Published in final edited form as:

Reprod Toxicol. 2016 April ; 60: 76–81. doi:10.1016/j.reprotox.2016.02.001.

Phthalate metabolite levels and menopausal hot flashes in midlife women

Ayelet Ziv-Gal^a, Lisa Gallicchio^b, Catheryne Chiang^a, Sara N. Ther^a, Susan R. Miller^c, Howard A. Zacur^c, Russell L. Dills^d, and Jodi A. Flaws^{a,*}

^aComparative Biosciences, University of Illinois, Urbana, IL, USA

^bMercy Medical Center, Baltimore, MD, USA

^cJohns Hopkins University School of Medicine, Baltimore, MD, USA

^dEnvironmental and Occupational Health Sciences, University of Washington, Seattle, USA

Abstract

During the menopausal transition, a woman's reproductive capacity declines, her hormone milieu changes, and her risk of hot flashes increases. Exposure to phthalates, which can be found in personal-care products, can also result in altered reproductive function. Here, we investigated the associations between phthalate metabolite levels and midlife hot flashes. Eligible women (45–54 years of age) provided detailed information on hot flashes history and donated urine samples (n=195). Urinary phthalate metabolite levels were measured by HPLC-MS/MS. A higher total sum of phthalate metabolites commonly found in personal-care products was associated with an increased risk of ever experiencing hot flashes (odds ratio (OR)=1.45; 95% confidence interval (CI)= 1.07–1.96), hot flashes in the past 30 days (OR=1.43; 95%CI=1.04–1.96), and more frequent hot flashes (OR=1.47; 95%CI=1.06–2.05). These data suggest that some phthalate exposures from personal care products are associated with menopausal hot flashes in women.

Keywords

hot flashes; menopause; phthalates; women

1. Introduction

Phthalates are a large class of ubiquitous synthetic chemicals, which are used as plasticizers and stabilizers in a myriad of consumer products, including shower curtains, children's toys, cosmetics, and personal care products such as perfumes, nail polishes, deodorants, and lotions [1–3]. Phthalates are also used in pesticides, wood finishes, adhesives, solvents,

*Corresponding Author: Jodi A. Flaws, Ph.D.; Department of Comparative Biosciences, University of Illinois, 2001 S. Lincoln Ave. Urbana, Illinois, 61802. Phone: 217-333-7933; Fax: 217-244-1652; jflaws@illinois.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of interest: The authors have no conflicts of interest to declare.

lubricants, and in medical devices including tubing, blood bags, surgical gloves, and dialysis equipment [1]. The wide range of products in which phthalates are incorporated results in a global production and use of phthalates that is greater than 18 billion pounds per year [1].

The chemical structures of phthalates consist of esters of ortho-phthalic acid and are named based on the alcohol that generates the varying lengths of the alkyl chain in a linear or branched format. Phthalate esters with long alkyl chains (more than 6 carbons) have higher molecular weights and are likely to undergo chemical modifications for renal excretion. At least six different parent phthalates are used in consumer products, including diethyl phthalate (DEP; short alkyl chain), di(2-ethylhexyl) phthalate (DEHP; long alkyl chain), dibutyl phthalate (DBP; short alkyl chain), diisobutyl phthalate (DiBP; short alkyl chain), diisononyl phthalate (DiNP; long alkyl chain), and butyl benzyl phthalate (BBzP; short alkyl chain) [1, 3]. The parent compounds can be converted mainly by the gastrointestinal tract or liver to various metabolites that can be more toxic than the parent compound [1, 3–5]. The ubiquity of phthalates and phthalate metabolites is further evidenced by their detection in nearly all tested human urine samples [6–10]. Interestingly, the measured levels of urinary phthalate metabolites are higher in women compared to men [1, 11]. This finding is possibly due to a greater use of personal care products by women compared to men.

Previous epidemiological studies indicate that phthalate metabolites can reach the reproductive tissues and adversely affect reproductive function [8, 12, 13]. For example, phthalate metabolites have been associated with an increased risk of endometriosis [10] and earlier age at menopause [14]. Further, animal studies indicate that exposure to phthalates inhibits ovarian synthesis of sex steroid hormones that are required for normal reproductive function [3, 4, 15–17], and epidemiological studies indicate that phthalate exposure is associated with reduced sex steroid hormone levels [8, 12, 18, 19]. It is not clear, however, whether and how phthalates impact reproductive function as women age and enter the menopausal transition.

During the menopausal transition, a woman's reproductive capacity declines, her hormone milieu changes, and her risk of hot flashes increases [20, 21]. Hot flashes are transient periods of intense heat in the upper parts of the body and are often followed by flushing of the skin, profuse sweating, chills, palpitations, and anxiety [22]. Despite the high prevalence of hot flashes among women undergoing the menopausal transition, little is known about the etiology or the risk factors for hot flashes. However, the predominant hypothesis is that drastic changes in estrogen levels lead to the onset of menopausal hot flashes [20, 22] and that low estradiol levels are associated with an increased risk of any, frequent, and severe hot flashes [20, 23–25].

