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Characterization of Individual Isopropylated and *tert*-Butylated Triarylphosphate (ITP and TBPP) Isomers in Several Commercial Flame Retardant Mixtures and House Dust Standard Reference Material SRM 2585

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Abstract

Since the phase-out of pentaBDE in the early 2000s, replacement flame-retardant mixtures including Firemaster 550 (FM 550), Firemaster 600 (FM 600), and organophosphate aryl ester technical mixtures have been increasingly used to treat polyurethane foam in residential upholstered furniture. These mixtures contain isomers of isopropylated and *tert*-butylated triarylphosphate esters (ITPs and TBPPs), which have similar or greater neuro- and developmental toxicity compared to BDE 47 in high-throughput assays. Additionally, human exposure to ITPs and TBPPs has been demonstrated to be widespread in several recent studies; however, the relative composition of these mixtures has remained largely uncharacterized. Using available authentic standards, the present study quantified the contribution of individual ITP and TBPP isomers in four commercial flame retardant mixtures: FM 550, FM 600, an ITP mixture, and a TBPP mixture. Findings suggest similarities between FM 550 and the ITP mixture, with 2-isopropylphenyl diphenyl phosphate (2IPPDPP), 2,4-diisopropylphenyl diphenyl phosphate (24DIPPDPP), and bis(2-isopropylphenyl) phenyl phosphate (B2IPPPP) being the most prevalent ITP isomers in both mixtures. FM 600 differed from FM 550 in that it contained TBPP isomers instead of ITP isomers. These analytes were also detected and quantified in a house dust standard reference material, SRM 2585, demonstrating their environmental relevance.

Graphical abstract

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b04179.

A figure showing a total ion chromatogram of the TBPP mixture. Two tables showing method detection limits and matrix spike recoveries. (PDF)

The authors declare no competing financial interest.

INTRODUCTION

In the early to mid-2000s, the use of polybrominated diphenyl ethers (PBDEs) was globally phased out due to concerns about their persistence, bioaccumulation, and potential toxicity (PBT). One PBDE commercial mixture, PentaBDE, was primarily used in furniture as a flame retardant to meet residential flammability standards; however, new flame retardant chemicals and mixtures have since entered the market. Recent studies by our group demonstrate that many of these new flame retardant mixtures contain alkylated organophosphate aryl esters and, in particular, several types of isopropyl- and *tert*-butyl-triphenyl phosphates (ITPs and TBPPs, respectively; Figure 1).^{1,2} In addition to being used as flame retardants, both ITPs and TBPPs are used in hydraulic fluids and as plasticizers in a variety of materials.^{3,4} In our previous research, we identified ITPs in a common commercial flame retardant mixture (Firemaster 550, FM 550), a mixture of organophosphate and brominated flame retardants, and an ITP mixture.⁵ Similarly, we identified TBPPs in Firemaster 600 (FM 600) and in a mixture with TPHP that we refer to as the TBPP mixture.² Despite their documented neuro- and developmental toxicity, there is currently very little information regarding human exposure to ITPs and TBPPs and limited documentation of the presence of these isomers in environmental samples.^{6,7} This pressing lack of data has been recognized by the U.S. Environmental Protection Agency (EPA), which recently prioritized ITPs as one of five fast-tracked PBT chemicals for further assessment under the 2016 amendment to the Toxic Substances Control Act (TSCA).⁸ Previous research conducted by our laboratory has demonstrated that exposure to ITP chemicals is very common in the U.S. population and that exposure ranges considerably among individuals, likely based on differential exposure to flame retardant and plasticizer-treated consumer products. For instance, Hammel *et al.* detected ITPs on silicone wristbands worn by human participants with 100% frequency ($n = 40$).⁹ Furthermore, out of five organophosphate flame retardant (PFR) metabolites measured in urine in this study, mono-isopropylphenyl phenyl phosphate (mono-ipPPP), a confirmed metabolite of ITPs, had the highest geometric mean concentration (2.6 ng/mL). Another recent study detected mono-ipPPP in 98% of all tested urine samples ($n = 48$) and found that, on average, levels of urinary mono-ipPPP were 1.2 times higher in children than in their mothers.¹⁰ Other recent studies have used commercial flame-retardant mixtures containing ITPs and TBPPs to assess toxicological endpoints, and yet the composition of these mixtures has not been well-described. For example, FM 550 has been shown to be endocrine disrupting and potentially obesogenic in rats, and exposure in fathead minnows resulted in significant DNA damage.^{11,12} The ITP and TBPP commercial mixtures have been found to disrupt *C. elegans* larval

