Title: Reducing Phthalate, Paraben, and Phenol Exposure from Personal Care Products in Adolescent Girls: Findings from the HERMOSA Intervention Study

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Abstract

Background: Personal care products are a major source of exposure to endocrine disrupting chemicals such as phthalates, parabens, triclosan, and benzophenone-3 (BP-3), particularly for adolescent girls.

Methods: We enrolled 100 Latina girls in a youth-led, community-based participatory research intervention study to determine whether using personal care products whose labels stated they did not contain these chemicals for three days could lower urinary concentrations. Pre- and post-intervention urine samples were analyzed for phthalate metabolites, parabens, triclosan and BP-3 using high-performance liquid chromatography/tandem mass spectrometry.

Results: Urinary concentrations of mono-ethyl phthalate (MEP) decreased by 27% from a geometric mean (GM) of 78.2 to 56.4 μg/L ($p<0.001$) over the 3 day intervention; no significant changes were seen in urinary concentrations of mono-n-butyl phthalate (MnBP) and mono-isobutyl phthalate (MiBP). Methyl and propyl paraben concentrations decreased by 44 - 45%, with the GM of methyl paraben decreasing from 77.4 to 43.2 μg/L ($p<0.01$) and propyl paraben decreasing from 22.6 to 12.3 μg/L ($p<0.01$). Unexpectedly, concentrations of ethyl and butyl paraben concentrations increased, although the absolute changes were small. Triclosan concentrations decreased by 36% (GM: 9.5 vs. 6.1 μg/L; $p=0.01$) and BP-3 concentrations decreased by 36% (GM: 173.8 vs. 113.4 μg/L; $p<0.01$).

Discussion: This study demonstrates that techniques available to consumers, such as choosing personal care products that are labelled to be free of phthalates, parabens, triclosan, and BP-3, can significantly reduce personal exposure to certain endocrine disrupting chemicals. Involving youth in the design and implementation of the study was key to recruitment, retention, compliance, and acceptability of the intervention.

Introduction

Cosmetics, fragrances, and other personal care products are a potential source of human exposure to endocrine disrupting chemicals, such as phthalates, parabens, and phenols (Braun et al. 2014; Meeker et al. 2013). Because women are the primary consumers of many personal care products, they are disproportionately exposed to these chemicals (Centers for Disease Control and Prevention 2012). Adolescent girls may be at particular risk of exposure through this route. For example, one small study found that the average adult woman uses approximately 12 individual personal care products each day, while the average teenage girl uses 17 (Environmental Working Group 2008).

Different types of phthalates, parabens, and phenols are used in a wide variety of consumer products. The three phthalates most commonly used in personal care products are diethyl phthalate (DEP), which is found in scented products, including perfumes, deodorants, soaps, and shampoo, and di-n-butyl phthalate (DnBP) and di-isobutyl phthalate (DiBP), which are used in nail polish and cosmetics (Dodson et al. 2012; Guo and Kannan 2011). *In vitro* and animal studies have shown DEP, DnBP, and DiBP to have estrogenic and anti-androgenic properties (Alam et al. 2010; Harris et al. 1997; Takeuchi et al. 2005; Zhang et al. 2011) and human studies link these phthalates to neurobehavioral problems, respiratory symptoms, and obesity in children (Braun et al. 2013). The parabens commonly used in personal care products include methyl, ethyl, butyl, and propyl paraben, which are used as preservatives and antibacterial agents in cosmetics (Soni et al. 2005). Parabens have demonstrated weak estrogenic and anti-androgenic activity (Chen et al. 2007; Routledge et al. 1998; Vo et al. 2010), although little is known about their health effects in humans. Two phenols are also commonly used in personal care products. Triclosan, an antimicrobial compound used in liquid soaps, acne cream, deodorants, shaving cream, and certain toothpastes, has been associated with alterations in thyroid hormone homeostasis in animal studies (Paul et al. 2010; Stoker et al. 2010). BP-3, also known as oxybenzone, is used in sunscreens, lip balm, and other sun protection products and is suspected to act as a weak estrogen (Schlumpf et al. 2001).

Personal care product use is widespread and human exposure to these chemicals is nearly ubiquitous, with mono-ester phthalate metabolites of DEP, DnBP, and DiBP detected in the urine of more than 96% of Americans participating in the 2009-2010 National Health and Nutrition Examination Survey (NHANES) (Zota et al. 2014). Methyl and propyl parabens were found in more than 90% of individuals, BP-3 in 97%, and triclosan in 75% (Calafat et al. 2008a; Calafat et al. 2010; Calafat et al. 2008b).

Awareness of endocrine disruptors in personal care products is increasing and some companies now advertise products that are “low chemical” or “phthalate- and paraben-free”. However, no studies have examined whether changing to low-chemical personal care products can lower levels of these potential endocrine disruptors in the body. Our community-based participatory research study was conducted by youth researchers in a primarily Mexican

American low-income community in Northern California. The intervention aimed to determine whether adolescent girls' urinary concentrations of metabolites of three phthalates (DEP, DnBP, and DiBP), four parabens (methyl, ethyl, butyl, and propyl paraben), and two phenols (triclosan and BP-3) decreased after switching to alternative personal care products. These nine compounds were targeted because they are widely used in personal care products; we expected to see no change in other phthalates and phenols (e.g. di-2-ethylhexyl phthalate (DEHP), bisphenol A (BPA)) whose use is primarily in plastics and other non-personal care product consumer goods.

Methods

The Health and Environmental Research on Makeup of Salinas Adolescents (HERMOSA) Study was a youth empowerment intervention study examining strategies to reduce personal care product chemical exposure to adolescent girls. HERMOSA means "beautiful" in Spanish, reflecting the study's focus on Latina teens. The study was designed in collaboration with fifteen local high school students (including three of the authors: MC, IV, GM) participating in the CHAMACOS Youth Community Council (Madrigal et al. 2014) who were hired as youth researchers and were involved in all aspects of the study, including study design, questionnaire development, identifying low-chemical replacement products, recruiting participants, collecting data, and returning results to the community.

