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Abstract: Identifying and characterizing sources of exposure to phthalates and bisphenol A (BPA) have proved challenging due to the presence of multiple co-exposures resulting from a wide variety of home environments and lifestyles. We hypothesized that the consistent lifestyle of an Old Order Mennonite (OOM) community would provide an ideal setting in which to characterize sources of exposure to BPA and phthalates. We obtained urine samples from ten mid-term pregnant OOM women (ages-21-39) to determine concentrations of 9 phthalate metabolites and BPA and collected a self-reported survey of participants' household environment, product use, and lifestyle within a 48-hour period prior to urine collection. We compared their metabolite concentrations to pregnant women included in the National Health and Nutrition Examination Survey (NHANES 2007-2008). Although OOM participants reported some use of plastic and fragranced household products, concentrations of metabolites were lower and significantly less for BPA ($p= 0.002$) and phthalate metabolites MEHP ($p= 0.0215$), MiBP ($p= 0.0020$) and MEP ($p= 0.021$), when compared to NHANES pregnant women. Levels of other phthalate metabolites were also lower in this population. Our data suggest three practices that may contribute to these lower levels: 1) consuming mostly homegrown produce (ingestion), 2) no cosmetics and limited use of personal care products; and 3) transportation primarily by sources other than automobiles.

Cover Letter

18 May 2012

Dear Chris,

Our revised manuscript is attached. In addition to the changes requested by the reviewers, I have also responded to your comments. Thank you.

Bernie

***Author Agreement**

This manuscript has not been submitted elsewhere.

***Revision Notes**

Ref.: Ms. No. NEUTOX-D-12-00046R1
Lifestyle behaviors associated with exposures to endocrine disruptors.
Neurotoxicology

Dear Dr Weiss,

This revision is being undertaken to allow you to correct the order of authors. There are a few minor revisions that you should consider, however.

I noticed that you increased the length of the introduction substantially in this version. Similarly with the methods where there is a longer history of the Mennonites than in the first version. Could I ask you to shorten these a bit?

I took out about a page of material.

As interesting as these are (to me at least) I think that most readers will find it a bit distracting. It's ok to keep the material that was requested by the reviewers, such as the justification for the use of pregnant women.

The tables are out of order. Table 1 appears last.

Will fix.

Under statistical procedures it is said that the sign test was conducted to compare medians. In the caption to Fig 1 the mean is referred to. Similarly on page 19, Line 1.

Corrected

Finally, Tables 2 through 4 have been moved from the results to the discussion. It seems to me that they belong in the results, unless there's some compelling reason to keep them in the discussion.

You're right. They have been moved.

*Conflict of Interest Statement

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Author name: BERNARD WEISS

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Please state any competing interests

NONE

Funding Source
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Bernard Weiss

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Signature (a scanned signature is acceptable, but each author must sign)

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Print name

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OOM MAY 16

Title: Lifestyle behaviors associated with exposures to endocrine disruptors.

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Abstract: Identifying and characterizing sources of exposure to phthalates and bisphenol A (BPA) have proved challenging due to the presence of multiple co-exposures resulting from a wide variety of home environments and lifestyles. We hypothesized that the consistent lifestyle of an Old Order Mennonite (OOM) community would provide an ideal setting in which to characterize sources of exposure to BPA and phthalates. We obtained urine samples from ten mid-term pregnant OOM women (ages-21-39) to determine concentrations of 9 phthalate metabolites and BPA and collected a self-reported survey of participants' household environment, product use, and lifestyle within a 48-hour period prior to urine collection. We compared their metabolite concentrations to pregnant women included in the National Health and Nutrition Examination Survey (NHANES 2007-2008). Although OOM participants reported some use of plastic and fragranced household products, concentrations of metabolites were lower and significantly less for BPA ($p= 0.002$) and phthalate metabolites MEHP ($p= 0.0215$), MiBP ($p= 0.0020$) and MEP ($p= 0.021$), when compared to NHANES pregnant women. Levels of other phthalate metabolites were also lower in this population. Our data suggest three practices that may contribute to these lower levels: 1) consuming mostly homegrown produce (ingestion), 2) no cosmetics and limited use of personal care products; and 3) transportation primarily by sources other than automobiles.

KEY WORDS:

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Plastics

Introduction

Endocrine disrupting chemicals (EDCs) provoke unusually challenging questions. They confound us because, rather than poisoning us overtly, as by killing cells, they distort the body's intrinsic hormonal mechanisms. They can displace, mimic, antagonize, or even amplify the vital processes governed by hormones, producing an array of subtle aberrations that are difficult to grasp if we seek answers by traditional methods.

The 27th International Neurotoxicology Conference addressed a variety of neurobehavioral outcomes associated with exposure to EDCs. One crucial element of this equation, however, was implicitly ignored in these presentations: exposure sources. The current report explores this question. It shows that individual behavioral choices and community lifestyle practices determine the sources of two important EDCs. Although it might be argued that the approach described here is more anthropological than toxicological, we view it instead as a means to help close a major gap in our comprehension of how exposure and consequences are intertwined.

