellular adhesion loss between Sertoli cells(103). Only few studies primarily focused on peri-pubertal DBP-exposure and observed changes in the expression of proteins involved in spermatogenesis and in the number and function of Sertoli and Leydig cells(40,41) and some studies suggested that DBP preferentially targets Sertoli cells in older animals (104,105).

Our results also suggest that high-DBP exposure in the newly exposed men decreased both testicular hormone and gonadotropin serum levels with disruption of the negative feedback of the gonadotropins in response to the changes in the testicular hormones. Similar results of suppression of both gonadotropins and testosterone as well as decreased semen quality were observed in the newly-exposed men to high-DBP mesalamine and were also observed in DEHP newly-exposed pre-pubertal and mature animals(130,131). In adult DEHP-exposed animals, pituitary LH and FSH protein expression decreased significantly(132,133). The testosterone decrease might indicate suppression of Leydig cell function and inhibin-B decrease might indicate suppression of Sertoli cell function as well as germ cell production in adult testis(134). Similarly, in humans, both inhibin-B and FSH decreased with DEHP exposure(135) suggesting that the normal endocrine communication between the pituitary and the testis might be disrupted(41,135) hence disruption of the hypothalamo-pituitary-testis axis (HPT). We speculate that high-DBP exposure from mesalamine was associated with HPT axis disruption targeting both the gonads and the pituitary in the newly exposed men. However, our results also suggest that the negative gonadotropin feedback response was functioning in men who were exposed to high-DBP before but for a short duration (<3yrs). This may suggest that after short exposure only the testicular hormones remain suppressed and the negative gonadotropin feedback might be regained. However, after longer duration of high-DBP exposure (≥3yrs), the reproductive hormones were suppressed and desensitized to exposure removal.

Recent epidemiological studies investigated the association between environmental (background) DBP-exposure on reproductive hormones and/or semen quality in adult men. However the results were inconsistent. A recent meta-analysis that included fourteen publications on phthalates and semen quality concluded that higher urinary monobutyl phthalate (MBP) concentration, the primary metabolite of DBP, was associated with reduced sperm concentration and straight line velocity(99). All fourteen publications

were cross-sectional with eleven conducted in infertility clinics. Two recent US studies(42,43) on fertile men not included in the meta-analysis did not find significant associations.

Also, urinary MBP was negatively correlated with FT among occupationally exposed Chinese men(136). Studies in infertility clinics observed that urinary MBP was associated with a significant testosterone decrease in men from Slovenia(49), China(57), and India (only in the unadjusted analysis)(50) but not in men from Poland(58). Both pooled and non-pooled analysis of infertile and fertile men in the US did not find any significant associations between urinary MBP and any of the reproductive hormones(44-46). Only a few studies included men from the general population. Urinary MBP was inversely associated with testosterone only in men aged 40-60 years from the National Health and Nutrition Examination Survey (NHANES)(47), but no strong evidence from studies in Denmark (only a significant increase in LH)(48), Sweden(137), or China(138).

Our study differs in many respects from the earlier cross-sectional studies, including all studies described above, that used a single semen sample or a single serum sample to measure the semen quality parameters or hormone concentrations, respectively at the same time when urinary MBP was measured. In addition, most of the studies were based in infertility clinics. To our knowledge, Janjua et al(139) conducted the only human study with some similarity to our design examining the association between DBP-exposure (dermally) and short term effects only on reproductive hormones and did not examine semen quality. It was a placebo controlled crossover study in which men were assigned to daily whole-body topical DBP-exposure for a week after a week of placebo exposure. They did not find associations of topical DBP-exposure with reproductive hormones. These results need to be interpreted cautiously due to a small sample size (n=26), non-randomization, likely too short washout period (one week), only assessing the short term effects, and considering only topical exposures with much lower anticipated exposure compared to our study. Our study assessed the chronic high-DBP exposure from oral mesalamine medications that was estimated to be approximately 1000 times higher than the median levels reported for men in the US general population(36) and was not investigated before. We used a more powerful

crossover-crossback prospective design and therefore had repeated blood and semen samples from men during both high and background DBP exposure. This allowed for within-person comparisons and was by design adjusted for non-time varying covariates(121). Furthermore, our design allowed us to assess the potential carryover effect.

