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Review

Recent advances in simultaneous analysis of bisphenol A and its conjugates in human matrices: Exposure biomarker perspectives

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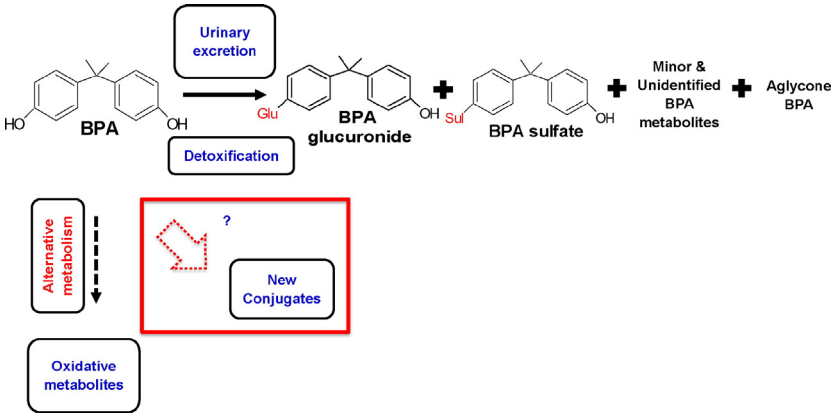
HIGHLIGHTS

- Bisphenol A conjugates require in vivo metabolism and not prone to external contamination.
- Analytical trends in the measurement of BPA conjugates are reviewed in this work.
- BPA conjugates can be potential additional biomarkers of human exposure to BPA.

GRAPHICAL ABSTRACT



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ABSTRACT

Human exposures to bisphenol A (BPA) has attained considerable global health attention and represents one of the leading environmental contaminants with potential adverse health effects including endocrine disruption. Current practice of measuring of exposure to BPA includes the measurement of unconjugated BPA (aglycone) and total (both conjugated and unconjugated) BPA; the difference between the two measurements leads to estimation of conjugated forms. However, the measurement of BPA as the end analyte leads to inaccurate estimates from potential interferences from background sources during sample collection and analysis. BPA glucuronides (BPAG) and sulfates (BPAS) represent better candidates for biomarkers of BPA exposure, since they require in vivo metabolism and are not prone to external contamination. In this work, the primary focus was to review the current state of the art in analytical methods available to quantitate BPA conjugates. The entire analytical procedure for the simultaneous extraction and detection of aglycone BPA and conjugates is covered, from sample pre-treatment, extraction, separation, ionization, and detection. Solid phase extraction coupled with liquid chromatograph and tandem mass spectrometer analysis provides the most sensitive detection and quantification of BPA conjugates. Discussed herein are the applications of BPA conjugates analysis in human exposure assessment studies. Measuring these potential biomarkers of BPA exposure has only recently become analytically feasible and there are limitations and challenges to overcome in biomonitoring studies.

