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Ruthann A. Rudel, Janet M Gray, Connie L. Engel,
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Food Packaging and Bisphenol A and Bis(2-Ethyhexyl) Phthalate Exposure: Findings from a
Dietary Intervention

Authors: Ruthann A. Rudel,^{1*} Janet M Gray,^{2,3} Connie L. Engel,² Teresa W. Rawsthorne,⁴
Robin E. Dodson,¹ Janet M Ackerman,¹ Jeanne Rizzo,² Janet L. Nudelman,² Julia Green Brody¹

¹Silent Spring Institute, Newton, MA 02458

²Breast Cancer Fund, San Francisco, CA 94109

³Vassar College, Poughkeepsie, NY 12604

⁴AXYS Analytical Services, Sidney, British Columbia V8L 5X2, Canada

*Address correspondence to Ruthann Rudel, Silent Spring Institute, 29 Crafts Street, Newton,
MA 02458; phone 617-332-4288 x214; fax 617-332-4284; rudel@silentspring.org

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Key words: canned foods, diet, endocrine disruptor, exposure, food packaging, intervention design, phthalates, plastics, pharmacokinetics

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Abbreviations:

BPA, bisphenol A

BBP, butyl benzyl phthalate

DBP, di-butyl phthalate

DEP, diethyl phthalate

DEHP, bis(2-ethylhexyl)phthalate

DMP, di-methyl phthalate

FDA, Food and Drug Administration

HPLC, high pressure liquid chromatography

MMEP, monomethyl phthalate

MEP, mono ethyl phthalate,

MBUP, mono butyl phthalate (n-and iso)

MBZP, mono benzyl phthalate

MEHP, mono-2-ethylhexyl phthalate

MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate

MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate

MS-MS, tandem mass spectrometry

MRL, method reporting limit

NHANES, National Health and Nutrition Examination Survey

PEM, phthalate ester metabolite

PVC, polyvinyl chloride

Abstract

BACKGROUND: Bisphenol A (BPA) and bis(2-ethylhexyl) phthalate (DEHP) are high-production-volume chemicals used in plastics and resins for food packaging. They have been associated with endocrine disruption in animals and in some human studies. Human exposure sources have been estimated, but the relative contribution of dietary exposure to total intake has not been studied empirically.

OBJECTIVES: To evaluate the contribution of food packaging to exposure, we measured urinary BPA and phthalate metabolites before, during and after a “fresh foods” dietary intervention.

METHODS: We selected 20 participants in five families based on self-reported use of canned and packaged foods. Participants ate their usual diet, followed by three days of “fresh foods” that were not canned or packaged in plastic, and then returned to their usual diet. We collected evening urine samples over eight days in January 2010 and composited them into pre-intervention, intervention, and post-intervention samples. We used mixed effects models for repeated measures and Wilcoxon signed rank tests to assess change in urinary levels across time.

RESULTS: Urine levels of BPA and DEHP metabolites decreased significantly during the fresh foods intervention (e.g., BPA geometric mean 3.7 ng/mL pre-intervention and 1.2 ng/mL during intervention; MEHHP geometric mean 57 ng/mL vs 25 ng/mL). The intervention reduced geometric mean concentrations of BPA by 66% and DEHP metabolites by 53-56%. Maxima were reduced by 76% for BPA and 93-96% for DEHP metabolites.

CONCLUSIONS: BPA and DEHP exposures were substantially reduced when participants’ diets were restricted to food with limited packaging.

Introduction

Bisphenol A (BPA) is a high-production volume industrial chemical used in the manufacture of polycarbonate and other plastic products and epoxy resin-based food can liners. It is present in both canned and plastic-packaged foods sold in the US (Schechter et al. 2010). Exposure is widespread, with detectable levels in urine samples from over 90% of the US population (Calafat et al. 2008). A wide body of evidence from *in vitro*, animal, and epidemiological studies indicates the potential for BPA-induced endocrine disruption in a number of organ systems. BPA uses, exposure, and health effects are reviewed elsewhere (NTP-CERHR 2008; Vandenberg et al. 2010).

Phthalates are another common class of endocrine disrupting chemicals (EDCs) produced in high volumes and widely used in consumer goods, including food packaging (EFSA 2005; Fromme et al. 2007; NTP-CERHR 2006; Wormuth et al. 2006). This family includes higher molecular weight phthalates such as bis(2-ethylhexyl)phthalate (DEHP), a common polyvinyl chloride (PVC) additive, di-butyl phthalate (DBP), and butyl benzyl phthalate (BBP); and also lower molecular weight phthalates such as dimethyl phthalate (DMP) and diethyl phthalate (DEP), commonly used as a solvent for fragrance. All of these are used in food packaging. The higher molecular weight phthalates DEHP, DBP, and BBP are identified as EDCs based on inhibition of testosterone synthesis and effects on the developing male reproductive system in rodents, while the lower molecular weight phthalates DEP and DMP did not induce these effects (Gray et al. 2000). Some epidemiologic evidence shows associations between urinary excretion of phthalate metabolites and effects on the developing male reproductive system (Swan 2008), male hormone levels and semen quality (Hauser 2008; Meeker et al. 2007; Meeker et al. 2009), and neurobehavioral endpoints (Engel et al. 2009).

Exposure estimates based on food, air, dust, and consumer product concentrations and intake rates indicate that diet is likely to be a major source of exposure for BPA and DEHP (Fromme et al. 2007; Lalkind and Naiman 2010; NTP-CERHR 2006; NTP-CERHR 2008) and an important source of exposure to BBP and DBP (NTP-CERHR 2003; NTP-CERHR No Date; Wormuth et al. 2006). Diet is expected to account for only a small fraction of exposure to DMP or DEP, which are predominantly from consumer product sources (Itoh et al. 2007; Wormuth et al. 2006). However, empirical data to verify these estimates are limited.

Better information about exposure sources, such as the role of diet, is needed to provide reliable information about opportunities to reduce exposure. Many individuals seek guidance to avoid exposures as a precaution while health effects remain under study. In addition, the US Food and Drug Administration (FDA) recently announced its support for “reasonable steps” by the agency to reduce BPA exposure (FDA 2010).

