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Bisphenol A alternatives in thermal paper from the Netherlands, Spain, Sweden and Norway. Screening and potential toxicity

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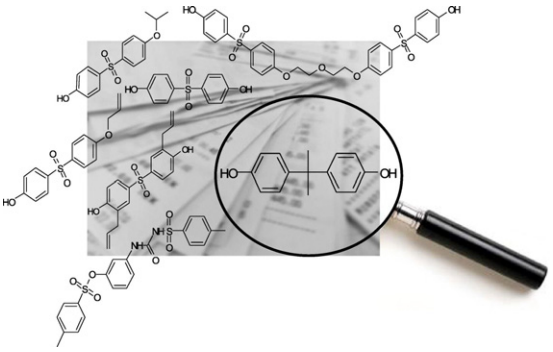
HIGHLIGHTS

- BPA, BPS, Pergafast 201, D-8, D-90, TGSA and BPS-MAE were detected in thermal paper.
- Unreported impurities related to developers were identified.
- BPA alternatives except BPS seem to be far less estrogenic than BPA.
- TGSA and D-8, like BPA, might exert developmental toxicity.

GRAPHICAL ABSTRACT



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ABSTRACT

Thermal paper contains potentially toxic additives, such as bisphenol A (BPA), as a common color developer. Because of its known endocrine disrupting effects, structural analogues to BPA, such as bisphenol S (BPS), D-8 and Pergafast 201, have been used as alternatives, but little is known about the presence and toxicological effects of alternatives other than BPS. In this study, thermal paper is screened by direct probe ambient mass spectrometry (rapid prescreening method not requiring sample preparation) and by liquid chromatography (LC) with high resolution time-of flight (TOF-MS) mass spectrometry. Cash receipts and other thermal paper products (cinema tickets, boarding passes and luggage tags) were analyzed. Besides BPA and BPS, other developers only recently reported (Pergafast 201, D-8) or to the best of our knowledge not reported before (D-90, TGSA, BPS-MAE) were frequently found as well as some related unreported impurities (2,4-BPS that is a BPS related impurity and a TGSA related impurity). To gain some insight into the potential estrogenicity of the detected developers, a selection of extracts was further analyzed using a LC-nanofractionation platform in combination with cell-based bioassay testing. These preliminary results seems to indicate very low or absence of estrogenic activity for Pergafast 201, D-8, D-90, TGSA and BPS-MAE in comparison to BPA and BPS, although further dose-response tests with authentic standards are required to confirm these results. Compounds for which standards were available were also tested for developmental toxicity and neurotoxicity using zebrafish (*Danio rerio*) embryos. TGSA and D-8 induced similar teratogenic effects as BPA in zebrafish embryos. BPS and 2,4-BPS did not induce any developmental effects but 2,4-BPS did alter the locomotor activity at the tested concentration. Our findings suggest that the alternatives used as alternatives to BPA (except BPS) might not be estrogenic. However, TGSA and D-8 showed abnormal developmental effects similar to BPA.

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1. Introduction

Bisphenol A (BPA) is a high production volume chemical and a well-studied endocrine disruptor with estrogenic effects widely reported and reviewed in the literature (Rochester, 2013). Although BPA was initially classified as a weak environmental estrogen, later research showed that it could also exert toxic effects at lower levels and with multidirectional effects (Michałowicz, 2014; Rubin, 2011). There is also emerging concern about BPA acting as a neurodevelopmental toxicant (Kinch et al., 2015; Saili et al., 2012) and that prenatal exposure to BPA may affect child behavior (Perera et al., 2012).

Food-packaging materials and bottles are thought to be the major route for human exposure to BPA due to its use in polycarbonate plastics and epoxy resins (EFSA, 2015). Recently, emphasis has been placed on identifying other possible sources for human exposure to BPA, thermal paper being one of them. BPA is used as a developer in thermal paper and reacts upon applied heat or pressure with a dye to produce the color in a reversible reaction. Since BPA is present in the paper as its unbound monomer it is readily released into the environment. Handling thermal paper products, such as cash receipts, has been suggested to be a potential source for human exposure through dermal transfer (Biedermann et al., 2010; Liao and Kannan, 2011; Mielke et al., 2011; Zalko et al., 2011; Geens et al., 2012; Rocha et al., 2015; Fan et al., 2015; Ehrlich et al., 2014; Hehn, 2016; Birnbaum et al., 2016).

Because of its known endocrine disrupting effects, the use of BPA in the manufacturing of consumer products has been debated. In 2011, the European Commission (EC) restricted the use of BPA for the manufacturing of baby bottles (COMMISSION DIRECTIVE 2011/8/EU, 2011). This resulted in the use of alternatives to BPA, but still little is known about the presence and toxicological effects of these compounds. Due to structural similarities, these alternatives might exhibit a similar toxicological behavior to BPA. In fact, the estrogenic activity of BPS was already reported in 2002 (Chen et al., 2002). Although the acute toxicity and estrogenic activity of BPS was determined as about ten times lower than that of BPA, more recent data suggest that it can also exhibit an estrogenic activity comparable to BPA as well through other mechanisms (Kitamura et al., 2005; Kuruto-Niwa et al., 2005; Grignard et al., 2012). BPS has been widely detected in thermal paper cash receipts (Birnbaum et al., 2016; Pivnenko et al., 2015; Goldinger et al., 2015; Liao et al., 2012) as well as in other paper products (e.g. mailing envelopes, airplane boarding passes and luggage tags) (Liao et al., 2012). BPS has been the most frequently reported replacement to BPA in thermal paper, however, other alternatives have also been very recently detected and reported in the environmental literature, namely Pergafast 201 and D-8 (Goldinger et al., 2015).