Participants were 100 adolescent girls living in Salinas, California, a small city in an agricultural region of Northern California with a predominantly Latino population. Participants were recruited through social media, word of mouth, and personal networks of the youth researchers. Girls were eligible to participate if they were between 14 and 18 years old, spoke English or Spanish, and had lived in the United States for at least one year. Data collection took place in June-July 2013. This study was approved by the Committee for the Protection of Human Subjects at UC Berkeley.

Intervention

Girls participating in the study were provided with low-chemical personal care products and asked to refrain from using their regular products for 3 days. Each girl was provided with small (2-4 oz) polyethylene containers of shampoo, conditioner, body wash, and moisturizing lotion; a bar of hand soap (for home); a container of liquid soap (to carry in her purse); and roll-on deodorant. Additionally, each girl was allowed to choose 4 items from among liquid or powder foundation, mascara, eyeliner, lipstick/lip gloss/lip balm, and sunscreen. Girls were encouraged to choose the beauty products they used most often to help them comply with the intervention. Participants were asked to avoid using any personal care products or cosmetics other than those provided by the study; if a particular type of product was not provided, participants were asked to forego using that item during the intervention period. Girls who

reported using Colgate Total toothpaste (the only brand that contains triclosan) were given alternate toothpaste; all others were allowed to use their regular toothpaste brand.

The replacement personal care products provided to participants were selected to be free of phthalates, parabens, triclosan, and BP-3. Products were not tested for the presence of these chemicals because we lacked the resources for laboratory testing and because we wanted to select products based on information available to consumers. Rather, we identified products whose ingredient lists did not include triclosan, BP-3/oxybenzone, or any parabens. Because phthalates are not listed on ingredient lists, we avoided any products containing parfum or fragrance unless they were specifically labelled as "phthalate-free". We identified a selection of eligible soaps, shampoos, lotions, sunscreens, and cosmetics through on-line searches, consumer databases (e.g. the Environmental Working Group's SkinDeep Database), and in-store research, with priority given to products that were affordable and available at local retail stores. The final products for use in the intervention were selected by the youth researchers, who tested them to identify those most likely to appeal to adolescents based on marketing, scent, and effectiveness.

Data collection

Participants were interviewed three times: during a home visit, and at pre- and post-intervention office visits. All interviews were conducted by the youth researchers. At the home visit, we obtained informed assent (ages 14-17) or consent (age 18) from the participant as well as informed permission from her parent or guardian. We collected information about family income, household habits, and the family's usual personal care and cleaning products from the parent/guardian using a brief survey. Additionally, at the home visit, we gave the participant four bins labeled "hair products", "face products", "body products", and "teeth products" and asked her to place all the personal care products that she regularly used in the appropriate bin. We took photographs of each bin to ascertain brand names.

The pre- and post-intervention visits occurred at the HERMOSA research office. At the pre-intervention visit, we measured participants' height using a wall-mounted stadiometer and measured their weight and percent body fat using a Tanita bio-impedance scale. Participants then completed a facilitated questionnaire including basic demographic questions and detailed questions about personal care product use in the previous 48 hours. The participant was shown the photographs we had taken at the home visit and asked to indicate which products she had used in the previous 48 hours and when. She was also asked if there were products she had used that were not in the pictures. She was then reminded not to use any of her usual personal care products during the intervention period. Participants provided a urine sample in a sterile polypropylene cup. At the conclusion of the visit, participants visited the study's "Beauty Bar" where they selected replacement personal care products, learned about the potentially harmful chemicals in makeup and personal care products, and received additional instructions about avoiding all personal care products except those provided by the study for the next 3 days.

The post-intervention visit was scheduled 3 days later at the same time of day as the pre-intervention visit to minimize diurnal variability in analyte concentrations. The participants provided a follow-up urine sample and completed a brief questionnaire about their knowledge, attitudes, and behaviors related to personal care product chemicals and their compliance with the intervention. They also answered open-ended questions about their experience with the intervention study.

All urine samples were aliquoted and frozen at -80°C until shipment on dry ice to the Environmental Health Laboratory of the California Department of Public Health in Richmond, California for analysis.

Laboratory Methods

Phthalate laboratory methods were adapted from Kato et al (2005). Ten phthalate urinary mono-ester metabolites were measured (MEP, MnBP, MiBP, MBzP, MCHP, MEHP, MEHHP, MECPP, MEOHP, MCPP), although only MEP, MnBP, and MiBP (metabolites of DEP, DnBP, and DiBP, respectively) were included in this study. Urine samples were spiked with a mixture of stable isotope-labeled internal standards (Cambridge Isotopes) and enzymatically digested with glucuronidase at 37ºC for 90 min. Five hundred microliters of digested sample solution were injected into an on-line solid-phase extraction (SPE) column and analyzed using a high-performance liquid chromatography/tandem mass spectrometer (HPLC-MS/MS) system (API 5000, AB Sciex). Target analytes were chromatographically separated on a Betasil™ phenyl column in a mobile phase consisting of acetonitrile and 0.1% acetic acid in gradient elution mode (Kato et al. 2005). Ionization of analytes was carried out with an electrospray ionization (ESI) source operating in negative mode. To enhance sensitivity, the mass spectrometer data was acquired using multi-period mode during chromatographic elution time.

The analytical method used to measure environmental phenols in urine has been previously described (Gavin et al. 2014). This method measures four parabens (methyl paraben, ethyl paraben, propyl paraben, and butyl paraben), BP-3, triclosan, and bisphenol A (BPA). Urine samples were spiked with stable isotope-labeled internal standards and enzymatically de-conjugated overnight at 37ºC. The digested samples were then processed by SPE using C18 cartridges and the eluents were evaporated and reconstituted with mobile phase immediately prior to the analysis using a reversed-phase HPLC-MS/MS system (API 5500 QTRAP, AB Sciex). Ionization of the analytes was carried out by atmospheric pressure chemical ionization (APCI).