With evidence from animal studies that phthalates reduce estradiol levels [3, 4, 15, 16], evidence from epidemiological studies that low estradiol levels are associated with an increased risk of hot flashes [20, 23, 25], and information that women commonly use personal care products containing phthalates [1, 2], we tested the hypothesis that higher urinary levels of phthalate metabolites, including those combinations of phthalate metabolites present in personal care products, are associated with an increased risk of midlife hot flashes.

2. Materials and Methods

2.1 Ethical approval

All participants gave written informed consent according to procedures approved by the University of Illinois and Johns Hopkins University Institutional Review Boards (file number: 06741).

2.2. Parent study design

Women (primarily Caucasian and African American) residing in Baltimore city (Maryland, USA) and its surrounding counties were enrolled in the Midlife Women's Health Study from 2007–2015. Specifically, women with and without natural hot flashes between the ages of 45 and 54 years were invited to participate in a study of women's midlife health by mail. To be eligible for the study, women must have had their ovaries and uterus, must have been late premenopausal, early or late perimenopausal, and not be pregnant. Women were excluded from the study if they were taking hormone therapy, herbal agents, or other natural agents for treatment of menopausal symptoms, taking oral contraceptives, being treated for any cancer, or postmenopausal. Menopausal status was defined as follows: pre-menopausal women were those who experienced their last menstrual period within the past 3 months and reported 11 or more periods within the past year. Perimenopausal women were those who experienced: 1) their last menstrual period within the past year, but not within the past 3 months or 2) their last menstrual period within the past 3 months and experienced 10 or fewer periods within the past year. Postmenopausal women were those women who had not experienced a menstrual period within the past year.

Eligible women were invited to the clinic site at Johns Hopkins University. At the clinic visit, women had their weights and heights recorded to calculate their body mass index (BMI). Women also donated spot urine samples for measurements of phthalate metabolites and they donated blood samples for measurements of sex steroid hormone levels. Further, women were asked to complete a detailed questionnaire that included questions regarding their hot flashes history along with additional demographic, medical and reproductive history, and lifestyle information. Hot flashes status was determined based on women's answers on the questionnaires (see below). Overall, in the current study, a sample of 195 participants (96 with hot flashes and 99 without hot flashes) was evaluated for urine phthalate metabolite levels. Additional details related to the study design, recruitment of study participants, and hormone levels measurements are described in Gallicchio *et al.* [23].

2.3 Assessment of Hot Flashes

On the study questionnaires, hot flashes were defined for participants as “a sudden feeling of heat in the face, neck, or upper part of the chest. Hot flashes are often accompanied by reddening or flushing of the skin followed by sweating and chills.” At baseline, a detailed hot flash history was obtained through a series of questions on the survey that asked for information on the following: whether the woman had ever experienced hot flashes, whether she had a hot flash in the past 30 days, the usual severity of hot flashes, the frequency of hot flashes, and the length of time that the woman experienced hot flashes. Women who responded no to ever experiencing hot flashes were prompted to skip the more detailed hot

flash questions and were categorized as “never experiencing hot flashes”. The selected hot flashes questions have been used to collect data on hot flashes in the Midlife Health Studies for over 10 years [23–27].

In terms of severity, each woman was asked to describe her hot flashes as: mild (sensation of heat without sweating), moderate (sensation of heat with sweating), or severe (sensation of heat with sweating that disrupts usual activity). In terms of frequency of hot flashes, each woman was asked to describe her hot flashes as occurring: every hour, every 2–5 hours, every 6–11 hours, every 12–23 hours, 1–2 days per week, 5–6 days per week, 2–3 days per month, 1 day per month, less than 1 day per month, or never. For analysis of the data, the following hot flash variables were examined as dependent variables (outcomes): ever experienced hot flashes (yes versus no); experienced any hot flashes in the past 30 days (yes versus no); moderate or severe hot flashes (yes versus no); and daily hot flashes (yes versus no).

2.4 Measurement of phthalate metabolites

Urine samples were analyzed blinded by isotope dilution high-performance liquid chromatography negative-ion electrospray ionization-tandem mass spectrometry (HPLC-MS/MS) at the Environmental Health Laboratory & Trace Organics Analysis Center, School of Public Health at the University of Washington as previously described [10, 28]. The following metabolites were measured: mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), monobutyl phthalate (MBP), monoethyl phthalate (MEP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-benzyl phthalate (MBzP), mono-isobutyl phthalate (MiBP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and mono-2-ethylhexyl phthalate (MEHP). These phthalate metabolites were selected because they are the major urinary metabolites of common phthalate parent compounds DEP, DEHP, DBP, DiBP, and BBzP [6, 8, 9, 29]. Further, the selected metabolites or their parent compounds have been associated with adverse reproductive outcomes in animal models and epidemiological studies [1, 3–5, 12, 16–18, 30–32]. All values (ng/mL) were normalized to the specific gravity value of the sample to account for any potential hydration differences between the participants and volume of the donated sample as described in other studies [33–35]. In cases in which values were lower than the limit of quantitation (LOQ) of the assay, a value of LOQ/square root 2 was assigned to the sample [36].

