A NOVEL BROMINATED TRIAZINE-BASED FLAME RETARDANT (TTBP-TAZ) IN PLASTIC CONSUMER PRODUCTS AND INDOOR DUST

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Introduction

Restrictions on the use of PBDEs have resulted in an increased use of alternative flame retardants (FRs). In recent years, a number of new FRs have been identified in consumer products and in environmental samples, e.g. "V6" (1), resorcinol bis-(diphenylphosphate) (PBDPP) and bisphenol A bis (diphenylphosphate) (BPA-BDPP) (2). The content of these compounds in daily products is still largely unknown. When conducting non-target analysis, alternative techniques can be used for screening and for providing information on the identity of the compound. In this study, we report for the first time the presence of 2,4,6-tris(2,4,6-tribromophenoxy)-1,3,5-triazine (TTBP-TAZ), CAS number 25713-60-4, in plastic parts of electrical and electronic equipment and in indoor dust. TTBP-TAZ is a novel brominated triazine-based FR that is used in acrylonitrile butadiene styrene (ABS) and high impact polystyrene (HIPS) and could be used as replacement of the banned PBDEs in these polymers. We identified this unknown brominated compound by untargeted screening using a fast screening method that is based on direct probe (DP) ambient mass spectrometry coupled to atmospheric pressure chemical ionization and high resolution mass spectrometry (APCI-HRTOF-MS). This method, recently developed by our research group (3), does not require sample preparation or chromatographic separation that could hamper the detection of potentially unknown compounds, since the solid sample is introduced directly into the MS source. Also, we employed a solvent extraction method followed by liquid chromatography (LC)-APCI-HR-TOF-MS analysis for the confirmation of the screening results and for quantifying the levels of TTBP-TAZ in plastic and in dust samples. Finally, we investigated the *in vitro* metabolism of TTBP-TAZ using human liver microsomes (HLM) to identify the oxidative metabolites formed and possible candidate biomarkers for human exposure. The main results of this study have been recently published (4) and disseminated by the scientific media (5,6).

Materials and methods

The target compound 2,4,6-tris(2,4,6-tribromophenoxy)-1,3,5-triazine was obtained from Accustandard (New Haven, CT, USA) in ampoules of ~100 µg mL^{-1} in toluene (97% purity). Another TTBP-TAZ standard, which was only tested for the presence of impurities, was obtained from TCI Europe N.V. (Tokyo, Japan). A microTOF II with resolution >16,500 FWHM equipped with an LC-APCI II source (Bruker Daltonics, Bremen, Germany) was used for TTBP-TAZ analysis. The source was also equipped with a direct probe assembly (Bruker Daltonics) which is mounted in the place of the nebulizer sprayer on top of the vaporizer heater. A Kinetex core-shell LC C_{18} column (2.1 mm x 100mm x 2.6 µm), obtained from Phenomenex (Torrance, CA, USA) was used.

Rapid screening of TTBP-TAZ in plastic samples

A small amount of plastic sample (few milligrams) was introduced directly into the MS source through a capillary glass probe by a commercial direct probe assembly. The glass probes were loaded with the sample by scratching the surface of the solid plastic product with the probe to release small particles. Main particles outside the probe were removed with a lint-free cotton cloth to prevent the contamination of the source. A drop of ~5 µL of calibration solution (Agilent APCI tune mix) was added at the outer surface of the probe before introducing it into the MS source for internal MS calibration.

