Toxicology Letters 245 (2016) 31–39

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Effects of primary metabolites of organophosphate flame retardants on transcriptional activity *via* human nuclear receptors

Hiroyuki Kojima[a,*], Shinji Takeuchi[a], Nele Van den Eede[b], Adrian Covaci[b]

[a] Hokkaido Institute of Public Health, Kita-19, Nishi-12, Kita-ku, Sapporo 060-0819, Japan
[b] Toxicological Center, Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Antwerp, Belgium

HIGHLIGHTS

- Nuclear receptor activities of 12 OPFR-metabolites were studied by cell-based assays.
- HO-*m*-TPHP and HO-*p*-TPHP showed more potent ERα/β agonistic activity than did TPHP.
- These HO-TPHPs also acted as PXR agonists as well as ERβ, AR and GR antagonists.
- Diester OPFR-metabolites and BCIPHIPP did not show any receptor activity.

ARTICLE INFO

Article history:
Received 13 September 2015
Received in revised form 1 December 2015
Accepted 11 January 2016
Available online 14 January 2016

Keywords:
Flame retardants
Human
Nuclear receptor
Organophosphate
Reporter gene assay
Urinary metabolite

ABSTRACT

Organophosphate flame retardants (OPFRs) have been used in a wide variety of applications and detected in several environmental matrices, including indoor air and dust. Continuous human exposure to these chemicals is of growing concern. In this study, the agonistic and/or antagonistic activities of 12 primary OPFR-metabolites against ten human nuclear receptors were examined using cell-based transcriptional assays, and compared to those of their parent compounds. As a result, 3-hydroxylphenyl diphenyl phosphate and 4-hydroxylphenyl diphenyl phosphate showed more potent estrogen receptor α (ERα) and ERβ agonistic activity than did their parent, triphenyl phosphate (TPHP). In addition, these hydroxylated TPHP-metabolites also showed ERβ antagonistic activity at higher concentrations and exhibited pregnane X receptor (PXR) agonistic activity as well as androgen receptor (AR) and glucocorticoid receptor (GR) antagonistic activities at similar levels to those of TPHP. Bis(2-butoxyethyl) 3′-hydroxy-2-butoxyethyl phosphate and 2-hydroxyethyl bis(2-butoxyethyl) phosphate act as PXR agonists at similar levels to their parent, tris(2-butoxyethyl) phosphate. On the other hand, seven diester OPFR-metabolites and 1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate did not show any receptor activity. Taken together, these results suggest that hydroxylated TPHP-metabolites show increased estrogenicity compared to the parent compound, whereas the diester OPFR-metabolites may have limited nuclear receptor activity compared to their parent triester OPFRs.

1. Introduction

The phasing-out of polybrominated diphenyl ethers (PBDEs) saw an increase in the use of organophosphate flame retardants (OPFRs) as additives to flame retardants and plasticizers in a variety of applications, such as building materials, textiles and electric appliances, resulting in their widespread environmental dispersion (Reemtsma et al., 2008; van der Veen and de Boer, 2012). Many studies have reported that various OPFRs are widely distributed in both indoor and outdoor environments (Hartmann et al., 2004; Marklund et al., 2003; Takigami et al., 2009; Stapleton et al., 2009). In particular, the presence of OPFRs in indoor dust has been worldwide reported, with several OPFRs, such as tris(2-butoxyethyl) phosphate (TBOEP), tris(1-chloro-2-propyl) phosphate (TCIPP), triphenyl phosphate (TPHP), tris(2-chloroethyl) phosphate (TCEP), tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), and tri-*n*-butyl phosphate (TNBP), predominately found in indoor dust from residential houses and offices (Tajima et al., 2014; Van den Eede et al., 2011). The concentrations of OPFRs detected in indoor dust in recent years have been higher than those of PBDEs

* Corresponding author. Fax: +81 11 736 9476.
E-mail address: kojima@iph.pref.hokkaido.jp (H. Kojima).

http://dx.doi.org/10.1016/j.toxlet.2016.01.004
0378-4274/

(Ali et al., 2012; Stapleton et al., 2012). As TCEP, TDCIPP, TCIPP and TBOEP have been shown to be carcinogenic or possibly carcinogenic in animal studies (WHO, 1998, 2000), continuous human exposure to these OPFRs is of growing concern.

Two previous studies using rodents showed that TDCIPP is quickly metabolized to bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), which is excreted primarily in the urine (Lynn et al., 1981; Nomeir et al., 1981). Recent human biomonitoring studies have also reported that OPFRs are readily metabolized, *via* hydrolysis or hydroxylation, and excreted in the urine as their dialkyl and diaryl compounds, which function as biomarkers for OPFR exposure (Butt et al., 2014; Cooper et al., 2011; Dodson et al., 2014; Van den Eede et al., 2013b). Van den Eede et al. (2015b) reported that 1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate (BCIPHIPP), diphenyl phosphate (DPHP), and BDCIPP were detected in >95% of human urine samples and bis(2-butoxyethyl) 2-hydroxyethyl phosphate (BBOEHEP) was found in >80% of those. In particular, DPHP, a primary metabolite of TPHP, was frequently detected at ppb levels in human urine (Cooper et al., 2011; Meeker et al., 2013; Reemtsma et al., 2011; Schindler et al., 2009). Thus, although various OPFR-metabolites are frequently detected in human urine and are probably quickly eliminated from the human body, it remains unclear whether these metabolites have adverse effects on human health during their residence time in the body.