Many studies identified DBP and other phthalates in coatings of many licensed including over-the-counter medications in the US(23-26), Canada(28,35), United Kingdom(30), Germany(27,32) and China(31). Phthalate concentrations in medications may not be openly displayed, due to proprietary formulations(23). Medications with phthalates in the coating include mesalamine, didanosine, omeprazole, theophylline(24) and others(23,27,32). Our research and others have shown that mesalamine medications with DBP contribute to a very high-DBP exposure as measured by urinary MBP concentration(24-26). The human daily DBP intake from the maximum recommended dose of Asacol® is 21 mg(34,114) and of Asacol®HD is 48 mg(115). Therefore, the estimated maximum daily DBP intake for a 60 kg person who is under high-DBP mesalamine (Asacol® or Asacol®HD) is 350 or 800 µg/kg/day(29), respectively. These exposures are considered very high-DBP exposures compared to the estimated average DBP human intake of 0.84 - 5.22 µg/kg/day(116), the US Environmental Protection Agency (EPA) reference dose of 100 µg/kg/day(26), and the European tolerable daily intake of 10 µg/kg/day(117).

The urinary MBP in persons under Asacol® was approximately 1000 times higher than the median levels reported for men in the US general population (NHANES)(36). Therefore, we used *a priori* medication type as assigned as a proxy for high-DBP exposure as an intention to treat analyseis(29) rather than measuring urinary MBP concentrations which represent only the past several hours of DBP-exposure due to its short half-life(139). Due to the unique crossover-crossback design and the well-documented very high exposure from Asacol®(23-27,32,34,114,115), that urinary MBP concentrations would have not added significant information beyond using the assigned medication. Also, statistically, we would

essentially dichotomize exposure according to the different mesalamine formulations (DBP versus non-DBP containing) even if we had the continuous urinary data and use the same modeling approach we used due to the expected non-overlapping large differences in urinary MBP. Thus, the effect estimates would have been completely dominated by the medication type(29).

Study limitations

Our study had several potential limitations including that we did not randomize the men to high-DBP or non-DBP containing mesalamine coatings at baseline. However physicians do not prescribe mesalamine based on DBP in the enteric coating nor on their hormonal concentrations or semen quality. Mesalamine medications (with or without DBP coating) for treating inflammatory bowel disease (IBD) have the same active ingredients, therefore confounding by indication was unlikely. Although our sample size was not very large due to the innovative study and participation length, the power of the study came from the unique design and the intra-person comparison rather than the inter-person variability avoiding the purely cross-sectional analysis that may be confounded and lack temporality. We also conducted a post-hoc power analysis and the study had a sufficient number of participants to provide 80% power for detecting at least 7% change in TT or FT, 8% in SHBG, 6% in inhibin-B and FSH, 9% in LH, 23% in sperm concentration and an absolute mean difference of at least 0.4 ml in ejaculate volume, 1% in morphologically normal sperm, 6% in motile sperm and 4% in progressive motile sperm, which are all reasonable for a study of this design. Although there may be a concern of generalizing results from men with IBD whose incidence is significantly increasing worldwide especially in industrialized countries(140), these results raise concerns about high-DBP exposure and reproductive hormones. In addition, there is no evidence that IBD or mesalamine is linked to male infertility(123,124).

Study strengths

Our study had several important strengths. MARS has a unique innovative design rarely conducted in environmental epidemiology. We were able to compare, within the same men, their hormone parameters

during periods of high-DBP to background-DBP exposure and vice versa accounting for confounding by measured and unmeasured non-time-varying characteristics(121). This is a major strength compared to all existing cross-sectional studies. We had repeated measures up to six time points per man which increased the reliability of the measures. The different orders of exposure and unexposure in the different arms gave us a unique opportunity to observe the potential reversibility and carryover effect of the changes under the study period. Our study was not restricted to infertile men unlike most of the previous literature. We used the gold standard methods for semen and hormone analyses especially testosterone that accurately and precisely measured total using LC-MS/MS methodology(141). We did not use the free androgen index (FAI) as a surrogate for FT as most of the studies did because the FAI correlates poorly with FT in men(141). Instead, we used the actual measured SHBG, albumin and TT applying Vermeulen et al.'s method which is considered the most useful estimate of FT in blood(129,141). We had the opportunity to examine high-DBP exposure (1000 times background) compared to environmental background exposure. Therefore, we had more power to detect significant changes.