development, zebrafish embryonic development, and zebrafish behavior at concentrations in the low- μ M range.^{6,13} The TBPP mixture has also been shown to elevate estradiol serum levels, alter reproductive cycles, and elicit cholesteryl lipidosis in adrenocortical and ovarian interstitial cells of exposed female rats.¹⁴ However, because there have been no studies that identify the composition and ITP & TBPP isomer profile of these commercial mixtures, it is difficult to attribute toxicological findings to a specific component(s) of the mixture. Such information will give context to toxicological studies using these mixtures, and give direction to future exposure studies. Moreover, there is considerable confusion in the flame retardant literature regarding the naming and acronyms associated with these compounds, and these mixtures also have a myriad of technical/brand names associated with them from multiple manufacturers (Table 1). For the purposes of this study and to avoid future confusion, individual ITP and TBPP isomer acronyms are used according to their practical abbreviations (PRABs).¹⁵ Individual standards for these isomers have become available in the past year, and the present study attempts to alleviate confusion regarding these compounds. Here, we characterize the ITP and TBPP isomer profiles present in FM 550 and FM 600 and two other types of organophosphate commercial mixtures that are currently available on the market.

EXPERIMENTAL SECTION

Materials

Individual, authentic standards of 2-isopropylphenyl diphenyl phosphate (2IPDP), 3-isopropylphenyl diphenyl phosphate (3IPDP), 4-isopropylphenyl diphenyl phosphate (4IPDP), 2,4-diisopropylphenyl diphenyl phosphate (24DIPDP), bis(2-isopropylphenyl) phenyl phosphate (B2IPDP), bis(3-isopropylphenyl) phenyl phosphate (B3IPDP), bis(4-isopropylphenyl) phenyl phosphate (B4IPDP), bis(2,4-diisopropylphenyl) phenyl phosphate (B24DIPDP), tris(3-isopropylphenyl) phosphate (T3IPDP), tris(4-isopropylphenyl) phosphate (T4IPDP), 4-*tert*-butylphenyl diphenyl phosphate (4tBPDP), bis(2-*tert*-butylphenyl) phenyl phosphate (B2tBPDP), bis(4-*tert*-butylphenyl) phenyl phosphate (B4tBPDP), ¹³C-TPHP, ¹³C-EH-TBB, and ¹³C-BEH-TEBP were purchased from or provided by Wellington Laboratories (Guelph, Ontario, Canada). TPHP (99% pure) and Tris(4-*tert*-butylphenyl) phosphate (T4tBPDP) were purchased from Sigma-Aldrich (Saint Louis, Missouri). High-performance liquid chromatography grade isooctane was purchased from Honeywell Burdick and Jackson (Muskegon, Michigan). FM 550 was provided by Chemtura (lot no. 77000DI8P), the commercial mixture containing only ITPs was purchased from Jinan Great Chemical Industry Co. (no lot information provided by manufacturer), and the TBPP mixture was produced by Ubichem and obtained from the National Toxicology Program (lot no. M062011NS) for research purposes as part of a materials transfer agreement. The commercial FM 600 mixture itself was not available for analysis; for this reason, a block of FM 600 treated polyurethane foam was obtained from a North Carolina foam manufacturer for analysis.

