

July 2020

A Cross-Sectional Study of Phthalate Exposure and Inflammation Biomarker Levels Among Postmenopausal Women

Avery Trim
University of Massachusetts Amherst

Follow this and additional works at: https://scholarworks.umass.edu/masters_theses_2



Part of the [Epidemiology Commons](#)

Recommended Citation

Trim, Avery, "A Cross-Sectional Study of Phthalate Exposure and Inflammation Biomarker Levels Among Postmenopausal Women" (2020). *Masters Theses*. 902.
https://scholarworks.umass.edu/masters_theses_2/902

This Open Access Thesis is brought to you for free and open access by the Dissertations and Theses at ScholarWorks@UMass Amherst. It has been accepted for inclusion in Masters Theses by an authorized administrator of ScholarWorks@UMass Amherst. For more information, please contact scholarworks@library.umass.edu.

A Cross-Sectional Study of Phthalate Exposure and Inflammation Biomarker Levels
Among Postmenopausal Women

A Thesis Presented

By

AVERY TRIM

Submitted to the Graduate School of
the University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

May 2020

School of Public Health and Health Sciences

Department of Biostatistics and Epidemiology

A Cross-Sectional Study of Phthalate Exposure and Inflammation Biomarker Levels
Among
Postmenopausal Women

A Thesis Presented

By

AVERY TRIM

Approved as to style and content by:

Katherine Reeves, Chair

Susan Hankinson, Member

Paula Stamps, Graduate Program Director
Department of Biostatistics and Epidemiology

ABSTRACT

A CROSS-SECTIONAL STUDY OF PHTHALATE EXPOSURE AND INFLAMMATION BIOMARKER LEVELS AMONG POSTMENOPAUSAL WOMEN

MAY 2020

AVERY TRIM, B.S., UNIVERSITY OF MASSACHUSETTS AMHERST

M.S., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed By: Dr. Katherine Reeves

Phthalates are industrial chemicals added to plastics found in products such as children's toys, cosmetics, and household items, and some laboratory studies suggest phthalates may increase levels of inflammation. Chronic inflammation is associated with many chronic health conditions, such as diabetes and rheumatoid arthritis. Although research is limited, recent studies suggest a strong positive relationship between mono-butyl phthalate (MBP), mono-isobutyl phthalate (MiBP), and monocarboxynonyl phthalate (MCNP) and c-reactive protein (CRP), as well as monoethyl phthalate (MEP) and mono-3-carboxypropyl phthalate (MCP) and interleukin-6 (IL-6). Additionally, this relationship has not been examined among postmenopausal women, a population that is at higher risk of developing chronic health conditions. Our aim was to examine the association between urinary phthalate biomarkers and inflammation biomarkers among postmenopausal women using baseline data from a subset of participants of the Women's Health Initiative (WHI) (n=443). Phthalate exposure was assessed using phthalate biomarkers (i.e. phthalate metabolites or their molar sum) from urine samples collected at WHI clinical centers from 1993-1998. We measured 13 phthalate metabolites: MEP, MBP, mono-hydroxybutyl phthalate (MHBP), MiBP, mono-hydroxyisobutyl phthalate

(MHiBP), monobenzyl phthalate (MBzP), MCPP, mono (2-ethylhexyl) phthalate (MEHP), mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono (2-ethyl-5-oxohexyl) phthalate (MEOHP), mono (2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-carboxyoctyl phthalate (MCOP), and MCNP. Serum and plasma inflammatory biomarker levels (i.e. CRP, IL-6) were measured in separate WHI ancillary studies, using blood samples collected at baseline. We used multivariable linear regression to analyze associations between each phthalate biomarker and inflammation biomarker, adjusting for important covariates. Phthalate biomarkers MCNP (Model 1: $\beta = 0.523$; Model 2: $\beta = 0.362$) and MCOP (Model 1: $\beta = 0.384$; Model 2: $\beta = 0.240$) were positively associated with CRP. Additionally, MCNP (Model 1: $\beta = 0.369$; Model 2: $\beta = 0.181$) was positively associated with IL-6. Statistically significant associations were not observed among the remaining phthalate biomarkers. Our findings suggest that certain phthalates may be related to increasing levels of inflammation.

TABLE OF CONTENTS

	Page
ABSTRACT.....	iii
LIST OF TABLES.....	vi
LIST OF FIGURES.....	vii
CHAPTER	
I. INTRODUCTION.....	1
II. SUBJECTS AND METHODS	3
A. Study Population	3
B. Phthalate Exposure Assessment.....	3
C. Inflammation Biomarker Assessment.....	5
D. Consolidation of Inflammation Biomarker Results.....	6
E. Covariate Assessment.....	6
F. Statistical Analyses.....	7
III. RESULTS	9
IV. DISCUSSION	11
APPENDIX: SUPPLEMENTAL TABLES	16
BIBLIOGRAPHY.....	26

LIST OF TABLES

Table	Page
1. Beta coefficients and 95% confidence intervals for predictors of inflammation and CRP and IL-6 levels.....	16
2. Distribution of sociodemographic and behavioral characteristics in the Women's Health Initiative Study (WHI); 1993-1998.....	19
3. Distribution of creatinine-standardized phthalate metabolite concentrations and inflammation biomarker levels in the Women's Health Initiative (WHI) Study (n=443); 1993-1998.....	20
4. Pearson correlation table for creatinine-standardized phthalate metabolite concentrations and inflammation biomarker levels in the Women's Health Initiative (WHI) Study; 1993-1998.....	21
5. Multiple linear regression and 95% confidence intervals for phthalate metabolite concentrations and CRP levels (n=414).....	22
6. Multiple linear regression and 95% confidence intervals for phthalate metabolite concentrations and IL-6 levels (n=177).....	23
7. Multiple linear regression and 95% confidence intervals for phthalate metabolite concentrations and CRP levels (n=258) among participants not currently using NSAIDs.....	24
8. Multiple linear regression and 95% confidence intervals for phthalate metabolite concentrations and IL-6 levels (n=105) among participants not currently using NSAIDs.....	25

LIST OF FIGURES

Figure	Page
1. Study population ascertainment for the analysis of phthalate exposure and inflammation biomarker levels in postmenopausal women.....	17
2. Venn diagram of inflammation biomarker results among participants.....	18

CHAPTER I

INTRODUCTION

Accumulating scientific evidence, especially in recent years, indicates a potentially harmful relationship between phthalates and inflammation. Phthalates are man-made chemicals added to plastics, and can be found in many everyday household, personal care, medical, and child products.¹ Detectable concentrations have been observed among the majority of the U.S. population in varying amounts.² Inflammation is a response to the presence of unknown substances within the body and is a natural defense mechanism that typically occurs in acute phases.³ However, chronic inflammation, which often goes unresolved,³ is associated with rheumatoid arthritis⁴, Alzheimer's disease,⁵ diabetes,^{5,6} cancer,⁵⁻⁷ cardiovascular disease^{6,8} and osteoporosis⁹, which are prevalent among postmenopausal women.

