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Migration of cyclo-diBA from coatings into canned food: Method of analysis, concentration determined in a survey and *in silico* hazard profiling

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ABSTRACT

Cyclo-diBA, the cyclic product formed from bisphenol A and bisphenol A diglycidyl ether during production of epoxy resins, was measured in canned food using reversed phase HPLC with fluorescence detection. Half (9 of 17) of the samples of canned fish in oil collected in April 2010 contained cyclo-diBA with an average concentration of 1025 μg/kg and a maximum of 1980 μg/kg. In September 2012, cyclo-diBA was detectable (>25 μg/kg) in merely 13 from 44 such products; the average concentration in these was 807 μg/kg and the maximum now reached 2640 μg/kg. Fish in brine contained far less cyclo-diBA. The majority of the canned meat products contained cyclo-diBA at a mean concentration of 477 μg/kg and a maximum of 1050 μg/kg. All prepared meals, such as ravioli or soups, contained cyclo-diBA, with a mean at 287 μg/kg. In canned tomatoes, peas and other vegetables in water or fruits in syrup, no cyclo-diBA was detected (<25 μg/kg). Since no experimental toxicity data are available except for its cytotoxicity, an *in silico* hazard profiling was performed. Cyclo-diBA seems to be stable and of low reactivity. There is indication for considerable oral bioavailability and for the potential to accumulate in the human body. Cyclo-diBA can be metabolized into cyclic and acyclic compounds. Based on SAR assessment for cyclo-diBA and read-across from BADGE to linear cyclo-diBA metabolites, genotoxic effects are improbable. Specific binding of cyclo-diBA to nuclear receptors, such as ERβ, can be predicted, indicating a potential endocrine-disrupting potency. The limit by the EFSA guidelines of 50 μg/person/d for compounds shown not to be genotoxic as well as the TTC-based Cramer structural class III value of 90 μg/person/d could be exceeded several fold by high consumers of canned fish in oil with high brand loyalty. As a consequence, risk reduction measures were taken.

1. Introduction

Cyclo-diBA (sometimes also termed cyclo-di-BADGE; CASRN 20583-87-3) is a cyclic compound formed from bisphenol A (BPA) and bisphenol A diglycidyl ether (BADGE; Fig. 1). It is a minor by-product from the manufacture of epoxy resins by the so-called advancing (Oldring, 1996). Cyclo-diBA can formally be regarded as cyclo-di(bisphenol-A-monoglycidylether) and exists as diastereomers (as *cis*- and *trans*-form).

Among many applications, epoxy resins are used to form coatings of food cans. Can coatings came to the fore of enforcement authorities in 1996 when it was detected that the migration of BADGE broadly exceeded the limit of the Council of Europe (Resolution AP 96/5) and national legislation of, e.g., Switzerland of 20 μg/kg. Based on *in vitro* tests, BADGE was considered genotoxic. Many products of the type fish in oil exceeded the limit around 100 times, some products more than 1000 times (Biedermann et al., 1996). BADGE migrated as a residual monomer from epoxy coatings, but even more when used as an additive (stabilizer) for organosol coatings (Biedermann et al., 1997). This initiated an *in vivo* toxicological evaluation from which it was concluded that BADGE is not genotoxic. A tolerable daily intake (TDI) for BADGE and its derivatives BADGE·H_2O and BADGE·$2H_2O$ of 0.15 mg/kg body weight (bw)/d was derived and a specific migration limit (SML) of 9 mg/kg introduced by EU-Regulation 1985/2005 (EC, 2005). EFSA's current TDI for BPA is at 0.05 mg/kg bw/d and its SML at 0.6 mg/kg (EFSA, 2010; EC, 2011).