EDCs are found in many chemical classes, including pharmaceuticals, pesticides, dioxins, PCBs, organic tin compounds, brominated flame retardants, perfluorinated coatings for cookware, and others. In this report, we focus on two EDCs that have provoked intense concern about their neurotoxic properties and a corresponding volume of research.

One, bisphenol A (BPA), is a single chemical. The other, phthalate esters, represents a large chemical class. Both agents are produced in massive

amounts, are used extensively in plastics manufacture, and are widely distributed in the environment and in human tissues (Koch and Calafat, 2009). In laboratory studies, BPA and phthalates are shown to produce adverse effects on a variety of organ systems, although the exposure levels at which they do so is debated. Like other EDCs, they seem to exert their most potent and permanent effects on the developing organism. Although both BPA and phthalates are assumed to be metabolized rapidly, there is also evidence that, because they are fat-soluble, some portion accumulates in fatty tissues from which they are slowly released (Stahlhut et al, 2009, for BPA; Frederiksen et al, 2007, for phthalates).

BPA is employed in the manufacture of polycarbonate plastics such as the epoxy resins found in can linings, and in thermal paper products, dental sealants, baby bottles, food containers, and other plastic products. The Centers for Disease Control and Prevention (CDC) reports that 93% of Americans aged 6-85+ show detectable levels of BPA metabolites in urine (Calafat et al, 2008) and an expanding literature demonstrates the presence of the parent compound and metabolites as well in various human tissues, blood, and even in newborns and breast milk (Vandenberg et al, 2010).

Foods and beverages are major exposure sources (Koch and Calafat, 2009; Wilson, 2007). Because residual BPA typically leaches from the can or container, stored food or beverage can become contaminated (vom Saal and Hughes, 2005). Carwile et al (2011) found that a daily serving of canned soup for five days raised BPA levels in urine by 1,200%. But BPA is also found in plastic cups, recycled cardboard (e.g., pizza boxes) and paper. The analysis by LaKind

and Naiman (2011), based on data from the 2005–2006 NHANES, found that soft drinks, meals outside the home, and--a special cause for concern--school lunches, were associated with higher urinary BPA values.

BPA in thermal paper such as store receipts, and in personal care products, can penetrate the dermis (Beidermann, 2010), raising the possibility that it then bypasses liver metabolism and enters the bloodstream directly. Inhalation of dust particles containing BPA is another potential exposure source (Geens et al, 2009).

As with other EDCs, early development is the phase of the life cycle that appears most sensitive to BPA exposure. In rodents, numerous toxic effects are seen in a variety of tissues and organ systems. Among the most prominent developmental effects are those affecting the brain and behavior. Earlier reviews by Vandenberg et al (2009) and vom Saal et al (2007) noted the wide scope of BPA neurotoxicity. Rubin (2011), Kundakovic and Champagne (2011) and Wolstenholme et al (2011) have addressed associated domains such as epigenetics and obesity. Because BPA research is such an active area, we can expect to see a substantial volume of new data in the next few years. For example, Braun et al (2009, 2011) reported a correlation between prenatal exposure levels and externalizing behavior (increased hyperactivity and aggression) in girls two years of age and poor emotional control and anxiety and depression at three years of age.

The estrogen label would also not have led to predictions about the obesogenic properties of BPA (Carwile and Michaels, 2011), its association with

heart disease (Melzer et al, 2010, 2012), or its effects on spermatogenesis (Mruk and Cheng, 2011; Meeker et al, 2010). The scope of adverse consequences associated with BPA means that exploration of its sources should be an important goal of environmental health research.

As with BPA, humans are exposed to phthalates by many routes: orally, dermally, through inhalation and even subdermally, the route varying with the particular phthalate. For example, exposure to diethylhexyl phthalate (DEHP), the only phthalate regulated in drinking water, occurs primarily through food and beverage consumption. It is also an important component of polyvinyl chloride (PVC), and soft plastics that find their way into children's mouths as from toys. It can enter the circulation dermally (as in personal care products), internally (as in medical tubing), and inhalation in the form of dust particles from sources such as vinyl flooring and upholstery (Afshari et al, 2004, Bornehag et al, 2005, Jaakkola and Knight, 2008). DEP is a component of many cosmetics we apply to the skin, including lotions for babies, shampoos, and aftershave products. DBP can be found in products that we inhale, such as hair spray and nail polish. BBzP can also be found in vinyl floor tiles and some cosmetics.

A large rodent literature testifies to the ability of several phthalates to interfere with the development of the male reproductive system (e.g., Foster, 2006). These phthalates produce a cluster of abnormalities in newborn males, stemming from prenatal exposure, labeled as the *Phthalate Syndrome*. The abnormalities are seen in reproductive tract structures, the external genitalia (in the form of

hypospadias), as cryptorchidism, and as testicular pathology. In addition, phthalates reduce anogenital distance (AGD) in males, which, in both rodents and humans, is greater (50%-100%) in males than in females. These changes result from a reduction in testicular testosterone production during a critical developmental period. A remarkably similar syndrome occurs in humans and has earned the term, *Testicular Dysgenesis Syndrome* (Skakkabaek, 2003; Sharpe & Skakkebaek, 2008; Wohlfahrt-Veje et al, 2009).