Implications of the study

Our results are of a potential high concern because testosterone deficiency in adult men is a risk factor not only for decreased semen quality as we observed but also for infertility, decreased libido, energy, intellectual capacity, bone mass, and muscle strength, and associated with erectile dysfunction, osteoporosis, abdominal obesity, type-2 diabetes, metabolic syndrome, and, cardiovascular disease, and hence increased mortality(142-145). Recently, there was a consensus that women with IBD who are pregnant or might become pregnant may need to avoid high-DBP mesalamine(28) leaving men in reproductive age with no recommendations and no guidance about any other medications that contain phthalate coating. Therefore, our results suggest the need to reconsider the use of DBP containing mesalamine and other DBP containing medications for men.

Future suggestions for a crossover-crossback design

Although our study had a unique innovative design with several strengths, we have some suggestions for future research or for those considering a similar crossover-crossback study. The following recommendations include enrolling more men within each arm so we gain more power to detect significant effects. We would recruit men taking DBP containing medications include mesalamine, didanosine, omeprazole and theophylline(24) with the same prescribed condition e.g., mesalamine for inflammatory bowel disease (IBD) or theophylline for asthma to investigate how generalizable the results are. We would recruit men taking each medication and with the same condition with enough power. We can always test in the analyses if the results are different within men with the same condition. Although the results are important even within these subgroups, however, it is important to know how much the results are generalizable to men from the general population with lower but ubiquitous exposure with potential high-DBP exposure from medications. This would have even more important implications for public health and for the regulatory agencies. We would recruit men in the B_1HB_2-arm as twice in number as in the H_1BH_2-arm so we have more power to examine effects in the newly exposed men who were more susceptible to changes than the ones with previous history of high-DBP exposure from medications. We would recruit half of men in the H_1BH_2-arm with a short high-DBP exposure history e.g., < 3 years and the second half of men in the H_1BH_2-arm with a long high-DBP exposure history e.g., ≥ 3 years. Thus, we have enough power to examine the heterogeneity of response to high-DBP exposure. We would also recruit men especially the ones with long exposure history preferably starting their high-DBP exposure from medications in their childhood i.e., pre-pubertal so we have the opportunity to examine the potential difference in response if the exposure started early in childhood and for longer duration versus if the exposure started after puberty and for shorter duration.

We would keep the number of the samples (urine, blood and semen) as six samples each similar to the original MARS study to keep the good compliance we had (average of five sample per man overall) however, we plan to change the sample spacing to get more information about the variability over time.

We would collect only one baseline sample (v1) instead of two. We also would choose *a priori* the 'crossover and 'crossback' periods to be six months. We would want to examine the reversibility of responses after enough washout periods of more than two spermatogenesis cycles instead of four months. However, instead of collecting the two crossover samples after the whole crossover period, here six months, we would collect one crossover sample (v2) after four months and the other crossover sample (v3) would be after additional two months. The participants will need to crossback to their original medications after v3 for another six months. The first crossback sample (v4) would be after four months of crossback then the second crossback sample would be after additional two months (v5). Then, we would plan to collect the last sample (v6) for each participant after one year from v5 while the participants are still on their original medications to be able to test for potential reversibility if any after one year. See (Figure 4.1) for our proposed new design.

