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Life without plastic: A family experiment and biomonitoring study

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ABSTRACT

Exposure to bisphenol-A (BPA) and phthalates has been associated with negative health outcomes in animal and human studies, and human bio-monitoring studies demonstrate widespread exposure in the US and Europe. Out of concern for the environment and health, individuals may attempt to modify their environment, diet, and consumer choices to avoid such exposures, but these natural experiments are rarely if ever quantitatively evaluated.

The aim of the study was to evaluate the difference in urinary concentrations of BPA and phthalate metabolites following an exposure reduction intervention among an Austrian family of five.

Urine samples were taken shortly after the family had removed all plastic kitchenware, toys, and bathroom products, and started a concerted effort to eat less food packaged in plastic. Two-months later, urine samples were collected at a follow-up visit, and concentrations of BPA and phthalate metabolites were compared.

Shortly after removal of plastic urinary concentrations of BPA were below limit of quantification in all samples. Phthalate concentrations were low, however, 10 of 14 investigated metabolites could be found above limit of quantification. After the two-month intervention, phthalate urinary concentrations had declined in some but not all family members. In the mother most phthalate metabolites increased.

The low levels might be partly due to the environmentally conscious lifestyle of the family and partly due to the fact that body levels had dropped already because of the delay of four days between finishing removal and first measurement. Further two months avoidance of dietary exposure and exposure to environmental plastics reduced urinary concentrations for all but one metabolite in the oldest son only, but decreased somewhat in all family members except the mother.

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1. Introduction

Concerns are growing regarding potential health effects from widespread human exposure to bisphenol A and phthalates (Woodruff et al., 2011; CDC, 2009; Danish, 2013; Rochester, 2013). In the United States, 93% of urine samples from 2517 participants

over the age of 6 contained detectable levels of BPA in 2003–4 (Calafat et al., 2008). While the dose at which BPA may induce harm is debated (Vandenberg, 2013), exposure is now linked via both animal and human studies to a variety of negative health outcomes. As BPA is an endocrine disruptor with estrogen mimicking activity, it can function through the estrogen-related receptor- γ to alter insulin production and release, contributing to the development of insulin resistance and type 2 diabetes (Nadal et al., 2009). It has also been related to increased weight gain and obesity in adults and children (Song et al., 2014; Carwile and Michels, 2011; Trasande et al., 2012). BPA was designated an ovarian toxicant (Peretz et al., 2014) as it interrupts early oogenesis and meiosis in women, and may impact male fertility via alterations in spermatogenesis (Vrooman et al., 2015), lower semen quality (Li et al., 2011; Lassen et al., 2014), and through alterations to reproductive hormones (Lassen et al., 2014).

BPA is a phenolic chemical which is used in the manufacture of

Abbreviations: 3-cx-MPP, 3-carboxyl-mono-propyl phthalate: secondary metabolite of di-*n*-octyl phthalate, di-*n*-butyl phthalate and/or di-isononyl phthalate; 5-cx-MEPP, Mono-(5-carboxy-2-ethylpentyl)phthalate: secondary metabolite of di-(2-ethylhexyl) phthalate; 5OH-MEHP, Mono-(2-ethyl-5-hydroxyhexyl) phthalate: secondary metabolite of di-(2-ethylhexyl) phthalate; 5oxo-MEHP, Mono-(2-ethyl-5-oxohexyl) phthalate: secondary metabolite of di-(2-ethylhexyl) phthalate; BPA, Bisphenol-A; LC-ESI-MS/MS, Liquid chromatography-electrospray ionization-tandem mass spectrometry; LOD, Limit of detection

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polycarbonate plastics and epoxy resins, and as a polymerization inhibitor in the formation of polyvinyl chloride plastics (CDC, 2009). It can be found in a wide range of consumer products including plastic dinnerware, metal cans (in the protective lining), eyeglass lenses, thermal paper, polycarbonate water jugs, baby bottles, and toys (CDC, 2009). Exposure typically occurs through ingestion, but small amounts are inhaled with dust. For small children, hand-mouth-contact may also play a role (CDC, 2009).

Phthalates are a group of chemicals used to increase the softness and flexibility of plastics, as stabilizing agents and as solvents. They are found in floorings and medical equipment (tubings), but also appear in a variety of cosmetics and personal care products, including fragrances, lotions, nail polish, and hair shampoo & conditioner, and also some insecticides (CDC, 2009; NIH U.S. National Library of Medicine/LoM, 2015; Centers for Disease Control and Prevention, 2015). Furthermore, phthalates are used, *inter alia*, in food packaging, raincoats and toys. Human exposure to phthalates can occur from food, air, dust, or contact to products which contain phthalates. At the highest risk of exposure are those who receive transfusions via plastic tubing and fluid which releases phthalates, such as dialysis patients or haemophiliacs.