Humans are exposed to mixtures of phthalates that can contain different parent compounds or metabolites. These parent compounds and metabolites often have different toxicities, depending on dose, tissue, type, and species [1, 3, 12, 37, 38]. Thus, it is important to estimate exposure to relevant phthalate mixtures. To do so, we used calculations that have been performed in other studies on phthalate exposures in humans [32, 39–42]. For the estimation of the phthalates present in women’s personal care products (PCP), we summed the metabolite molar concentrations (normalized to their respective specific gravity values) of MBP (MW 222 $\mu\text{g}/\mu\text{mol}$) and MEP (MW 194 $\mu\text{g}/\mu\text{mol}$). This provides an estimate of phthalate exposure from pharmaceutical agents, shampoos, conditioners, perfumes, nail polishes, and other personal care products [39, 41, 43]. For the calculation of the sum of DEHP metabolites (sum DEHP) per sample, we added the metabolite concentrations

(normalized to their respective specific gravity values) of MECPP (MW 308 $\mu\text{g}/\mu\text{mol}$), MEHHP (MW 294 $\mu\text{g}/\mu\text{mol}$), MEOHP (MW 292 $\mu\text{g}/\mu\text{mol}$), and MEHP (MW 278 $\mu\text{g}/\mu\text{mol}$) per sample. This provides an estimate of phthalate exposure from DEHP-containing products such as polyvinylchloride plastics, building products, and medical devices [39, 41, 44–46]. Finally, we calculated the sum phthalate metabolites based on their anti-androgenic activity (sum AA) by adding the DEHP metabolite concentrations (normalized to their respective specific gravity values) of MBP, MBzP, and MiBP. This provides an estimate of exposure to phthalates with known anti-androgenic activity in experimental and/or epidemiological studies [3, 8, 47].

After conducting analyses using the various mixture formulas described above, we examined the associations between individual phthalate metabolites and the hot flashes outcomes. This was done to determine which phthalate metabolite in the mixture may be responsible for an observed association between a summary measure and a hot flashes outcome.

2.5 Statistical analysis

Phthalate concentration data were log-transformed as data for these variables were not normally distributed. Differences in demographic, reproductive, and health habit characteristics by hot flash status (ever versus never) were examined using chi-square tests for categorical variables. Logistic regression was carried out to calculate odds ratios (ORs) and 95% confidence intervals (95% CI) for the associations between phthalate metabolite concentrations and the hot flash outcomes, adjusted for age, race, and education. Age, race, and education were included in the regression models as they have been shown to be significantly associated with hot flashes in either this study or the literature [48]. Menopausal status was not entered into the logistic regression models, as it was strongly associated with age (and, thus, collinear). All analyses were performed with SAS, version 9.2 (SAS Institute, Inc., Cary, North Carolina). A two-sided p -value of equal or less than 0.05 was considered statistically significant.

3. Results

3.1 Study sample characteristics

Baseline characteristics for women with and without hot flashes are presented in Table 1. Women who ever experienced hot flashes were more likely to be of older age ($p = 0.0005$), to report less than some college education ($p = 0.01$), and to be perimenopausal ($p < 0.0001$) than those who did not experience hot flashes. Further, women who ever experienced hot flashes had lower levels of estradiol ($p < 0.0001$) and progesterone ($p = 0.001$) compared to women who never experienced hot flashes.

3.2 Phthalate metabolite levels in the study sample

The majority of the phthalate metabolites detected in our study were above the limit of quantification (LOQ) in most tested samples (Table 2). Further, the metabolite levels in our study sample were similar to those published from the National Health and Nutrition Examination Survey 2011–2012 [18].

3.3 Phthalate metabolite levels and the association with hot flashes

Sum PCP was significantly and positively associated with ever experiencing hot flashes (Table 3). Further, the associations between both sum DEHP and sum AA with ever experiencing hot flashes were of borderline statistical significance (Table 3). In addition, some individual phthalate metabolite levels in the mixtures were significantly and positively associated with ever experiencing hot flashes. Specifically, MECPP (OR =1.37; 95% CI=1.03, 1.81), MEHHP (OR=1.40; 95% CI=1.03, 1.91), and MEP levels (OR=1.39; 95% CI=1.07, 1.82) were significantly associated with ever experiencing hot flashes.

Sum PCP was significantly associated with experiencing hot flashes in the past 30 days (Table 4). Only one individual phthalate metabolite in the mixture, MEP, was significantly associated with having hot flashes in the past 30 days (OR=1.36; 95% CI=1.03, 1.79); however, the associations between both MECPP (OR=1.27; 95% CI=0.95, 1.70) and MEHPP (OR=1.30; 95% CI=0.94, 1.79) and having hot flashes in the past 30 days were of borderline statistical significance.

Although none of the phthalate mixtures were significantly associated with moderate/severe hot flashes, the associations between sum PCP, sum DEHP, and sum AA with this outcome were all of borderline statistical significance (Table 5). MECPP (OR=1.45; 95% CI=1.06, 1.99) and MEHPP levels in the mixtures (OR=1.41; 95% CI=1.01, 1.97) were significantly associated with moderate/severe hot flashes; and the association between MEP (OR=1.26; 95% CI=0.95, 1.68) and moderate/severe hot flashes was of borderline statistical significance.