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) is suggested as a potential mechanism behind the association between phthalates and inflammation. It is hypothesized that phthalate exposure leads to the activation of NF- κ B, which signals the production of pro-inflammatory cytokines, such as interleukin-6 (IL-6).¹⁰ NF- κ B is also shown to stimulate production of c-reactive protein (CRP), however this process is suggested to occur through IL-6 and interleukin 1 beta (IL-1 β).¹¹

Animal and cellular studies indicate that phthalate exposure can increase the production of pro-inflammatory cytokines, although this positive relationship is typically observed in higher phthalate metabolite concentrations.^{10,12-17} Prior epidemiological studies also indicate positive associations between phthalate metabolites MBP, MiBP, and MCNP and CRP, as well as MEP and MCPP and IL-6.¹⁸⁻²⁰ Directionality among

other phthalate metabolites is inconsistent and may be due to insufficient power from small sample sizes, or the use of a single phthalate urinary sample.¹⁸⁻²¹

Prior research studies have not examined the relationship between phthalate metabolite concentrations and inflammation biomarker levels among postmenopausal women only. We investigated the associations between 13 phthalates metabolite and 2 inflammation biomarkers using 1993-1998 baseline data from a subset of Women's Health Initiative (WHI) participants.

CHAPTER II

SUBJECTS AND METHODS

A. Study population

WHI is a large-scale national study evaluating potential strategies to prevent and minimize causes of morbidity and mortality among postmenopausal women.²² WHI conducted three clinical trials as well as a separate observational study from 1993-1998, enrolling a total of 161,808 postmenopausal women from 50-79 years old.^{23,24} Written informed consent was provided upon enrollment.²⁴ In addition, WHI conducted a bone density substudy at three clinical sites, which included 11,020 participants from any of the clinical trials or the observational study. Our nested case-control study selected breast cancer cases and 2:1 matched controls from among WHI bone density substudy participants.^{23,25} A number of ancillary studies also occurred throughout the WHI study and measured inflammatory biomarkers on selected participants. Our cross-sectional study included selected participants from the nested-case control study with inflammatory biomarker values measured at baseline from overlapping ancillary studies (n=443). Participants were excluded if they 1) were missing baseline covariate information, and 2) had results measured using unreliable assays (i.e. those with high CV). Figure 1 shows the study population ascertainment for this analysis.

B. Phthalate exposure assessment

Phthalate exposure was assessed using biomarkers (i.e. urinary phthalate metabolites). First morning void urinary samples were conducted by participants at home and

refrigerated until their baseline visit at a WHI clinical center. During this visit samples were acquired and frozen by trained personnel until they were ready for shipment to McKesson Bioservices, where they were stored at -80°C. Participant samples used for the WHI nested-case control study were taken from McKesson Bioservices and sent to the CDC for processing and analysis. Urine samples were analyzed for 13 phthalate metabolites (MEP, MBP, MHBP, MiBP, MHiBP, MBzP, MCPP, MEHP, MEHHP, MEOHP, MECPP, MCOP, and MCNP) using enzymatic deconjugation of the glucuronidated analytes, followed by assessment of exposure levels using on-line solid phase extraction and high performance liquid chromatography-electrospray ionization-tandem mass spectrometry. Each phthalate metabolite (or their molar sum) were considered as continuous variables in the analyses.

Phthalate exposure was assessed using an objective measure (i.e. urine sample). In addition, laboratory workers were not privy to any information that could identify participants based on their urinary sample, such as disease status. Coefficient of variation (CV), which uses blinded duplicate samples to examine potential differences in lab values, was used on 10% of phthalate metabolite samples to assess validity. CVs were 5.4% for MBP, 6.1% for MBzP, 4.7% for MCNP, 6.3% for MCOP, 5.8% for MCPP, 4.3% for MECPP, 5.4% for MEHHP, 19.5% for MEHP, 6.0% for MEOHP, 3.1% for MEP, 9.0% for MHBP, 21.9% for MHiBP, and 10.3% for MiBP.²³ Phthalate metabolite concentrations below the limit of detection (LOD) were given a value equal to $\text{LOD} / \sqrt{2}$.²³ Five phthalate metabolites had samples whose concentrations were below the LOD (MBP = 0.07%, MEHP = 0.63%, MHBP = 0.43%, MHiBP = 1.56%, MiBP = 0.46%).²³ All samples from the eight other phthalate metabolites were above the LOD.

C. Inflammation biomarker assessment

Blood samples were collected at WHI clinical centers during the first screening visit, prior to which participants were required to fast for at least 12 hours. Participants were also asked to refrain from smoking, taking aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs), or partaking in strenuous physical activity prior to their visit. To separate plasma (ethylenediaminetetraacetic acid (EDTA) and citrate) and serum from the blood, samples were left to clot at 4°C for approximately one hour and then centrifuged for 10 minutes. Separated serum and plasma were then placed into multiple smaller vials and frozen at -70°C for 2 hours until they were ready to be shipped to McKesson Bioservices for permanent storage at -80°C. Samples were then shipped from McKesson Bioservices to a laboratory for analysis. Among the 22 ancillary studies included in our final sample, inflammation biomarker levels were measured at approximately 10 different laboratories using around 6 different assay methods (Immulite Immunoassay Analyzer, Enzyme Linked Immunosorbent Assay (ELISA), Quantikine High Sensitivity Immunoassay, Luminex Multiplex Cytokine Assay, Roche Modular P Chemistry Analyzer, Behring Nephelometer II Analyzer). Inflammation biomarkers were measured as continuous variables in our analyses.