The contribution of different sources and the effectiveness of exposure reduction strategies can be efficiently evaluated through longitudinal studies of small numbers of participants in interventions designed to alter exposure. BPA and phthalates are suited to this design, because they have short biological half-lives, non-invasive exposure biomarkers, and sources that can be modified by individual behaviors. The value of this design has been demonstrated in studies that showed an increase in urinary BPA in students using polycarbonate drinking water bottles (Carwile et al. 2009); reductions in urinary pesticide metabolites in children provided with an organic diet (Lu et al. 2006); and reduced urinary excretion of antibiotics and phthalates following a five-day Buddhist “temple stay” that involved a vegetarian diet (Ji et al. 2010).

In the present study, we assessed changes in urinary BPA and phthalate metabolite levels during and after a three-day dietary intervention designed to minimize exposure to food packaged in plastic or cans by substituting a “fresh-foods” diet. We measured phthalate metabolites that we expected to have substantial dietary sources and, for comparison, some metabolites for which diet is not expected to be a major source. We expected to see large reductions in BPA and the DEHP metabolites mono-2-ethylhexyl phthalate (MEHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-(2-ethyl-5-hydroxy hexyl) phthalate (MEHHP). We expected smaller reductions in monobutyl phthalate (MBUP, a metabolite of DBP and BBP) and monobenzyl phthalate (MBZP, a metabolite of BBP), and little or no reductions in monoethyl phthalate (MEP, a metabolite of DEP) and monomethyl phthalate (MMEP, a metabolite of DMP).

Methods

Participants

We selected five families to participate in a study to assess BPA and phthalate urine levels at three time-periods: pre-intervention, while eating their typical diet; intervention, on a special diet of fresh foods (no canned foods) prepared and packaged almost exclusively without contact with plastic; and post-intervention, after ending the special diet.

Sixty-three families in the greater San Francisco Bay Area responded to letters on five listservs by completing a brief online survey about demographic characteristics and diet over the previous two days (see Supplemental Material, Initial Recruitment Survey). In order to identify families whose diet included sources of BPA and phthalates, we asked families to complete a survey on certain dietary practices. Eligible families had two adults and two toilet-trained

children between the ages of 3-12 years, lived in the San Francisco Bay Area, had no significant dietary restrictions, and indicated either the consumption of canned foods, or exposure to at least two of these potential sources of dietary BPA and phthalates: drank from personal water bottles; drank from large polycarbonate 2-5 gallon water bottles in office coolers, ate meals outside of the home, or microwaved in plastic. Of 63 families that completed the survey, 20 met the criteria for study inclusion. Three of these families could not participate due to logistical concerns (e.g., travel). Another three did not return calls. Based on phone interviews with the remaining 14, we selected the five families who reported the most frequent consumption of canned foods and who seemed likely to be able to comply with the study protocol (e.g., we excluded potential participants who worked night shift, ate low-carbohydrate diet, etc). The age, family composition, and geographic location of the 9 non-participant families were similar to the 5 families who were enrolled. The Vassar College Institutional Review Board approved the study protocol.

Dietary Intervention

A caterer, whom the research team had informed about possible sources of BPA and phthalates to avoid, developed an initial set of menu options. After reviewing these options and sharing them with participants to learn their preferences, the research team selected a final menu.

All families received the same foods for the three-day meal intervention in January 2010. Intervention-period foods were prepared almost exclusively from fresh and organic fruits, vegetables, grains and meats (Supplemental Material, Table 1). Preparation techniques avoided contact with plastic utensils and non-stick coated cookware, and foods were stored in glass containers with BPA-free plastic lids. Containers were filled to below the top so foods did not

contact the lids. Researchers instructed families to store foods only in these containers during the intervention and to avoid microwaving the lids. Participants received stainless steel water bottles and lunch containers to avoid other common sources of BPA and phthalates. Participants were encouraged to eat only the food provided during the intervention, but were advised that if they had to depart from the provided foods, they could use fresh foods, such as fruits, vegetables, eggs, peanut butter and jelly from glass jars, and milk and orange juice (from glass containers or LDPE plastic if glass was not available). Coffee drinkers were advised to use a French press or ceramic drip rather than using a plastic coffee maker or buying coffee from a café.

Sample Collection

Prior to sample collection, all adult participants gave informed consent for themselves and their children. Families received pre-labeled 125 mL amber glass urine sampling containers (EC Scientific Products), a daily checklist of study activities, and guidelines for storing and heating foods during the intervention. The field director spoke with families daily to address questions and concerns and remind the families of study requirements for the day. We recorded any reported deviations from the intervention diet at this time. Families also completed food questionnaires to characterize potential dietary sources of BPA and phthalates during the pre- and post-intervention periods. Data collection spanned eight consecutive days. On days 1 and 2, families ate their normal diet, and on day 2, the researchers delivered food for days 3-5 prepared by a local caterer. On days 6-8, families returned to preparing their own food.

Each participant provided a urine sample in the evening, usually after dinner, of days 1 and 2 (pre-intervention), 4 and 5 (intervention), and 7 and 8 (post-intervention) (Figure 1). No samples were collected on days 3 and 6, while participants transitioned onto and off of the

intervention. Families double-bagged urine specimen jars and stored them in their freezers until pickup within a week of the study's conclusion. After pickup, urine samples were stored in a freezer overnight and shipped overnight on blue ice to the laboratory for processing and analysis. Samples were stored frozen at -20C (BPA < 2 weeks, phthalate ester metabolite (PEM) < 8 weeks) before being thawed for analysis. After thawing, the laboratory archived aliquots of each individual urine sample at -20C for possible future analysis.

Laboratory analysis

For each study phase (pre-, during, and post-intervention) we combined the two urine samples collected from each individual. Both urine samples were thawed and equal 40 mL volumes were combined in a clean 120 mL amber glass jar. Once mixed, a 2 mL subsample was taken for creatinine measurement and 1 mL subsamples were taken for the BPA and PEM analytical methods. Analysis was by HPLC/MS/MS using isotope dilution quantification. See Supplemental Material for detailed extraction, analysis and quantification methods.

Samples were analyzed in batches including quality control samples: a procedural blank, one spiked reference sample, and a reference sample in duplicate using laboratory stock urine for inter- and intra- batch comparisons. All analyte detection limits were ≤ 1 ng/mL, except that MMEP in one sample had a 2.15 ng/mL detection limit. All quality control samples were within specifications for each batch. The laboratory was blind to the identity of the samples, including which ones represented intervention or non-intervention collections.