Because the bulk of the rodent literature had been based on exposure levels far greater than those experienced by humans, phthalates had not, until recently, been viewed as threats to human health. That perception was overthrown by a publication (Swan et al, 2005) indicating that AGD in boys was inversely related to urinary concentrations of phthalate metabolites during pregnancy. It has led to a surge in publications supporting and expanding the original observations (Swan, 2008; Meeker et al, 2009; Hauser et al, 2006).

Nonreproductive behaviors are another target of phthalates. Swan et al (2010) measured play behavior in young children with a questionnaire, completed by the parents, whose items asked about behaviors such as toy preferences (e.g., trucks vs. dolls). Higher concentrations of phthalate metabolites during pregnancy reduced masculine play behavior in boys.

Other nonreproductive behaviors affected by phthalates include cognitive function (Engel et al, 2010; Kim et al, 2009; Cho et al, 2010; Whyatt et al, 2012), and social responsiveness (Miodovnik et al, 2011). Asthma and allergy in children (Bornehag and Nanberg, 2010) are also associated with phthalate

exposure as measured by phthalates in household dust. And, as with BPA, phthalate exposures are associated with obesity (Hatch et al 2010; Stahlhut et al 2007), male fertility (Hauser 2008), and heart disease (Lind and Lind 2011).

The exposure conundrum

Despite attempts to estimate the relative contributions of different sources of exposure to these two agents, ambiguity persists. It persists largely because of differences between various populations in geography, life style choices, and dietary practices. The latter's importance was underscored by Ji et al (2010), who reported that a five-day period in a Buddhist temple, while eating a vegetarian diet, led to significant decreases in urinary phthalate metabolites. But this ancient Buddhist temple also lacked many contemporary sources of exposure such as vinyl floors and wall coverings, as well as various other plastic products.

The task of identifying and characterizing sources of exposure to phthalates and BPA confronts a variety of challenges. First, we have to contend with the wide variety of home environments and lifestyles in the U.S. population. This assortment of variables limits our ability to link them to health effects and, ultimately, to develop recommendations about exposure levels. Such data uncertainty is greater in the U.S. heterogeneous environment, which also contains multiple confounders such as income.

A frequent tactic of environmental research to gain information about exposure sources is to study special or unique environments or populations. In the present instance, our study participants came from an Old Order Mennonite

(OOM) community near Rochester, New York. The members adhere to a simpler lifestyle than the general U.S. population. They grow most of their own food, do not apply pesticides, consume few processed foods, use many fewer household chemicals and cosmetics, and depend on automobiles for transportation much less than the general population.

It appeared to us that the simpler and consistent lifestyles and rural environment of this Old Order Mennonites (OOM) community might yield more limited and less intense exposures to BPA and phthalates, which would make them an ideal study population in which to begin to identify common exposure sources in the general population. Because many of the concerns generated by phthalates and BPA arise from gestational exposures, we focused on this period for our assessment, and elected to study a sample of pregnant women. Pregnancy is a period during which significant hormonal changes occur, so it is likely that EDC pharmacokinetics are significantly changed as well.

Methods

Selection of target chemicals

Bisphenol A (BPA) and nine phthalate analytes were selected for detailed study. The latter included mono-2-ethyl-5-carboxypentyl phthalate (mecpp), mono-2-ethyl-5-hydrohexyl phthalate (mehhp), mono-2-ethyl-5-oxohexyl phthalate (meohp), mono-2-ethylhexyl phthalate (mehp), mono-3-carboxypropyl phthalate (mcpp), mono-isobutyl phthalate (mibp), mono-n-butyl phthalate (mbp), monobenzyl phthalate (mbzp), monoethyl phthalate (mep). It is common practice

in epidemiological studies to measure a suite of phthalate metabolites because different phthalate esters are used for different purposes and their toxic effects differ in scope and intensity. Some, such as metabolites of DEHP, come mostly from food and medical tubing, but this particular ester is use in a variety of other applications as well. Others, such as metabolites of DBP and MEP, come from a variety of products, including cosmetics. BzBP is used in vinyl tiles for flooring, vinyl foam, and even in some perfumes.

Study population, sample collection and questionnaire

The community we enlisted is one whose lifestyle choices, environment, and diet contrasts markedly with most other U.S. communities. This community, consisting of Old Order Mennonites (OOM), depends largely on local, home-grown food rather than on processed commercial products, and embraces a much simpler, less materialist lifestyle. It also has the virtue of being a population with less genetic and cultural variability than the population at large.

