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Quantitative determination of nine urinary metabolites of organophosphate flame retardants using solid phase extraction and ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS)

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ABSTRACT

Over the last few years, the use of organophosphate flame retardants (OPFRs) has been on the rise; however, there are knowledge gaps in both the human health effects of OPFRs and levels of human exposure. Currently, human biomonitoring data on the levels of OPFR metabolites in the Canadian population are still non-existent. Herein we describe a novel method to measure nine urinary OPFR metabolites using solid phase extraction and ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS). The method detection limits were between 0.08 and 0.25 ng/mL for target metabolites. The newly developed and validated method was applied to the analysis of 24 urine samples collected in 2010–12 from pregnant Canadian women. The most frequently detected OPFR metabolite in urine of study participants (detection frequency: 97%) was diphenyl phosphate (DPHP), with concentrations ranging between <0.13–25.2 ng/mL, followed (75%) by the sum of two metabolites (D*o*CP: Di-*o*-cresyl phosphate and D*p*CP: Di-*p*- cresyl phosphate) of tricresyl phosphate, with concentrations between <0.13–4.38 ng/mL. With the exception of desbutyl-tris-(2-butoxy-ethyl) phosphate which was not detected in any of the samples, all other OPFR metabolites measured were found among study participants with variable detection frequency, suggesting pregnant Canadian women may be exposed to OPFRs.

1. Introduction

For many decades now, halogenated flame retardants have been extensively used in consumer and industrial products to impart flame retardant properties to those products. Polybrominated diphenyl ether (PBDE) flame retardants, in particular, have been heavily used in numerous applications. As a result of their widespread use, chemical persistence and bioaccumulation, number of PBDE congeners have been detected in a variety of environmental and human matrices [1–4]. Epidemiological data and animal studies highlight the adverse health effects arising from exposure to these chemicals with the result that the majority of PBDE formulations are banned in several countries [5]. In addition, an increased scientific interest as well as concern over the widespread exposure to PBDEs has resulted in the inclusion of certain brominated flame retardants in the Stockholm Convention List of Persistent Organic Pollutants (POPs) [6]. Voluntary phase-out by some manufacturers of PBDEs has seen the increase in the use of alternative flame retardants, including organophosphate and non-PBDE halogenated flame retardants in order to meet stringent fire safety codes imposed by regulators [7,8].

Organophosphate flame retardants (OPFRs) have been used commercially since the 1970s in a variety of products [9] including: plasticizer formulations, hydraulic fluids, as additives in lubricants, adhesives and polyurethane foam, rubber, and textile coatings [10,11]. There are two major organophosphate formulations in use: the halogenated compounds used mainly as flame retardants and the non-halogenated forms used mainly as plasticizers [12], although the latter are also used in certain flame retardant applications. Current industrial market reports indicate that OPFRs are being employed at an increased rate in consumer products due to the fact that they are perceived by the industry to be more environmentally friendly and safer than the halogen-based flame

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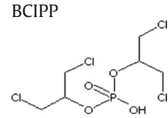
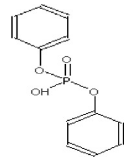
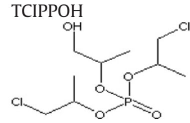
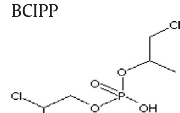
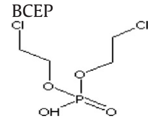
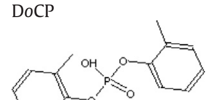
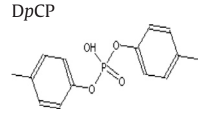
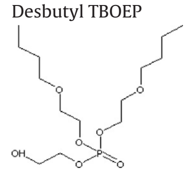
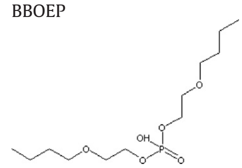
Table 1
List of parent OPFR compounds and their metabolites analyzed.

OPFR parent compounds			OPFR metabolites		
CAS no.	Acronym	Chemical name	Acronym/structure	Chemical name	CAS no.
13674-87-8	TDCIPP	Tris(1,3- dichloro- 2- propyl) phosphate	BCIPP 	Bis(1,3-dichloro-2-propyl) phosphate	72236-72-7
115-86-6	TPHP	Triphenyl phosphate	DPHP 	Diphenyl phosphate	838-85-7
13674-84-5	TCIPP	Tris(1-chloro-2-propyl) phosphate	TCIPPOH 	1-Hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate	Not available
			BCIPP 	Bis(2-chloropropyl) phosphate	789440-10-4
115-96-8	TCEP	Tri(2-chloroethyl) phosphate	BCEP 	Bis(2-chloroethyl) hydrogen phosphate	3040-56-0
1330-78-5	TCrP	Tricresyl phosphate	D*o*CP 	DI-*o*-cresyl phosphate or Di-*o*-tolyl phosphate	3587-74-7
			D*p*CP 	Di-*p*-cresyl phosphate or Di-*p*-tolyl-phosphate	843-24-3
78-51-3	TBOEP	Tributoxyethyl phosphate	Desbutyl TBOEP 	Desbutyl-tris-(2-butoxy-ethyl) phosphate	Not available
			BBOEP 	Bis-2(butoxyethyl) phosphate	14260-97-0
1241-94-7	EHDPHP	2-Ethylhexyl diphenyl phosphate	DPHP	Diphenyl phosphate	838-85-7
56803-37-3	BPDP	*tert*-Butylphenyl diphenyl phosphate	DPHP	Diphenyl phosphate	838-85-7
125997-21-9	RDP	Resorcinol bis(diphenylphosphate)	DPHP	Diphenyl phosphate	838-85-7
5945-33-5	BPA-BPDP	Bisphenol A bis(diphenylphosphate)	DPHP	Diphenyl phosphate	838-85-7

retardants [13]. According to the same market report, in 2013 OPFR market volume had increased to 620,000 t, representing 30% of the total global market volume of flame retardants. Quantification of OPFRs in indoor environments [8,14–17] suggest that human exposure to these compounds is likely. Although OPFRs have been present in industrial formulations for several decades, information on their fate in the environment as well as toxicological effects in humans and biota are scarce [18]. According to the few reported animal studies [19–21], OPFRs tend to be readily absorbed and rapidly metabolized by biological systems into compounds which in a majority of cases are dialkyl and diaryl phosphate analogs. Biomonitoring of such compounds in humans in particular, is complicated by the absence of information on the biotransformation of OPFRs in humans. Consequently, biomarkers are generally assumed to be the same as those observed in animal models. Moreover, metabolites such as diphenyl phosphate (DPHP) have been shown to be a metabolite of multiple OPFR compounds, including triphenyl phosphate (TPHP) [22], 2-ethylhexyl diphenyl phosphate (EHDPP) [20], and *tert*-butylphenyl diphenyl phosphate (BPDP) [23]. Furthermore, DPHP itself has also been used as an additive substance in industrial applications, typically as a catalyst for resin manufacturing. Therefore the presence of DPHP