Several phthalates have endocrine disrupting properties (WHO/UNEP, 2013). Phthalates have been associated with obesity (Smerieri et al., 2015; Buser et al., 2014) and with other negative health outcomes like asthma (Bornehag et al., 2004; Bertelsen et al., 2013; Shu et al., 2014). Furthermore, Di(2-ethylhexyl) phthalate (DEHP) is listed as "reasonably anticipated to be a human carcinogen" in the 13th Report on Carcinogens published by the U.S. Department of Health and Human Services (NTP (National Toxicology Program), 2014).

In light of the accumulating evidence that BPA and phthalates may be capable of inducing harmful health effects in humans, individuals may want to take care to avoid unnecessary exposure, but the extent to which we can control our exposures through consumer, lifestyle, and dietary choices is unclear. In a study of 894 pregnant women in the United States, 60% agreed environmental chemicals are dangerous, but 25% felt they were impossible to avoid (Barrett et al., 2014).

Out of a concern for the environment and for their personal health (inspired by Werner Boote's documentary "Plastic Planet"), a family of five in Austria set out to dramatically lower their exposure to chemicals emanating from plastic products. In their blog, they explained: "This isn't about denial or rigor. There are items that we don't and won't do without, such as computers, refrigerators, TV and mobile phones. But for other items, there is usually a natural alternative, and most plastics can be replaced by wood, glass, metal, ceramic or plant fibers" (<http://www.keinheimfuerplastik.at/about>, accessed, 2015).

As far as we know, changes in urinary concentrations of phthalates and BPA from the intentional restriction of exposure to plastics has not been biologically quantified in a natural human environment. The uniqueness of this project provided an opportunity to evaluate the hypothesis that reduced daily micro-exposures will result in lower overall body burden of common metabolites of substances emitted from plastics. The paper describes the family experiment and its results.

2. Material and methods

The family experiment started on 14th of November 2009, by removing most products made of plastics from the household. When possible, removed items were replaced with non-plastic alternatives. Except for products like refrigerator and oven containing plastic sealing, plastic items were removed from the kitchen and the bathroom, including storage containers, kitchen

appliances (scoops, spoons etc.) and cups. Equally, two-thirds of toys were removed. Only items that were indispensable, or for which replacement would have been too costly, remained in the house. Directly after removing plastic items, the house (especially the kitchen and hall) were thoroughly mopped with water. Generally, floors were mopped once weekly. The only time the family was absent during the study period was for a 6-day holiday at a local winter sports resort.

The lifestyle of the family can be characterized by a high degree of environmental awareness. They did not make any substantial changes to their diet during the study period. However, they consciously abstained from snacks such as dried fruit and nuts, as these typically are packaged in plastic. They equally eliminated sweets and ready meals from their diet, although they very occasionally indulged in frozen pizza (which comes wrapped in plastic). In general, their attitude to food only changed in so much as they actively tried to avoid anything that had been in contact with plastics.

The family's consent to participate in the current interventional biomonitoring study was obtained by telephone. At the same time, they were provided with information outlining the aims of the biomonitoring study and associated potential health risks. After obtaining consent, dates for home visits were arranged.

2.1. Sample collection

Two home visits were arranged for sample collection, one at the beginning of the study four days after finishing the removal and cleaning process (29th of November 2009), and a follow-up visit 2 months into the study period (25th of January 2010). The first visit could not be scheduled earlier because of time constraints of the family and organizational reasons.

All five family members provided fasting morning urine samples, for which they were issued with glass containers and instructions for adequate collection. Samples were transported and stored under chilled conditions and arrived at the Austrian Federal Environmental Agency within 12 h for processing. During the first home visit, a full medical history was recorded for each participant and an interview about eating and drinking habits, about school/working conditions and leisure time activities was conducted.

2.2. Urine analysis

Morning urine samples were tested for primary and secondary phthalate metabolites, as well as bisphenol A (BPA) (see Table 1). Concentrations of creatinine were also determined.

Analysis of phthalate metabolites was performed by means of liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) after enzymatic hydrolysis. 800 μ L of urine sample were pipetted into a centrifuge vessel and were spiked with a mix of isotopically labeled surrogates. Sodium buffer was added (0.1 M, pH 5) and the solution was shaken well. 100 μ L of β -Glucuronidase (*Helix pomatia*, approx. 16,700 units per mL) was added and the mixture was shaken again. The mixture was incubated for 2 h at 37° C on a water shaking bath. The sample was centrifuged, and the supernatant was transferred into brown glass vial for instrumental analysis. Analysis was performed on a HP 1200 HPLC system consisting of a membrane degasser, an automatic sampler, a column heater that was coupled to a 4000 QTRAP tandem mass spectrometer (Applied Biosystems). Separation of analytes was performed using a 150 \times 2 mm Luna Phenyl-Hexyl column with 3 μ m pore size. Eluents were water acetonitrile 9/1 v/v, and acetonitrile/water 9/1 v/v, each modified with 1% acetic acid. The column heater was operated at 25 °C. The mass spectrometer source was operated in the negative ESI mode. Quantitation was performed in the multiple reaction monitoring (MRM)

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