The nuclear receptor superfamily is comprised of ligand-inducible transcription factors that specifically regulate the expression of target genes involved in metabolism, development, and reproduction (McKenna et al., 1999). Their primary function is to mediate the transcriptional response in target cells to hormones, such as sex steroids, adrenal steroids, vitamin D3, and thyroid and retinoid hormones, in addition to a variety of other metabolic ligands. Recent *in vitro* studies have suggested that several OPFRs act as endocrine disruptors *via* nuclear receptors, including hormone receptors (Kojima et al., 2013; Suzuki et al., 2013). In particular, our previous study using cell-based transactivation assays revealed that several OPFRs have agonistic and/or antagonistic activity against estrogen receptors (ERs), androgen receptor (AR), glucocorticoid receptor (GR) and pregnane X receptor (PXR) (Kojima et al., 2013). In addition, other studies have shown that TPHP could act as an activator of the peroxisome proliferators-activated receptor (PPAR)γ to induce adipogenesis (Belcher et al., 2014; Pillai et al., 2014). In the present study, we characterized the agonistic and antagonistic activity of twelve metabolites of TBOEP, TCIPP, TPHP, TCEP, TDCIPP, and TNBP, which are predominately found in indoor dust (Tajima et al., 2014; Van den Eede et al., 2011), against the following human nuclear receptors; ERα/β, AR, GR, $TR_{\alpha 1}$, retinoic acid receptor (RAR)α, retinoic X receptor (RXR)α, PPARα/γ and PXR. In this article, we provide the first evidence that hydroxylated TPHP-metabolites might possess multiple effects on

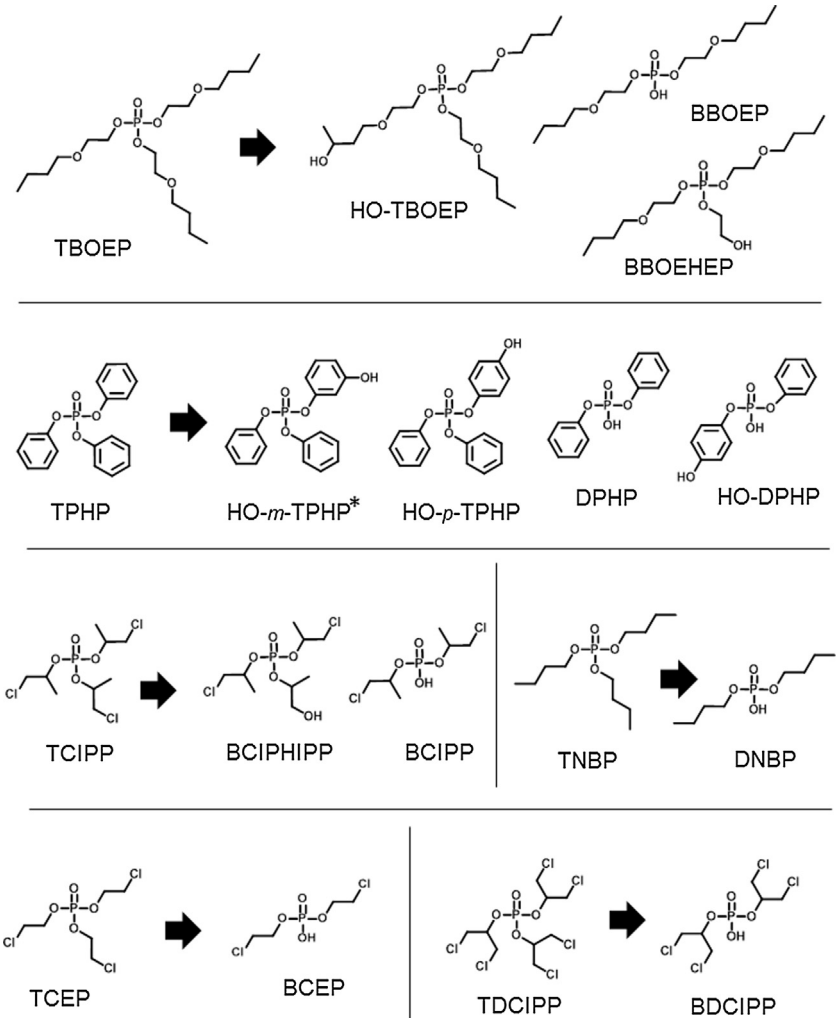

Fig. 1. Chemical structures of the 12 OPFR-metabolites used in the present study.
*HO-*m*-TPHP was confirmed as a metabolite of TPHP in chicken embryonic hepatocytes (Su et al., 2015), but not in *in vitro* metabolizing study using human liver preparations.