An average CV percentage was calculated based on groupings by biomarker, sample type, testing method, and lab.²⁶ Average CVs ranged from 1.9% to 9.2% among CRP values,²⁷ and 4% to 42% among IL-6 values.²⁷ The correlation coefficient, which measures the degree of association between blinded duplicate sample pairs, was calculated and averaged based on groupings by biomarker, sample type, testing method,

and lab.²⁶ Average correlation coefficients ranged from 0.9-1.0 among CRP values and 0.75-1.0 among IL-6 values.²⁷

D. Consolidation of inflammation biomarker results

Some participants had multiple CRP and/or IL-6 results from various WHI ancillary studies. Among participants with more than one specimen type (i.e. citrate, serum, EDTA) per inflammation biomarker, a single result was selected based on the frequency of specimen types within the total sample. Random sampling was used in order to select one result per biomarker and participant. Additionally, to harmonize inflammation biomarker results across ancillary studies, predicted values of each inflammatory biomarker were calculated from linear regression models using strong predictors including age, smoking status, BMI, use of anti-inflammatory medication 48 hours prior to blood draw, ancillary study ID (i.e. assay method and lab), and storage time (i.e. the time between blood draw and assay). Predicted CRP levels equal to zero were given a value of 0.025 (ng/mL or pg in order to allow for log transformation of values).

E. Covariate assessment

Sociodemographic and behavioral characteristics, as well as medical history were ascertained from a self-report questionnaire completed at first screening visit. Physical measurements (ex. Height, blood pressure) were taken at first screening visit. Current medication information was determined by collection of participant medications at first screening visit and later recording prescription information. We included age,^{18,21,28} creatinine,¹⁸ race/ethnicity,^{18,20} socioeconomic status,²¹ smoking status,²¹ alcohol intake,²¹

and BMI¹⁸⁻²¹ as covariates based on prior epidemiological studies. Total physical activity was also included as a covariate, as prior literature indicates confounding between phthalate exposure and inflammation.^{23,29}

F. Statistical analysis

Phthalate biomarkers (i.e. individual metabolites or their molar sums) and inflammation biomarkers were natural log transformed for data to follow a normal distribution. Demographic and behavioral characteristics of the study population were summarized by inflammation biomarker and compared to distributions in the nested case-control study. T-tests or chi-square tests were used to examine potential differences in characteristics between participants sample sizes for each inflammation biomarker and the nested case-control study. Distributions (i.e. mean, standard deviation, range) of phthalate biomarker (creatinine-standardized) and inflammation biomarker levels were calculated. Pearson's correlations were calculated to assess the level of dependence of each phthalate biomarker and inflammation biomarker.

Two multivariable linear regression models were used to examine the relationship between each phthalate biomarker and inflammation biomarker. Covariates which produced at least a 10% change in inflammation biomarker level estimates were included in both models. Model 1 was adjusted for age, creatinine, socioeconomic status, alcohol intake, and smoking status as covariates. Model 2 was adjusted for covariates included Model 1 as well as BMI. Among most phthalate biomarkers, race/ethnicity and total physical activity had little impact on the estimated association with inflammation biomarkers (<10% change) in both models, and therefore were not included as covariates.

Our analyses included 443 participants (CRP, n=414; IL-6, n=177) who had complete data on covariates, exposure, and outcome. As a sensitivity analysis, we repeated our multivariable linear regression analyses using participants who were not identified as current NSAID users based on medication data collection at baseline. For all of our analyses, a p-value < 0.05 was considered statistically significant.

Power calculations were based on a Type III F test within a multiple linear regression model. Among participants with CRP values (n=414), we had >80% power to explain 10-20% of variability (i.e. standard error) in values with 95% confidence, based on the inclusion of 6 predictors and use of continuous phthalate metabolites. Using the same criteria, we had >80% power among participants with IL-6 values (n=177).

CHAPTER III

RESULTS

Table 2 describes the distribution of sociodemographic and behavioral characteristics of the study participants in this cross-sectional study by inflammation biomarker, in comparison to the distribution within the nested case-control study. Compared to participants within the nested case-control study, participants with CRP values were more likely to be non-white, non-drinkers, have a higher annual income and lower socioeconomic status. Similarly, participants with IL-6 values were more likely to be older, non-white, past-smokers, and consume less than 1 drink per week.

Table 3 describes the distribution of creatinine-standardized phthalate biomarker concentrations and inflammation biomarker levels among all study participants. MBzP, MCPP, MCOP and MCNP concentrations have similar values ranging from 0.007 $\mu\text{g/g}$ to 2.558 $\mu\text{g/g}$. ΣDEHP , ΣDBP , and ΣDiBP concentrations have similar values ranging from 0.00003 $\mu\text{g/g}$ to 0.083 $\mu\text{g/g}$. MEP concentrations range from 0.088 $\mu\text{g/g}$ to 130 $\mu\text{g/g}$, with an average value of 3.8 $\mu\text{g/g}$. The distribution of CRP levels ranges from 0.025 mg/L to 16.4 mg/L, with a mean of 14.9 mg/L and a standard deviation of 3.0 mg/L. IL-6 levels range from 0.029 pg/mL to 11.2 pg/mL, with a mean of 2.9 pg/mL and a standard deviation of 2.0 pg/mL.

Table 4 describes the relationship between each creatinine-standardized phthalate biomarker and inflammation biomarker using Pearson's correlation. Overall, results indicate a weak, non-significant correlation between each phthalate biomarker and inflammation biomarker. However, we observed statistically significant, positive

correlation between MCNP and IL-6 ($r = 0.20$, $p = 0.01$).