Data analysis

Urinary concentrations are reported as analyte mass per volume (ng/mL), unadjusted for creatinine. Adjustment for creatinine is commonly used to reduce the impact of varying dilution

on urinary biomarker concentrations. However, we addressed the influence of urine dilution by including creatinine as a variable in our model, as recommended in Barr et al. (2005), and we conducted confirmatory analyses using both unadjusted and creatinine-adjusted concentrations. These approaches were selected for a number of reasons. Creatinine concentrations have been shown to vary with protein content of the diet (Kesteloot and Joossens 1993; Neubert and Remer 1998), and therefore might be altered during the dietary intervention. Furthermore, since creatinine is associated with age and gender (Barr et al. 2005), adjusting for it might therefore bias associations between urine metabolite concentrations and age or gender.

We calculated a method reporting limit (MRL) as the maximum of the sample-specific method detection limit and the 90th percentile of the four lab blanks. We used all reported data including measurements below the MRL. Twelve percent of MMEP measurements were reported as nondetect, and to these, we assigned the sample-specific MRL (USEPA 2006). MRLs ranged from 0.25 ng/mL (BPA) to 7 ng/mL (MEP). Concentrations were not normally distributed but were approximately log-normal; therefore, we log-transformed concentrations for mixed effects modeling and used nonparametric tests.

We used mixed effect models for repeated measures, with family and participant included as multilevel random effects and creatinine as a fixed effect, to evaluate changes in concentrations over time. Specifically, we used a linear spline model with one knot placed at the middle time point, during the intervention. The impact of age (adult/child as a categorical variable) and gender were evaluated as fixed effects. To corroborate our findings, we used Wilcoxon signed rank tests on paired data to compare concentrations across two time periods (e.g. pre- and during intervention). Wilcoxon comparison of pre- and during intervention urine concentrations used both unadjusted and creatinine adjusted concentrations.

We evaluated the influence of being in the same family on exposure. To evaluate effects over the course of the study, we used variance estimates from the mixed effects model. Specifically, we estimated the correlation among participants within the same family (the intraclass correlation, ICC) as the variance attributable to the random effect of being in the same family divided by total variance (family, participant and residual). We also estimated the percent variance explained by being in the same family by finding the difference in residual variance between models with and without family. In addition, to compare inter- and intra-family variability at each time period, we used the nonparametric Kruskal-Wallis test, which evaluates the ratio of between- and within-group variability. Differences among families during the intervention are of particular interest, because when diet is held constant, exposure variation due to other sources, some of which may be shared by families living together, can be observed.

We conducted data management and analysis in R (R Development Core Team 2010). All statistical tests were conducted at the 0.05 significance level.

Results

Twenty participants (4 members in each of 5 families) completed the dietary intervention study and provided a total of 6 urine samples, including two samples collected during each phase of the study. These were later combined to make one sample per phase for each participant (Figure 1). The median age of the 10 adults was 40.5 years; the median age of the 10 children was 7 years. Characteristics of study participants are provided in Table 1.

All analytes were detected in 100% of the samples except MMEP, which was detected in 88% of samples. Compared to the 2007-08 NHANES sample of 2604 individuals age 6 years and older (CDC 2009), pre-intervention medians and 95th percentile estimates for adults and

children combined were higher in this study for BPA and metabolites of DEHP (MEHP, MEHHP and MEHOP), and for MBUP (a metabolite of DBP and BBP) and MMEP (a metabolite of DMP); much lower for the DEP metabolite MEP; and similar for the BBP metabolite MBZP (Figure 2 and Table 2). Higher overall median values for BPA and DEHP were due to higher median values in adult study participants than in NHANES adults, while children's levels were similar to NHANES median values for children. The pre-intervention creatinine medians were similar to those derived from the 1988-1994 NHANES sample of 22,245 individuals (Barr et al. 2005).

Urinary geometric mean values of BPA and of the DEHP metabolites MEHP, MEHHP, and MEOHP were significantly lower during the intervention than before the intervention (Figure 2, Table 3). Geometric means were reduced 66%, 53%, 55% and 56% for BPA, MEHP, MEOHP and MEHHP, respectively. Similar findings were observed with the paired Wilcoxon signed rank tests for unadjusted (Supplemental Material, Figure 1) and creatinine-adjusted (Supplemental Material, Figure 2) concentrations for BPA and the three DEHP metabolites, although the decrease was not statistically significant for creatinine-adjusted MEHP and MEOHP. Reductions in the upper ends of the exposure distributions were larger than corresponding reductions in the geometric mean values (Figure 2 and Supplemental Material, Table 2). For example, the 90th percentiles of BPA and MEHP were reduced by 73% and 84% respectively, and maxima were reduced by 76% and 96%. Consistent with the greater reduction at the tops of the exposure distributions, the lower geometric means during the intervention were accompanied by smaller interquartile ranges (reductions of 75%, 48%, 64%, 68% for BPA, MEHP, MEOHP and MEHHP, respectively) (Supplemental Material, Table 2). Among the

phthalates other than DEHP metabolites, we observed a nonsignificant 25% reduction in MBUP and no clear differences for other analytes (Table 3).

After the return to regular diets, BPA levels increased to approximately pre-intervention levels ($p < 0.01$) (Figure 2, Table 3). A significant increase in BPA was also observed in paired Wilcoxon signed rank tests using adjusted and unadjusted concentrations (Supplemental Material, Figures 1-2). The geometric means of DEHP metabolites increased by 16-22% following the intervention, although this change was not statistically significant (Figure 2, Table 3). Creatinine concentrations were reduced by the intervention (geometric means 94 mg/dL vs 76 mg/dL, paired Wilcoxon signed rank test: $p = 0.04$).

Urinary concentrations did not differ significantly between adults and children for BPA, but some differences were observed for phthalate metabolites. In the mixed effects model, there were significant differences in urinary concentrations between adults and children across the study period for MEP, MBUP, MBZP and MMEP ($p < 0.05$; results not shown). Adults had significantly higher concentrations than children for MEP (geometric means adults vs. children; pre: 78 vs. 21 ng/mL, during: 92 vs. 27 ng/mL, post: 98 vs. 29 ng/mL), while children had significantly higher concentrations for MBUP (geometric means adults vs. children; pre: 34 vs 53 ng/mL, during: 35 vs. 29 ng/mL, post: 32 vs 38 ng/mL), MBZP (pre: 9.3 vs. 14 ng/mL, during: 11 vs 9.3 ng/mL, post: 8.3 vs 16 ng/mL) and MMEP (pre: 11 vs 13 ng/mL, during: 10 vs 13 ng/mL, post: 8.7 vs 10 ng/mL (Supplemental Material, Table 2). Males and females did not differ significantly, and therefore gender was included in the final model (data not shown).