The determination of BADGE also revealed that epoxy coatings released many more compounds into food. The overall migrates were often close to 10 mg/dm^2 and their fractions of a molecular weight (MW) < 1000 D in the range of 0.5–5.1 mg/dm^2 (Bronz et al., 1998). Despite considerable efforts, only a minor part of this migrate, primarily the easily predictable components, could be

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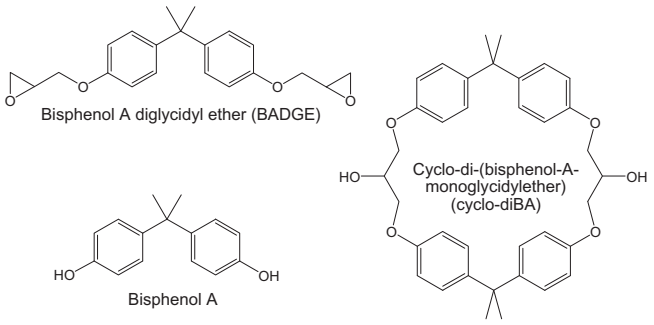

Fig. 1. Structure of cyclo-diBA as well as of BADGE and BPA it is formed from.

identified, such as oligomeric material and adducts of solvents, water, chloride and chain stoppers (Biedermann et al., 1998; Grob et al., 1999). No information about the safety of this migrating material was available.

From 1999 to 2004, at the Council of Europe a committee formed by delegates from regulatory authorities and industry as well as scientific experts discussed the ways how the legal requirement to ensure the safety of the migrates from coatings could be fulfilled. The Framework Resolution AP(2004)1 (Council of Europe, 2004) was issued. It took into account that the migrates from most coatings almost exclusively consisted of reaction products and that reaction products commonly have properties different from the starting materials, i.e. that a toxicological evaluation of the starting materials does not cover the reaction products. It was stated in Section 3.4 of the Resolution that the coatings "should not transfer migrating components not listed in 'Technical document No. 1 – list of substances to be used in the manufacture of coatings intended to come into contact with foodstuffs' which have MW < 1000 D in quantities which could endanger human health. These non-listed substances of MW < 1000 D should be subjected to appropriate risk assessment, taking into account dietary exposure as well as toxicological and structure activity considerations." Safety must be demonstrated through the investigation of the migrate rather than solely by the starting substances – which repeats Article 3 of the EU framework Regulation 1935/2004 and was legally required already for a long time. Exposure estimates can be used as well as structure activity considerations, which ease the requirements.

Shortly later, the association of the coating industry (European Council of producers and importers of paints, printing inks and artists' colors, CEPE) issued a "Code of Practice for Coated Articles" (CEPE, 2007) largely returning to the position that the use of authorized monomers and starting substances is sufficient to demonstrate compliance, i.e. safety of the migrate. In 2008, the French authority for risk assessment (AFSSA) reacted to this by an opinion stating that "the evaluation of the starting materials does not enable to rule out the presence of reaction products in the migrates, the potential danger of which for human health should be considered to ensure the safety of a food contact material (FCM) made from resins" (AFSSA, 2008; Grob et al., 2008).

Cyclo-diBA was identified as a dominating component in the migrate (Biedermann and Grob, 1998). Low reactivity prevents its integration into the polymeric structure. In 1997, it reached a concentration of 2.2 mg/kg in acetonitrile extracts of cans simulating migration into oily foods sterilized at high temperature (Bronz et al., 1998) and of 1.6 mg/kg in food (Biedermann and Grob, 1998).

Migrates from epoxy can coatings are mostly analyzed by high performance liquid chromatography (HPLC) with fluorescence detection (FD), since FD is highly sensitive and the response is similar for all bisphenol-type compounds (Paseiro Losada et al., 1997; Biedermann and Grob, 1998; Berger et al., 2001; Sendòn Garcìa et al., 2003; Schaefer and Simat, 2004; Sun et al., 2006). Alternatively detection by mass spectrometry (MS) was applied (Berger and Oehme, 2000; Sendòn Garcìa and Paseiro Losada, 2004).

In 2010, in order to check the compliance work on the migration from epoxy coatings, the Official Food Control Authority of the Canton of Zurich asked the can producers of a number of products to send in documentation showing the safety of cyclo-diBA. Cyclo-diBA was selected because of its substantial migration, but also because of *in vitro* tests suggesting a significant contribution to the total toxicity of the migrates (Mittag et al., 2005, 2006; Mittag and Simat, 2007). Food toxicologists from the Swiss Federal Office of Public Health evaluated these documents and gave their own opinion. In the first part of this paper, the analytical method for determining cyclo-diBA in foods and the concentrations found are described. In the second part, it reports an *in silico* hazard profiling, a preliminary hazard assessment and, finally, the conclusions drawn by the Swiss food safety authorities.