Multivariable linear regression models assessing the relationship between each creatinine-standardized phthalate biomarker and inflammation biomarker are shown in Tables 5 and 6. Coefficients represent the change in inflammatory biomarker per 10-unit change in the phthalate metabolite on the natural scale. Model 1 is adjusted for age, creatinine, alcohol intake, socioeconomic status, and smoking status. Model 2 is adjusted for covariates in Model 1 as well as BMI. We observed statistically significant, positive associations between MCNP and CRP in both models: Model 1 ($\beta = 0.523$; $p = 0.0002$), Model 2 ($\beta = 0.362$; $p = 0.004$). Similarly, we found a statistically significant positive association between MCNP and IL-6 in Model 1 ($\beta = 0.369$, $p = 0.01$), but not in Model 2. We also observed a statistically significant positive association between MCOP and CRP in Model 1 ($\beta = 0.384$, $p = 0.01$) and a borderline significant positive association in Model 2 ($\beta = 0.240$, $p = 0.05$). Similar results were observed when restricting these analyses to participants not currently using NSAIDs (Tables 7 and 8).

CHAPTER IV

DISCUSSION

Overall, we did not observe strong, statistically significant associations between urinary phthalate biomarkers and CRP or IL-6 in our sample of postmenopausal women from WHI. We found a significant positive association between MCNP and CRP when including and excluding BMI as a covariate, with comparable findings between MCOP and CRP. A similar trend is observed among participants not currently using NSAIDs, indicating that NSAID use does not affect the relationship between phthalates and inflammation. Ferguson et al. observed positive associations for MCNP, and positive and negative associations for MCOP.^{18,19} We also found a statistically significant, positive association between MCNP and IL-6 when excluding BMI as a covariate, which was attenuated and not statistically significant when including BMI as a covariate. Ferguson et al. 2014 found an increase in IL-6 levels in association with an interquartile range increase in MCNP ($\% \Delta = 16.8$, 95% CI: 2.69, 32.9, $p=0.02$) while adjusting for BMI.

Our results suggest a positive association between MCNP and inflammation, as this relationship was observed among both inflammation biomarkers (CRP and IL-6). Although a statistically significant relationship was observed between MCOP and CRP, this was not observed with IL-6, which is potentially the result of reduced sample size and statistical power. Our results also show an attenuation in strength of the association between phthalate biomarkers and inflammation biomarker levels in models including BMI as a covariate, compared to models excluding BMI as a covariate. However, this trend is not consistent across all phthalate metabolites. A potential explanation for the

conflicting findings is the varying sources of phthalate exposure. Diet is a common source of phthalate exposure among the majority of individuals.¹ DiNP, which metabolizes into MCOP can be found in food packaging,³⁰ which often contain unhealthy food items. Although information is not readily available, as of March 2020 past federal regulations from the U.S Food & Drug Administration cite DiDP (i.e. MCNP) as a substance used in food packaging.³¹ Furthermore, obesity is an established risk factor for inflammation.⁶ As our sample population has an average BMI of 28 kg/m² and a standard deviation of roughly 6 kg/m², indicating an overweight population, it is possible that the attenuation in strength of association among some phthalates is the result of correctly adjusting for confounding due to BMI. It is also a possibility that the relationship between some phthalates and inflammation occurs indirectly through BMI. In this case, linear regression models excluding BMI as a covariate more accurately reflect the true association as compared to models including BMI as a covariate. The increased in strength of association among certain phthalate biomarkers may be the result of originating from an exposure source unrelated to BMI, such as indoor air or dust.¹ This could produce an overestimation of the association between phthalate exposure and inflammation, as BMI is adjusted for unnecessarily. Our findings also suggest that NSAID does not confound the relationship between phthalate exposure and inflammation, as we observed similar associations among our restricted sample as compared to our full sample.

A potential explanation for differences in findings across studies is the sample population. A total of 5 prior epidemiological studies examined the relationship between individual urinary phthalate metabolites and inflammation biomarkers. Sample

populations include pregnant women and men and women of varying ages. Our study is the first to examine the relationship between phthalate exposure and inflammation biomarker levels among postmenopausal women only. It possible that certain populations are impacted differently by inflammation, resulting in conflicting results across studies. We excluded participants with missing exposure, outcome and covariates as well as participants with inflammatory biomarker values obtained from unreliable assays (i.e. those with high CV). The reduced sample size and lowered statistical power may have contributed to differences between our findings compared to those from prior studies.

We observed null finding across all other phthalate metabolites, which differs from prior epidemiological studies. A potential explanation for these null associations could be differences in sample size and phthalate exposure assessment. Our study uses a single urine sample to assess phthalate exposure in comparison to prior studies which use up to 4 samples in a repeated cross-sectional study to measure change in phthalate exposure over time. Phthalates quickly pass through the body and reduce to half their original amount anywhere from 3 to 18 hours following exposure.³² As a result, a single urine sample may not accurately reflect participants long-term exposure. This can also create high within-person variation as phthalate metabolites concentrations can change daily, leading to nondifferential misclassification among participants in our sample. As a result, our observed association may be attenuated. Our sample sizes for CRP (n=414) and IL-6 (n=177) are generally smaller compared to the sample sizes of prior studies. The use of a single measurement as well as a small sample size increases the variability of values and decreases the power, therefore reducing the ability to observe an association.

Our study is limited by the reliance on inflammatory biomarker levels that were

combined from multiple WHI ancillary studies. Inflammatory biomarker levels varied by specimen type, lab, assay method, and storage time, leading to differences in measurement among our sample. Additionally, as inflammation biomarker levels were selected based on availability from other studies, it is possible that by design, our sample population is different (i.e. sicker, older) from the WHI study population. To minimize potential measurement error, we used strong predictors of inflammation (i.e. age, smoking status, BMI, use of anti-inflammatory medication 48 hours prior to blood draw, study ID, and storage time) to estimate CRP and IL-6 values and standardize our results. Results from our prediction model were in line with our expectations regarding directionality (Table 1). However, we did not observe a statistically significant relationship between predictors age and anti-inflammatory medication use and CRP levels. We also did not observe a statistically significant relationship between predictors age, smoking status, and anti-inflammatory medication use and IL-6 levels. As a result, it is likely that some measurement error remains among our predicted CRP and IL-6 values. To understand the level of bias that may have occurred through sample selection, we compared the distribution of characteristics within our sample sizes for CRP (n=414) and IL-6 (n=177) values to participants within the nested case-control study (n=1,257). Although our samples differed by age, race/ethnicity, and SES index, the remaining characteristics were not statistically significantly different from the nested case-control sample.