Sum PCP was significantly associated with more frequent (daily) hot flashes (Table 6). Only one individual phthalate metabolite in the mixture, MEP, was significantly associated with more frequent hot flashes (OR=1.42; 95% CI=1.16, 1.91), but the associations between both MECPP (OR=1.37; 95% CI=0.99, 1.92) and MEHPP (OR=1.40; 95% CI=0.98, 2.01) and more frequent hot flashes were of borderline statistical significance.

4. Discussion

In the current study, we examined the associations between phthalate metabolite levels and menopausal hot flashes in generally healthy midlife women. Our data suggest that some, but not all, phthalate metabolite mixtures and individual metabolites may be associated with menopausal hot flashes.

Interestingly, the PCP summary variable, which estimates phthalate exposure from women's personal care products, was associated or borderline associated with most of the hot flashes outcomes (ever experiencing hot flashes, experiencing hot flashes in the past 30 days, and more frequent hot flashes). These findings add to the current literature that correlates exposure to phthalates and their metabolites with aberrant reproductive function [1, 3, 38]. Additionally, studies in mice indicate that phthalate exposure may result in early reproductive senescence. Specifically, in adult mice, oral exposure to DEHP accelerates primordial follicle recruitment [16] and this may accelerate reproductive aging [49]. Moreover, *in utero* MEHP exposure in mice accelerates folliculogenesis and causes

premature reproductive senescence in the female offspring [50]. These studies are important in the light of epidemiological studies such as that by Gibson-Helm *et al.* [51] indicating that women diagnosed with premature ovarian failure or medically induced ovarian failure report hot flashes more frequently than women prior to their menopausal transition. Strikingly, Grindler *et al.* reported significant associations between higher levels of DEHP metabolites (i.e., MEHHP and MEOHP) and early age at menopause [14]. Similarly, Hart *et al.* reported a significant association between higher exposure levels of MEP during pregnancy and lower anti-Müllerian hormone levels in the adolescent daughters [52]. Interestingly, lower anti-Müllerian hormone levels were reported as a marker for reduced ovarian reserve in adolescent girls [53] and reduced ovarian reserve can result in early age at menopause [54]. Further, previous studies indicate that early age at menopause is associated with hot flashes severity [55] and our study suggests that MEHHP is associated with hot flashes severity. Hence, it is possible that exposure to some of the phthalates increases the risk for early menopause and thus, the likelihood to experience menopausal hot flashes and in a more severe form.

The underlying mechanism by which phthalate metabolites are associated with hot flashes is unknown. However, it likely involves hormone levels. In our current study and in other epidemiological studies, low levels of estradiol and progesterone are associated with hot flashes [20, 23, 24, 56]. Further, several experimental studies indicate that phthalate exposures decrease both estradiol and progesterone levels [3, 4, 15–17]. Thus, future studies should examine whether the observed associations between urinary phthalate metabolites and hot flashes are mediated by low estradiol and/or progesterone levels.

In our current study, not all phthalate metabolites were associated with hot flashes. It is possible that some of the phthalate metabolites were not associated with hot flashes because they do not play a role in the etiology of hot flashes. It is also possible that certain aspects of the study limited our ability to detect real associations between certain phthalate metabolites and hot flashes. For example, our study examined single urine samples of 195 women and this only provides a snap shot of phthalate exposure. However, all samples were collected under similar conditions around the same time of the day and the levels that were measured in our study are similar to the levels published by other epidemiological studies [18]. We collected blood samples without considering the day of the menstrual cycle because women undergoing the menopausal transition have extremely variable cycles; however, we used the average of the four blood draws per participant to account for potential variation. Second, there may be additional factors that were not assessed in the current study that may mask some of the potential associations between phthalate metabolites and hot flashes. Such factors may include differences in the metabolic capacity of the women, environmental sources of phthalate exposures such as house dust, medical issues such as consumption of medications that are coated with phthalates, exposure to additional endocrine disrupting chemicals, or even a potential combined effect of several metabolites/chemicals that were out of the scope of our current study.

5. Conclusion

Our study shows that a phthalate mixture and some individual phthalate metabolite levels are associated with hot flashes in midlife women. This observation is consistent with studies indicating that some, but not all phthalate metabolites, are associated with selected reproductive outcomes. For example, Meeker *et al.* showed that some, but not all, phthalate metabolites are associated with decreased testosterone levels [18]. Even though not all phthalate mixtures and individual metabolites were associated with hot flashes, our study provides important information on which phthalate metabolites should be of concern and to which we should consider limiting human exposure. In our study, most of the metabolites that were positively associated with hot flashes were derived from the parent phthalate DEP. Thus, it could be that exposure to DEP poses a greater health risk in women than other parent compounds. However, future studies are needed to determine whether this is the case and to determine the potential mechanisms by which phthalates may increase the risk of hot flashes.

Acknowledgments

The authors thank Judith Keifer and Teresa Greene for their help with recruitment and sample collection. This work was supported by the National Institute on Aging (AG18400) and a Research Board grant from the University of Illinois.