Effects of family membership on exposure

Variance estimates from the mixed effects model were used to estimate the correlations among participants within the same family (ICC) and the percent of total variance explained by inclusion of family as a random effect. The estimated ICCs range from approximately zero for MEP to 0.27 for MMEP, indicating that there was substantial variation within families (data not shown). The only analyte with a substantial percent of variance explained by family membership was BPA (11%). In contrast, the percent of variance explained by family membership was only 3.6% for MMEP, 2.6% for MBUP, and near zero for other metabolites.

Variation in urinary BPA was greater among families than within families during the intervention (Kruskal-Wallis test, $p < 0.01$) but not at any other time. Urinary concentrations during the intervention of individuals grouped by family are shown in Supplemental Material, Figure 3. Significant variation in MEOHP and MEHHP was observed among families after the intervention (data not shown), and variation in MMEP among families was observed during and after the intervention (Kruskal-Wallis test, $p < 0.05$).

Dietary sources

Based on daily contacts with the research staff, participant-reported compliance with the intervention protocol was high, and the few substitutions reported by participants were within the options specified in the instructions. Reported deviations from the intervention diet are presented in Supplemental Material, Table 3.

Potential exposure sources recorded during the 2 days before and 3 days after the intervention included meals prepared outside the home, canned foods, canned soda, frozen dinners, drinking from polycarbonate water bottles, and microwaving in plastic. All families

reported using canned foods or having at least one meal outside the home during the pre-or post-intervention phases of the study. Two families reported microwaving frozen meals in plastic. Seven of 10 adults and 5 of 10 children had canned soda. One participant reported repeated use of a polycarbonate beverage container and one family reported drinking from a multi-gallon polycarbonate drinking water container on one occasion. The self-reported diet data we collected (Supplemental Material, Table 4) were too limited to support statistical analysis of dietary predictors of high BPA and PEM levels.

Discussion

In this study, geometric mean urinary BPA concentrations fell by 66% and geometric mean DEHP metabolite concentrations by 53-56% when participants began a “fresh foods” diet, suggesting that the majority of BPA and DEHP intake came from food packaging or meals outside the home. Maxima declined 76% for BPA and 93-96% for DEHP metabolites, showing a dramatic reduction in the range of exposures while participants were eating “fresh foods.” In contrast, the DBP metabolite MBUP decreased nonsignificantly and other phthalates showed little or no effect.

Participants’ reports of their food practices suggested that consumption of canned foods and beverages and restaurant meals were the most likely sources of exposure to BPA and DEHP in their usual diets, since they reported limited use of polycarbonate water bottles, frozen prepared foods, and microwaving in plastic. This inference is consistent with NHANES data showing higher BPA levels associated with consumption of meals prepared out of the home, sodas, and school lunches (canned foods were not assessed) (Lakind and Naiman 2010). Exposure to PVC film, commonly used in food storage at home and in restaurants, may be

another important exposure source, since these films are known to contain BPA and DEHP (Lopez-Cervantes and Paseiro-Losada 2003; Petersen and Jensen 2010) and were not used during the intervention in this study. Our intervention limited exposures to canned foods and plastic food packaging by substituting fresh foods prepared from basic ingredients; however it is difficult to determine exactly which of these changes in food sourcing and handling were responsible for the significant exposure reductions we observed.

Our findings are consistent with estimates that predict dietary intake as a major source of BPA and DEHP exposure (NTP-CERHR 2006; Willhite et al. 2008; Wormuth et al. 2006). Although DBP and BBP exposure are also predicted to be substantially from diet, we observed relatively little or no change in their metabolites MBUP and MBZP. For DEP, diet is not expected to be a major source, and we saw no reduction in its metabolite MEP.

Our intervention did not eliminate all dietary sources of exposure. Food contamination may occur during pre-market processing of whole foods or from the presence of phthalates and BPA in the environment from which the food originates. One example, among others, is the migration of DEHP into milk from PVC tubing used in the milking process (Feng et al. 2005), and BPA, DBP and DEHP have been detected in whole eggs sold in Asia, demonstrating the possibility for contamination prior to preparation and packaging (Shao et al. 2007 and others). Thus, we are not surprised that exposure reductions in this study were not as large as predicted from the NTP exposure assessments for BPA and DEHP, which estimate diet as the source of 99% and 90% of exposure, respectively (NTP-CERHR 2006; NTP-CERHR 2008). Our findings of little or no influence of the intervention on DBP and BBP could mean that these compounds enter food upstream of our intervention, packaging formulations have changed between the NTP estimates and our 2010 intervention, or that non-dietary sources are a larger component of

exposure than predicted. The report by Colacino (2010) showing that NHANES participants with higher vegetable intake had higher urinary MEP suggests the possibility that the nonsignificant increase in DEP during our intervention was due to higher vegetable intake, or our finding could be due to chance.

Implications of Chemical Differences in Clearance Time

While BPA levels increased between the intervention and post-intervention samples, DEHP metabolites did not. This could reflect a longer clearance time for DEHP than for BPA. Estimates of elimination half-lives in primates or humans are 3-6 hours for BPA (Doerge et al. 2010; Taylor et al. 2010; Willhite et al. 2008) and 15-24 hours for DEHP (Koch et al. 2005). Thus, the one-day lags between sampling periods may have been well-suited for BPA pharmacokinetics but too short to fully capture changes in DEHP intake. This problem is more likely to affect the post-intervention “rebound,” because the effective clearance time was shorter. The time lag from pre- intervention to intervention samples (when DEHP metabolites decreased) was 48 hours (from dinner on day 2 to the 1st collection of intervention urine on day 4), while the lag from intervention to post-intervention was effectively about 34 hours (probably from breakfast or lunch on day 6 to the 1st collection of post-intervention urine on day 7). This ~14-hour discrepancy may explain the absence of noticeable “rebound” effects in the levels of DEHP metabolites. This explanation is also supported by the non-significant increase in DEHP metabolite levels between intervention and post-intervention collections, and by the nonsignificant decrease in post-intervention levels compared to pre-intervention. Alternatively, persistent (after the intervention) participant changes in behaviors that affect DEHP but not BPA exposure could potentially explain the pattern of DEHP levels after the intervention.