The r^2 of calibration curves for all target analytes for both methods were ≥ 0.99. The limits of detection (LOD) of both methods were sufficiently low to allow exposure assessment for the general population. LOD values are presented in Table 2. Randomly selected samples (~5%) were analyzed in duplicate and the relative percent differences (RPD) between duplicate results were ≤20%. Quality control samples were included in every analytical run and the

recoveries were all within 30% of the respective target values. Precision for each quality control level was good, with coefficients of variation (CV) for all analytes ≤15%. Field quality control included collection of 20 field blanks using highly purified water treated as urine samples, including contact with all field collection materials (urine cups, aliquotting materials, vials, etc). All target analytes were below the respective LODs.

Chemical analyte concentrations were reported in ng/mL of urine. Concentrations below the limit of detection were assigned the value of LOD divided by the square root 2 (Hornung 1990).

To account for urinary dilution, urine specific gravity was measured in the field using a handheld refractometer (PAL-10S, Atago USA Inc). Because NHANES does not report specific-gravity corrected concentrations, we also measured creatinine in the laboratory to facilitate comparison with NHANES. Creatinine was measured using applications of a colorimetric method known as the Jaffe reaction, and loosely based on a method commercially available (BioAssay Systems QuantiChrom Creatinine Assay Kit DICT-500)

Statistical Analysis

We examined distributions of all the urinary analytes and compared them with those of all females aged 14-18 in the 2011-2012 wave of NHANES (N = 108). NHANES data were downloaded from the Centers for Disease Control and Prevention website (http://www.cdc.gov/nchs/tutorials/NHANES/Preparing/Download/Frame2.htm). All analytes were approximately log-normally distributed so analyte concentrations were $\log_{10}$ transformed for analysis. Geometric mean concentrations of creatinine-corrected and uncorrected analytes were compared between HERMOSA and NHANES using t-tests.

We used paired t-tests to compare pre- and post-intervention urinary concentrations of the HERMOSA participants. The primary analyses used specific-gravity corrected concentrations, calculated using the formula: (analyte concentration * 0.024)/(sample specific gravity – 1) (Mahalingaiah et al. 2008) although we also compared creatinine-corrected values. Mixed effects models were used to quantify average within-individual percent change in specific-gravity corrected and creatinine-corrected concentrations before and after the intervention, controlling for time of urine collection. We also examined uncorrected analyte concentrations while controlling for specific gravity or creatinine. In sensitivity analyses, we ran separate mixed effects models that excluded 1) participants on their menses (N=17), in case this contaminated the urine samples and 2) participants who were also youth research assistants (N=5) in case their involvement in the study resulted in different behaviors.

In exploratory analyses, we compared pre- and post-intervention urinary concentrations of BP-3 among sunscreen users and triclosan among Colgate Total toothpaste users. We

hypothesized that sunscreen would be the primary source of BP-3 exposure and Colgate Total would be a significant source of triclosan exposure; thus, we expected to see larger decreases when we restricted the analyses to users of these products than in the study population as a whole. Because parabens and phthalates are found in a wider range of products, we did not conduct similar exploratory analyses for these analytes.

All analyses were conducted using Stata version 13 (Stata Statistical Software, College Station, TX).

Results

Demographics: The characteristics of the 100 young women enrolled in the study are shown in Table 1. All were between the ages of 14 and 18 and self-identified as Mexican or Mexican American. Over half (57%) of participants reported speaking mostly Spanish at home and 19% were born in Mexico. Only a third (33%) of participants reported that at least one of their parents had completed a high school education, and 38% of participants lived in households with an annual income less than the U.S. federal poverty threshold for a family of four ($24,250).

At the time of the pre-intervention interview, 67% of participants were wearing makeup and 30% were wearing sunscreen. Half of the study participants (50%) reported using makeup at least four days per week and 65% reported wearing fragrance at least four days per week. Skin moisturizer use was common, with 65% of participants reporting daily use and 84% using moisturizer at least four times per week.

Comparison with NHANES: Exposure to the phthalates, parabens, and phenols of interest was common, with more than 90% of participants having detectable concentrations of MEP, MnBP, MiBP, methyl paraben, propyl paraben, triclosan, or BP-3 in their urine at the pre-intervention visit (Table 2). Creatinine-corrected urinary concentrations of all analytes except triclosan were higher among HERMOSA participants than among 14-18 year-old girls in NHANES. Urinary concentrations of all three phthalate metabolites were slightly higher in HERMOSA than NHANES but these differences were only statistically significant for MnBP (geometric means: 15.8 vs. 8.0 µg/g) and MiBP (8.5 vs. 6.4 µg/g). Geometric mean urinary concentrations of methyl, ethyl, butyl, and propyl paraben were significantly higher in HERMOSA participants than in NHANES (43.4 vs. 11.0; 1.6 vs. 0.9, 0.5 vs. 0.1; 12.7 vs. 1.4 µg/g, respectively). Concentrations of BP-3 were considerably higher in HERMOSA participants (97.4 µg/g) than in NHANES adolescent girls (14.7 µg/g), although it should be noted that the HERMOSA samples were collected in June and July when sunscreen use is most common whereas NHANES was conducted throughout the year. Geometric mean concentrations of triclosan were slightly lower in HERMOSA compared to NHANES (5.3 vs.8.0 µg/g) but these differences were not statistically significant. Results were similar when non-creatinine-corrected concentrations in HERMOSA and NHANES were compared, except that the

difference in triclosan concentrations became statistically significant while the differences in MEP, MnBP, MiBP, and ethyl paraben were no longer statistically significant. Concentrations of BPA and the phthalates that were not the primary focus of this analysis tended to be higher in NHANES girls compared to HERMOSA (Supplemental Materials, Table S2).