References

1. Heudorf U, Mersch-Sundermann V, Angerer J. Phthalates: toxicology and exposure. *Int J Hyg Environ Health*. 2007; 210:623–634. [PubMed: 17889607]
2. Koo HJ, Lee BM. Human monitoring of phthalates and risk assessment. *J Toxicol Environ Health A*. 2005; 68:1379–1392. [PubMed: 16009652]
3. Hannon PR, Flaws JA. The effects of phthalates on the ovary. *Front Endocrinol (Lausanne)*. 2015; 6:8. [PubMed: 25699018]
4. Lovekamp TN, Davis BJ. Mono-(2-ethylhexyl) phthalate suppresses aromatase transcript levels and estradiol production in cultured rat granulosa cells. *Toxicol Appl Pharmacol*. 2001; 172:217–224. [PubMed: 11312650]
5. Wang W, Craig ZR, Basavarajappa MS, Hafner KS, Flaws JA. Mono-(2-ethylhexyl) phthalate induces oxidative stress and inhibits growth of mouse ovarian antral follicles. *Biol Reprod*. 2012; 87:152. [PubMed: 23077170]
6. Hogberg J, Hanberg A, Berglund M, Skerfving S, Remberger M, Calafat AM, et al. Phthalate diesters and their metabolites in human breast milk, blood or serum, and urine as biomarkers of exposure in vulnerable populations. *Environ Health Perspect*. 2008; 116:334–339. [PubMed: 18335100]
7. James-Todd T, Stahlhut R, Meeker JD, Powell SG, Hauser R, Huang T, et al. Urinary phthalate metabolite concentrations and diabetes among women in the National Health and Nutrition Examination Survey (NHANES) 2001–2008. *Environ Health Perspect*. 2012; 120:1307–1313. [PubMed: 22796563]
8. Marsee K, Woodruff TJ, Axelrad DA, Calafat AM, Swan SH. Estimated daily phthalate exposures in a population of mothers of male infants exhibiting reduced anogenital distance. *Environ Health Perspect*. 2006; 114:805–809. [PubMed: 16759976]
9. Silva MJ, Barr DB, Reidy JA, Malek NA, Hodge CC, Caudill SP, et al. Urinary levels of seven phthalate metabolites in the U.S. population from the National Health and Nutrition Examination Survey (NHANES) 1999–2000. *Environ Health Perspect*. 2004; 112:331–338. [PubMed: 14998749]
10. Upson K, Sathyanarayana S, De Roos AJ, Thompson ML, Scholes D, Dills R, et al. Phthalates and risk of endometriosis. *Environ Res*. 2013; 126:91–97. [PubMed: 23890968]