There are several strengths to our cross-sectional study. First, this is the first study to assess this association among postmenopausal women only. As this population is at higher risk for developing inflammation-related chronic conditions (diabetes, rheumatoid

arthritis, etc.), our findings could provide insight into the mechanism between phthalate exposure and inflammation and could lead to improvements in disease prevention. Second, although residual confounding is a potential concern in any study, we were able to adjust for a large number of confounders in our analyses. As a result, we were able to assess the potential impact of certain covariates on the relationship between urinary phthalate biomarkers and inflammation biomarker levels. Third, we expect our results to be generalizable to all postmenopausal women. Although the biological mechanism between phthalate exposure and inflammation is not established, we do not expect that mechanisms linking phthalate exposure to inflammation would vary by age, race/ethnicity, or geographic location.

In conclusion, our study indicates an overall positive association between MCNP and inflammation biomarkers CRP and IL-6. Although the relationship between MCNP and IL-6 is borderline significant when including BMI as a confounder ($p=0.05$), it is possible that BMI is instead an intermediary step between phthalate exposure and inflammation. Further research should examine this relationship as it relates to BMI. Additionally, future studies should aim to use a larger sample size and include additional inflammation biomarkers (ex. TNF- α and IL-8).

APPENDIX

SUPPLEMENTAL TABLES

Table 1. Beta coefficients and 95% confidence intervals for predictors of inflammation and CRP and IL-6 levels

	CRP		IL-6	
	Beta (95% CI)	p-value	Beta (95% CI)	p-value
Age	0.02 (-0.05, 0.09)	0.56	0.002 (-0.07, 0.08)	0.95
Smoking status				
Never smoked	Reference	--	Reference	--
Past smoker	2.25 (1.32, 3.19)	<0.0001 ^b	0.44 (-0.49, 1.37)	0.93
Current smoker	2.62 (0.76, 4.47)	0.005 ^b	0.21 (-1.93, 2.35)	0.19
BMI	0.45 (0.37, 0.53)	<0.0001 ^b	0.15 (0.07, 0.23)	0.0003 ^b
Anti-inflammatory medication use ^a				
No	Reference	--	Reference	--
Yes	-0.81 (-1.74, 0.11)	0.08	-0.53 (-1.43, 0.37)	0.25

^aanti-inflammatory medication use in the last 48-hours prior to blood draw

^bp<0.05

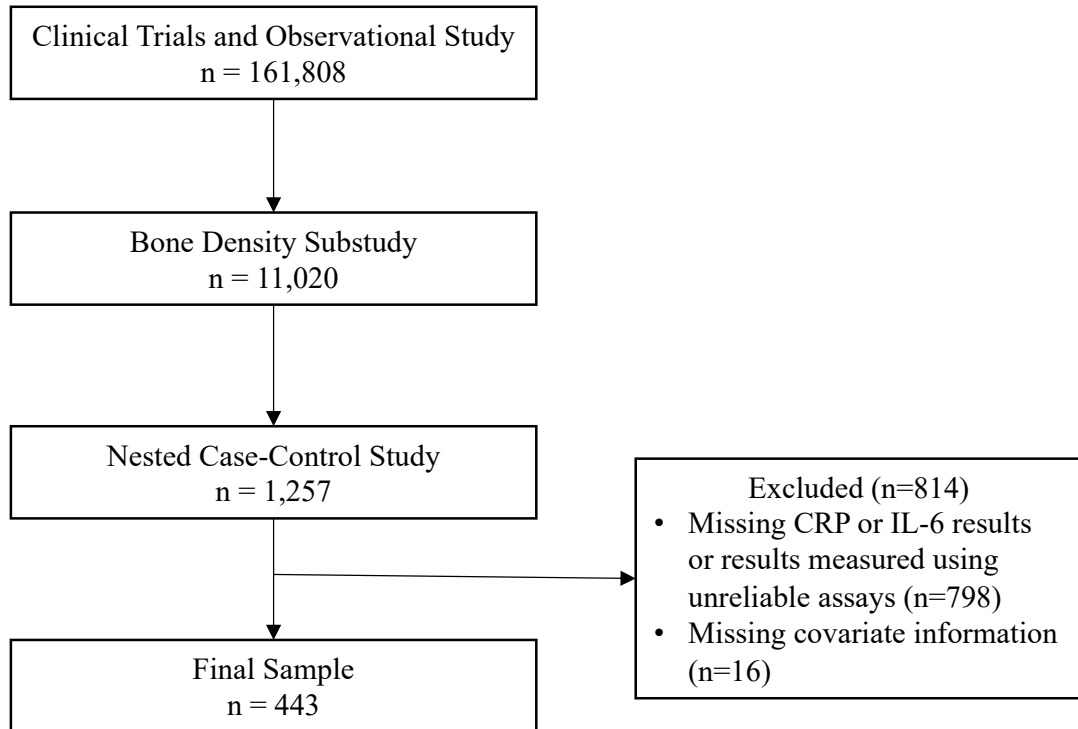


Figure 1. Study population ascertainment for the analysis of phthalate exposure and inflammation biomarker levels in postmenopausal women

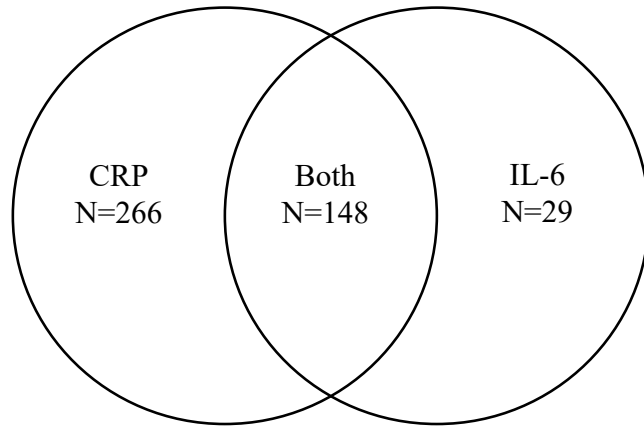


Figure 2. Venn diagram of inflammation biomarker results among participants

Table 2. Distribution of sociodemographic and behavioral characteristics in the Women's Health Initiative Study (WHI); 1993-1998