Impact of intervention on chemical exposure: Urinary concentrations of phthalate metabolites, paraben, triclosan, and BP-3 all decreased over the course of the 3-day intervention (Table 3 and Figure 1). We observed a 27% decrease in MEP metabolite concentrations (pre-intervention GM = 78.2 μg/L vs. post-intervention GM = 56.4 μg/L; $p<0.001$) and a non-significant 11% decrease in MnBP concentrations (pre-intervention GM = 28.3 μg/L vs post-intervention GM = 25.1 μg/L; $p=0.07$). There were no changes in MiBP urinary concentrations.

Methyl and propyl paraben, the most commonly detected parabens, decreased by 44-45%, with the GM of methyl paraben decreasing from 77.4 μg/L to 43.2 μg/L ($p<0.01$) and propyl paraben decreasing from 22.6 μg/L to 12.3 μg/L ($p<0.01$). Unexpectedly, ethyl and butyl paraben concentrations both increased over the course of the intervention period, although the absolute changes were small. Butyl paraben increased by 101.7% (pre-intervention GM = 0.8 μg/L vs post-intervention GM = 1.7 μg/L, $p<0.01$) and ethyl paraben increased by 47.3% (pre-intervention GM = 2.9 μg/L vs post-intervention GM = 4.2 μg/L; $p=0.054$).

Triclosan concentrations decreased by 35.7% (pre-intervention GM = 9.5 μg/L vs post-intervention GM = 6.1 μg/L; $p=0.01$) over the 3-day intervention (Table 3). The decrease was larger when we restricted our analysis to the 32 girls who reported using triclosan-containing toothpaste at the pre-intervention visit; among these girls, triclosan concentrations decreased by 70% (pre-intervention GM = 36.4 μg/L vs post-intervention GM = 11.0 μg/L; $p<0.01$) (not shown). BP-3 concentrations decreased by 36.0% overall (pre-intervention GM = 173.8 μg/L vs post-intervention GM = 113.4 μg/L; $p<0.01$). When we restricted the analysis to the 30 girls who reported using sunscreen at the pre-intervention visit, BP-3 concentrations decreased by 52% (pre-intervention GM = 434.4 μg/L vs post-intervention GM = 203.0 μg/L; $p=0.02$).

Results comparing pre- and post-intervention analyte concentrations were similar using creatinine-corrected rather than specific-gravity corrected values and the decrease in MnBP became statistically significant (Supplemental Material, Table S1). Results did not change substantially when creatinine or specific gravity was included as a covariate in the model rather than using creatinine- or specific-gravity-corrected values or when girls on their menses or girls employed as youth research assistants were excluded. Changes in analyte concentrations for each individual are shown in Supplemental Materials, Figure S1.

Urinary concentrations of other measured analytes that are not primarily found in personal care products, such as the phthalate metabolites MBzP, MCHP, MEHP, MEHHP, MECPP, MEOHP, MCPP and BPA, did not change during the course of the intervention (Supplemental Material, Table S2).

Participant compliance: Compliance with the intervention varied for different product types (Table 4). At the post-intervention visit, more than 90% of participants reported that they only used the low-chemical replacement hair, makeup, and body products they were given. However, 45% of participants stated that, at least once during the three day intervention, they had washed with soaps other than the replacement soaps provided by the study. The main reasons for non-compliance were forgetting (32%), being away from home (12%), and using their regular product in cases when they did not choose a replacement among the additional 4 products allowed (21%).

Changes in knowledge, attitudes, and behaviors: Most girls stated that they had learned something new about chemicals in cosmetics because of the study (66%) and that they would buy products without phthalates, parabens, triclosan, or BP-3/oxybenzone (77%). At the post-intervention visit, 23% of girls reported that they had checked the ingredients of their regular products for these chemicals. Participants seemed to enjoy being in the study. Several girls stated that they appreciated learning about chemical exposures in personal care products and being taught methods for reducing their exposure (Appendix 1).

Discussion

The adolescent girls in this study experienced a 27-45% decline in urinary concentrations of certain phthalates, certain parabens, triclosan, and oxybenzone after three days of abstaining from conventional personal care products and using replacement products with labels indicating they did not contain these chemicals.

Of the classes of chemicals studied, we found that phthalate concentrations decreased the least, with a 27% decrease in MEP, an 11% non-significant decrease in MnBP, and no change in MiBP. The relatively modest change in these compounds over the intervention period may be due to the presence of these phthalates in other scented products in the home that were not replaced during the intervention, including air fresheners, fabric softeners, and cleaning products (Buckley et al. 2012; Cacho et al. 2015; Vinas et al. 2015)as well as ingestion of phthalates in food (Colacino et al. 2010) and medications (Hernandez-Diaz et al. 2013; Kelley et al. 2012). It is estimated that the majority of an adult woman's cumulative exposure to DEP is through personal care product use, but that her cumulative exposure to DnBP and DiBP may also reflect significant uptake from food and house dust (Guo and Kannan 2013). This is consistent with our finding of a decrease in MEP but not the other two metabolites. Additionally, MnBP is slower to clear from the body after exposure than MEP (Janjua et al. 2008a), so it is possible three days was not long enough to detect statistically significant differences in MnBP concentrations. Finally, there are indications that DEP and DnBP use in personal care products is decreasing, presumably due to consumer advocacy (Campaign for Safe Cosmetics 2011). Several cosmetics companies have pledged to remove phthalates from their products (Kessler 2015) and urinary

concentrations of MEP and MnBP have decreased substantially in the general U.S. population since 2004 (Zota et al. 2014), suggesting that the contribution of personal care products to Americans' overall phthalate exposure may be declining.

The compounds that declined the most during the intervention were methyl and propyl paraben, which decreased by 44- 45% over the course of our study. Parabens can be found in a wide variety of personal care products including cosmetics, lotions, soaps, and shampoos (Guo and Kannan 2013). Although several parabens are also used as preservatives in baked goods and processed food (Soni et al. 2005), levels are lower than in personal care products and diet is not considered to be a major source of exposure (Liao et al. 2013). Dermal absorption from personal care products is likely the primary route of exposure (Guo and Kannan 2013; Liao et al. 2013), which is consistent with the reductions in urinary paraben concentrations seen in this study.

