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Influence of storage vial material on measurement of organophosphate flame retardant metabolites in urine

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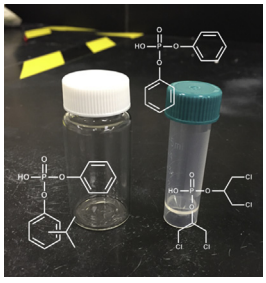
HIGHLIGHTS

- We measured PFR metabolites in duplicate urine samples stored in glass and plastic vials.
- Concentrations were highly correlated between duplicates.
- ip-PPP was slightly but significantly higher using glass compared to plastic vials for storage.
- DPHP and BDCIPP showed no storage difference between the glass and plastic vials.

GRAPHICAL ABSTRACT



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ABSTRACT

Use of organophosphate flame retardants (PFRs) has increased over the past decade with the phase out of polybrominated diphenyl ethers. Urinary metabolites of PFRs are used as biomarkers of exposure in epidemiologic research, which typically uses samples collected and stored in polypropylene plastic cryovials. However, a small study suggested that the storage vial material may influence reported concentrations. Therefore, we aimed to examine the influence of the storage vial material on analytical measurement of PFR urinary metabolites. Using urine samples collected from participants in the Environment and Reproductive Health (EARTH) Study, we analyzed the PFR metabolites in duplicate aliquots that were stored in glass and plastic vials ($n = 31$ pairs). Bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), diphenyl phosphate (DPHP) and isopropyl-phenyl phenyl phosphate (ip-PPP) were detected in 98%, 97% and 87% of duplicates. We observed high correlations between glass-plastic duplicates for BDCIPP ($r_s = 0.95$), DPHP ($r_s = 0.79$) and ip-PPP ($r_s = 0.82$) ($p < 0.0001$). Urinary ip-PPP was an average of 0.04 ng/ml ($p = 0.04$) higher among samples stored in glass, with a mean relative difference of 14%. While this difference is statistically significant, it is small in magnitude. No differences were observed for BDCIPP or DPHP, however future research should seek to reduce the potential for type II error (false negatives). We conclude that storing urine samples in polypropylene plastic cryovials may result in slightly reduced concentrations of urinary ip-PPP relative to storage in glass vials and future research

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should seek to increase the sample size, reduce background variability and consider the material of the urine collection cup.

Abbreviations

BDCIPP	Bis(1,3-dichloro-2-propyl) phosphate
BCIPP	bis(1-chloro-2-propyl) phosphate
DPHP	diphenyl phosphate
EARTH	Environment and Reproductive Health
GM	Geometric mean
ip-PPP	isopropyl-phenyl phenyl phosphate
MDL	Method detection limit
PFRs	Organophosphate flame retardants
SG	Specific gravity
tb-PPP	tert-butyl-phenyl phenyl phosphate

1. Introduction

Organophosphate flame retardants (PFRs) have been used in the polyurethane foam of upholstered furniture (Stapleton et al., 2009) with increasing prevalence over the past decade following the phase out polybrominated diphenyl ethers (PBDEs) (Stapleton et al., 2012). Flame retardants used in polyurethane foam are additives that are not chemically bound, and therefore migrate into the air and dust of indoor environments (van der Veen and de Boer, 2012) and lead to human exposure. Two commonly used PFRs are triphenyl phosphate (TPHP) and tris(1,3-dichloro-2-propyl)phosphate (TDCIPP), which after intake are excreted within hours primarily as the urinary metabolites diphenyl phosphate (DPHP) and bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), respectively (Nomeir et al., 1981; Sasaki et al., 1984; Van den Eede et al., 2013). TPHP is often used in the Firemaster® 550 mixture in combination with two brominated flame retardants as well as various mono-substituted triphenyl phosphate isomers (Fig. 1). In addition to being used as a flame retardant, TPHP is also used as a plasticizer and is found in nail polish, hydraulic fluids and polyvinyl chloride (PVC) (Mendelsohn et al., 2016; van der Veen and de Boer, 2012). Additionally, the primary metabolite of TPHP, diphenyl phosphate (DPHP), is also sold and used as a plasticizer, although production volumes of DPHP are significantly lower than TPHP. TCIPP in contrast is used as a flame retardant primarily in rigid polyurethane foam for insulation and construction (80% of use) as well in flexible polyurethane foam (e.g., furniture cushions) (European Union Risk, 2008).