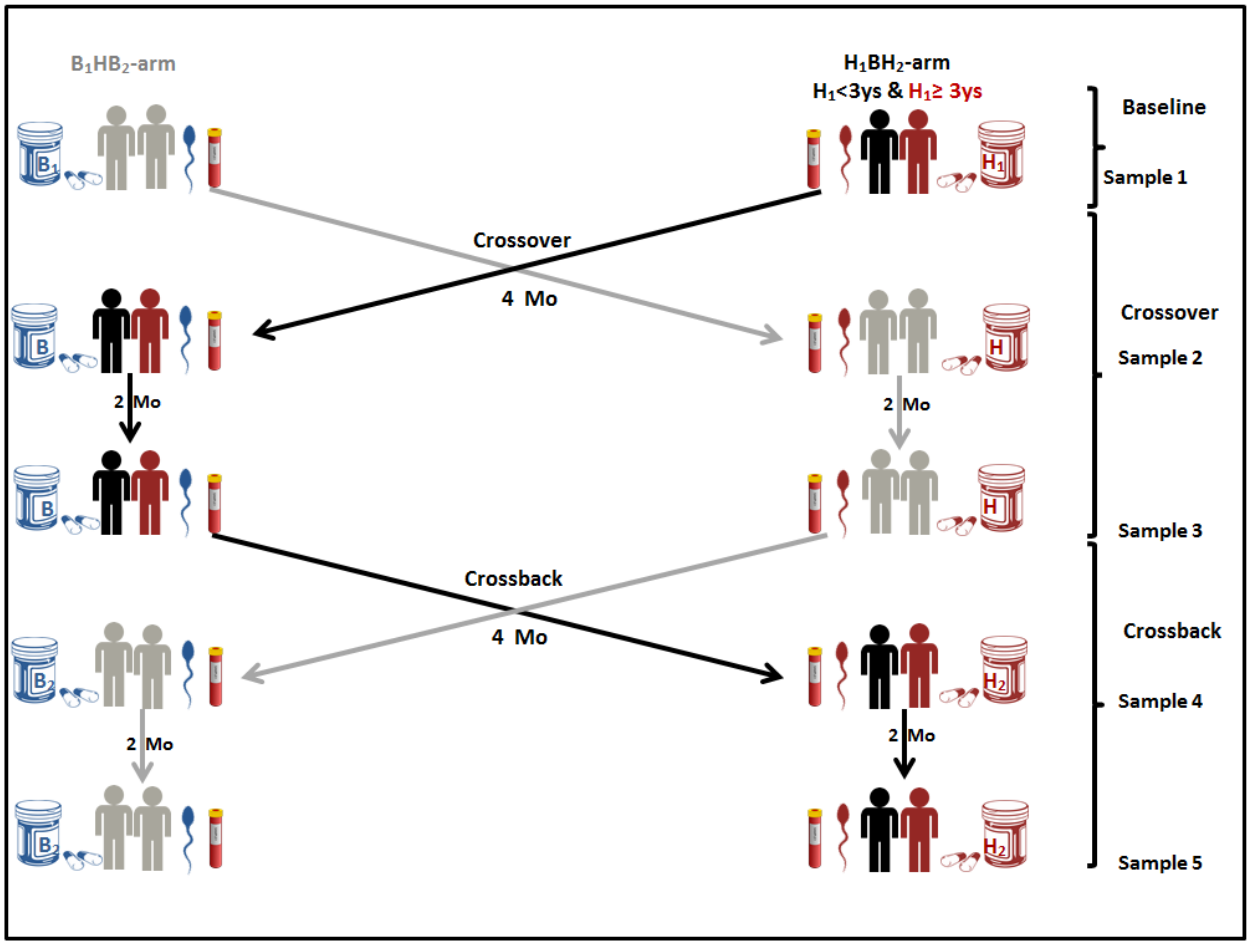
Conclusions of Chapters 1 and 2

Although, some medications are a high-exposure source especially for certain subpopulations such as IBD patients, this source is relatively overlooked and the focus has been more on background low environmental exposure. To our knowledge, MARS is the only and the first study that examined the association between such high-DBP exposure and health outcomes and we used an innovative crossover-crossback design.