Contrary to expectations, we observed increases in urinary concentrations of ethyl and butyl parabens during the intervention, although the absolute levels of these compounds remained low. The ingredient lists of all the replacement beauty products indicated that they were paraben-free, however it is possible that some of these products contained ethyl and butyl paraben either as an unintentional contaminant or as an unlabeled substitute for methyl and/or propyl paraben. A limitation of our study is that we were not able to test the replacement products to ensure that they did not contain the chemicals of concern. Exploratory analyses of our data show that the girls who selected the replacement liquid foundation had higher urinary ethyl and butyl paraben concentrations at the post-intervention visit than girls who did not receive this product. However, other products, such as shampoo, conditioner, and body wash were received by all girls so we were unable to identify the sources of ethyl and butyl paraben exposure with certainty.

We also observed decreases of 36% in triclosan and BP-3 concentrations. Triclosan exposure is thought to occur by ingestion among users of triclosan-containing toothpaste and by dermal absorption among users of antibacterial soaps, skin cleansers, and other triclosan-containing personal care products. We found that the decrease in triclosan concentrations was largest among girls who had been using toothpaste containing triclosan but changed brands during the study, suggesting that toothpaste may be a significant source of triclosan exposure. BP-3 is a UV-protection agent found mainly in sunscreens and cosmetics offering sun protection, which is consistent with our finding of the largest decrease in BP-3 levels among sunscreen users.

The phthalates, parabens, and phenols examined in this study are non-persistent compounds with short half-lives that are cleared from the body within 24 – 48 hours following exposure (Gonzalez et al. 2006; Janjua et al. 2008a; Janjua et al. 2008b; Sandborgh-Englund et al. 2006), which allowed us to observe differences in urinary concentrations after just three days. We measured the analytes in spot urine samples, which reflect very recent exposure, likely over the past 12 to 24 hours. Although urinary metabolites tend to be highly variable within subjects,

concentrations in single spot urine samples have been shown to be moderately-to-highly correlated with 24-hour urine samples (Bradman et al. 2013; Fisher et al. 2015; Preau et al. 2010). Collecting 24-hours urine samples from the participants in this study would have yielded a more comprehensive picture of exposure over that entire day, but would have imposed considerably more burden on the participants. By collecting the spot samples at the same time of day before and after the intervention, we minimized within-day variability while assessing the impact of the intervention on recent exposure.

Although urinary concentrations of MEP, methyl and propyl paraben, triclosan, and BP-3 decreased over the course of the intervention, they were not eliminated. Even after changing to low chemical products, the majority of girls (>90%) continued to have detectable concentrations of these compounds in their urine. One possibility is that not all of the pre-intervention exposure was cleared from the body during the intervention period; particularly for BP-3, there is evidence that low levels of the chemical may remain in the body for up to 5 days after dermal exposure (Gonzalez et al. 2006). Thus, it is possible that the 3-day intervention period was too short and that we might have observed larger decreases in urinary metabolite concentrations if the girls had used the replacement personal care products for a longer period of time. We selected the 3-day intervention period because it was long enough to detect changes in urinary metabolite levels but short enough to maximize participant compliance. It is also likely that other sources of exposure beyond personal care products exist, and/or that the replacement products were not completely free of these compounds. Although on average most analytes decreased, a number of participants experienced increases in some analyte levels over the course of the intervention. Upon receiving individual results, some of these girls were surprised that their levels had risen despite their best efforts to comply. Their experience highlights the difficulty of reducing exposure to these ubiquitous compounds.

HERMOSA Study participants had urinary concentrations of the chemicals of interest that were similar, but slightly higher, than adolescent girls participating in NHANES. The NHANES and HERMOSA samples were analyzed by different laboratories, which may affect comparability. However, the California Department of Public Health laboratory that analyzed the HERMOSA samples has participated in and passed the proficiency test program administrated by Centers for Disease Control and Prevention every year (two rounds per year) since 2012 to ensure that their results are comparable to NHANES. Additionally, although concentrations of parabens, MiBP, MnBP, and BP3 were higher in HERMOSA participants, concentrations of other phthalates and phenols not primarily found in personal care products were higher in NHANES girls, suggesting that the differences were due to exposure patterns rather than systematic analytical differences between laboratories. We compared the HERMOSA urinary concentrations to those of all adolescent girls in NHANES, rather than Latina adolescents, because of NHANES sample size limitations. The HERMOSA Study participants were all Latina and mostly low-income and their personal care product use patterns may differ from than the general U.S. population, which may impact the generalizability of our

findings. The finding of higher analyte concentrations in our population are consistent with studies in NHANES showing that levels of phthalates and parabens are higher among African Americans and Mexican Americans than whites (Centers for Disease Control and Prevention 2012).

Overall, compliance was good in this study, although most girls found it difficult to completely comply with the protocol, particularly regarding use of hand soap. Presumably decreases in exposure would have been larger with perfect compliance. Most girls stated that they would like to use products without potentially harmful chemicals. However, identifying these products often requires a considerable amount of effort and expense on the part of the consumer. In our experience, we had little trouble purchasing low-chemical hand soaps and shampoos even in mainstream retailers, but found that low-chemical make-up was hard to identify, not sold many places, and often expensive. Although many stores have dedicated displays for low-chemical personal care products, these still represent only a small portion of the personal care product market and are particularly difficult to find in low income communities. Future studies should look at the long-term effects of education about endocrine disruptors in personal care products on girls' product choices, behavior changes, and exposure levels.

Finally, a major strength of this study was that it was rooted in the tenets of community-based participatory research, including bidirectional learning between the researchers and community youth. Involving the youth research assistants in the study design and implementation allowed us to create an intervention that was culturally appropriate, scientifically valid, and relevant and interesting to the adolescent participants. The 100% retention, good compliance, and high quality data collection were likely due to the connection that the youth research assistants were able to forge with the study participants.