The Mennonites arrived and established a community in the United States before the American Revolution (1681-83), in Germantown, Pennsylvania. Our study population's genealogy can be traced to these founders (Global Anabaptist Mennonite History, 2009).

Our participants came from a group of Mennonites belonging to the Horse and Buggy Groffdale Conference (Wenger) OOM group. In 2008, there were 447 Groffdale Conference OOM households in the Finger Lakes Region of Western New York State.

On the basis of what was known about their life style and dietary practices, we hypothesized that the OOM would be less exposed than the general population to the two environmental chemicals, BPA and phthalates, of our concern. Such a natural comparison group could allow us to more explicitly identify environmental sources and pathways (ingestion, dermal, and inhalation) that present the highest level of exposure risk to pregnant women.

Study Design.

A total of 10 pregnant OOM women (ages-21-39) were recruited over a period of one week (August 25 through September 2, 2009). They had been pregnant for 14-26 weeks at study inception. None of the participants withdrew from the study. The Institutional Review Board at the University of Rochester approved the study, and statements of written informed consent were obtained from all subjects. After informed consent was obtained, all ten OOM study subjects were given a participant's 24-hour data collection kit that included the following items:

1. Labeled and sealed envelope containing home urine collection and survey study kit (1)
2. Household Environment Survey (1)
3. Two (pre-labeled) freezer bags to store urine (2)

4. A polypropylene plastic urine collection cup (1) prescreened for phthalates.
5. One sealed packet of 4 extra labels (in the event labels are damaged)

Participants were instructed to collect their first void urine, document the time and date of collection and time of last void and meal, and to immediately store the urine in the sealed labeled container, within two sealed freezer bags (double bag), in their home freezer and to notify the PI the day of collection for sample retrieval. Within 24 hours or less, the study PI picked up the samples and surveys from the individual participants' homes and transported them back to the University of Rochester Medical Center. Urine samples were shipped to and analyzed at the CDC. Although multiple urine samples would have offered an estimate of consistency, we wished to avoid any barriers to recruitment in a community unaccustomed to such participation. In addition, others have found single spot samples for BPA and phthalates to be reasonable estimates of longer term values (Mahalingaiah 2008, Braun 2012), and Boas et al (2010) found interday variation to be < 10%.

Urine sample collection followed CDC guidelines for collection, storing and shipping. All urine samples were analyzed at the CDC according to CDC protocols (Calafat et al 2008). Analyses for BPA used a modification of the method reported in Ye et al (2005) for both free and conjugated species. For comparisons with other US populations, we employed previously published data for urinary concentration in mid-pregnancy for phthalates (Swan, 2005) and BPA (Braun, 2009).

The participants completed a 14-category household exposure history questionnaire within the 48-hour period preceding urine collection. The 20-minute self-report survey targets the following categories: demographic data; urine collection time of day and date; water source; modes of transportation; occupation(s); home materials; stress and health self-report; personal hygiene and cosmetic products; household cleaners; other household products; pest reduction chemicals; medications and supplements. The questions provide identification of possible target chemical products, reveal lifestyle choices, and environmental and social stressors of health that may enhance or negate vulnerability to exposure within that 48-hour period.

Analysis of urinary Bisphenol A

The Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention (CDC), conducted the urine analyses using a modification of the method reported in Ye (2005). β-glucuronidase/sulfatase was used to hydrolyze conjugated BPA species. Following hydrolysis, the urine samples were acidified. Next, using online solid-phase extraction, the BPA was concentrated. BPA concentrations were quantified using high-performance liquid chromatography–isotope-dilution tandem mass spectrometry. The limit of detection (LOD) was 0.4 μg/L (Calafat, 2008). Concentrations below the LOD were given a value of LOD/√2 for statistical analyses (Hornung and Reed 1990).

Analysis of urinary phthalates

Because different phthalates are used for different purposes, it was important to determine a variety of different metabolites. as listed in Table 1. Some, such as metabolites of DEHP, come from food. Others, such as metabolites of DBP, come from cosmetics. It is now standard practice to analyze a whole suite of metabolites.

TABLE 1 HERE

Urinary phthalate metabolite analyses were carried out by the Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention (CDC). The analysis of urinary phthalate metabolites (Silva et al, 2007) is a modification of previously published methods (Silva et al, 2004). The analysis entails the enzymatic deconjugation of the phthalate metabolites from their glucuronidated form, automated on-line solid-phase extraction, separation with high-performance liquid chromatography, and detection by isotope-dilution tandem mass spectrometry. LODs are in the low nanogram per milliliter range. Concentrations below the LOD were given a value of LOD/√2, as was done for BPA.

Statistical procedures

For our statistical analysis, designed to compare the OOM urine metabolites to those of pregnant women in the general population (NHANES 07-08), we used

the non-parametric Sign Test to assess the hypothesis that there is no difference in medians between our two random variables OOM (X) and NHANES (*Y)*. We compared the median urine metabolite values of the OOM samples to those from the NHANES (07-08) samples using Version 9 of the SAS System for Windows, specifically, PROC UNIVARIATE, with the LOCCOUNT option, for BPA, MEHP, MEHHP, MEOHP, MECPP MBP, MiBP, MBzP, MEP and MCPP (see Table 1). This option allows us to see the number of OOM values above, below and equal to the corresponding NHANES median for a given metabolite.