While population-wide data are limited, studies suggest that exposure to these PFRs is likely widespread in the U.S. as DPHP and BDCIPP have been detected in over 90% of adult urine samples (Butt et al., 2014; Carignan et al., 2013; Hoffman et al., 2014; Meeker et al., 2013a). This is of concern because TDCIPP and TPHP are suspected endocrine disrupting chemicals that have been shown to disrupt thyroid hormone and estrogen signaling as well as to reduce reproductive performance in zebrafish and chickens (Farhat et al., 2013; Liu et al., 2013; Wang et al., 2015). TPHP is a suspected obesogen that can initiate adipocyte differentiation and antagonize osteogenesis (Belcher et al., 2014; Pillai et al., 2014). TDCIPP is considered a carcinogen under Proposition 65 regulated by the State of California (Evidence on the Carcinoge, 2011) and is a potential developmental neurotoxicant (Dishaw et al., 2014). TCIPP can also disrupt the endocrine system, with in vitro evidence of antiandrogenic and antiestrogenic activity (Ohyama et al., 2006). In vivo studies report morphological changes in the thyroid and adverse effects on reproduction including changes to the estrous cycle, increased uterine weights, low birth weight, and delayed hatching (TNO Quality of Life (2007); Freudenthal and Henrich, 1999; Farhat et al., 2013; U.S. EPA, 2014). Little is known regarding toxicity of the mono-substituted triphenyl phosphate isomers. Few epidemiologic studies have investigated PFRs, however an exploratory analysis of 33 men found that urinary BDCIPP and DPHP were associated with reductions in sperm motility and increased total T_3 (Meeker et al., 2013b).

Epidemiologic studies investigating PFRs are needed and may utilize urinary metabolites as biomarkers of exposure. Typically, urine samples are collected in plastic (polypropylene) specimen cups and frozen in plastic cryovials. However, preliminary results have suggested that PFR metabolites may adhere to these collection and storage containers (Cooper et al., 2011). Therefore, our objective was to determine whether the material of the storage vial material biases analytical determination of PFR urinary metabolites using a subset of aliquots from a U.S. preconception cohort that were stored both in glass and plastic vials.

2. Materials and methods

2.1. Participants

Study participants were women recruited into the Environment and Reproductive Health (EARTH) study between November 2005 and October 2015 from patients undergoing assisted reproductive technologies at the Massachusetts General Hospital Fertility Center. Female participants must be between the ages of 18 and 46 to enroll in the study. The EARTH study was approved by the Human Studies Institutional Review Boards of Massachusetts General Hospital and Harvard T.H. Chan School of Public Health. Participants signed an informed consent after the study procedures were explained by a research nurse and any questions were answered.

2.2. Urine samples

Urine was collected in a sterile polypropylene cup and specific gravity (SG) was measured using a handheld refractometer (National Instrument Company, Inc.). Each sample was divided into aliquots (2.5–5 ml) and stored at −80 °C. We randomly selected duplicate samples collected between 2008 and 2009 that were stored in glass vials (Shorty Vials®, Borosilicate Glass, PTFE lined Screw Cap, Wheaton) and plastic cryovials (Nalgene® Cryogenic Vials, Polypropylene, Sterile, External Thread with Screw Cap, Thermo Scientific) (glass-plastic duplicates). While the main objective of our analysis was to compare analytical results from duplicates stored in glass and plastic storage vials, we also evaluated analytical variability from duplicates stored only in plastic storage vials. To do so, we selected duplicate samples collected between 2005 and 2015 that were stored in plastic cryovials (plastic-plastic duplicates). The glass-plastic (n = 31 pairs) and