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The association between urinary concentrations of phosphorous-containing flame retardant metabolites and semen parameters among men from a fertility clinic

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ABSTRACT

Background: The use of PFRs has steadily increased as brominated compounds have been or are being phased out. Human exposure is widespread and animal studies have shown adverse impacts on male reproduction, but human data are lacking.

Objective: To study the associations between urinary concentrations of phosphorous-containing flame retardant (PFR) metabolites and semen parameters.

Methods: A subset of 220 men from an existing longitudinal cohort of couples were recruited from Massachusetts General Hospital fertility clinic between 2005 and 2015. Semen parameters included sperm count, concentration, motility, and morphology; some men had samples measured from multiple clinic visits (up to five visits; n = 269 semen samples). Metabolites [bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), diphenyl phosphate (DPHP), isopropylphenyl phenyl phosphate (ip-PPP), tert-butylphenyl phenyl phosphate (tb-PPP) and bis(1-chloro-2-propyl) phosphate (BCIPP)] were measured in urine samples (between one and five urine samples per participant; n = 355 urine samples). Semen parameters were evaluated continuously and dichotomized for models. Metabolites were assessed for associations with semen parameters as continuous and categorized into quartiles using multivariable generalized mixed models, adjusted for specific gravity, age, BMI, smoking, and abstinence period.

Results: Metabolites BDCIPP, DPHP, and ip-PPP were detected in a high proportion of urine samples (85%, 86%, and 65% respectively). Concentrations varied by season of collection, particularly for BDCIPP where samples collected in the summer were approximately 2-fold higher than concentrations of other seasons ($p < 0.0001$). The odds of having a sperm count less than 39 mil/ejaculate decreased by 20% for increasing BDCIPP concentrations ($p = 0.04$). When regressing semen parameters on PFR metabolite quartiles, some negative associations were observed for individual quartiles among sample volume and morphology, but overall associations were weak and inconsistent.

Conclusion: Detection rates were high for BDCIPP, DPHP, and ip-PPP. We did not observe consistent associations between PFR metabolites and semen parameters. Due to the high prevalence of exposure, further investigation of other potential health effects should be conducted.

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1. Introduction

Infertility, the inability to conceive after one year of unprotected intercourse, affects approximately one out of every six couples (Meacham et al., 2007). In 2002, a national survey estimated two million couples in the U.S. suffer from infertility (Chandra et al., 2002). An increase in infertility is partially related to the postponement of first birth (Dunson et al., 2004; Sharma et al., 2013). However, aside from advanced age, genetic risk factors, psychosocial factors, and environmental agents can also impair fertility (Chalupka and Chalupka, 2010; Macaluso et al., 2010).

The underlying cause of infertility may be related to female or male factors or a combination of both. In 2002, approximately 20% of men reported fertility problems (Hotaling et al., 2012). However, a national survey study suggests this to be an underestimate for the U.S. population as male factor infertility is likely to be underdiagnosed (Hwang et al., 2011; Hotaling et al., 2012) Although, a recent meta-analysis found an approximate 50% reduction in total sperm count and sperm concentration among men from Western countries over the last several decades, irrespective of fertility diagnosis (Levine et al., 2017). The cost of male factor infertility alone was $17 million US dollars in the year 2000, which does not include the additional $18 billion for assisted reproductive technology treatment (Meacham et al., 2007). To date, a semen analysis measuring sperm count, concentration, morphology, and volume remains the primary evaluation for male factor infertility (World Health Orginization (WHO), 2010; Hwang et al., 2011). Semen quality is also associated with other various health outcomes. A study of Finnish men found an increase risk in testicular cancer among those with poor semen quality (Jørgensen et al., 2011), while a Danish study found subpar semen associated with a shorter life span (Jensen et al., 2009). Many environmental agents such as glycol ethers, pesticides, and phthalates are also known to impact semen quality (Chalupka and Chalupka, 2010).

Among possible environmental chemicals of concern for reproductive health are organophosphate esters, which are increasingly being used as flame retardants (PFRs). The use of PFRs has grown due to their use as replacement chemicals for the phased-out of polybrominated diphenyl ethers. As their prevalence rose, PFRs became and remain a high production volume chemical. Today they are commonly applied to materials for use as either a flame retardant, or as a plasticizer, therefore are common in polyvinyl chloride (PVC), hydraulic fluids, and polyurethane foam (PUF) in cars and furniture (Marklund et al., 2003; van der and de Boer, 2012; Tajima et al., 2014). PFRs include both chlorinated alkyl esters such as tris(2-chloroisopropyl) phosphate (TCIPP) and tris(1,3-dichloroisopropyl) phosphate (TDCIPP), and non-halogenated aryl phosphates such as triphenyl phosphate (TPHP) (Marklund et al., 2003; Brommer and Harrad, 2015). Often considered 'additive' compounds, the weak bonds allow volatilization into air and settlement in dust. PFRs have been detected in the dust of homes, cars, and offices (Brommer and Harrad, 2015; Ali et al., 2016). Unlike brominated flame retardants, PFRs are considered non-persistent, with a short half-life in humans, yet they are detected in nearly 100% of urine samples from men (Meeker et al., 2013a), pregnant women (Hoffman et al., 2014), and children (Cequier et al., 2015).