11. Hatch EE, Nelson JW, Qureshi MM, Weinberg J, Moore LL, Singer M, et al. Association of urinary phthalate metabolite concentrations with body mass index and waist circumference: a cross-sectional study of NHANES data, 1999–2002. *Environ Health*. 2008; 7:27. [PubMed: 18522739]
12. Hauser R, Skakkebaek NE, Hass U, Toppari J, Juul A, Andersson AM, et al. Male reproductive disorders, diseases, and costs of exposure to endocrine-disrupting chemicals in the European Union. *J Clin Endocrinol Metab*. 2015; 100:1267–1277. [PubMed: 25742517]
13. Adibi JJ, Hauser R, Williams PL, Whyatt RM, Calafat AM, Nelson H, et al. Maternal urinary metabolites of Di-(2-Ethylhexyl) phthalate in relation to the timing of labor in a US multicenter pregnancy cohort study. *Am J Epidemiol*. 2009; 169:1015–1024. [PubMed: 19251754]
14. Grindler NM, Allsworth JE, Macones GA, Kannan K, Roehl KA, Cooper AR. Persistent organic pollutants and early menopause in U.S. women. *PLoS One*. 2015; 10:e0116057. [PubMed: 25629726]
15. Hannon PR, Brannick KE, Wang W, Flaws JA. Mono(2-ethylhexyl) phthalate accelerates early folliculogenesis and inhibits steroidogenesis in cultured mouse whole ovaries and antral follicles. *Biol Reprod*. 2015; 92:120. [PubMed: 25810477]
16. Hannon PR, Brannick KE, Wang W, Gupta RK, Flaws JA. Di(2-ethylhexyl) phthalate inhibits antral follicle growth, induces atresia, and inhibits steroid hormone production in cultured mouse antral follicles. *Toxicol Appl Pharmacol*. 2015; 284:42–53. [PubMed: 25701202]
17. Gupta RK, Singh JM, Leslie TC, Meachum S, Flaws JA, Yao HH. Di-(2-ethylhexyl) phthalate and mono-(2-ethylhexyl) phthalate inhibit growth and reduce estradiol levels of antral follicles in vitro. *Toxicol Appl Pharmacol*. 2010; 242:224–230. [PubMed: 19874833]
18. Meeker JD, Ferguson KK. Urinary phthalate metabolites are associated with decreased serum testosterone in men, women, and children from NHANES 2011–2012. *J Clin Endocrinol Metab*. 2014; 99:4346–4352. [PubMed: 25121464]
19. Mendiola J, Meeker JD, Jorgensen N, Andersson AM, Liu F, Calafat AM, et al. Urinary concentrations of di(2-ethylhexyl) phthalate metabolites and serum reproductive hormones: pooled analysis of fertile and infertile men. *J Androl*. 2012; 33:488–498. [PubMed: 21597090]
20. Ziv-Gal A, Flaws JA. Factors that may influence the experience of hot flashes by healthy middle-aged women. *J Womens Health (Larchmt)*. 2010; 19:1905–1914. [PubMed: 20831431]
21. Freeman EW, Sammel MD, Gracia CR, Kapoor S, Lin H, Liu L, et al. Follicular phase hormone levels and menstrual bleeding status in the approach to menopause. *Fertil Steril*. 2005; 83:383–392. [PubMed: 15705379]
22. Kronenberg F. Hot flashes: epidemiology and physiology. *Ann N Y Acad Sci*. 1990; 592:52–86. discussion 123–33. [PubMed: 2197954]
23. Gallicchio L, Miller SR, Kiefer J, Greene T, Zacur HA, Flaws JA. Risk factors for hot flashes among women undergoing the menopausal transition: baseline results from the Midlife Women's Health Study. *Menopause*. 2015
24. Gallicchio L, Miller SR, Visvanathan K, Lewis LM, Babus J, Zacur H, et al. Cigarette smoking, estrogen levels, and hot flashes in midlife women. *Maturitas*. 2006; 53:133–143. [PubMed: 16368467]
25. Visvanathan K, Gallicchio L, Schilling C, Babus JK, Lewis LM, Miller SR, et al. Cytochrome gene polymorphisms, serum estrogens, and hot flashes in midlife women. *Obstet Gynecol*. 2005; 106:1372–1381. [PubMed: 16319265]
26. Cochran CJ, Gallicchio L, Miller SR, Zacur H, Flaws JA. Cigarette smoking, androgen levels, and hot flashes in midlife women. *Obstet Gynecol*. 2008; 112:1037–1044. [PubMed: 18978103]
27. Ziv-Gal A, Gallicchio L, Miller SR, Zacur HA, Flaws JA. Genetic polymorphisms in the aryl hydrocarbon receptor signaling pathway as potential risk factors of menopausal hot flashes. *Am J Obstet Gynecol*. 2012; 207:202.e9–202.e18. [PubMed: 22840970]
28. Sathyanarayana S, Barrett E, Butts S, Wang CW, Swan S. Phthalate Exposure and Reproductive Hormone Concentrations in Pregnancy. *Reproduction*. 2013
29. Christensen KL, Makris SL, Lorber M. Generation of hazard indices for cumulative exposure to phthalates for use in cumulative risk assessment. *Regul Toxicol Pharmacol*. 2014; 69:380–389. [PubMed: 24815596]