Commercial Mixture Preparation

Each flame retardant mixture preparation was weighed using a Mettler Toledo A21 Comparator microbalance and dissolved in isooctane to make three low-concentration (~1

µg/mL) and three high-concentration (~5 µg/mL) solutions. FM 600-treated foam was weighed (100 mg) and extracted in triplicate using dichloromethane according to previously published methods.¹⁶ For the analysis here, the concentration of FM 600 in the foam was assumed to be 4% by weight, similar to measurements made previously in polyurethane foam.¹⁶ Prior to analysis, each solution was spiked with the appropriate internal standards.

SRM 2585 Analysis

Approximately 300 mg of SRM 2585 (National Institute of Standards and Technology, Gaithersburg, MD) was spiked with ¹³C-TPHP and extracted three times in dichloromethane with sonication. SRM extracts ($n = 4$) were cleaned using a Supelclean ENVI-Florisor SPE cartridges (6 mL; 1.0 g; Supelco, Bellefonte, Pennsylvania) using previously published methods.¹⁷ ITP and TBPP isomers were eluted using 10 mL of ethyl acetate. Eluents were concentrated under nitrogen and solvent-exchanged to hexane prior to analysis.

Component Quantification

EH-TBB and BEH-TEBP were quantified with previously described GC/ENCI-MS methods using ¹³C-EH-TBB and ¹³C-BEH-TEBP as internal standards.⁵ TPHP and individual ITP and TBPP isomers were quantified with the previously described GC/EI-MS methods using ¹³C-TPHP as an internal standard.¹⁶ Briefly, the quantification of TPHP, ITP, and TBPP isomers was performed using an Agilent (Wilmington, DE) gas chromatograph (model 7890A) mass spectrometer (model 5975C) operating in electron-impact (EI) mode. Pressurized temperature vaporization (PTV) injection was employed in the inlet, and a 0.25 mm (I.D.) × 30 m fused silica capillary column coated with 5% phenyl methylpolysiloxane (J&W Scientific, 0.25 µm film thickness) was used in the GC to resolve the analytes. Helium was used as the carrier gas in the GC with a constant flow rate of 1.3 mL/min. The inlet was set to a temperature of 80 °C for 0.3 min and then ramped to 300 °C at a rate of 600 °C/min to efficiently transfer samples to the head of the GC column. The GC oven was held at 80 °C for 2 min, ramped to 250 °C at 20 °C/min, ramped to 260 °C at 1.5 °C/min, then ramped to 300 °C at 25 °C/min and held at 300 °C for 20 min. The transfer line temperature was held at 300 °C, and the ion source was maintained at 200 °C. Table 2 outlines the m/z ions and retention times used for the quantification of ITP and TBPP isomers.

Quality Assurance and Quality Control

Laboratory blanks were included in both the commercial mixture preparation ($n = 3$) and the SRM 2585 analysis ($n = 6$). All of the samples were blank-corrected using the average blank level. Method detection limits (MDLs) were calculated using 3 times the standard deviation of the average lab blanks and were normalized to the amount of dust extracted for the SRM 2585 analysis. MDLs are reported in Table S1 for each isomer. Linear calibration curves were constructed using a five-point calibration for the quantification of each isomer, with r^2 values ranging from 0.9985 to 0.9999. To evaluate the recoveries of ITP and TBPP isomers in house dust, a matrix spike experiment was performed using a low and a high dose of isomers. Samples of SRM 2585 were spiked with either a low dose (50 ng) or a high dose (500 ng) of all ITP and TBPP isomers and extracted, in triplicate, according to the methods detailed above. ¹³C TPHP was added prior to GC-MS analysis to quantify recovery of each

analyte. Recoveries ranged from $72.4 \pm 1.0\%$ to $109.9 \pm 10.7\%$ (see Table S2) for the various isomers.