	CRP sample (n=414)	p-value ^a	IL-6 sample (n=177)	p- value ^a	Nested case control (n=1,257)
Age, years; Mean(SD)	63.1(7.0)	0.10	64.0(6.5)	0.01 ^b	62.9(6.9)
Race/ethnicity; N(%)		<0.0001 ^b		0.01 ^b	
White	247(59.7)		133(75.1)		1,045(83.1)
Non-white	167(40.3)		44(24.9)		212(16.9)
Education level; N(%)		0.47		1.00	
Less than high school degree	121(29.5)		48(27.8)		345(27.6)
Post high school/some college	155(37.8)		63(36.4)		456(36.5)
College degree or higher	134(32.7)		62(35.8)		450(36.0)
Income level, yearly; N(%)		0.06		0.39	
<35,000	214(54.6)		88(52.7)		585(49.1)
>=35,000	178(45.4)		79(47.3)		606(50.9)
SES index - inflation adjusted; Mean(SD)	70.4(10.5)	<0.0001 ^b	72.5(10.0)	0.46	73.1(8.7)
Alcohol intake; N(%)		0.05		0.61	
0 drinks per week	164(39.6)		60(33.9)		412(33.0)
<1 drink per week	141(34.1)		68(38.4)		431(34.5)
1-6 drinks per week	76(18.4)		35(19.8)		288(23.1)
7+ drinks per week	33(8.0)		14(7.9)		117(9.4)
Smoking status; N(%)		0.63		0.35	
Never smoked	241(58.2)		91(51.4)		698(56.4)
Past smoker	144(34.8)		76(42.9)		461(37.3)
Current smoker	29(7.0)		10(5.7)		78(6.3)
Body mass index, kg/m ² ; Mean(SD)	28.7(6.0)	0.06	28.2(5.6)	0.84	28.1(5.8)
Physical activity level, MET hrs/week; Mean(SD)	11.5(13.5)	0.52	12.1(13.3)	0.96	12.0(14.4)
Current NSAID use; N(%)		0.82		0.35	
Yes	258(62.3)		105(59.3)		791(62.9)
No	156(37.7)		72(40.7)		466(37.1)

Abbreviations: NSAID, nonsteroidal anti-inflammatory drug; SES, socioeconomic; MET, metabolic equivalent

^ap-values are for the comparison of CRP and IL-6 samples to the nested case-control sample

^bp<0.05

Table 3. Distribution of creatinine-standardized phthalate metabolite concentrations and inflammation biomarker levels in the Women's Health Initiative (WHI) Study (n=443); 1993-1998

Phthalate metabolites	Mean	SD	Min	25th percentile	75th percentile	Max
DEHP, umol/g ^a	0.0033	0.0050	0.0002	0.0016	0.0038	0.0831
DBP, umol/g ^b	0.0025	0.0029	0.0001	0.0010	0.0031	0.0359
DiBP, umol/g ^c	0.0003	0.0004	0.00003	0.0001	0.0003	0.0046
MEP, ug/g	3.758	12.566	0.088	0.544	2.588	130.046
MBzP, ug/g	0.232	0.240	0.013	0.099	0.270	2.149
MCPP, ug/g	0.061	0.087	0.007	0.028	0.062	1.152
MCOP, ug/g	0.084	0.185	0.009	0.032	0.077	2.558
MCNP, ug/g	0.059	0.136	0.007	0.023	0.056	2.372
CRP (mg/L) ^d	4.885	2.995	0.025	2.836	6.652	16.415
IL-6 (pg/mL) ^e	2.941	2.016	0.029	1.650	3.354	11.227

^asum of MEHP, MEHHP, MEOHP, and MECPP

^bsum of MBP and MHBP

^csum of MiBP and MHiBP

^dn=414

^en=177

Table 4. Pearson correlation table for creatinine-standardized phthalate metabolite concentrations and inflammation biomarker levels in the Women's Health Initiative (WHI) Study; 1993-1998

	CRP (n=414)		IL-6 (n=177)	
	r	p	r	p
DEHP ^b	0.020	0.69	0.031	0.68
DBP ^c	-0.037	0.46	0.070	0.93
DiBP ^d	0.009	0.86	0.019	0.81
MEP	0.008	0.88	-0.017	0.83
MBzP	0.023	0.64	-0.088	0.24
MCPP	0.015	0.76	-0.028	0.72
MCOP	0.039	0.43	0.082	0.28
MCNP	0.080	0.12	0.202	0.007 ^e

^aphthalate metabolite concentrations and inflammation biomarker levels were log-transformed

^bsum of MEHP, MEHHP, MEOHP, and MECPP

^csum of MBP and MHBP

^dsum of MiBP and MHiBP

^ep<0.05

Table 5. Multiple linear regression and 95% confidence intervals for phthalate metabolite concentrations and CRP levels (n=414)^a

	Model 1 ^b		Model 2 ^c	
	Beta (95% CI)	p-value	Beta (95% CI)	p-value
DEHP ^d	0.248 (-0.061, 0.557)	0.11	0.103 (-0.142, 0.348)	0.41
DBP ^e	-0.009 (-0.275, 0.256)	0.95	-0.071 (-0.280, 0.139)	0.51
DiBP ^f	0.103 (-0.173, 0.380)	0.46	0.002 (-0.217, 0.221)	0.98
MEP	0.040 (-0.164, 0.243)	0.70	0.010 (-0.151, 0.170)	0.91
MBzP	0.110 (-0.164, 0.384)	0.43	-0.079 (-0.297, 0.139)	0.48
MCCP	0.218 (-0.099, 0.534)	0.18	0.136 (-0.115, 0.386)	0.29
MCOP	0.384 (0.076, 0.692)	0.01 ^g	0.240 (-0.004, 0.485)	0.05
MCNP	0.523 (0.276, 0.890)	0.0002 ^g	0.362 (0.116, 0.607)	0.004 ^g

^aphthalate metabolite concentrations and inflammation biomarker levels were log-transformed. coefficients shown are based on a 10-unit change on the natural scale

^badjusted for age, creatinine, alcohol intake, socioeconomic status, and smoking status

^cadjusted for model 1 + BMI

^dsum of MEHP, MEHHP, MEOHP, and MCCP

^esum of MBP and MHBP

^fsum of MiBP and MHiBP

^gp<0.05

Table 6. Multiple linear regression and 95% confidence intervals for phthalate metabolite concentrations and IL-6 levels (n=177)^a

