Food and Chemical Toxicology 83 (2015) 68–75

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Monitoring of bisphenols in canned tuna from Italian markets

Margherita Fattore [a], Giacomo Russo [b], Francesco Barbato [b], Lucia Grumetto [a, b, *], Stefania Albrizio [a, b]

[a] Consorzio Interuniversitario INBB, Viale Medaglie d'Oro, 305, I-00136 Rome, Italy
[b] Dipartimento di Farmacia, Università degli Studi di Napoli Federico II, Via D. Montesano, 49, I-80131 Naples, Italy

ARTICLE INFO

Article history:
Received 16 December 2014
Received in revised form
19 May 2015
Accepted 20 May 2015
Available online 10 June 2015

Keywords:
Bisphenols
Endocrine disruptors
Canned tuna
Migration
Italian population

ABSTRACT

Monitoring of food contamination from bisphenols is a necessary process for the consumers' risk assessment. A method for the quali-quantitative analysis of Bisphenol A (BPA), Bisphenol B (BPB), Bisphenol A Diglycidyl Ether (BADGE), and Bisphenol F Diglycidyl Ether (BFDGE), by liquid chromatography with fluorescence detection (LC-FD), was performed and validated for their determination in 33 samples of tuna fish, canned in either oil or aqueous medium. Samples were collected in Italian markets. Tuna and the correspondent preservation medium were analyzed separately. Detected levels of bisphenols ranged from 19.1 to 187.0 ng/g in tuna matrix and from 6.3 to 66.9 ng/mL in oil medium. No bisphenols were found in aqueous medium. At least one of the analytes was found in 83% of the tuna samples in oil medium, whereas tuna samples in aqueous medium showed BPA alone in 67% of samples. 21% of the oil medium samples resulted positive for at least one bisphenol. On the basis of measured concentrations and general daily ingestion rate of canned tuna fish, the probable daily intake of BPA for Italian population was calculated.

1. Introduction

Bisphenol A (BPA) and its various analogs are important chemical starting substances in the production of polycarbonate (PC) plastics and epoxy resins, with multiple industrial applications (Glausiusz, 2014). PC is widely used in manufacturing food containers, whereas epoxy resins are used as interior protective lining for food and beverage cans (Sendon Garcia and Paseiro Losada, 2004).

Due to an incomplete polymerization process, residues of bisphenol monomers in PC food containers or epoxy resin coatings can migrate into foods, especially oily food, during storage and processing at high temperatures (Brede et al., 2002; Cao et al., 2011; Geens et al., 2010; Grumetto et al., 2008; EFSA, 2006; Noonan et al., 2011; Rauter et al., 1999; Simoneau et al., 1999; Sungur et al., 2014; Theobald et al., 2000). Furthermore, migration from parts of facilities and/or utensils routinely employed during the food production process may occur under certain conditions (Casajuana and Lacorte, 2003, 2004; Goodson et al., 2004; Grumetto et al., 2013; Guart et al., 2011).

The migration limits fixed by the European Commission are 0.6 mg/kg of food for BPA, 9.0 mg/kg of food for BADGE and its hydroxyl derivatives and 1.0 mg/kg of food for BADGE and its chlorinated derivatives (European Commission, 2005; European Commission, 2011). Moreover, the use of BFDGE has been forbidden (European Commission, 2005) and no information exists about BPB migration limits.

In the January of 2015 EFSA has published a re-evaluation of BPA exposure from diet and other sources (EFSA, 2015). EFSA experts have reduced the safe level of BPA from 50 μg per kilogram of body weight per day (μg/kg of bw/day) to 4 μg/kg of bw/day, although, on the basis of collected data, the exposure risk is clearly under the "tolerable daily intake" (TDI).

The presence of bisphenols (BPs) in food represents one of the most serious chemical contamination problems due to their properties as Endocrine Disrupting Chemicals (EDCs). EDCs are substances that influence synthesis, transport, secretion, action, binding or elimination of natural hormones in the body (García-Arevalo et al., 2014; Jeng, 2014; Le Corre et al., 2015).

Actually, food, and especially canned food, is considered the predominant source of contamination from BPs, mainly from BPA (Le Corre et al., 2015). Due to its large number of applications, BPA is

* Corresponding author. Dipartimento di Farmacia, Via D. Montesano, 49, I-80131 Naples, Italy.
E-mail address: grumetto@unina.it (L. Grumetto).

http://dx.doi.org/10.1016/j.fct.2015.05.010
0278-6915/

ubiquitously present in the environment so that human exposure routes are multiple. For example, it is present in the fragments of PC or other plastics that represent the majority of anthropogenic debris in watersheds and in the marine environment (Crain et al., 2007; Moore, 2008; Engler, 2012; Richard et al., 2009; Teuten et al., 2009a,b). Therefore, plastic fragments ingested by fishes can represent another contamination source from BPA along the food chain. As further consequence, bisphenol presence in canned fish may occur apart from packaging migration (Mita et al., 2011; Wei et al., 2011; Staniszewska et al., 2014). It is widely recognized that canned tuna is one of the most widespread fish commodities in the world. Europe is the world's largest canned tuna market, and Italy is one of the main European Union (EU) country for tuna consumption, with 2.33 kg/year/inhabitant of canned tuna (Market and Industry Dynamics in the Global Tuna Supply Chain).