Residual shared family exposures

In general, membership in a family did not have a large effect on exposure over the entire study period. However, during the intervention, when many dietary sources were controlled, we found significant between-family variation for BPA, suggesting that other key exposures are shared within a family. These shared exposures likely occur in the home and may be due to direct contact with BPA-containing materials or exposure to BPA in house dust or indoor air (Rudel et al. 2003; Rudel et al. 2010). For the phthalates, we did not observe significant between-family variation during the intervention, except for MMEP, suggesting that individual behaviors are relatively more important than the shared home environment.

Differences between adults and children and by gender

Mixed effects models and Wilcoxon tests indicated significantly higher levels of the DEP metabolite MEP in adults compared to children. Mixed effects models also indicated significantly higher levels of the other phthalate metabolites (MBUP, MBZP, and MMEP) in children compared to adults across the study period. The difference between DEP and other phthalates may originate from differences in intake rate or exposure sources. No significant gender differences were observed.

Limitations

Although effect estimates were statistically significant using multiple approaches, our sample size was small and we cannot rule out the possible role of chance in our findings. We note, however, that an intervention study, where individuals serve as their own controls, avoids many sources of variation that can confound findings in cross-sectional studies.

Generalizability from this sample to the US population is limited, because the relatively small number of participants in a particular geographic location may not be representative. In addition, we intentionally selected participants who reported consuming packaged and prepared foods expected to contain BPA and DEHP. If these participants consumed more packaged and prepared foods than typical Americans, our results could overstate the role of these sources in overall exposure. However, our observation that exposures prior to the intervention were generally in the range of those reported for the US population by CDC (Calafat et al. 2008) suggests that our findings are likely to be broadly relevant to American diets.

Although participants reported high compliance with the study intervention, we cannot be sure that all deviations from the intervention diet were reported. The consumption of non-approved foods during the intervention might have reduced the effect of the intervention. In addition, families may have responded to study information by lowering their intake of BPA- or phthalate-containing foods at any time prior to or during the study, reducing the effect of the intervention.

Conclusions

Three days of eating food with limited food packaging was associated with substantial reductions in BPA and DEHP exposures. Results of this study suggest that removing BPA and DEHP from food packaging will significantly decrease exposure for adults and children. More generally, these results illustrate how intervention studies of chemicals in consumer products can inform regulatory decision-making, product formulation, and consumer choices.

References

- Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. 2005. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. *Environ Health Perspect* 113:192-200.
- Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL. 2008. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003-2004. *Environ Health Perspect* 116:39-44.
- Carwile JL, Luu HT, Bassett LS, Driscoll DA, Yuan C, Chang JY, et al. 2009. Polycarbonate bottle use and urinary bisphenol A concentrations. *Environ Health Perspect* 117:1368-1372.
- CDC. 2009. Fourth National Report on Human Exposure to Environmental Chemicals. Available: http://www.cdc.gov/exposurereport/data_tables/index.html#DataTablesByChemicalGroup [accessed 10 March, 2011].
- Colacino JA, Harris TR, Schechter A. 2010. Dietary intake is associated with phthalate body burden in a nationally representative sample. *Environ Health Perspect* 118:998-1003.
- Doerge DR, Twaddle NC, Woodling KA, Fisher JW. 2010. Pharmacokinetics of bisphenol A in neonatal and adult rhesus monkeys. *Toxicol Appl Pharmacol* 248:1-11.
- EFSA. 2005. Opinion of the scientific panel on food additives, flavorings, processing aids and materials in contact with food (AFC) on a request from the commission related to bis (2-ethylhexyl) phthalate (DEHP) for use in food contact materials. *EFSA J* 243:1-20.
- Engel SM, Zhu C, Berkowitz GS, Calafat AM, Silva MJ, Miodovnik A, et al. 2009. Prenatal phthalate exposure and performance on the Neonatal Behavioral Assessment Scale in a multiethnic birth cohort. *Neurotoxicology* 30:522-528.
- FDA. 2010. Update on Bisphenol A for Use in Food Contact Applications: January 2010. Available: <http://www.fda.gov/NewsEvents/PublicHealthFocus/ucm197739.htm> [accessed 27 October 2010].

- Feng YL, Zhu JP, Sensenstein R. 2005. Development of a headspace solid-phase microextraction method combined with gas chromatography mass spectrometry for the determination of phthalate esters in cow milk. *Anal Chim Acta* 538:41-48.
- Fromme H, Gruber L, Schlummer M, Wolz G, Bohmer S, Angerer J, et al. 2007. Intake of phthalates and di(2-ethylhexyl)adipate: Results of the Integrated Exposure Assessment Survey based on duplicate diet samples and biomonitoring data. *Environ Int* 33:1012-1020.
- Gray LE, Jr., Ostby J, Furr J, Price M, Veeramachaneni DN, Parks L. 2000. Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. *Toxicol Sci* 58:350-365.
- Hauser R. 2008. Urinary phthalate metabolites and semen quality: a review of a potential biomarker of susceptibility. *Int J Androl* 31:112-117.
- Itoh H, Yoshida K, Masunaga S. 2007. Quantitative identification of unknown exposure pathways of phthalates based on measuring their metabolites in human urine. *Environ Sci Technol* 41:4542-4547.
- Ji K, Lim Kho Y, Park Y, Choi K. 2010. Influence of a five-day vegetarian diet on urinary levels of antibiotics and phthalate metabolites: a pilot study with "Temple Stay" participants. *Environ Res* 110:375-382.
- Kesteloot HE, Joossens JV. 1993. Relationship between dietary protein intake and serum urea, uric acid and creatinine, and 24-hour urinary creatinine excretion: the BIRNH Study. *J Am Coll Nutr* 12:42-46.
- Koch HM, Bolt HM, Preuss R, Angerer J. 2005. New metabolites of di(2-ethylhexyl)phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP. *Arch Toxicol* 79:367-376.
- Lakind JS, Naiman DQ. 2010. Daily intake of bisphenol A and potential sources of exposure: 2005-2006 National Health and Nutrition Examination Survey. *J Expo Sci Environ Epidemiol*. 10.1038/jes.2010.9 [Online 17 March 2010]