Results

The BPA, MEHP, MiBP and MEP distributions were all significantly different ($p < 0.05$) from the NHANES values. The p-values were 0.0020, 0.0215, 0.0020 and 0.0215, respectively. The other six metabolites, although not meeting the $p<0.05$ criterion, reflected the same direction of difference.

Table 1 contains the results of the chemical analyses. Figure 1 shows individual OOM participants (N=10) in comparison to the median NHANES (07-08) data of pregnant women in that sample. Urinary concentrations are reported as analyte mass per volume (ng/mL) unadjusted for creatinine.

FIGURE 1 HERE

Bisphenol A

Seven out of 10 of the OOM women had urinary BPA values above the limit of detection (LOD = 0.4), with three participants below LOD. Both field blanks were negative. Their median BPA concentration was 0.71 ng/ml, (range 0.28 ng/mL to 1.7 ng/mL) and all values were lower than the median of 1.80 ng/mL reported in Braun et al (2009). The CDC Fourth Chemical Report of the U.S. population from the National Health and Nutrition Examination Survey (NHANES) (2009) gives a reference range for the 2003-2004 BPA data as 2.7-15.9 ng/mL (LOD = 0.4 UNITS).

Phthalates

All 10 samples from this group of women showed detectable levels of the DEHP metabolites MEHP, MEOHP and MEHHP. Neither of the 2 blanks had detectable levels. For DEHP/MEHP the LOD was 1.2 ng/mL and 80% of the women had values below detection. For MEHHP the LOD was 0.7 ng/mL and all of the women had levels below detection. For MEOHP the LOD was 0.7 ng/mL and 10% of the women had detectable levels. For the sum DEHP (MEHP+ MEOHP+MEHHP) the LOD was 2.6 ng/mL; one of the 10 women had a value at 2.6 ng/mL while the other nine values were above the LOD. The median values for SUM (DEHP) for OOM women was 17.45 ng/mL compared to 25.1 ng/mL for the women surveyed in Swan et al (2005) and 22.80 ng/mL for NHANES (CDC, 2009). In almost all cases, the OOM values fell below the NHANES medan. In a departure from this pattern, one of the women (Participant 10) showed markedly higher levels of MEP than the others, suggesting recent use of a fragranced

product or a medication coated with DEP (Duty, 2005). A subsequent analysis of her questionnaire responses revealed that she had used hairspray and perfume while the other nine subjects had not.

Influences on exposures via self-report survey

Questionnaire Responses. Participants reported consuming mostly homegrown produce along with some processed food. These data are shown in Table 2. The primary mode of transportation in order of prevalence from greatest to least is horse and buggy, walking, bicycle and car or truck within the 48-hour period prior to urine collection (Table 3). Three participants reported that they had been in a car or truck within the 48 hour period. For these participants, Sum DEHP levels were above those who had not. Use of plastic food storage containers was high, as were household products containing fragrances. The questionnaire responses indicated no use of cosmetics and low personal hygiene product use (deodorant, shampoo), as seen in Table 4. The one participant who used hairspray and perfume had very high levels of MEP (1,500 ng/mL creatinine corrected) while all the others had levels below detection.

TABLE 2 HERE

TABLE 3 HERE

TABLE 4 HERE

Discussion

Urinary levels of BPA and phthalates

We chose, for comparison, the figures reported in the NHANES tables. Although these data may have limitations arising from technical considerations such as which subsamples of the total sample are chosen for chemical analyses, they still represent the best estimate we have for levels prevailing in the aggregate U.S. population.

In comparing the metabolic profiles of our ten OOM participants to that of NHANES, we found statistically significant differences in levels of exposures to BPA and three phthalate metabolites: MEHP (a metabolite of DEHP), MiBP (a metabolite of DBP) and MEP (a metabolite of DEP). All the figures, however, show the same pattern; namely, lower exposures in the OOM women. Other statistical models, based on parametric tests, might have shown a higher number of statistically significant results, but the small number of subjects made such models questionable.

As a whole, the phthalate levels in Table 1 and Figure 1 offer a sharp contrast to those seen in the Study for Future Families (Swan et al, 2005) and NHANES. The former reported a mean sum DEHP of 25.1ng/mL, and the latter, based on the NHANES samples taken in 2005-2006, reported a value of 22.80 ng/mL. Our OOM sample gave a median sum DEHP of 17.45 ng/mL. Compared to median BPA data in Braun et al (2009) of 1.80 ng/mL and the NHANES value of 1.7 ng/mL, the OOM median of 0.65 ng/mL is remarkably low. The data from Carwile et al (2011) suggest that consumption of canned foods might account for much of this difference while the analyses of LaKind and Naiman (2011) point to restaurant meals and soft drinks as additional contributions.