To date studies assessing the health effects of PFRs are limited, yet animal and in vitro studies suggest these compounds act as endocrine disrupting chemicals. A study of TPHP and tris(2-chloroethyl) phosphate (TCEP) in mice found a disruption of gene expression for testosterone synthesis and oxidative stress (Chen et al., 2015), while an in vitro study of mouse Leydig cells found a disruption in steroid production (Schang et al., 2016). A small study of U.S. men detected inverse relationships of bis(1,3-dichloropropyl) phosphate (BDCPP) and diphenyl phosphate (DPHP) concentrations in urine with sperm concentration and motility (Meeker et al., 2013b). To the best of our knowledge, this prior analysis is the only human study to date to assess the relationship of PFRs with semen parameters. In our present work, we expand upon this preliminary evidence with a larger cohort to characterize the relationship between five PFR metabolites: bis(1-chloro-2-propyl) phosphate (BCIPP), bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), diphenyl phosphate (DPHP), isopropylphenyl phenyl phosphate (ip-PPP), tert-butylphenyl phenyl phosphate (tb-PPP) with semen parameters in men attending a fertility center.

2. Methods

2.1. Participant recruitment

Participants from this analysis are a subset of men from the Environment and Reproductive Health (EARTH) study, a larger cohort assessing the impact of environmental agents on reproductive health. Participation and recruitment have been described elsewhere (Meeker et al., 2006). Briefly, men (18–54 years of age) attending the Massachusetts General Hospital fertility clinic between 2005 and 2015 were eligible. Participants originated from couples whose infertility diagnosis was either male factor, female factor, or a combination of both. Prior vasectomy or hormone supplementation were the only exclusion criteria. Informed consent was signed by each participant and Institutional Review Board approval was received by all institutions.

2.2. Semen collection and analysis

Semen collection and analysis have been previously described (Meeker et al., 2006; Lewis et al., 2017). Briefly, men abstained from ejaculation for 48 h prior to sample collection into plastic specimen cup. Men provided up to five samples depending on the number of fertility treatments, additional fertility evaluation, or a combination of both. An andrologist quantified sample volume (mL) with a graduated pipet. Sperm concentration (mil/mL) and motility (% motile) was determined using a computer-aided semen analyzer (CASA, version 10 HTM-IVOS; Hamilton Thorne Research, Beverly, MA). Samples (5 μL) were collected on a disposable Leja Slide (Spectrum Technologies, CA, USA) and placed into a pre-warmed (37 °C) counting chamber (Sefi-Medical Instruments, Haifa, Israel) before assessing concentration and motility. Among each sample, at least 200 sperm cells were analyzed from four different fields. Progressive motility was graded in accordance to the WHO's assessment criteria of active movement (linearly or in a large circle), regardless of velocity (World Health Organization (WHO), 2010). The product of sperm concentration and sample volume determined sperm count (mil/ejaculate) while progressive motility count (mil/ejaculate) was calculated by multiplying progressive motility and total sperm count. Fresh semen samples were allowed to dry on two prepared slides and prepared for morphology (% normal) assessment with a microscope using an oil-immersion 100 × objective (Nikon, Tokyo, Japan). A minimum of 200 cells per slide were analyzed for each specimen. Classification of normal or subnormal morphology was determined using strict Kruger scoring criteria (Kruger et al., 1988). Quality assurance and control procedures in the laboratory were conducted for sperm morphology smears weekly, as well as quarterly and biannual evaluations for technicians.

2.3. Urine collection and analysis

Urine samples (up to five cycles) were collected in sterile polypropylene cups on the day of oocyte retrieval for each cycle per participant. Prior to being frozen (−80°) and stored, specific gravity (SG) was measured using a handheld refractometer (National Instrument Company, Inc., Austin, TX). For metabolite analysis, samples were shipped overnight on dry ice to Dr. Stapleton's lab at Duke University (Durham, NC).

Analytic methods for metabolites: BCIPP, BDCIPP, DPHP, ip-PPP, and tb-PPP have been previously described (Butt et al., 2014). Briefly, 5 ml aliquots were thawed and transferred to test tubes and spiked with