- Lopez-Cervantes J, Paseiro-Losada P. 2003. Determination of bisphenol A in, and its migration from, PVC stretch film used for food packaging. *Food Addit Contam* 20:596-606.
- Lu C, Toepel K, Irish R, Fenske RA, Barr DB, Bravo R. 2006. Organic diets significantly lower children's dietary exposure to organophosphorus pesticides. *Environ Health Perspect* 114:260-263.
- Meeker JD, Calafat AM, Hauser R. 2007. Di(2-ethylhexyl) phthalate metabolites may alter thyroid hormone levels in men. *Environ Health Perspect* 115:1029-1034.
- Meeker JD, Calafat AM, Hauser R. 2009. Urinary metabolites of di(2-ethylhexyl) phthalate are associated with decreased steroid hormone levels in adult men. *J Androl* 30:287-297.
- Neubert A, Remer T. 1998. The impact of dietary protein intake on urinary creatinine excretion in a healthy pediatric population. *The Journal of Pediatrics* 133:655-659.
- NTP-CERHR. 2003. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Butyl Benzyl Phthalate (BBP). NIH 03-4487. National Toxicology Program Center for the Evaluation of Risks to Human Reproduction. Available: <http://cerhr.niehs.nih.gov/evals/phthalates/bb-phthalate/bb-phthalate.html> [accessed 3 September 2010].
- NTP-CERHR. 2006. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di(2-Ethylhexyl) Phthalate. NIH 06-4476. National Toxicology Program Center for the Evaluation of Risks to Human Reproduction. Available: <http://cerhr.niehs.nih.gov/evals/phthalates/dehp/dehp.html> [accessed 3 September 2010].
- NTP-CERHR. 2008. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Bisphenol A. NIH 08-5994. National Toxicology Program Center for the Evaluation of Risks to Human Reproduction. Available: <http://cerhr.niehs.nih.gov/evals/bisphenol/bisphenol.html> [accessed 3 September 2010].
- NTP-CERHR. No Date. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-*n*-Butyl Phthalate (DBP). National Toxicology Program

- Center for the Evaluation of Risks to Human Reproduction. Available:
<http://cerhr.niehs.nih.gov/evals/phthalates/dbp/dbp.html> [accessed 3 September 2010].
- Petersen JH, Jensen LK. 2010. Phthalates and food-contact materials: enforcing the 2008 European Union plastics legislation. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*:1-9.
- R Development Core Team. 2010. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.
- Rudel RA, Camann DE, Spengler JD, Korn LR, Brody JG. 2003. Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. *Environ Sci Technol* 37:4543-4553.
- Rudel RA, Dodson RE, Perovich LJ, Morello-Frosch R, Camann DE, Zuniga MM, et al. 2010. Semivolatile Endocrine-Disrupting Compounds in Paired Indoor and Outdoor Air in Two Northern California Communities. *Environ Sci Technol* 44:6583-6590.
- Schechter A, Malik N, Haffner D, Smith S, Harris TR, Paepke O, et al. 2010. Bisphenol A (BPA) in U.S. Food. *Environ Sci Technol* doi: 10.1021/es102785d [Online 2 October 2010].
- Shao B, Han H, Tu X, Huang L. 2007. Analysis of alkylphenol and bisphenol A in eggs and milk by matrix solid phase dispersion extraction and liquid chromatography with tandem mass spectrometry. *J Chromatogr B* 850:412-416.
- Swan SH. 2008. Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans. *Environ Res* 108:177-184.
- Taylor JA, Vom Saal FS, Welshons WV, Drury B, Rottinghaus G, Hunt PA, et al. 2010. Similarity of Bisphenol A Pharmacokinetics in Rhesus Monkeys and Mice: Relevance for Human Exposure. *Environ Health Perspect*.
- USEPA. 2006. Data Quality Assessment: Statistical Methods for Practitioners. US EPA QA/G-9S. Available: <http://www.epa.gov/QUALITY/qs-docs/g9s-final.pdf> [accessed 20 January, 2011].

- Vandenberg LN, Chahoud I, Heindel JJ, Padmanabhan V, Paumgartten FJ, Schoenfelder G. 2010. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. *Environ Health Perspect* 118:1055-1070.
- Willhite CC, Ball GL, McLellan CJ. 2008. Derivation of a bisphenol A oral reference dose (RfD) and drinking-water equivalent concentration. *J Toxicol Environ Health B Crit Rev* 11:69-146.
- Wormuth M, Scheringer M, Vollenweider M, Hungerbuhler K. 2006. What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? *Risk Anal* 26:803-824.

Table 1 Characteristics of the 20 participants

Characteristic	n (%)
Age	
<6	3 (15)
6 - <12	7 (35)
12 - <20	0 (0)
20 - <40	4 (20)
40 - <60	6 (30)
60+	0 (0)
Ethnicity	
White	14 (70)
Hispanic	1 (5)
Asian	1 (5)
Mixed	4 (20)
Gender	
Male	9 (45)
Female	11 (55)
Urinary creatinine	
<i>Pre-intervention</i>	
<118.6 mg/dL ^a	11 (55)
>118.6 mg/dL	9 (45)
<i>Intervention</i>	
<118.6 mg/dL	11 (55)
>118.6 mg/dL	9 (45)
<i>Post-intervention</i>	
<118.6 mg/dL	13 (65)
>118.6 mg/dL	7 (35)

^a creatinine data classified as above or below median reported in Barr et al. 2005 for a 1998-1994 sample of 22,245 individuals (ages 6-90)

Table 2. Pre-intervention concentrations of urinary analytes

Analyte	MRL ^a	Adults (n = 10)			NHANES adult median ^b	Children (n = 10)			NHANES child median ^c	Study combined median	NHANES overall median ^d
		Min	Median	Max		Min	Median	Max			
Creatinine (mg/dl)		58	150	220	119-128.8	33	68	160	98.09	100	118.6
BPA (ng/mL)	0.25	1.0	4.9	11	2	1.2	2.6	16	2.4	3.4	2.1
MEHP (ng/mL)	1	3.3	7.4	190	2.1	2.1	3.9	15	2.2	4.5	2.2
MEOHP (ng/mL)	1	9.5	24	630	10.7	11	16	66	16.5	17	11.4
MEHHP (ng/mL)	1	22	50	1400	19.6	15	35	150	27	42	20.7
MEP (ng/mL)	7	32	73	340	128	6.7	25	39	68.7	34	124
MBUP ^e (ng/mL)	1	18	30	160	26	34	46	160	40.1	39	28
MBZP (ng/mL)	1	2.9	8.0	32	9.9	3.9	12	82	24.2	8.3	11.7
MMEP (ng/mL)	5	<MRL ^f	8.1	34	<1.1	5.7	15	38	1.2	13	<1.1