To date no information is available on the exposure of Italian population to bisphenols by this foodstuff. For these reasons the objective of this study was to determine the concentrations of BPA and its analogs Bisphenol B (BPB), Bisphenol A Diglycidyl Ether (BADGE), and Bisphenol F Diglycidyl Ether (BFDGE), in tuna, canned in either oil or aqueous medium, retailed in Italian markets. We applied and validated a simple and effective method for their extraction, from tuna and correspondent preservation media separately, and for their quantitative analysis. The levels of bisphenols were determined by liquid chromatography/fluorescence detection (LC-FD). Fluorescence detection was chosen because it is sensitive and easy to perform; furthermore it is cheaper than other detection techniques, such as mass spectrometry.

2. Materials and methods

2.1. Reagents and chemicals

All chemicals and reagents were of either analytical or LC grade and were purchased from Sigma–Aldrich (Dorset, UK). BPA and BADGE standards (minimum purity of 99%), BFDGE (minimum purity of, 99% as mixture of three positional isomers *ortho–ortho*, *ortho–para*, and *para–para*) were purchased from Sigma–Aldrich (Dorset, UK), while BPB standard (minimum purity of 99%) was purchased from TCI Europe (Zwijndrecht, Belgium). Stock solutions (1 mg/mL) of the four BPs were prepared in acetonitrile as solvent. Standard solutions containing the analytes were prepared just before use by mixing appropriate quantities of individual stock solutions and diluting with acetonitrile. All solutions were stored at 4 °C for not more than 3 months.

2.2. Sample preparation

The analyses were performed on 33 tuna fish samples, canned in either oil or aqueous medium, all of different brands retailed in Italian markets. Twenty four tuna samples were in oil medium, while nine were in aqueous medium. For each brand the analysis was performed on the content of two cans with the same batch number. The expiration date of samples ranged from two to five years at the moment of the analyses. Samples were stored sealed at room temperature until analysis. Oil or water from the tuna cans were poured off and analyzed separately. Throughout the analyses glassware and plastic equipment was properly treated to avoid any possible BPs background contamination (Ballesteros-Gómez et al., 2009). Pure water was also verified as bisphenol-free, since it was reported that it may contain detectable levels of BPA (Gallart-Ayala et al., 2010).

2.2.1. Analyte extraction from tuna

The solid content of each can was homogenized by stainless steel hand blender (700 W, Moulinex) for 5 min and an aliquot was taken for analysis. The remaining content of each can was frozen and stored at −20 °C. Ten g of tuna paste were added of 40 mL of a mixture *n*-hexane/acetonitrile 1:1 (v/v) and extracted in an ultrasound bath at room temperature (Branson ultrasonic 2210, frequency 40 kHz). After sonication, the sample was stirred for 15 min, using a polytetrafluoroethylene (PTFE) stir bar, left in contact with the solvent for further 15 min, and centrifuged in polypropylene (PP) tube at 3500 rpm for 15 min. Finally, the acetonitrile phase was filtrated through a 0.45 μm pore size PTFE microfilter and analyzed by LC-FD.

2.2.2. Analyte extraction from oil of canned tuna

500 μl of oil were added of 1.0 mL of *n*-hexane, sonicated for 15 min and left in contact with the solvent for further 15 min. The mixture was loaded onto a Florisil cartridge (Chromabond, Florisil, Macherey–Nagel, Düran, Germany) previously conditioned with 2.0 mL of *n*-hexane. After cartridge washing with 10.0 mL of *n*-hexane and 20.0 mL of *n*-hexane/ethylacetate 95:5 (v/v), the sample was eluted with 10.0 mL of *n*-hexane/ethylacetate 50:50 (v/v). The eluate was evaporated at dryness and the residue reconstituted with 5.0 mL of acetonitrile for the LC-FD analysis.

2.2.3. Analyte extraction from aqueous medium of canned tuna

The aqueous medium samples were centrifuged for 20 min at 3500 rpm in order to eliminate particulate and analyzed by LC-FD after filtration through 0.45 μm pore size PTFE microfilters.

2.3. Equipment and chromatographic conditions

The liquid chromatograph was a LC-10AD VP (Shimadzu Corp., Kyoto, Japan) equipped with a 7725 Rheodyne injection valve fitted with a 20 μl loop. The stainless steel column was a reversed-phase Ascentis C18 HPLC column (250 mm × 4.60 mm i.d., 5 μm particle size) with a Supelguard Ascentis C18 guard column (both from Supelco, Bellefonte, PA, USA). The mobile phase was acetonitrile-water 60:40 (v/v). Analyses were carried out at room temperature (20 ± 2 °C) at a flow rate of 0.5 mL/min in isocratic mode. The fluorescence detector was a model 20A (Shimadzu Corp., Kyoto, Japan) set at 273 nm excitation wavelength and 300 nm emission wavelength. The FD signals were processed with a personal computer software (Chromatoplus 2008, Shimadzu Corp., Kyoto, Japan). Each sample was analyzed in triplicate.

2.4. Mass spectrometry

All the samples found positive for at least one of the four bisphenols under investigation were also analyzed by mass spectrometry to confirm the identity of the peaks.

Table 1
Summary of LC-MS/MS optimized conditions. CE: Collision Energy, CXP: Collision Cell Potential.

Compound	Precursor ion $[M-H]^-$ *m/z*	Product ion *m/z*	CE	CXP
Bisphenol A	227.2	212.2	−25	−13
		133.2	−38	−10
Bisphenol B	241	212	−15	−15
		226	−20	−15
BADGE	341.2	191.2	18	13
		173.1	22	12
BFDGE	313.1	189.2	17	10
		163.1	15	10