Chemosphere 93 (2013) 2814–2822

Contents lists available at ScienceDirect

Chemosphere

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

Chlorination of bisphenol A: Non-targeted screening for the identification of transformation products and assessment of estrogenicity in generated water

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HIGHLIGHTS

- Bisphenol A (BPA) was completely removed in 10 min by chlorination in our conditions.
- A non-targeted approach was developed to reveal 21 transformation products.
- Brominated intermediates were reported for the first time for BPA chlorination.
- We evidenced a decrease of estrogenic activity after chlorination by ER-CALUX.

ARTICLE INFO

Article history:
Received 18 April 2013
Received in revised form 15 September 2013
Accepted 23 September 2013
Available online 25 October 2013

Keywords:
Chemical food safety
Bisphenol A
Chlorination
Transformation products
High-resolution mass spectrometry

ABSTRACT

Besides the performance of water treatments on the removal of micropollutants, concern about the generation of potential biologically active transformation products has been growing. Thus, the detection and structural elucidation of micropollutants transformation products have turned out to be major issues to evaluate comprehensively the efficiency of the processes implemented for drinking water treatment. However, most of existing water treatment studies are carried out at the bench scale with high concentrations and simplified conditions and thus do not reflect realistic conditions. Conversely, this study describes a non-targeted profiling approach borrowed from metabolomic science, using liquid chromatography coupled to high-resolution mass spectrometry, in order to reveal potential chlorination products of bisphenol A (BPA) in real water samples spiked at 50 $\mu g\,L^{-1}$. Targeted measurements first evidenced a fast removal of BPA (>99%) by chlorination with sodium hypochlorite (0.8 $mg\,L^{-1}$) within 10 min. Then, the developed differential global profiling approach enabled to reveal 21 chlorination products of BPA. Among them, 17 were brominated compounds, described for the first time, demonstrating the potential interest of this innovative methodology applied to environmental sciences. In parallel to the significant removal of BPA, the estrogenic activity of water samples, evaluated by ER-CALUX assay, was found to significantly decrease after 10 min of chlorination. These results confirm that chlorination is effective at removing BPA in drinking water and they may indicate that the generated compounds have significantly lower estrogenic activity.

1. Introduction

Bisphenol A (BPA), 4,4′-(propane-2,2-diyl)diphenol, is commonly used in the manufacture of polycarbonate plastics and epoxy resins (Staples et al., 1998). It is also used to produce paper, such as thermal paper and carbonless copy paper (Fukazawa et al., 2001). BPA is a high volume production chemical with a global production estimated to over 3 Mt in 2006.

In recent years, BPA has received a great attention due to its extensive industrial use and its potential estrogenic activity (Segner et al., 2003; Kang et al., 2006). Studies reported the occurrence of BPA in some surface waters (Fromme et al., 2002; Stachel et al., 2003), even at $\mu g\,L^{-1}$ levels. Consequently, the removal of BPA in aqueous solutions has been recently extensively investigated (Liu et al., 2009).

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0045-6535/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.chemosphere.2013.09.080

Accordingly, BPA was reported to strongly react with chlorinating agents (Fukazawa et al., 2001; Hu et al., 2002; Yamamoto and Yasuhara, 2002; Gallard et al., 2004; Lee et al., 2004), widely used as disinfectants of finished drinking waters. Indeed, due to its phenolic structure, chlorination takes place on the aromatic moiety by electrophilic substitution at *ortho*-position (Rebenne et al., 1996; Lee et al., 2004) and leads to the formation of different chlorinated BPA congeners, from mono- to tetra-chloroBPA. The formation of other chlorinated products, like trichlorophenol and polychlorinated phenoxy-phenols, was also observed (Hu et al., 2002; Yamamoto and Yasuhara, 2002). However, chlorination experiments, relative to the publications mentioned above, were generally carried out at bench- and/or pilot-scale at high concentration levels (ppm) and in simplified conditions (e.g. deionized, ultrapure or buffered water). More recently, a study investigated the removal of BPA in a drinking water treatment plant and the formation of transformation products (Dupuis et al., 2012). In this case, only a target screening was implemented to detect the presence of chlorinated congeners of BPA. However, in real conditions (real water and ppb or ppt concentration range), the presence of dissolved natural organic and inorganic compounds like bromine (Von Gunten, 2003; Von Gunten and Salhi, 2003) may induce competitive reactions and may lead to the formation of a wider range of transformation products. To reveal these potential chlorination products generated after water treatment, liquid chromatography coupled to high resolution mass spectrometry (LC–HRMS) can be used. The acquisition of full-scan MS profiles with high resolution and high mass accuracy and the subsequent post-acquisition data processing were the basis of this non-targeted screening of compounds generated after treatment. Such an approach has been basically used in the field of metabolomics to reveal unknown metabolites (Courant et al., 2009; Antignac et al., 2011) and has appeared as very promising for environmental analyses (Krauss et al., 2010). A metabolomic-like approach was notably implemented to identify microbial transformation products of pharmaceuticals and pesticides (Helbling et al., 2010). In our group, we have also applied this approach to identify previously unreported transformation products of ethinylestradiol and estrone-sulfate in drinking water (Gervais et al., 2011; Bourgin et al., 2013).