BPA, bisphenol A, MBUP, mono butyl phthalate (n-and iso); MBZP, mono benzyl phthalate, MEHP, mono-2-ethylhexyl phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MMEP, monomethyl phthalate; NHANES, National Health and Nutrition Examination Survey

^a MRL (method reporting limit), defined as the maximum of analytical detection limit and the 90th percentile of lab blank concentrations. Data below the MRL are included in later analysis; ^b creatinine: medians for 30-39-year-olds and 40-49-year-olds from Barr et al. (2005) analysis of 1988-1994 NHANES data (n= 3259 and 2542); BPA and phthalates: medians for "20 and over" from 2007-08 data (CDC 2009), (n = 1814); ^c creatinine: medians for 6-11-year-olds from Barr et al. (2005) (n=3078); BPA and phthalates: medians for 6-11-year-olds from 2007-08 data (CDC 2009) (n = 389); ^d creatinine: median for 6-90-year olds from Barr et al. (2005) (n =22,245); BPA and phthalates: medians for 6-85+-year-olds from 2007-08 data (CDC 2009) (n = 2604); ^e this study did not distinguish between mono n-butyl phthalate and mono iso-butyl phthalate. NHANES medians presented for MBUP are the sum of these 2 forms; ^f three samples were below the MRL for MMEP, and one could not be analyzed for MMEP.

Table 3. Mixed effects model results for multi-level spline model

Analyte	Variable	% change in GM (GMs; ng/ml) ^a	95% CI for slope estimate ^b
BPA	pre- to during intervention change ^c	-66 (3.7 to 1.2)	(-1.6, -0.55)**
	during to post-intervention change ^d	202 (1.2 to 3.8)	(0.61, 1.6)**
MEHP	pre- to during intervention change	-53 (7.1 to 3.4)	(-1.2, -0.16)*
	during to post-intervention change	21 (3.4 to 4.1)	(-0.32, 0.74)
MEOHP	pre- to during intervention change	-55 (27 to 12)	(-1.2, -0.2)*
	during to post-intervention change	16 (12 to 14)	(-0.35, 0.69)
MEHHP	pre- to during intervention change	-56 (57 to 25)	(-1.3, -0.25)*
	during to post-intervention change	22 (25 to 31)	(-0.3, 0.72)
MEP	pre- to during intervention change	23 (41 to 50)	(-0.059, 0.7)
	during to post-intervention change	7 (50 to 53)	(-0.28, 0.47)
MBUP	pre- to during intervention change	-25 (43 to 32)	(-0.44, 0.043)
	during to post-intervention change	11 (32 to 35)	(-0.12, 0.36)
MBZP	pre- to during intervention change	-12 (12 to 10)	(-0.38, 0.36)
	during to post-intervention change	13 (10 to 11)	(-0.22, 0.51)
MMEP	pre- to during intervention change	-4 (12 to 12)	(-0.28, 0.32)
	during to post-intervention change	-19 (12 to 9.3)	(-0.5, 0.091)

BPA, bisphenol A; MBUP, mono butyl phthalate (n-and iso); MBZP, mono benzyl phthalate; MeHP, mono-2-ethylhexyl phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MMEP, monomethyl phthalate; SE, standard error

** indicates statistical significance (p<0.005); *indicates statistical significance (p <0.05)

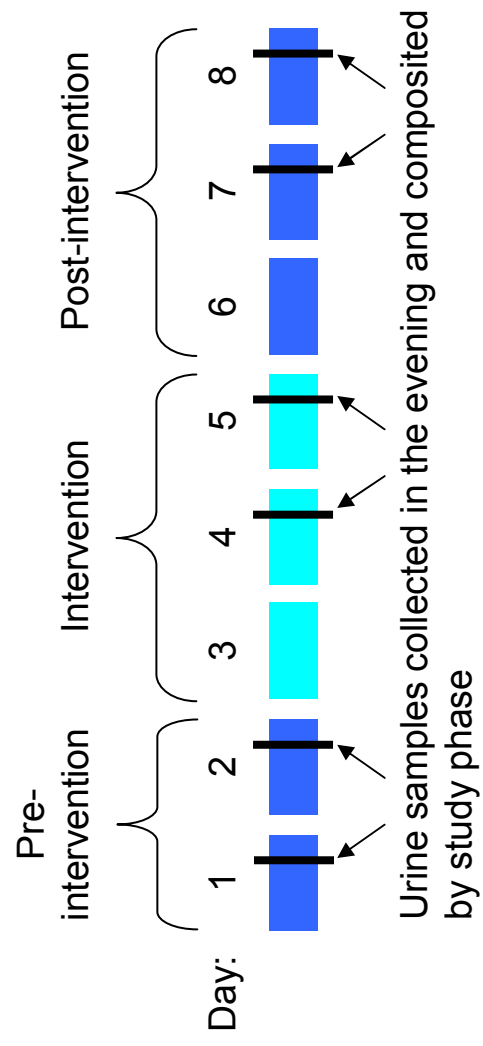
^apercent change in the geometric mean between the two time periods with geometric means of the two time periods in parentheses; ^b95% confidence interval for slope estimate; ^cpre- to during intervention change estimate is the slope between the two time periods; ^dduring to post-intervention change estimate is the slope between the two time periods.

Intercept, which represents the log concentration during the intervention, significant for all models except BPA; creatinine and age (adult v child) included in all models; creatinine significant for MEOHP, MEP, MBUP, MBZP, MMEP; age significant for MEP, MBUP, MBZP, MMEP; gender not significant in any of the models and not included in final models.