Influences of lifestyle

Our data indicate how OOM lifestyle may explain the lower exposure levels. Three important differences are apparent: 1) consuming mostly fresh, homegrown produce (ingestion), 2) the absence of cosmetics and low use of personal hygiene products (dermal) and 3) transportation choices that limit the use of automobiles (inhalation). Such behavioral practices surely underlie much of the contrasts with other populations in exposure levels.

4.3. Food

Although many of the OOM use plastic products (freezer bags, plastic wrap and containers) for storing food, they still exhibit lower levels of exposure to BPA and phthalates. The participants consumed mostly fresh produce, either local or homegrown, and not prepackaged or processed, which has been shown to influence exposures to these chemicals (Rudel et al, 2011). Moreover, because both BPA and especially phthalates are fat-soluble, they tend to concentrate in materials such as butter, milk, and cheese. The OOM community provides much of these foods from their own farms so that outside environmental sources of contamination are minimized.

Transportation

The preferred modes of transportation of our participants are horse and buggy, walking, and bicycle, which may also help explain lower levels of DEHP exposure than the NHANES (07-08) sample. Yet half the participants acknowledged some use of a car or truck within the 48 hours prior to urine

collection, and those participants had higher levels of Sum DEHP. This may suggest the presence of phthalates and volatile organic compounds in the cabin air of private-use cars and trucks (Fromme etal, 2004; Rudel et al, 2003). In a study of 23 private motor vehicles (Geiss et al, 2009), concentrations of chemical compounds in cabin air were 40% higher in the summer months when cabin temperatures can reach 70° C. The study sampled vehicle cabin air for phthalates, which were detected in some, but not all of the vehicles, suggesting the concentrations may vary with the age, condition and materials used in the interior of the car. Our study was conducted in late summer, when the internal temperature of the car or truck cabin is elevated, creating conditions for compounds found in the vinyl interior trim of some vehicles to deteriorate and decompose, allowing the release of phthalate particles (inhalation).

Personal Care and Cosmetics

In general, urinary concentrations of MEP were significantly lower than NHANES (2007-2008), even though some OOM women reported using personal care products. Personal care products contribute to phthalate body burden (Schettler et al, 2006; Romero-Franco, 2009), as do synthetically fragranced products (Koo and Lee, 2004). Only one participant had high levels of MEP and she reported using hairspray within the 48 hours prior to urine collection. However, hairspray by nature is highly volatilized and has adhesive qualities, leaving residues on surfaces in the bathroom that may have contributed to this high level.

Conclusions

Despite the small sample size of this study, the results are remarkably robust and consistent. They underscore the degree to which the home environment determines exposure levels to many toxic or potentially toxic chemicals. They also argue for a much greater emphasis on individual lifestyle factors in assessing variations in exposure values that obscure attempts to link health effects to particular chemical agents.

Although this report was not aimed directly at strategies for reducing exposure to BPA and phthalates, and other EDCs as well, it does provide clear guidance for doing so. Avoiding processed foods, especially canned foods (as documented in Carwile et al, 2011), and foods with plastic packaging (Rudel et al, 2011) will prove helpful. Soft plastic toys purchased before phthalates were banned from such products will help decrease children's exposure. Cosmetics seem to be major contributors, especially those containing fragrances. And, as shown by Bornehag et al (2005) adequate ventilation will help to reduce phthalate dust particles arising from vinyl tiles and furniture coverings.

Our data also provide guidance for studies directed at the neurobehavioral consequences of exposure to environmental chemicals. Just as studies of exposed populations take into account the social and psychological aspects of the home environment, the identification of exposure sources would amplify and extend the results based on outcomes.

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FIGURE LEGENDS

FIGURE 1 TOP. Urinary metabolite values for BPA, mehp, meohp, mehhp, and mbp. BOTTOM. Urinary metabolite values for mibp, mep, mcpp, mbzp, and meccp. Each of the ten subjects is depicted individually as (◆). Concentrations are given as ng/ml. The line depicts the median NHANES value. Statistical analyses (Sign Test) are based on the number of subjects above and below the NHANES value.

Table 1. Urine metabolites for BPA and 9 phthalates in Pregnant Old Order Mennonites and Pregnant Women from the General Population NHANES 2007-2008.