On April 2016, the EC proposed a draft amendment to the REACH (Regulation (EC) No. 1907/2006) to set a limit of 0.02% in weight for BPA in thermal paper. As a consequence, the use of alternatives is expected to increase in the market. Concern has also been raised about BPS, for which the EC demands more data on its use in thermal paper and its toxicity. The US Environmental Protection Agency (EPA) recently proposed a list of 19 BPA alternatives for use in thermal paper (US environmental Protection Agency, 2015). The list includes BPS and some compounds that have been already used as alternatives for BPA in the market.

In the present study, paper products from The Netherlands, Norway, Spain and Sweden ($n = 141$) is screened by using different analysis techniques in order to identify and discuss the main developers used in the market and to which extent new alternatives are used in comparison with BPA. High resolution TOF-MS with direct probe injection-atmospheric pressure chemical ionization (DIP-APCI) and with LC-TOF-MS with both electrospray (ESI) and APCI ionization sources were employed to cover a wide polarity range of developers. DIP-APCI, as other ambient mass spectrometry techniques, does not require a time-consuming sample preparation or chromatographic separation and allows rapid screening. Both suspect and non-target screening approaches were employed for the identification of developers. Together with BPA and BPS, other developers only recently reported (Pergafast 201, D-8) or to the best of our knowledge not reported before (D-90, TGSA, BPS-MAE) were frequently found as well as some related impurities. Finally, to gain insight into the potential toxicity of the BPA alternatives, estrogenic activity tests were carried out. For this purpose, a LC-nanofractionation-MS platform combined with bioassay testing using an ER-LUC gene reporter assay was used (Jonker et al., 2015). The method was applied to some representative samples containing the main developers detected. Furthermore, the developmental and neuro- toxicity of the identified developers (BPA, BPS, 2,4-BPS, TGSA and D-8) were tested as individual compounds using zebrafish embryos.

2. Experimental section

2.1. Chemical and reagents

Methanol and acetone were acquired from J.T Baker® (Center Valley, USA), acetonitrile from Sigma Aldrich (Zwijndrecht, The Netherlands), tetrahydrofuran from Biosolve (Valkenswaard, The Netherlands), dichloromethane (Picograde) from Promochem® (Wesel, Germany) and dimethyl sulfoxide (AcroSeal™) from ACROS Organics, Fisher Scientific (Loughborough, UK). Milli-Q water was obtained from ultrapure water purification Q-Pod system (Millipore, Bedford, USA). Bisphenol A (BPA) (CAS 80-05-7, purity ≥ 99%) and Bisphenol S (BPS) (CAS 80-09-1, purity 98%) were obtained from Sigma Aldrich. 2,4-Bisphenol S (2,4-BPS) (CAS 5397-34-2, purity 98%), 4,4′-Sulfonylbis(2-allylphenol) (TGSA) (CAS 41481-66-7, purity 98%) and 4-Hydroxyphenyl 4-Isopropoxyphenyl Sulfone (D-8) (CAS 95235-30-6, purity 98%) were obtained from Toronto Research Chemicals (Toronto, Canada). The internal MS calibration in APCI-mode was made with an APCI-TOF tuning-mix provided by Supelco (Bellefonte, PA, USA). Dulbecco's modified eagle medium (DMEM)/F12 with glutamax, low glucose, phenol free DMEM and charcoal stripped fetal calf serum (FCS) were from Thermofisher (Landsmeer, The Netherlands). Phosphate buffered saline (PBS), streptomycin, penicillin, fetal calf serum (FCS), ethylenediaminetetraacetic acid (EDTA), tris(hydroxymethyl)aminomethane (TRIS), dithiotreitol (DDT), 1,2-cyclohexyldinitrilo-tetraacetic acid (CDTA), glycerol, triton-X100, Co-enzyme A, Luciferin, adenosine triphosphate (ATP) and G-418 were obtained from Sigma Aldrich (Zwijndrecht, The Netherlands).

2.2. Apparatus and sample analysis

The LC system used was an Agilent 1290 infinity LC. An InertSustain C_{18} column (2.1 mm i.d., 100 mm length, 3.0 μm particle size) and an InertSustain C_{18} cartridge guard column (1.5 mm i.d., 10 mm length, 3.0 μm particle size) were obtained from GL Sciences, Inc., USA. The mobile phase consisted of MilliQ (A) and methanol (B) at a flow rate of 0.25 mL min^{-1}. The gradient was as follows: initial 25% B, increased to 95% in 15 min and hold for 10 min and finally re-conditioning for 10 min. The MS system was a microTOF II with resolving power > 16,500 FWHM equipped with LC-ESI, LC-APCI II or direct probe injection (DIP)-APCI source (Bruker Daltonics, Bremen, Germany). The source parameters for ESI and APCI were optimized for a m/z range of 100–1000 and are given in Table S-1. Mass analyzer parameters were set as following: Capillary exit, 90 V; skimmer 1, 30 V; hexapole 1, 23 V; hexapole RF, 120 Vpp; skimmer 2, 23 V; transfer time, 50 μs; pre pulse storage time, 5 μs. Confirmation MS/MS experiments were done on a high resolution QTOF instrument (maxis 4G upgraded with HD collision cell) with resolving power up to 80,000 FWHM equipped with LC-ESI and DIP-APCI source (Bruker Daltonics, Bremen, Germany). Source parameters are the same as described for LC-ESI-MS-HR-TOF and DIP-APCI-HR-TOF. Mass analyzer settings were as follows: funnel 1 RF, 200 Vpp; multipole RF, 400 Vpp; quadrupole ion energy, 5.0 eV; collision RF, 330 and 750 Vpp for m/z below and above 500, respectively; transfer time, 50 and 75 μs for m/z below and above 500, respectively