RESULTS AND DISCUSSION

Firemaster 550 and ITP Mixture

The percent composition of each ITP isomer present in the FM 550 and ITP mixture, respectively, is shown in Table 3 and is presented on a percent *w/w* basis. The FM 550 mixture consisted of approximately 44% of the brominated components, with the remaining mass composed of TPHP and the ITPs. Like the FM 550 mixture, the ITP mixture consisted of very similar ITP isomers, particularly TPHP, 2IPDP, and B2IPDP, which were the dominant compounds in the mixture. Notably, the contribution of the individual organophosphate components in the ITP mixture is roughly twice that of their contribution in FM 550, suggesting a common ITP formulation and/or synthesis (Figure 2). T4IPDP was not detected in either of the mixtures. Our analyses accounted for $97.8 \pm 1.5\%$ of FM 550 and $97.0 \pm 5.3\%$ of the ITP mixture.

Firemaster 600 and TBPP Mixture

The percent composition of each TBPP isomer present in the FM 600 and TBPP mixture is shown in Table 4 and is presented on a percent *w/w* basis. In the case of the FM 600 analysis, we used foam as a standard, which could be a limitation affecting the accuracy of our characterization. We assumed that the foam was treated with 4% FM 600 by weight.¹⁶ Using that assumption, the foam treated with FM 600 contained approximately 40% of the brominated compounds, with the remaining mass being composed of TPHP and TBPP isomers. The organophosphate formulation of FM 600 was dominated by B4tBPP and T4tBPP isomers, with relatively little TPHP or 4tBPDPP present in the mixture. In contrast, the TBPP mixture was composed of higher percentages of TPHP and 4tBPDPP (Figure 3). In total, we could account for $95.3 \pm 5.2\%$ of the FM 600 mixture and $79.5 \pm 1.1\%$ of the TBPP mixture. 2tBPDPP and B2tBPP were not detected in either FM 600 or the TBPP mixture, but chromatographic peaks for 3tBPDPP and B3tBPP were observed for both mixtures (Figure S1). These peaks were presumed to be the *meta*-isomers based on their mass spectra ($m/z = 382$ for 3tBPDPP and $m/z = 438$ for B3tBPP), retention times between *ortho*- and *para*-substituted isomers, and lack of matching with the *ortho*- and *para*-substituted isomers. Standards of *meta*-TBPP isomers are not commercially available, so quantification of 3tBPDPP and B3tBPP was not possible.

Concentrations in SRM 2585

Levels of ITP and TBPP isomers in house dust standard reference material 2585 are shown in Table 5. Deuterated TPHP was used as a recovery standard, and the percent recovery of ¹³C-TPHP was $95.7 \pm 6.2\%$. Measured concentrations of TPHP were 1002.13 ± 52.88 ng/g, similar to those reported previously.^{17,18} 2IPDP, 4IPDP, 4tBPDPP, and B4BPDPP were the most prevalent ITP and TBPP isomers in SRM 2585, all of which had concentrations of >200 ng/g. In contrast, T3IPDP, T4IPDP, 2tBPDPP, and B2tBPP were not detected in SRM 2585 above MDL levels (0.67, 1.07, 0.61, and 0.98 ng/g, respectively.) While individual isomer levels are lower than that of other organophosphate flame retardants, ΣITP and

ΣTBPP levels (1098.28 and 762.09 ng/g, respectively) approach and, in some cases, exceed levels that have been reported for TPHP, TCEP, and TCPP previously.^{18,19} These concentrations suggest the potential for chronic human exposure to ITP and TBPP isomers via inadvertent dust ingestion and hand-to-mouth contact. In addition, the fact that SRM 2585 was prepared from dust collected in 1993 and 1994 suggests that ITP and TBPP use had been ongoing before PentaBDE was phased out. Interestingly, it has been reported that the ITP isomers were originally developed as tricresyl phosphate replacements due to the erratic supply and increasing cost of cresols in the 1970s. Similarly, TBPP isomers were introduced in the 1970s for use in hydraulic applications.⁴ However, to the authors' knowledge, this is the first demonstration of their presence in SRM 2585.