In parallel, the biological activity of the transformation products may be evaluated and compared to that of the parent compound (BPA), in order to address the growing concern about the efficiency of water chlorination in terms of toxicity and estrogenic activity reduction (Rizzo, 2011). Concerning the chlorination of BPA, it appeared that its chlorinated congeners – detected in some chlorinated wastewaters – individually showed estrogen-agonist activities greater than the parent compound (Fukazawa et al., 2002; Kuruto-Niwa et al., 2002; Takemura et al., 2005). This was confirmed in other studies as the estrogenic activity of chlorinated aqueous BPA solution was found to increase in the very first minutes of treatment (Hu et al., 2002; Lee et al., 2004), probably corresponding to the time necessary to form chlorinated BPA congeners, but finally tended to considerably decrease after a long chlorination time (Lee et al., 2004). Consequently, though carried out in particular conditions (high concentration of BPA, ca 10^{-5} M, dissolved in ultrapure water), this latter result allowed to corroborate the effectiveness of a chlorination treatment in reducing the estrogenic activity of BPA solution.

The objective of the present study was to investigate the degradation of BPA by chlorination in drinking waters under treatment conditions as close to reality as possible. To meet this goal, we (i) monitored the decreasing concentration level of BPA after chlorination; (ii) revealed the generated treatment products and identify them by LC–(HR)MS and (HR)MS^n; (iii) proposed a BPA degradation pathway; and (iv) monitored the estrogenic activity of BPA-spiked drinking waters during the chlorination process.

2. Materials and methods

2.1. Reagents and chemicals

Methanol, acetonitrile, dichloromethane, ethanol and glacial acetic acid were HPLC grade and purchased from Carlo Erba Reactif (Val de Reuil, France). Ultra-pure water was produced with a Thermo Scientific Brandstread Nanopure system (Thermo Fischer Scientific, Whaltman, MA, USA). BPA was purchased from Sigma (St. Louis, MO, USA) and deuterated BPA-d_4 was from Riedel-De Haen (Seelze, Germany). Individual stock solutions at 100 mg L^{-1} were prepared in methanol and stored at −20 °C. A working solution at 1 mg L^{-1} was prepared by dilution of the stock solution with ultrapure water and was used to spike water samples with a minimum of organic solvent.

2.2. Water samples and chlorination treatment

The water samples used for the chlorination experiments were collected from a Drinking Water Treatment Plant (DWTP), pre-ozonated then filtered through granular activated carbon. This water was considered free of BPA as no trace was detected above the limit of quantification (10 ng L^{-1}).

Before spiking, the collected water was characterized by pH, Total Organic Carbon (TOC), concentration of bromide ions and alkalinity values of 7.8, 1.3 mg L^{-1}, 50 μg L^{-1} and 4.4 meq L^{-1}, respectively. Once in the lab, samples were prepared by diluting working solution in the collected water to get a final concentration at 50 μg L^{-1}. This concentration was selected as a compromise since it is low enough to be environmentally relevant and high enough to determine the chlorination products.

Both spiked and unspiked water samples were chlorinated in duplicate at 0.8 mg L^{-1} using a sodium hypochlorite commercial solution during 10–120 min in a 20-L glass reactor. This chlorination dose is commonly used in drinking water production. The reaction was conducted at room temperature (20–22 °C) at pH 7.5 adjusted with hydrochloric acid addition, as this pH value is usually measured for natural waters. The samples were quenched using sodium thiosulfate and frozen at −20 °C until analysis.

2.3. Targeted analysis of BPA

For the direct analysis of BPA in water samples, a specified protocol at the sub μg L^{-1} level was implemented (0.01 μg L^{-1}). 1 L of water was submitted to a liquid–liquid extraction. Deuterated BPA-d_4 was added as internal standard. Three successive extractions were operated with respectively 50 mL, 30 mL and 30 mL of dichloromethane. The extracts were gathered and evaporated to dryness. Extracts were finally reconstituted in acetone (200 μL) and finally derivatized with MSTFA (25 μL) for 30 s to get ready for GC–MS analysis.

An Agilent 6890 series GC coupled with an Agilent 5975 mass spectrometer was used. Chromatographic separation was achieved with a DB-5HT column (15 m × 0.25 mm i.d., d_f: 0.1 μm) (Agilent Scientific, USA). Helium was used as carrier gas at a constant flow of 1 mL min^{-1}. Injections (1 μL) were performed using splitless liner with double taper operating in the splitless pulse mode (103 kPa during 1 min). Inlet temperature was fixed at 300 °C. The oven was configured as follows: initial temperature: 50 °C, 40 °C min^{-1} to 285 °C, 10 °C min^{-1} to 295 °C, 50 °C min^{-1} to 320 °C. GC–MS transfer line, source and quadrupole were heated at 300, 200 and 150 °C, respectively. The electron voltage was set at 70 eV. BPA was detected and quantified in single ion monitoring acquisition mode, using the *m/z* 357 ion as diagnostic signal for