Chemical[3]	***Metabolite***	***LOD***[2] ng/mL	***OOM Range*** ng/mL	***OOM Median*** ng/mL	**NHANES 2007-2008 Median** ng/mL	**P-value for Sign Test** [1]
BPA		0.4	0.3 - 1.7	0.7	2.8	**0.0020**
DEHP	**MEHP**	1.2	0.8 - 4.8	0.9	3.3	**0.0215**
	MEHHP	0.7	1.3 - 18.3	9.3	17.2	0.1094
	MEOHP	0.7	0.5 - 18.7	7.4	11.7	0.1094
	MECPP	0.6	2.2 - 39.3	9.9	23.8	0.3438
DBP	**MBP**	0.6	0.9 - 38.1	13.6	17.3	0.1094
	MiBP	0.3	0.2 - 3.1	1.1	9.4	**0.0020**
DBzP	**MBzP**	0.3	0.2 - 48.8	7.4	8.4	1.0000
DEP	**MEP**	0.8	4.8 - 1410.	7.9	131.3	**0.0215**
DnOP	**MCPP**	0.2	0.6 - 4.0	1.20	2.0	0.5078

[1] *Sign test for number of subjects with analyte value <= comparison population's median*
[2] *Limit of detection/LOD*
[3] *Parent compounds: DEHP= Di(2-ethylhexyl) phthalate, DBP= Di-n-butyl phthalate, DBzP=Dibenzyl phthalate, DEP= Diethyl phthalate, DnOP= Di(n-octyl) phthalate*

Table 2: N=10 Food Consumption (ranked by the participant) within the 48-hour Time Period before Urine Collection

#	Homegrown	Store bought	Water Source
1	3:Most	2:Some	Private well
2	3:Most	2:Some	Private well
3	2:Some	2:Some	Private well
4	3:Most	2:Some	Private well
5	3:Most	2:Some	Private well
6	3:Most	2:Some	Public/Municipal
7	3:Most	2:Some	Private well
8	3:Most	2:Some	Private well
9	3:Most	2:Some	Private well
10	3:Most	2:Some	Private well

Legend: 1= None, 2= Some, 3= Most and 4= All

Table 3: N=10 Mode of Transportation (ranked by the participant as top 3 methods of transportation) within the 48-hour Time Period before Urine Collection

#	Walking	Bicycle	Horse & Buggy	Car or Truck
1	2:Some	3:Most	1:Least	0:No
2	2:Some	1:Least	3:Most	0:No
3	0:No	0:No	0:No	0:No
4	1:Least	0:No	3:Most	2:Some
5	0:No	2:Some	3:Most	1:Least
6	1:Least	2:Some	3:Most	0:No
7	2:Some	0:No	3:Most	1:Least
8	2:Some	1:Least	3:Most	0:No
9	1:Least	0:No	3:Most	2:Some
10	0:No	3:Most	2:Some	1:Least

Legend
0 = No or none, 1 = Least (least often), 2 = Some, 3 = Most

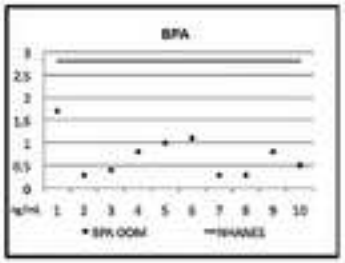
Table 4: N=10 Use of Personal Products within the 48-hour Time Period before Urine Collection

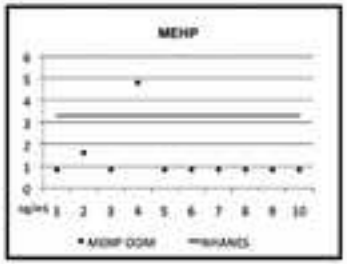
#	Bar Soap	Shampoo	Lotion	Deodorant	Hair Spray	Perfume	Body Wash
1	1:Yes	0:No	0:No	0:No	0:No	0:No	0:No
2	1:Yes	1:Yes	1:Yes **(NF)**	0:No	0:No	0:No	1:Yes
3	1:Yes	0:No	0:No	1:Yes	0:No	0:No	0:No
4	0:No	1:Yes **(NF)**	0:No	0:No	0:No	0:No	1:Yes **(NF)**
5	1:Yes	0:No	0:No	0:No	0:No	0:No	0:No
6	1:Yes **(NF)**	0:No	0:No	1:Yes	0:No	1:Yes	0:No
7	1:Yes	0:No	0:No	0:No	0:No	0:No	0:No
8	1:Yes **(NF)**	1:Yes **(NF)**	0:No	0:No	0:No	0:No	1:Yes **(NF)**
9	0:Yes	1:Yes	0:No	1:Yes	0:No	0:No	1:Yes
10	1:Yes	1:Yes	0:No	1:Yes	1:Yes	0:No	1:Yes

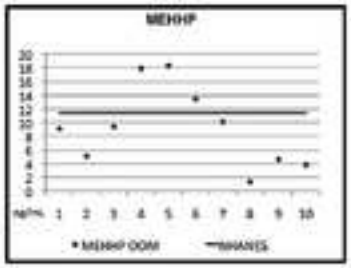
NF= no fragrance

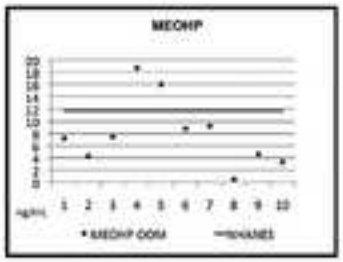
Figure

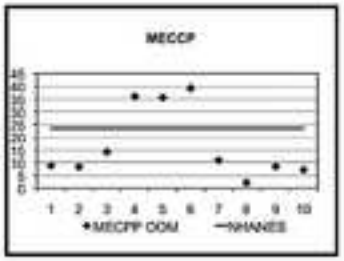
Click here to download high resolution image