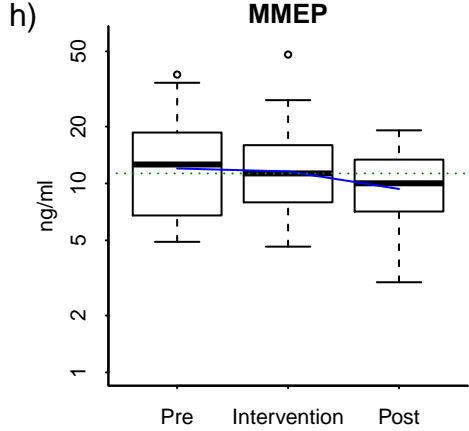
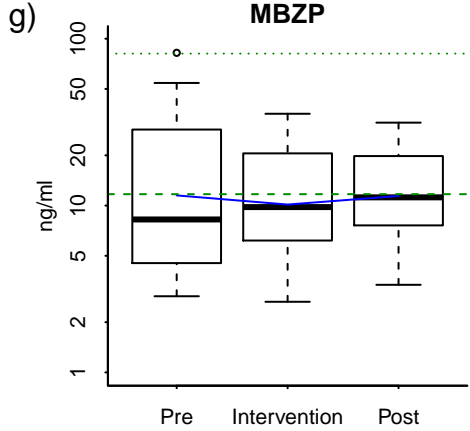
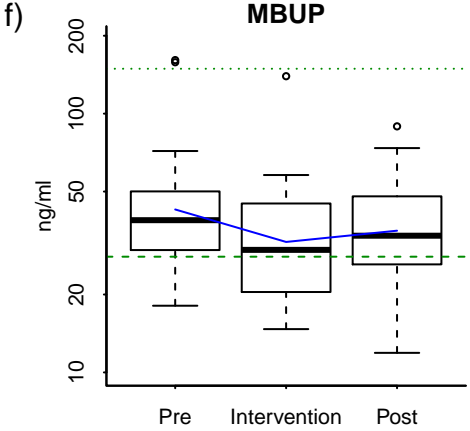
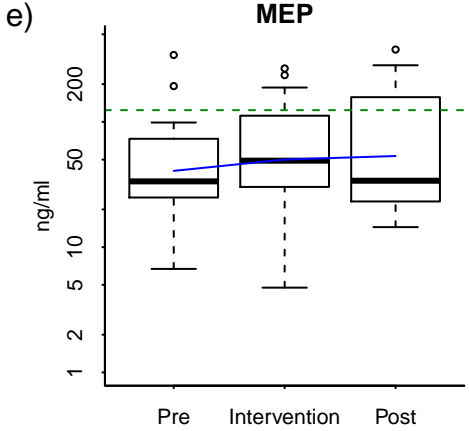
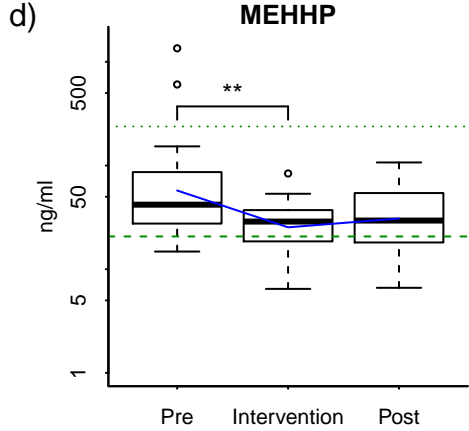
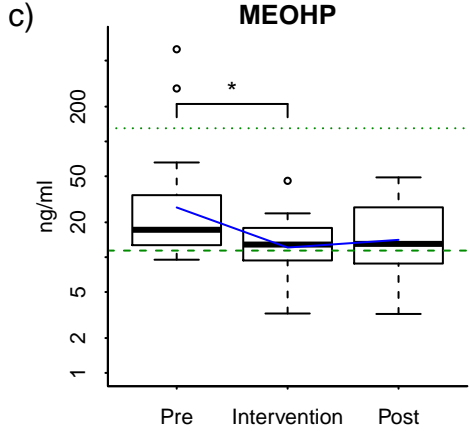
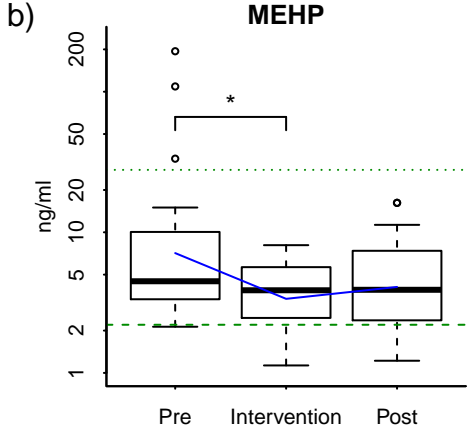
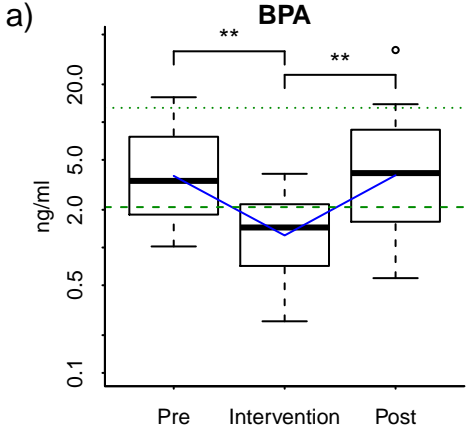
Figure legends

Figure 1 Intervention study design; n = 20 individuals from 5 families. Each participant provided a total of six urine samples (two/phase). Paired samples collected from each individual during each phase were combined for analysis.

Figure 2 Distributions of urinary levels of BPA and phthalate metabolites at three time points. Box and whiskers plots show the distribution of urinary levels for each analyte in the pre-intervention, intervention, and post-intervention samples. The blue line shows the geometric means. The fresh food intervention was associated with significant reductions in urinary excretion of bisphenol A (a) and metabolites of DEHP (b-d). No significant change was seen in the other analyzed phthalate metabolites, although there was a small reduction in DBP (e-h). Asterisks indicate significant reductions or increases between intervention phases as determined by p-value for slope in the mixed effects model: * indicates $p < 0.05$; ** indicates $p < 0.005$. Compared to the 2007-08 NHANES sample of 2604 individuals ages 6 years and older (CDC 2009), the pre-intervention medians and 95th percentile estimates for adults and children combined were higher for BPA (a) and metabolites of DEHP (b-d), MBUP (f), and MMEP (h), much lower for MEP metabolite (e), and similar for MBZP (g). NHANES median for MMEP was $< DL$ of 1.1 ng/ml. NHANES 95th percentile for MEP was 2140 ng/ml.



DEHP metabolites



— Geometric mean
- - - NHANES 2007-2008 Median
- - - NHANES 2007-2008 95th %tile