	Model 1 ^b		Model 2 ^c	
	Beta (95% CI)	p-value	Beta (95% CI)	p-value
DEHP ^d	0.096 (-0.195, 0.387)	0.52	-0.044 (-0.291, 0.202)	0.72
DBP ^e	0.081 (-0.194, 0.356)	0.56	0.076 (-0.154, 0.307)	0.51
DiBP ^f	0.137 (-0.142, 0.416)	0.34	0.069 (-0.166, 0.304)	0.56
MEP	0.010 (-0.168, 0.189)	0.91	0.008 (-0.142, 0.157)	0.92
MBzP	-0.088 (-0.365, 0.190)	0.53	-0.150 (-0.382, 0.082)	0.20
MCCP	-0.048 (-0.359, 0.263)	0.76	-0.067 (-0.328, 0.194)	0.61
MCOP	0.172 (-0.089, 0.433)	0.20	0.014 (-0.209, 0.237)	0.90
MCNP	0.369 (0.081, 0.658)	0.01 ^g	0.181 (-0.068, 0.431)	0.15

^aphthalate metabolite concentrations and inflammation biomarker levels were log-transformed. coefficients shown are based on a 10-unit change on the natural scale

^badjusted for age, creatinine, alcohol intake, socioeconomic status, and smoking status

^cadjusted for model 1 + BMI

^dsum of MEHP, MEHHP, MEOHP, and MCCPP

^esum of MBP and MHBP

^fsum of MiBP and MHiBP

^gp<0.05

Table 7. Multiple linear regression and 95% confidence intervals for phthalate metabolite concentrations and CRP levels (n=258) among participants not currently using NSAIDs^a

	Model 1 ^b		Model 2 ^c	
	Beta (95% CI)	p-value	Beta (95% CI)	p-value
DEHP ^d	0.376 (0.005, 0.747)	0.047 ^g	0.236 (-0.058, 0.530)	0.12
DBP ^e	-0.033 (-0.372, 0.306)	0.85	-0.050 (-0.317, 0.216)	0.71
DiBP ^f	0.034 (-0.298, 0.367)	0.84	0.045 (-0.217, 0.307)	0.74
MEP	0.092 (-0.168, 0.352)	0.49	0.061 (-0.144, 0.266)	0.56
MBzP	0.282 (-0.065, 0.629)	0.11	0.10 (-0.176, 0.376)	0.48
MCPP	0.263 (-0.111, 0.637)	0.17	0.181 (-0.114, 0.476)	0.23
MCOP	0.553 (0.171, 0.934)	0.0005 ^g	0.382 (0.079, 0.685)	0.01 ^g
MCNP	0.584 (0.210, 0.957)	0.002 ^g	0.345 (0.046, 0.644)	0.02 ^g

^aphthalate metabolite concentrations and inflammation biomarker levels were log-transformed. coefficients shown are based on a 10-unit change on the natural scale

^badjusted for age, creatinine, alcohol intake, socioeconomic status, and smoking status

^cadjusted for model 1 + BMI

^dsum of MEHP, MEHHP, MEOHP, and MECPP

^esum of MBP and MHBP

^fsum of MiBP and MHiBP

^gp<0.05

Table 8. Multiple linear regression and 95% confidence intervals for phthalate metabolite concentrations and IL-6 levels (n=105) among participants not currently using NSAIDs^a

	Model 1 ^b		Model 2 ^c	
	Beta (95% CI)	p-value	Beta (95% CI)	p-value
DEHP ^d	0.084 (-0.261, 0.429)	0.63	-0.069 (-0.363, 0.225)	0.64
DBP ^e	-0.090 (-0.447, 0.267)	0.62	0.009 (-0.294, 0.312)	0.95
DiBP ^f	0.224 (-0.145, 0.593)	0.23	0.130 (-0.183, 0.443)	0.41
MEP	0.096 (-0.152, 0.344)	0.44	0.122 (-0.086, 0.330)	0.25
MBzP	0.050 (-0.321, 0.421)	0.79	-0.048 (-0.362, 0.265)	0.76
MCPP	-0.075 (-0.465, 0.315)	0.70	-0.045 (-0.373, 0.283)	0.78
MCOP	0.223 (-0.125, 0.570)	0.21	0.006 (-0.297, 0.309)	0.97
MCNP	0.401 (0.017, 0.784)	0.04 ^g	0.193 (-0.142, 0.527)	0.26

^aphthalate metabolite concentrations and inflammation biomarker levels were log-transformed. coefficients shown are based on a 10-unit change on the natural scale

^badjusted for age, creatinine, alcohol intake, socioeconomic status, and smoking status

^cadjusted for model 1 + BMI

^dsum of MEHP, MEHHP, MEOHP, and MECPP

^esum of MBP and MHBP

^fsum of MiBP and MHiBP

^gp<0.05

BIBLIOGRAPHY

1. Department of Health and Human Services, Center for Disease Control and Prevention. *Fourth National Report National Report on Human Exposure to Environmental Chemicals.*; 2009.
2. Silva MJ, Barr DB, Reidy JA, et al. Urinary levels of seven phthalate metabolites in the U.S. population from the National Health and Nutrition Examination Survey (NHANES) 1999-2000. *Environ Health Perspect.* 2004;112(3):331-338. doi:10.1289/ehp.6723
3. Minihane AM, Vinoy S, Russell WR, et al. Low-grade inflammation, diet composition and health: Current research evidence and its translation. *Br J Nutr.* 2015;114(7):999-1012. doi:10.1017/S0007114515002093
4. Centers for Disease Control and Prevention. Rheumatoid Arthritis (RA). <https://www.cdc.gov/arthritis/basics/rheumatoid-arthritis.html>. Accessed October 1, 2019.
5. Singh T, Newman AB. Inflammatory markers in population studies of aging. *Ageing Res Rev.* 2011;10(3). doi:10.1016/j.arr.2010.11.002
6. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity & inflammation: The linking mechanism & the complications. *Arch Med Sci.* 2017;13(4):851-863. doi:10.5114/aoms.2016.58928
7. Crusz SM, Balkwill FR. Inflammation and cancer: Advances and new agents. *Nat Rev Clin Oncol.* 2015;12(10):584-596. doi:10.1038/nrclinonc.2015.105
8. Willerson JT, Ridker PM. Inflammation as a cardiovascular risk factor. *Circulation.* 2004;109(21):II-2-II10.
9. Ginaldi L, Mengoli LP, De Martinis M. Osteoporosis, inflammation and ageing. *Handb Immunosenescence Basic Underst Clin Appl.* 2009;2(14):1-5. doi:10.1007/978-1-4020-9063-9-64
10. Nishioka J, Iwahara C, Kawasaki M, et al. Di-(2-ethylhexyl) phthalate induces production of inflammatory molecules in human macrophages. *Inflamm Res.* 2012;61(1):69-78. doi:10.1007/s00011-011-0390-x
11. Agrawal A, Cha-Molstad H, Samols D, Kushner I. Overexpressed nuclear factor- κ B can participate in endogenous C-reactive protein induction, and enhances the effects of C/EBP β and signal transducer and activator of transcription-3. *Immunology.* 2003;108(4):539-547. doi:10.1046/j.1365-2567.2003.01608.x
12. Zhou L, Chen H, Xu Q, et al. The effect of di-2-ethylhexyl phthalate on