Implications

There is growing evidence that the organophosphate components in these mixtures may elicit adverse health effects, and human exposure to them has been demonstrated to be widespread in recent years.^{9,20–22} A recent epidemiological study found a significant association between the urinary metabolite of ITPs and decreases in successful pregnancy outcomes.²³ Identification of the predominant ITP and TBPP isomers in commonly used commercial flame retardant mixtures will aid in hazard characterization and the ongoing, prioritized risk assessment of these compounds by the U.S. EPA. With the exception of FM 600, all of these mixtures contain ~20% TPHP, a compound with known toxicological activity.^{24–26} Interestingly, FM 600 has very low TPHP content, potentially due to distillation or other treatment following isomerization. The four mixtures in this study are complex, containing many different components, each with potentially unique health hazards and physicochemical properties. These findings should be taken into account when designing and interpreting toxicological studies. Furthermore, it should be recognized that there are many different manufacturers of these mixtures, and mixture formulations may differ among manufacturers and across lots. It is possible that the minor components of the ITP mixture (<1% w/w) are synthesis byproducts and might also vary from lot to lot. In addition, the flame retardant mixtures analyzed here are not the only sources of ITP and TBPP isomers to the environment; ITP and TBPP isomers also have uses as plasticizers and in hydraulic fluids.^{27–29} Future work should focus on identifying sources of these isomers, the degree of human exposure to these isomers, and the toxicological assessment of individual isomers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
 (A) General structure of an ITP isomer. (B) General structure of a TBPP isomer.
 Isopropylation and *tert*-butylation can occur at the *ortho*, *meta*, or *para* position and the degree of substitution varies among isomers.

Figure 2.

A comparison of the contributions of individual components in the FM 550 and ITP mixture suggests a common ITP formulation. FM 550 is shown in black, and the ITP mixture is shown in white. The two brominated components of FM 550, EH-TBB and BEH-TEBP, comprise ~43.6% of the mixture and are absent in the ITP mixture.

Figure 3.

A comparison of the contributions of individual components in the FM 600 and TBPP mixture suggests a disparate TBPP formulation. FM 600 is shown in gray, and the TBPP mixture is shown in white. Strikingly, FM 600 contained relatively little TPHP and mono-substituted TBPP isomer compared to the TBPP mixture. The concentration of FM 660 in the foam was assumed to be 4% by weight.¹⁶

Table 1

Acronyms and Brand Names Associated with ITP and TBPP Isomers

mixture	acronym or brand name	reference or manufacturer
ITP	ITP	30–32
ITP	IPP	13, 33, 34
ITP	IPTP	35
ITP	IPDP	36
ITP	PIP	37, 38
ITP	IPTPP	28
ITP	IPPP	15, 39
ITP	Durad 110, 150, 220, and 300	Lanxess ^a
ITP	Reofos 35, 50, 65, and 95	Great Lakes Solutions ^b
ITP	Phosflex 31L	ICL-IP ^c
ITP	CELLTECH IPPP	Cellular Technologies International, Inc. ^d
ITP	TBPP	1, 2
TBPP	BPDP	40–42
TBPP	BTP	3, 14
TBPP	Durad 110B, 150B, and 220B	Lanxess ^a
TBPP	Phosflex 71B	ICL-IP ^c

^aFrom http://add.lanxess.com/fileadmin/user_upload/Synthetic_base_fluids_lubricant_additives_04-2017.pdf.

^bFrom <http://greatlakes.com/deployedfiles/ChemturaV8/GreatLakes/Flame%20Retardants/FR%20Brochures/Flame%20Retardants%20Product%20Guide.pdf>.

^cFrom <http://icl-ip.com/wp-content/uploads/2013/08/FR-Brochure-2013-web.pdf>.

^dFrom <http://cellulartechology.us/flame-retardant-plasticizers-puviny/>.