In summary, this is the first study to show that techniques available to consumers, such as choosing personal care products that are labelled to be free of phthalates, parabens, triclosan, and oxybenzone, can significantly reduce personal exposure to these potentially endocrine-disrupting chemicals. Our study did not test for the presence of these chemicals, but simply used techniques available to the average consumer: reading labels and investigating product safety through web-based databases. Consumers hoping to reduce exposure to these potential endocrine disruptors should consider seeking out these lower chemical products.

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584 Table 1. Characteristics of adolescent girls participating in the HERMOSA Study, Salinas, CA
585 2013 (n=100)

	N (%)
Age	
14	11 (11)
15	22 (22)
16	29 (29)
17	30 (30)
18	8 (8)
Country of birth	
United States	81 (81)
Mexico	19 (19)
Language spoken at home	
Mostly Spanish	57 (57)
Spanish and English equally	29 (29)
Mostly English	14 (14)
Highest parental education	
Less high school	57 (57)
High school graduate	33 (33)
Unknown	10 (10)

Annual household income	
≤ $24,000	38 (38)
$24,001 - $36,000	29 (29)
>$36,000	25 (25)
Unknown	8 (8)
Frequency of makeup use	
Every day	27 (27)
4-6 times per week	23 (23)
2-3 times per week	20 (20)
Once a week or less	30 (30)
Frequency of moisturizer use	
Every day	65 (65)
4-6 times per week	19 (19)
2-6 times per week	8 (8)
Once a week or less	8 (8)
Frequency of fragrance use	
Every day	47 (47)
4-6 times per week	18 (18)
2-6 times per week	17 (17)
Once a week or less	18 (18)
Used makeup today?	
Yes	67 (67)
No	33 (33)
Used sunscreen today?	
Yes	30 (30)
No	70 (70)
Used triclosan-containing toothpaste today?	
Yes	32 (32)
No	68 (68)

586
587

Table 2. Comparison of creatinine-corrected urinary analyte concentrations (µg/g) in adolescent girls participating in HERMOSA (N=100) and NHANES (N=108)

	HERMOSA Study Pre-Intervention 2013							NHANES 2011-2012 (Girls 14-18)							
	LOD	DF	GM	Percentile (µg/g)				LOD	DF	GM	Percentile (µg/g)				
	(µg/L)	(%)	(µg/g)	25%	50%	75%	95%	(µg/L)	(%)	(µg/g)	25%	50%	75%	95%	p-value[a]
Phthalates:															
MEP[b]	0.5	100	43.9	21.33	36.4	83.7	237.9	0.4	100	29.0	15.2	31.2	62.7	267.9	0.16
MnBP[c]	0.9	97	15.9	11.0	15.0	22.3	46.6	1.0	96	8.0	5.1	8.8	14.6	34.0	<0.01
MiBP[d]	0.4	99	8.5	5.1	7.5	13.0	32.1	0.2	100	6.4	4.3	6.7	9.9	18.7	<0.01
Parabens:															
Methyl paraben	0.5	93	43.4	13.4	47.9	121.7	1,013.7	1.0	96	11.0	4.0	12.1	54.0	493.5	<0.01
Ethyl paraben	0.5	55	1.6	<LOD	1.25	3.75	62.18	1.0	25	0.9	0.4	0.6	1.3	20.1	<0.01
Butyl paraben	0.2	49	0.5	<LOD	<LOD	1.3	20.2	0.2	8	0.1	<LOD	<LOD	<LOD	0.6	<0.01
Propyl paraben	0.2	90	12.7	2.83	15.35	79.7	270.71	1.0	89	1.4	0.5	1.2	6.2	89.2	<0.01
Phenols:															
Triclosan	0.2	93	5.3	1.0	3.7	17.5	579.2	2.3	82	8.0	2.0	5.0	26.7	261.6	0.17
BP-3	0.5	97	97.4	26.6	117.2	434.3	2,938.2	0.4	100	14.7	3.8	9.2	30.1	375.7	<0.01

[a]Comparison of HERMOSA and NHANES geometric means

[b]Metabolite of diethyl phthalate

[c]Metabolite of di-n-butyl phthalate

[d]Metabolite of di-isobutyl phthalate

Abbreviations: LOD = Limit of detection, DF = Detection Frequency, GM = Geometric Mean

Table 3. Specific-gravity-corrected concentrations (ng/mL) of urinary analytes before and after the HERMOSA intervention.

	Pre-intervention GM (SE)	Post-intervention GM (SE)	% change[a]	p-value
Phthalates:				
MEP	78.2 (1.1)	56.4 (1.1)	-27.4	<0.001
MnBP	28.3 (1.1)	25.1 (1.1)	-11.3	0.07
MiBP	15.2 (1.1)	15.2 (2.3)	-.46	0.95
Parabens:				
Methyl paraben	77.4 (1.2)	43.2 (1.2)	-43.9	<0.01
Ethyl paraben	2.9 (1.2)	4.2 (1.2)	47.3	0.05
Butyl paraben	0.8 (1.2)	1.7 (1.2)	101.7	<0.01
Propyl paraben	22.6 (1.3)	12.3 (1.2)	-45.4	<0.01
Phenols:				
Triclosan	9.5 (1.3)	6.1 (1.2)	-35.7	0.01
BP-3	173.8 (1.2)	113.4 (1.2)	-36.0	<0.01

[a]Adjusted for time of urine collection

Table 4. Participants' compliance and behaviors related to personal care product use in HERMOSA Study

Used only HERMOSA replacement products during intervention period:	Percent
Hair Products	91
Makeup	91
Face Products	90
Scented Products	94
Body Products	94
Soap Products	65
Among participants with any non-compliance during intervention (N=57): Why did you use non-HERMOSA products?	
Forgot	32
Was away from home	12
Didn't get a replacement product	21
Replacement product didn't work	2
Didn't like look	0
Didn't like smell	0
Knowledge and attitudes:	
Learned something new about chemicals in cosmetics	66%
Has checked beauty products for phthalates, parabens, triclosan, oxybenzone	23%
Will buy products without phthalates, parabens, triclosan, oxybenzone	71%

Appendix 1. Comments from HERMOSA Participants

What did you enjoy most about participating in the HERMOSA study?