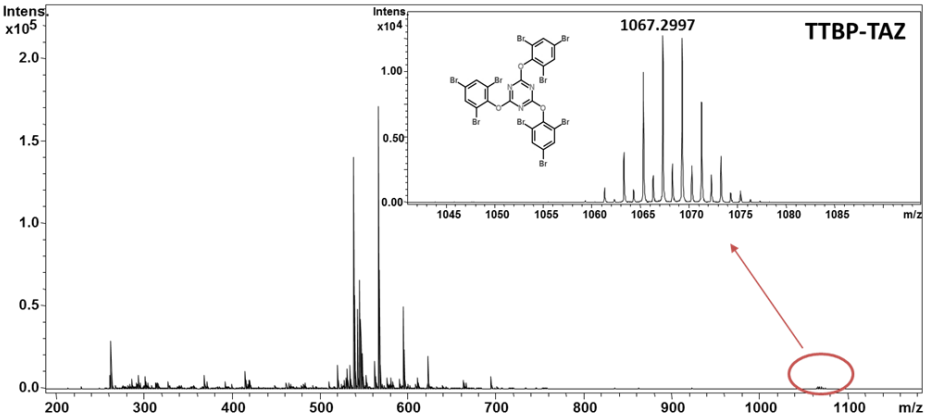

Figure 1. DP-APCI(+)-HRTOF-MS spectra of a power board sample containing TTBP-TAZ.

Confirmation and quantification method

Small pieces of the plastic samples (~50 mg) were extracted with 20 mL dichloromethane by shaking for 24 h and sonication for 10 min. The extracts were diluted with methanol:THF 90:10 v/v for 10-100 times as required. The diluted extracts were ultra-centrifuged in Eppendorf microtubes (10,000 rpm, 5 min) for precipitation of solids and aliquots of 2.5 µL were injected in the LC system. The extraction of the dust (~ 50 mg) was performed in two solvent extraction steps, first with acetone (10 mL) and then with toluene (10 mL). Each extraction step consisted of vortexing for 1 min and 15 min of ultrasonication followed by centrifugation (3000 rpm, 5 min) to precipitate solids. The combination of solvents was selected on the basis of our previous experience for obtaining good extraction efficiency for PBDEs, new BFRs and PFRs. The combined clear supernatant was evaporated almost until dryness and reconstituted in 2 mL methanol:THF 90:10 v/v (vortexing 1 min). The final extracts were ultracentrifuged (10,000 rpm, 5 min) in Eppendorf microtubes to precipitate remaining particles and aliquots of 2.5 µL were injected in the LC-APCI-HRTOFMS system. TTBP-TAZ was quantified both in plastic and in dust samples by external solvent-based calibration (standards prepared in methanol:THF 90:10 v/v).

Table 1. Main ions of TTBP-TAZ in the APCI source

APCI mode	Ion	Ion molecular formula	[a]Most intense isotopomer	[a]Monoisotopic neutral mass
Positive	$[M+H]^+$ (quantifier)	$C_{21}H_7Br_9N_3O_3$	1067.3052	1058.3059
Negative	$[M-C_6H_2Br_3-H]^-$ (qualifier)	$C_{15}H_4Br_6N_3O_3$	753.5287	747.5352

[a]Values calculated with Compass Isotope pattern tool of Data analysis software (Bruker Daltonics)

***In vitro* metabolism of TTBP-TAZ**

The reaction mixture consisting of 100 mM phosphate buffer (pH 7.4), pooled HLM (0.5 mg/mL, final protein concentration) and TTBP-TAZ dissolved in acetonitrile:THF 85:15 v/v (10 µM, final concentration) in a final volume of 0.99 mL was prepared on ice. Tubes were pre-incubated in a shaking water bath at 37ºC for 5 min.

Reaction was initiated by addition of 10 µL of NADPH diluted in buffer (1 mM final concentration) and quenched after 1h by using 1.0 mL of ice-cold methanol. Tubes were vortexed for 30 s. Formed metabolites were liquid-to-liquid extracted twice using 4 mL of methyl-t-butyl ether and hexane (1:1, *v/v*). Pooled extracts were evaporated to dryness and resuspended in 100 µL of methanol. Blank and negative control samples were routinely prepared. The formation of glucuronidated and sulfated metabolites of hydroxylated metabolites of TTBP-TAZ was also investigated. After incubating TTBP-TAZ as described above, the supernatant was transferred into a new set of tubes containing either human liver microsomes (0.5 mg mL^{-1}, final protein concentration) and UDPGA (for glucuronidation) or human liver cytosol (0.5 mg mL^{-1}, final protein concentration) and PAPS (for sulfation). Samples were incubated for 1 h and processed as described above. Cofactor negative control samples were also prepared.