Table 2*m/z* Ions and Retention Times Used for Quantification of ITP and TBPP Isomers

ITP or TBPP isomer	<i>m/z</i> quantifier	<i>m/z</i> qualifier	retention time (min) ^a
TPHP	325	326	14.49
2IPDPDP	251	368	15.82
3IPDPDP	353	368	17.09
4IPDPDP	353	368	17.71
24DIPDPDP	145	160	18.21
B2IPPPP	251	410	17.46
B3IPPPP	395	410	19.04
B4IPPPP	395	410	20.18
B24DIPPPP	145	160	21.78
T3IPPP	452	438	21.10
T4IPPP	452	438	23.56
2tBPDPP	367	382	17.03
4tBPDPP	367	382	18.64
B2tBPPP	423	438	19.38
B4tBPPP	423	438	21.52
T4tBPP	479	494	26.14

^a As observed on a 30 m DB5-MS Agilent J&W column.

Table 3Percent Composition (*w/w*) of Individual ITP Isomers in FM 550 and the ITP Mixture^a

Component Name	Structure	Mass Fraction (<i>w/w</i>) in FM 550	Mass Fraction (<i>w/w</i>) in ITP Mix
EH-TBB		29.7 ± 0.3%	none
BEH-TEBP		13.9 ± 0.1%	none
TPHP		19.8 ± 0.1%	44.6 ± 0.9%
2IPDPDP		11.8 ± 0.2%	26.9 ± 1.1%
3IPDPDP		1.7 ± 0.1%	0.3 ± 0.1%
4IPDPDP		2.3 ± 0.2%	4.9 ± 0.8%
24DIPDPDP		11.0 ± 0.1%	7.2 ± 0.9%
B2IPPPP		5.1 ± 0.2%	11.1 ± 0.9%
B3IPPPP		2.1 ± 0.1%	0.8 ± 0.2%
B4IPPPP		0.3 ± 0.1%	1.1 ± 0.3%
T3IPPP		0.1 ± 0.04%	0.1 ± 0.05%

^aThe total percentage accounted for was $97.8 \pm 1.5\%$ for FM 550 and $97.0 \pm 5.3\%$ for the ITP mix.

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Table 4Percent Composition (*w/w*) of Individual TBPP Isomers in FM 600-Treated Foam and the TBPP Mixture^a

Component Name	Structure	Mass Fraction (<i>w/w</i>) in FM 600-treated foam	Mass Fraction (<i>w/w</i>) in TBPP Mix
EH-TBB		25.0 ± 1.4%	none
BEH-TEBP		16.3 ± 0.6%	none
TPHP		1.7 ± 0.1%	23.7 ± 0.3%
4tBPDPP		2.6 ± 0.4%	35.5 ± 0.3%
B4tBPPP		26.4 ± 1.9%	17.8 ± 0.4%
T4tBPP		22.0 ± 0.7%	2.5 ± 0.1%

^aThe total percentage accounted was 95.3 ± 5.2% for FM 600-treated foam and 79.5 ± 1.1% for the TBPP mixture. The concentration of FM 600 in the foam was assumed to be 4% by weight¹⁶

Table 5ITP and TBPP Levels in House Dust SRM 2585 ($n = 4$)

ITP or TBPP isomer	Avg. (ng/g dust)	Std. Dev. (ng/g dust)
TPHP	1002.13	52.88
2IPDPDP	475.48	12.20
3IPDPDP	32.28	3.79
4IPDPDP	224.75	22.56
24DIPDPDP	120.29	15.15
B2IPPPP	110.25	9.55
B3IPPPP	88.76	14.04
B4IPPPP	30.21	14.06
B24DIPPPP	16.26	3.38
T3IPPP	N.D.	--
T4IPPP	N.D.	--
ΣITPs	1098.28	56.40
2tBPDPP	N.D.	--
4tBPDPP	405.27	92.28
B2tBPPP	N.D.	--
B4tBPPP	332.79	32.59
T4tBPP	24.03	2.24
ΣTBPPs	762.09	122.33

^aNot detected.