30. Lovekamp-Swan T, Davis BJ. Mechanisms of phthalate ester toxicity in the female reproductive system. *Environ Health Perspect.* 2003; 111:139–145. [PubMed: 12573895]
31. Wang W, Craig ZR, Basavarajappa MS, Gupta RK, Flaws JA. Di(2-ethylhexyl) phthalate inhibits growth of mouse ovarian antral follicles through an oxidative stress pathway. *Toxicol Appl Pharmacol.* 2012; 258:288–295. [PubMed: 22155089]
32. Meeker JD, Hu H, Cantonwine DE, Lamadrid-Figueroa H, Calafat AM, Ettinger AS, et al. Urinary phthalate metabolites in relation to preterm birth in Mexico city. *Environ Health Perspect.* 2009; 117:1587–1592. [PubMed: 20019910]
33. Ehrlich S, Williams PL, Hauser R, Missmer SA, Peretz J, Calafat AM, et al. Urinary bisphenol A concentrations and cytochrome P450 19 A1 (Cyp19) gene expression in ovarian granulosa cells: an in vivo human study. *Reprod Toxicol.* 2013; 42:18–23. [PubMed: 23850856]
34. Ehrlich S, Williams PL, Missmer SA, Flaws JA, Berry KF, Calafat AM, et al. Urinary bisphenol A concentrations and implantation failure among women undergoing in vitro fertilization. *Environ Health Perspect.* 2012; 120:978–983. [PubMed: 22484414]
35. Ehrlich S, Williams PL, Missmer SA, Flaws JA, Ye X, Calafat AM, et al. Urinary bisphenol A concentrations and early reproductive health outcomes among women undergoing IVF. *Hum Reprod.* 2012; 27:3583–3592. [PubMed: 23014629]
36. Finkelstein MM, Verma DK. Exposure estimation in the presence of nondetectable values: another look. *Aihaj.* 2001; 62:195–198. [PubMed: 11331991]
37. Christiansen S, Kortenkamp A, Axelstad M, Boberg J, Scholze M, Jacobsen PR, et al. Mixtures of endocrine disrupting contaminants modelled on human high end exposures: an exploratory study in rats. *Int J Androl.* 2012; 35:303–316. [PubMed: 22372636]
38. Kay VR, Chambers C, Foster WG. Reproductive and developmental effects of phthalate diesters in females. *Crit Rev Toxicol.* 2013; 43:200–219. [PubMed: 23405971]
39. Kobrosly RW, Parlett LE, Stahlhut RW, Barrett ES, Swan SH. Socioeconomic factors and phthalate metabolite concentrations among United States women of reproductive age. *Environ Res.* 2012; 115:11–17. [PubMed: 22472009]
40. Koo JW, Parham F, Kohn MC, Masten SA, Brock JW, Needham LL, et al. The association between biomarker-based exposure estimates for phthalates and demographic factors in a human reference population. *Environ Health Perspect.* 2002; 110:405–410. [PubMed: 11940459]
41. Schettler T. Human exposure to phthalates via consumer products. *Int J Androl.* 2006; 29:134–139. discussion 81–5. [PubMed: 16466533]
42. Ferguson KK, McElrath TF, Meeker JD. Environmental phthalate exposure and preterm birth. *JAMA Pediatr.* 2014; 168:61–67. [PubMed: 24247736]
43. Braun JM, Just AC, Williams PL, Smith KW, Calafat AM, Hauser R. Personal care product use and urinary phthalate metabolite and paraben concentrations during pregnancy among women from a fertility clinic. *J Expo Sci Environ Epidemiol.* 2014; 24:459–466. [PubMed: 24149971]
44. Ferguson KK, McElrath TF, Chen YH, Mukherjee B, Meeker JD. Urinary phthalate metabolites and biomarkers of oxidative stress in pregnant women: a repeated measures analysis. *Environ Health Perspect.* 2015; 123:210–216. [PubMed: 25402001]
45. Braun JM, Smith KW, Williams PL, Calafat AM, Berry K, Ehrlich S, et al. Variability of urinary phthalate metabolite and bisphenol A concentrations before and during pregnancy. *Environ Health Perspect.* 2012; 120:739–745. [PubMed: 22262702]
46. Wolff MS, Teitelbaum SL, McGovern K, Windham GC, Pinney SM, Galvez M, et al. Phthalate exposure and pubertal development in a longitudinal study of US girls. *Hum Reprod.* 2014
47. Marie C, Vendittelli F, Sauviant-Rochat MP. Obstetrical outcomes and biomarkers to assess exposure to phthalates: A review. *Environ Int.* 2015; 83:116–136. [PubMed: 26118330]
48. Miller SR, Gallicchio LM, Lewis LM, Babus JK, Langenberg P, Zacur HA, et al. Association between race and hot flashes in midlife women. *Maturitas.* 2006; 54:260–269. [PubMed: 16423474]
49. Hannon PR, Niermann S, Flaws JA. Acute exposure to di(2-ethylhexyl) phthalate in adulthood causes adverse reproductive outcomes later in life and accelerates reproductive aging in female mice. *Toxicol Sci.* 2015

50. Moyer B, Hixon ML. Reproductive effects in F1 adult females exposed in utero to moderate to high doses of mono-2-ethylhexylphthalate (MEHP). *Reprod Toxicol*. 2012; 34:43–50. [PubMed: 22401849]
51. Gibson-Helm M, Teede H, Vincent A. Symptoms, health behavior and understanding of menopause therapy in women with premature menopause. *Climacteric*. 2014; 17:666–673. [PubMed: 24742007]
52. Hart R, Doherty DA, Frederiksen H, Keelan JA, Hickey M, Sloboda D, et al. The influence of antenatal exposure to phthalates on subsequent female reproductive development in adolescence: a pilot study. *Reproduction*. 2014; 147:379–390. [PubMed: 24025997]
53. Hagen CP, Aksglaede L, Sorensen K, Main KM, Boas M, Cleemann L, et al. Serum levels of anti-Mullerian hormone as a marker of ovarian function in 926 healthy females from birth to adulthood and in 172 Turner syndrome patients. *J Clin Endocrinol Metab*. 2010; 95:5003–5010.
54. Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF. Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. *Hum Reprod*. 1992; 7:1342–1346. [PubMed: 1291557]
55. Edwards BJ, Li J. Endocrinology of menopause. *Periodontol 2000*. 2013; 61:177–194. [PubMed: 23240949]
56. Gallicchio L, Visvanathan K, Miller SR, Babus J, Lewis LM, Zacur H, et al. Body mass, estrogen levels, and hot flashes in midlife women. *Am J Obstet Gynecol*. 2005; 193:1353–1360. [PubMed: 16202725]

Highlights

- A higher sum of phthalate metabolites in personal care products (sum PCP) was associated with an increased risk of ever experiencing hot flashes.
- Sum PCP was significantly and positively associated with hot flashes in the past 30 days.
- Sum PCP was significantly and positively associated with daily hot flashes.