2. Experimental

Ethanol (8462) and acetonitrile (9012) were from J.T. Baker (Deventer, The Netherlands), BADGE ⩾ 95% from Fluka (Buchs, Switzerland). An Agilent Technologies HPLC instrument 1290 Infinity was used with a 1260 fluorescence detector. A BADGE standard solution of 1 mg/ml in acetonitrile was prepared (avoiding an alcohol because of potential reaction with the epoxy group).

Of products in oil or sauces, the whole can content was analyzed. From products in water (such as vegetables or mushrooms), the water was removed before analysis. Relevant can contents were homogenized, extracted with ethanol and analyzed by reversed phase HPLC (RPLC) with fluorescence detection (FD). Of the homogenates, 5 g were weighed into a 25 ml centrifuge tube and shaken with 8 ml ethanol. After 10 min, the suspension was centrifuged. The ethanol and possibly a supernatant oil phase were transferred into a 25 measuring flask. The residue was shaken with another 8 ml ethanol and allowed to stand during 2 h for the extraction of particles. This extract was combined with the previous one, filled up with water to the 25 ml mark and extracted 1–2 times with approximately 2 ml hexane for the removal of the fat. If necessary, the extract was completed to the 25 ml mark again with water.

Of the ethanol extract, 2 µl were injected into a 2.1 mm × 50 mm i.d. Zorbax Eclipse Plus C18/1.8 µm column kept at 60 °C and chromatographed at 0.6 ml/min with a gradient of water (A) and methanol (B). After 2 min, B was increased from 65% to 85%. After 4 min, the column was rinsed with ethanol at 1.2 ml/min. FD occurred at 225/295 nm. Cyclo-diBA was eluted after about 2.8 min.

The FD response of cyclo-diBA was determined by GC–FID analysis of an extract of a can coating. A can with internal epoxy coating was extracted with acetonitrile overnight at 60 °C. To 10 ml of this extract, 10 µl BADGE 1 mg/ml was added (1 µg/ml). After reconcentration to 1 ml, this extract was mixed with 1 ml 15% dichloromethane/hexane. The phases were separated by addition of 10 ml water. Of the supernatant, 10 µl were injected on-column into a 1.5 m × 0.32 mm i.d. deactivated precolumn followed by a 7 m × 0.25 mm i.d. separation column coated with a 0.13 µm film of PS-255 (a dimethyl polysiloxane from Fluka). The oven temperature was programmed at 55°/min from 45 °C to 350 °C. The carrier gas (hydrogen) was supplied at a constant pressure of 80 mbar. The detector block was heated to 350 °C. The concentration of cyclo-diBA measured in the can extract was 1.25 µg/ml (±0.04 µg/ml; $n = 3$). The extract with the added BADGE was also analyzed by RPLC-FD. The area ratios resulted in response factors ranging between 0.97 and 1.04.

Can coatings were identified by Fourier transform infrared spectroscopy (FT-IR) on a Spectrum One FT-IR spectrometer (Perkin Elmer, Schwerzenbach, Switzerland). Pieces of the lid, the side wall and the bottom were mounted on the Golden Gate Attenuated Total Reflection (ATR) insert of the spectrometer with a diamond crystal and single reflection (Specac, Orpington, UK). For identification, the IR spectra obtained were compared to in-house spectral libraries with the Perkin Elmer software Spectrum v5.3.1. Coatings were also tested by the Beilstein method: some coating was picked up onto the tip of a hot cupper wire; the wire was kept into the flame of a Bunsen burner, where chlorine turns the flame green.

3. Results

3.1. Method of analysis

Strong fluorescence accompanied by high selectivity favored the use of an HPLC method. Cyclo-diBA can be analyzed by GC, but the low volatility and the presence of two hydroxyl groups presuppose careful optimization of the injection technique and column. For the analysis of oily products, such as fish in oil, normal