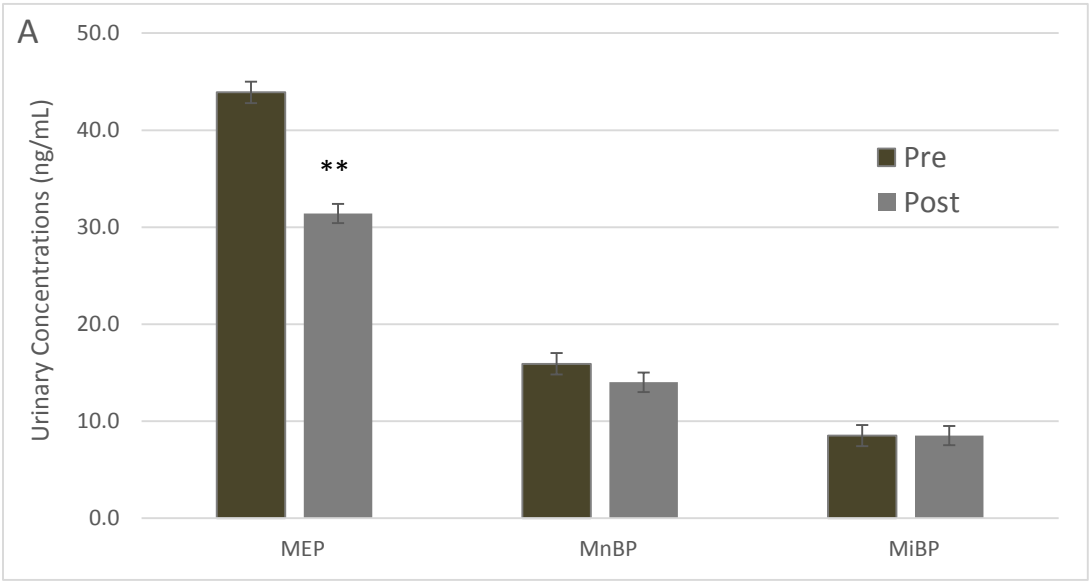
- I enjoyed meeting the members of HERMOSA study and getting introduced to other products of makeup that can be healthier to your skin.
- Realizing that the powder HERMOSA study provided me was not as heavy as the makeup brand I usually used and looked more natural.
- What I enjoyed the most was challenging myself to not use my products and just the products I was given.
- Trying more natural products and learning about the harsh chemicals found in the usual products I use.
- I enjoyed learning [about] low-chemical products. This was a good experience and a cool challenge most people should participate in to become informed on how their everyday products are affecting them.
- I enjoy getting FREE makeup.
- I enjoyed feeling naturally beautiful.

How has the study been beneficial to you?

- The study has taught to make smarter choices by choosing healthier and low chemical products.
- I learned that you can look good and still keep track of what your products contain.
- It has been beneficial because I learned that there are harmful things in the products we use every day.
- The study [was] beneficial to me because now I know what products I can buy for better health.
- This study has been beneficial to me in that it made me aware that products we have at home serve the same function as other products that are found in the store.
- The study has been beneficial to me because now I'm more aware of the chemicals my makeup has.
- It actually made me want to buy these [low chemical] products on my own because I really liked them.

Figure 1. Pre- and post-intervention geometric means (± SE) of urinary concentrations (ng/mL) of A) Phthalate, B) Paraben, C) Triclosan, and D) BP-3 analytes.

* $p<0.05$ ** $p < 0.01$


A
50.0
40.0
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Urinary Concentrations (ng/mL)
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Pre
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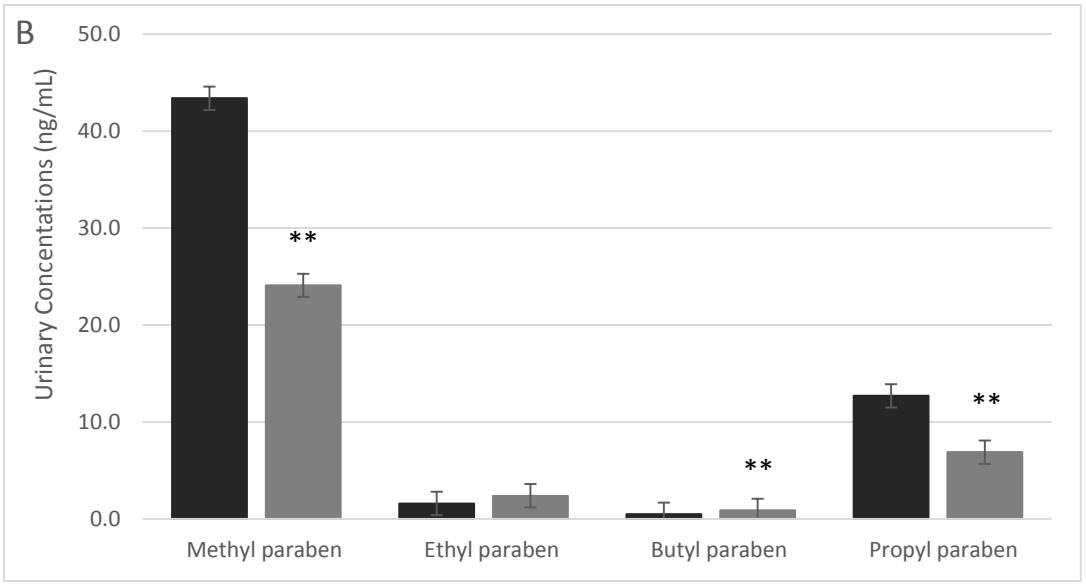
MiBP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Urinary Concentations (ng/mL)
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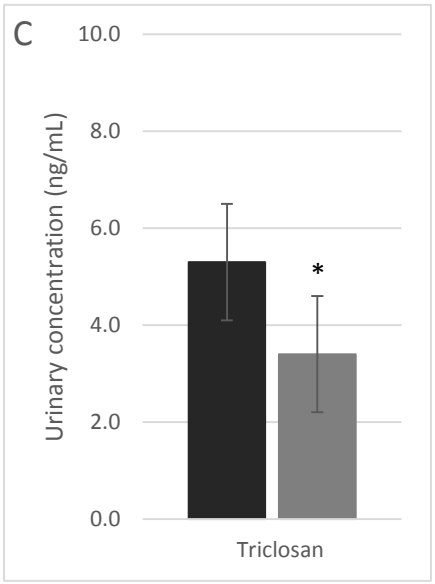
Methyl paraben
Ethyl paraben
Butyl paraben
Propyl paraben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Urinary concentration (ng/mL)
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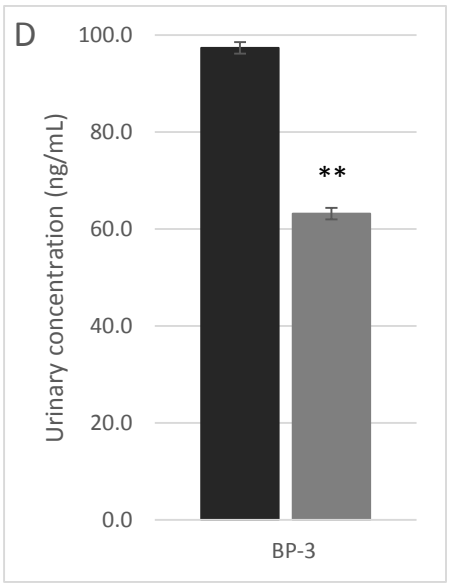
Triclosan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Urinary concentration (ng/mL)
**
BP-3