Demographic, biological, and hormonal characteristics of women in the Midlife Health Study, 2015 (n = 195)

Table 1

Characteristics	Hot flashes		No hot flashes		p-value
	n	%	n	%	
Age Group (years)					0.0005
	53	(55)	78	(79)	
	43	(45)	21	(21)	
Education					0.01
	45	(47)	29	(29)	
	51	(53)	69	(70)	
Race					0.2
	70	(73)	64	(65)	
	26	(27)	35	(35)	
Smoking status					0.7
	10	(10)	11	(11)	
	37	(39)	33	(33)	
	49	(51)	55	(56)	
Menopausal status					<0.0001
	45	(47)	80	(81)	
	51	(53)	19	(19)	
Body mass index					0.3
	41	(43)	32	(32)	
	24	(25)	30	(30)	
	31	(32)	37	(37)	
Hormone levels					
	GM	95% CI: n = 96	GM	95% CI: n = 99	
Estradiol (pg/mL)	41.6	(36.0, 48.0)	64.2	(55.7, 73.8)	<0.0001
Progesterone (ng/mL)	0.68	(0.50, 0.94)	1.43	(1.05, 1.95)	0.0001
Testosterone (ng/mL)	0.34	(0.34, 0.38)	0.305	(0.27, 0.34)	0.2

GM = geometric mean. Bold *p* values indicate significant difference between women with hot flashes and women without hot flashes

Table 2 Range of phthalate metabolites in study participants in the Midlife Health Study[/]

Phthalate metabolite (major parent phthalate)	% below LOQ	Median (Range)	Median (25 th , 75 th percentiles) reported in NHANES 2011–2012 ¹⁸
MBP (DBP)	1	15.9 (0.7–125.5)	9.89 (6.06, 18.6)
MBzP (BBzP)	14	5.1 (0.7–446.6)	5.34 (3.09, 9.91)
MECPP (DEHP)	9	13.3 (0.7–824.1)	16.10 (10.4, 23.9)
MEHHP (DEHP)	3	13.5 (0.7–461.6)	11.80 (5.82, 15.4)
MEHP (DEHP)	40	2.5 (0.7–41.0)	1.49 (0.90, 2.90)
MEOHP (DEHP)	6	8.5 (0.7–334.4)	6.73 (3.73, 10.0)
MEP (DEP)	0	56.2 (1.3–4,064.5)	54.0 (22.9, 124)
MiBP (DiBP)	14	8.4 (0.7–76.5)	7.63 (4.81, 13.4)

[/] ng/mL adjusted to specific gravity; LOQ = limit of quantification; % below limit of detection is calculated out of the total samples (n=195)

Table 3

Phthalate metabolite mixtures and odds of ever experiencing hot flashes in participants in the Midlife Women's Health Study

Summary Phthalate Measures	Ever had hot flashes
	OR (95% CI) ^I
Sum PCP	1.45 (1.07 – 1.96)
Sum DEHP	1.36 (0.99 – 1.88)
Sum AA	1.41 (0.98 – 2.03)

^I Adjusted for age, race, and education level. Sum PCP is the sum of phthalate metabolites present in women's personal care products: MBP+MEP. Sum DEHP is the sum of DEHP metabolites: MECPP+MEHHP+MEHP+MEOHP levels. Sum AA is the sum of phthalate metabolites with known androgenic activity: DEHP metabolites+MBP+MBzP+MiBP.

Table 4

Phthalate metabolite mixtures and odds of experiencing hot flashes in the past 30 days in participants in the Midlife Women's Health Study

Summary Phthalate Measures	Hot flashes in last 30 days
	OR (95% CI) ^I
Sum PCP	1.43 (1.04 – 1.96)
Sum DEHP	1.27 (0.90 – 1.75)
Sum AA	1.33 (0.90 – 1.96)

^I Adjusted for age, race, and education level. Sum PCP is the sum of phthalate metabolites present in women's personal care products: MBP+MEP. Sum DEHP is the sum of DEHP metabolites: MECPP+MEHHP+MEHP+MEOHP levels. Sum AA is the sum of phthalate metabolites with known androgenic activity: DEHP metabolites+MBP+MBzP+MiBP.

Table 5

Phthalate metabolite mixtures and odds of experiencing moderate/severe hot flashes in participants in the Midlife Women's Health Study

Summary Phthalate Measures	Moderate or Severe Hot flashes
	OR (95% CI) ^I
Sum PCP	1.31 (0.95 – 1.82)
Sum DEHP	1.38 (0.98 – 1.96)
Sum AA	1.39 (0.94 – 2.06)

^I Adjusted for age, race, and education level. Sum PCP is the sum of phthalate metabolites present in women's personal care products: MBP+MEP. Sum DEHP is the sum of DEHP metabolites: MECPP+MEHHP+MEHP+MEOHP levels. Sum AA is the sum of phthalate metabolites with known androgenic activity: DEHP metabolites+MBP+MBzP+MiBP.

Table 6

Phthalate metabolite mixtures and odds of experiencing more frequent (daily) hot flashes in participants in the Midlife Women's Health Study

Summary Phthalate Measures	Daily Hot Flashes
	OR (95% CI) ^I
Sum PCP	1.47 (1.06 – 2.05)
Sum DEHP	1.35 (0.93 – 1.98)
Sum AA	1.37 (0.89 – 2.10)

^I Adjusted for age, race, and education level. Sum PCP is the sum of phthalate metabolites present in women's personal care products: MBP+MEP. Sum DEHP is the sum of DEHP metabolites: MECPP+MEHHP+MEHP+MEOHP levels. Sum AA is the sum of phthalate metabolites with known androgenic activity: DEHP metabolites+MBP+MBzP+MiBP.