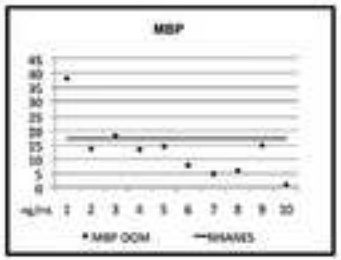


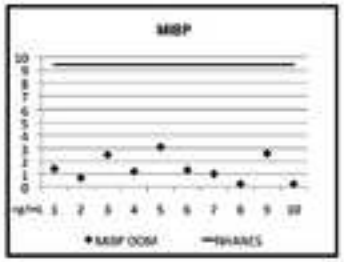


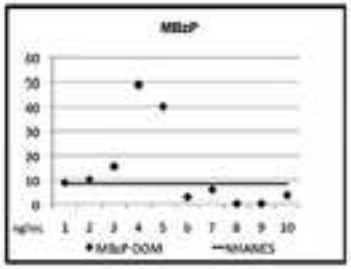


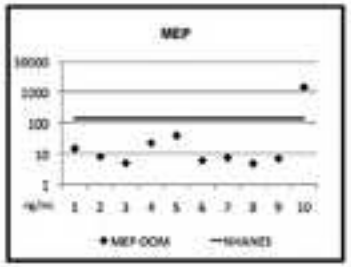


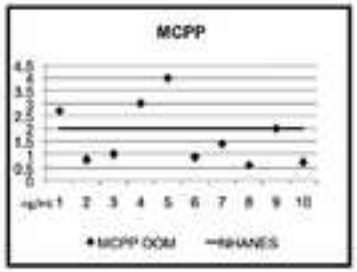












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[1] *Sign test for number of subjects with analyte value <= comparison population's median*
[2] *Limit of detection/LOD*
[3] *Parent compounds: DEHP= Di(2-ethylhexyl) phthalate, DBP= Di-n-butyl phthalate, DBzP=Benzybutyll phthalate, DEP= Diethyl phthalate, DnOP= Di(n-octyl) phthalate*

Tables

Table 2: N=10 Food Consumption (ranked by the participant) within the 48-hour Time Period before Urine Collection

#	Homegrown	Store bought	Water Source
1	3:Most	2:Some	Private well
2	3:Most	2:Some	Private well
3	2:Some	2:Some	Private well
4	3:Most	2:Some	Private well
5	3:Most	2:Some	Private well
6	3:Most	2:Some	Public/Municipal
7	3:Most	2:Some	Private well
8	3:Most	2:Some	Private well
9	3:Most	2:Some	Private well
10	3:Most	2:Some	Private well

Legend: 1= None, 2= Some, 3= Most and 4= All

Tables

Table 3: N=10 Mode of Transportation (ranked by the participant as top 3 methods of transportation) within the 48-hour Time Period before Urine Collection

#	Walking	Bicycle	Horse & Buggy	Car or Truck
1	2:Some	3:Most	1:Least	0:No
2	2:Some	1:Least	3:Most	0:No
3	0:No	0:No	0:No	0:No
4	1:Least	0:No	3:Most	2:Some
5	0:No	2:Some	3:Most	1:Least
6	1:Least	2:Some	3:Most	0:No
7	2:Some	0:No	3:Most	1:Least
8	2:Some	1:Least	3:Most	0:No
9	1:Least	0:No	3:Most	2:Some
10	0:No	3:Most	2:Some	1:Least

Legend
0 = No or none, 1 = Least (least often), 2 = Some, 3 = Most

Tables

Table 4: N=10 Use of Personal Products within the 48-hour Time Period before Urine Collection

#	Bar Soap	Shampoo	Lotion	Deodorant	Hair Spray	Perfume	Body Wash
1	1:Yes	0:No	0:No	0:No	0:No	0:No	0:No
2	1:Yes	1:Yes	1:Yes **(NF)**	0:No	0:No	0:No	1:Yes
3	1:Yes	0:No	0:No	1:Yes	0:No	0:No	0:No
4	0:No	1:Yes **(NF)**	0:No	0:No	0:No	0:No	1:Yes **(NF)**
5	1:Yes	0:No	0:No	0:No	0:No	0:No	0:No
6	1:Yes **(NF)**	0:No	0:No	1:Yes	0:No	1:Yes	0:No
7	1:Yes	0:No	0:No	0:No	0:No	0:No	0:No
8	1:Yes **(NF)**	1:Yes **(NF)**	0:No	0:No	0:No	0:No	1:Yes **(NF)**
9	0:Yes	1:Yes	0:No	1:Yes	0:No	0:No	1:Yes
10	1:Yes	1:Yes	0:No	1:Yes	1:Yes	0:No	1:Yes

NF= no fragrance