Supplemental Material, Table S1. Pre- and post-intervention creatinine-corrected urinary concentrations (ng/g)

	Pre-intervention GM (SE)	Post-intervention GM (SE)	% change	p-value
Phthalates:				
MEP	43.9 (1.1)	31.4 (1.1)	-28.2	<0.001
MnBP	15.9 (1.1)	14.0 (1.1)	-11.9	0.02
MiBP	8.5 (1.1)	8.5 (2.3)	-0.2	0.96
Parabens:				
Methyl paraben	43.4 (1.2)	24.1 (1.2)	-44.5	<0.01
Ethyl paraben	1.6 (1.2)	2.4 (1.2)	43.5	0.07
Butyl paraben	0.5 (1.2)	0.9 (1.2)	100.1	<0.01
Propyl paraben	12.7 (1.2)	6.9 (1.2)	-45.6	<0.01
Phenols:				
Triclosan	5.3 (1.3)	3.4 (1.2)	-36.3	<0.01
BP-3	97.4 (1.2)	63.2 (1.2)	-35.1	<0.01

Supplemental Material, Table S2. Creatinine-corrected urinary concentrations (μg/g) of other phthalates and phenols not primarily found in personal care products, comparing adolescent girls participating in HERMOSA (N=100) and NHANES (N=108)

	HERMOSA Study Pre-Intervention 2013							NHANES 2011-2012 (Girls 14-18)							
	LOD	DF	GM	Percentile (μg/g)				LOD	DF	GM	Percentile (μg/g)				
	(μg/L)	(%)	(μg/g)	25%	50%	75%	95%	(μg/L)	(%)	(μg/g)	25%	50%	75%	95%	p-value[a]
Phthalates:															
MBzP	0.2	100	4.9	2.8	4.6	7.8	22.2	0.3	99	5.3	3.2	5.5	10.1	20.6	0.50
MEHP	0.2	88	1.4	.72	1.6	2.4	6.1	0.5	78	1.4	.61	1.2	2.8	10.2	0.96
MEHHP	0.2	98	4.7	3.0	4.3	7.9	15.9	0.2	100	8.4	4.6	7.0	10.6	53.9	<0.01
MECPP	0.2	100	8.7	5.7	8.1	12.3	33.6	0.2	100	13.6	7.2	10.5	18.1	65.1	<0.01
MEOHP	0.1	99	3.7	2.4	3.6	5.5	12.5	0.2	100	5.3	3.0	4.3	7.1	31.6	0.02
MCPP	0.1	97	1.4	.9	1.4	1.9	4.3	0.2	98	2.9	1.1	1.8	4.8	45.7	<0.01
Phenols:															
BPA	0.2	81	0.8	.45	.9	1.5	4.3	0.4	91	1.6	.74	1.2	2.3	8.7	<0.01

[a]Comparison of HERMOSA and NHANES geometric means

Abbreviations: LOD = Limit of detection, DF = Detection Frequency, GM = Geometric Mean

Supplemental Material, Table S3. Pre- and post-intervention specific-gravity-corrected urinary concentrations (ng/mL) of other phthalates and phenols not primarily found in personal care products

	Pre-intervention GM (SE)	Post-intervention GM (SE)	p-value
Phthalates:			
MBzP	8.7 (1.1)	8.8 (1.1)	0.80
MEHP	2.4 (1.1)	2.8 (1.1)	0.11
MEHHP	8.3 (1.1)	8.4 (1.1)	0.88
MECPP	15.6 (1.1)	16.5 (1.1)	0.49
MEOHP	6.7 (1.1)	6.8 (1.1)	0.84
MCPP	2.6 (1.1)	2.4 (1.1)	0.44
Phenols:			
BPA	1.4 (1.1)	1.6 (1.1)	0.40

Supplemental Material, Figure S1. Individual pre- and post-intervention specific-gravity-corrected urinary concentrations (ng/mL) of A) mono-ethyl phthalate (MEP), B) MEP removing 3 influential individuals, C) propyl paraben C) triclosan and D) benzophenone-3 (BP-3) analytes for each participant in the study.