- inflammation and lipid metabolic disorder in rats. *Ecotoxicol Environ Saf.* 2019;170:391-398. doi:10.1016/j.ecoenv.2018.12.009
13. Zheng SJ, Tian HJ, Cao J, Gao YQ. Exposure to di(n-butyl)phthalate and benzo(a)pyrene alters IL-1 β secretion and subset expression of testicular macrophages, resulting in decreased testosterone production in rats. *Toxicol Appl Pharmacol.* 2010;248(1):28-37. doi:10.1016/j.taap.2010.07.008
 14. Jepsen KF, Abildtrup A, Larsen ST. Monophthalates promote IL-6 and IL-8 production in the human epithelial cell line A549. *Toxicol Vitro.* 2004;18(3):265-269. doi:10.1016/j.tiv.2003.09.008
 15. Rael LT, Bar-Or R, Ambruso DR, et al. Phthalate esters used as plasticizers in packed red blood cell storage bags may lead to progressive toxin exposure and the release of pro-inflammatory cytokines. *Oxid Med Cell Longev.* 2009;2(3):166-171. doi:10.4161/oxim.2.3.8608
 16. Coleman-Jensen A, Gregory C, Singh A. *Household Food Security in the United States in 2017.*; 2018. doi:10.2139/ssrn.2504067
 17. Kocbach Bølling A, Ovrevik J, Samuelsen JT, et al. Mono-2-ethylhexylphthalate (MEHP) induces TNF- α release and macrophage differentiation through different signalling pathways in RAW264.7 cells. *Toxicol Lett.* 2012;209(1):43-50. doi:10.1016/j.toxlet.2011.11.016
 18. Ferguson KK, Loch-Carusio R, Meeker JD. Urinary phthalate metabolites in relation to biomarkers of inflammation and oxidative stress: NHANES 1999-2006. *Environ Res.* 2011;111(5):718-726. doi:10.1016/j.envres.2011.02.002
 19. Ferguson KK, Cantonwine DE, Rivera-González LO, et al. Urinary phthalate metabolite associations with biomarkers of inflammation and oxidative stress across pregnancy in Puerto Rico. *Environ Sci Technol.* 2014;48(12):7018-7025. doi:10.1021/es502076j
 20. Ferguson KK, McElrath TF, Mukherjee B, Loch-Carusio R, Meeker JD. Associations between maternal biomarkers of phthalate exposure and inflammation using repeated measurements across pregnancy. *PLoS One.* 2015;10(8). doi:10.1371/journal.pone.0135601
 21. Choi YJ, Ha KH, Kim DJ. Exposure to bisphenol A is directly associated with inflammation in healthy Korean adults. *Environ Sci Pollut Res.* 2017. doi:10.1007/s11356-016-7806-7
 22. Women's Health Initiative. About WHI. [https://www.whi.org/about/SitePages/About WHI.aspx](https://www.whi.org/about/SitePages/About%20WHI.aspx). Accessed October 10, 2019.

23. Reeves KW, Santana MD, Manson JAE, et al. Predictors of urinary phthalate biomarker concentrations in postmenopausal women. *Environ Res.* 2019;169:122-130. doi:10.1016/j.envres.2018.10.024
24. Hays J, Hunt JR, Hubbell FA, et al. The Women's Health Initiative recruitment methods and results. *Ann Epidemiol.* 2003;13(9):S18-S77. doi:10.1016/S1047-2797(03)00042-5
25. Reeves K, Dias S, Manson JE, et al. Urinary Phthalate Biomarker Concentrations and Postmenopausal Breast Cancer Risk. *J Natl Cancer Inst.* 2019;111(10):1059-1067. doi:10.1093/jnci/djz002
26. Women's Health Initiative. *Quality Assurance (QA) Procedures in WHI Specimen Testing.*; 2012.
https://www.whi.org/researchers/data/_layouts/15/WopiFrame.aspx?sourcedoc=/researchers/data/Documents/WHIQA_121312.docx&action=default.
27. Women's Health Initiative. *Blood and Urine Collection, Processing and Shipment.*; 1997.
28. Bai PY, Wittert G, Taylor AW, et al. The association between total phthalate concentration and non-communicable diseases and chronic inflammation in South Australian urban dwelling men. *Environ Res.* 2017;158:366-372. doi:10.1016/j.envres.2017.06.021
29. Hamer M, Sabia S, Batty GD, et al. Physical activity and inflammatory markers over 10 years: Follow-up in men and women from the whitehall II cohort study. *Circulation.* 2012;126(8):928-933. doi:10.1161/CIRCULATIONAHA.112.103879
30. Centers for Disease Control and Prevention. Di-isononyl Phthalate. National Biomonitoring Program.
31. U.S. Food & Drug Administration. *Code of Federal Regulations.* Silver Spring; 2019:175.
<https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=175.105>.
32. Johns LE, Cooper GS, Galizia A, Meeker JD. Exposure assessment issues in epidemiology studies of phthalates. *Environ Int.* 2015;85:27-39. doi:10.1016/j.envint.2015.08.005