Results and discussion

TTBP-TAZ was found in most of the electric/electronic equipment purchased in 2012 (8 of 9 samples) and not detected in any of the children toys samples (Table 2). Concentrations ranged from 0.01-1.9 % weight/weight (w/w). The highest level was found in a television. A strong correlation was found between the concentration of TTBP-TAZ and 2,4,6-tribromophenol (2,4,6-TBP) in the plastics (Pearson correlation, $r = 0.951$, $p < 0.001$, n=8). This may indicate that the use of TTBP-TAZ is a potential source of contamination of 2,4,6-TBP, for which toxic properties have been already reported (7).

Table 2. TTBP-TAZ concentration ranges in weight percentage (% w/w) in plastic consumer products purchased in 2012

Product	Number of samples containing TTBP-TAZ	Concentration range (% w/w)
Electrical power boards and adaptors (*n*=4)	4	(0.01-0.8)
Children toys (*n*=4)	0	-
Televisions (*n*=2)	2	(0.06, 1.9)
Other household appliances (n=3)	2	(0.3, 0.6)

TTBP-TAZ was present in 9 of 17 house dust samples collected in the Netherlands, with the highest concentrations in the dust collected from the surfaces of electronic equipment (mainly computers and televisions; Table 2). Concentrations were in the range of 1,070-22,150 ng-g^{-1} on equipment and between 220 and 3950 ng-g^{-1} around the equipment. These findings suggest that the compound may be released from electronic equipment into the environment.

Table 3. TTBP-TAZ average concentrations and ranges in ng g^{-1} in house dust

Sampling site	Number of samples containing TTBP-TAZ	Median Concentration (ng g^{-1})	[a]Range (ng g^{-1})
On electronics (*n*=8)	4	535	(1070-22150)
Around electronics (*n*=7)	4	220	(220-3950)
In floor dust (*n*=2)	1	-	(<20-160)

[a]Quantitation limit 60 ng g^{-1}

The levels of TTBP-TAZ in house dust collected in 2011 (<20-3950 ng g^{-1}, samples collected on electronics not included) were lower than those reported of TBBPA (535–9730 ng g^{-1};2008 in Belgium, ref. 8) or of TPHP (<150-1798000 ng g^{-1}; 2002-2006 in US, ref. 9) but higher than those of DBDPE (<n.d.- 151 ng g^{-1}; 2007 in Sweden, ref. 10) and of BTBPE (<2.5.- 8.1 ng g^{-1}; 2007 in Sweden, ref. 10) and similar to the levels of V6 (<5-1117 ng g^{-1}; 2009 in US, ref. 1), that are the other alternative flame retardants. The levels of TTBP-TAZ were similar than those recorded in indoor dust for the pentaBDE congeners (e.g. 230-3000 ng g^{-1} of BDE 47; 70-3700 ng $g^{-1}$of BDE 99, ref. 11) but lower than those for decaBDE (120-21000 ng $g^{-1}$of BDE 209; ref. 11).

These results show that TTBPA-TAZ may be ubiquitous in dust from indoor environments in the Netherlands and that people could be exposed to this compound via dust inhalation and ingestion. Plastic products with electronic and/or electrical components, in which TTBPA-TAZ was detected in 8 of the 13 of the samples purchased in supermarkets in 2012, could be a major source of emission to the environment. Further research is needed to provide a more accurate estimation of the human exposure to TTBPA-TAZ through dust ingestion and source apportionment since we only report a limited number of samples.

Finally, a monohydroxylated metabolite (OH-TTBP-TAZ) and 2,4,6-TBP could be detected incubating TTBP-TAZ with human liver microsomes. These preliminary data suggest that the *in vitro* metabolism of TTBP-TAZ proceeds at low pace and produces a very limited number of metabolites, which suggest that TTBP-TAZ might be highly bioaccumulative in humans. While OH-TBBP-TAZ could be used as specific biomarker for human exposure in future biomonitoring studies, the formation of 2,4,6-TBP is relevant due to its reported toxicity (7).

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