Our results present evidence that high-DBP exposure disrupted serum levels of pituitary-gonadal hormones that largely reversed after exposure removal, but only in men with no history of or a short duration (<3yrs) of previous high-exposure. Paradoxically, among men with longer duration of high-DBP exposure, exposure removal did not change hormone levels, suggesting that long-term high-DBP exposure may alter the pituitary-gonadal axis and make it insensitive to changes in exposures.

We believe that our research should be of relevance for the regulatory agencies such as FDA and EPA as well as the clinical community and the general population.

Figure 4.1 Mesalamine And Reproductive health Study (MARS) new proposed design.



B_1HB_2-arm: B_1 represents background-exposure at baseline, **H** represents high-DBP exposure after crossover and **B_2** represents background-exposure after crossback; includes men taking mesalamine that did not contain DBP at baseline. **H_1BH_2-arm: H_1** represents high-DBP exposure at baseline, **B** represents background-exposure after crossover and **H_2** represents high-DBP exposure after crossback; includes men taking mesalamine-containing DBP at baseline. **Abbreviations: MARS**, Mesalamine And Reproductive health Study; **DBP,** dibutyl phthalate; **Mo**, month.

Discussion of Chapter 3

In Chapter 3, we examined the association between self-reported use of fourteen personal care products (PCPs) and urinary concentrations of nine urinary phthalate metabolites and three parabens in men participated in a prospective cohort (Environment And Reproductive Health (EARTH)) study. We found that self-reported PCP use among men was associated with higher urinary concentrations of monoethyl phthalate (MEP) and three parabens (methyparaben, propylparaben and butylparaben). Due to the short half-lives of phthalates and parabens(62,65) and the episodic use of PCPs, observed associations were stronger for PCP use within six hours of urine collection as compared with use twenty four hours prior to collection. We showed that ten PCPs contributed to at least 70% of the weighted score and explained more than a 250% increase in the urinary concentrations of MEP and the three parabens. Mono-isobutyl phthalate (MiBP) or the other phthalate metabolite urinary concentrations did not predict PCP use, consistently with infrequent use of these phthalates in PCPs(59).

In this work, men's self-reported information on PCP use was a useful tool for assessing mainly diethyl phthalate (DEP) and paraben exposures. Our recommendation for refining the questionnaire includes focusing on the following ten PCPs: cologne/perfume, deodorant, suntan/sunblock lotion, hand/body lotion, aftershave, other hair care products, mouthwash, conditioner/crème rinse, hairspray/hair gel, and liquid soap/body wash.

To our knowledge only two studies(68,83) investigated the association between PCP use and urinary phthalate metabolites and parabens in men. Our research is the first to use repeated collection of self-reported PCP use and urinary concentrations of phthalate metabolites and parabens from the same man for a large number of men over a ten year period and to use detailed information on time of PCP use. Another novel methodological aspect of this work was the use of a weighted PCP score to predict urinary concentrations in the multi-PCP approach. The weighted binary score (WBS) analyses yielded different weights for each PCP depending on the association with each urinary biomarker. In addition, the multi-

PCP approach could adjust for the other correlated PCPs and estimate the joint effect of multiple PCP use, rather than simply using the crude sum of the PCPs or modeling each PCP separately. A similar approach has been used in linear regression settings(167-170,181), and we extended the basic weighted quantile strategy in earlier work to the repeated measures data and in Tobit regression framework.

Conclusions of Chapter 3

In this work, we identified PCP predictors of urinary concentrations of MEP and three parabens in a large cohort of men. The results will be useful in future exposure assessment studies in men on PCP use as an important source of exposure to phthalates (mainly DEP) and parabens. Collecting concise PCP use information can be achieved by only asking specific questions about the use of the most relevant "important" PCPs within a narrow exposure window (six hours). Shortening the questionnaire would likely decrease missingness and improve participant's response rate. In addition, restricting the duration of inquiry to the six hours prior to urine collection would provide a more precise estimate of exposure to phthalates and parabens since the biomarkers urinary concentrations are a better reflection of exposure in the last 6 hours than in 24 hours. It is also likely that participants will better recall their PCP use within a shorter time frame hence decreasing misclassification while also optimizing research cost and time.

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