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1. Background

Bisphenol A (BPA) is a phenolic compound in wide use with innumerable industrial, commercial, consumer, and domestic applications. It is a monomer used in polycarbonate and epoxy resins that are used in the production of food, water and beverage packaging material (Geens et al., 2011, 2012a, 2012b). Human exposure to BPA primarily occurs from oral ingestion and dietary sources such as canned foods, water bottles and other food contact material (Kang et al., 2003; Takao et al., 2002; Cao et al., 2008; Cao and Corriveau, 2008a, 2008b; Cao et al., 2009a, 2009b, 2010a, 2010b; Brede et al., 2003; Santhi et al., 2012; Amiridou and Voutsa, 2011; Carwile et al., 2009, 2011), and through inhalation and dermal routes as well (Geens et al., 2012b; Wilson et al., 2003; Liao and Kannan, 2011; Schwartz and Landrigan, 2012; Dalhamn et al., 1968; Loganathan and Kannan, 2011; Rudel et al., 2003; Mielke et al., 2011). The bioavailability of BPA is dependent on the exposure route and is therefore an important factor for assessing BPA exposure risks in humans (Vandenberg et al., 2010, 2013). A schematic for the general exposure sources and routes in humans are presented in Fig. SI-1. Upon oral ingestion in humans, BPA is mostly absorbed and undergoes fast and almost complete conversion to conjugates by uridine diphosphate and glucuronosyltransferase (UGT) isoforms, and sulfotransferase in the gastrointestinal track and liver (Volkel et al., 2002). Within 24 h BPA is almost completely eliminated via urine with 84–97% of absorbed BPA excreted there within the first 5–7 h following ingestion (Volkel et al., 2002, 2005). The major fraction of BPA gets excreted in conjugated forms namely bisphenol A glucuronide (BPAG) and bisphenol A sulfate (BPAS). A minor fraction, usually <1% of total (aglycone and conjugated forms) BPA measured, circulates as the aglycone (Volkel et al., 2002, 2005, 2008), and absolute bioavailability is <0.1–0.2% (Teeguarden et al., 2011). BPA pharmacokinetics and bioavailability vary by route of exposure in humans (vom Saal and Welshons, 2014). For example, BPA exposure via dermal absorption (Liao and Kannan, 2011) resulted in a longer half-life and increased bioavailability (Birnbaum et al., 2012; Stahlhut et al., 2009). Associations between human exposures to BPA and several health outcomes were reviewed (Mustieles et al., 2015; Ranciere et al., 2015; Oppeneer and Robien, 2015; Vom Saal et al., 2012; Bodin et al., 2015; Mileva et al., 2014; Peretz et al., 2014; Rutkowska and Rachon, 2014; Gonzalez-Parra et al., 2013; Vaidya and Kulkarni, 2012; Stein et al., 2015; Fenichel et al., 2013).

2. Conventional practice of BPA exposure assessment: measuring total and aglycone BPA

Biomonitoring of BPA in various human matrices was recently reviewed (Asimakopoulos et al., 2012). Urine has traditionally been the most preferred matrix to study because BPA is extensively conjugated via glucuronidation and excreted in urine and sampling is minimally invasive. However given the short half-life of BPA in humans (~6 h), the observed levels in urine can only reflect recent exposure to BPA, limiting its scope as a biomarker (Calafat et al., 2015, 2016; Stahlhut et al., 2016). An overview of the common analytical workflow for the determination of total and aglycone BPA in human matrices is outlined in Fig. SI-2. Details of the analytical steps followed for the determination of total BPA, and BPA structural analogs and chlorinated derivatives in human matrices were recently reviewed (Andra et al., 2015). Current analytical methods determine the total concentration of BPA after enzymatic hydrolysis preferably at 37 °C for a few hours and in some cases overnight. Typically only the β-glucuronidase enzyme is used for the deconjugation step because of the predominant presence of the glucuronide conjugate form of BPA, while few have additionally used sulfatase enzyme for the release of BPA from the sulfate conjugate that occurs as a very minor fraction. In addition to the glucuronide form, sulfatase is also deconjugated if the source of β-glucuronidase is from *Helix pomatia*-H1 compared to *Escherichia coli*-K12 (Ye et al., 2005). Some issues that lead to inaccurate measurement or underestimation due to suboptimal conditions of total BPA arises from insufficient enzyme concentrations, inappropriate choice of enzymes, incomplete deconjugation, unfavorable hydrolysis conditions and overall suboptimal deconjugation protocol. In cases where only aglycone BPA was measured the enzymatic deconjugation step was skipped. Representative sample preparation, analyte separation and detection approaches for the analysis of total and aglycone BPA in human matrices are presented in Table SI-1.

3. Rationale to study BPA conjugates

A major concern in using BPA concentrations in bio-matrices for assessing human exposure is the potential post-exposure specimen contamination from external sources. Such contaminations result in elevation of aglycone BPA that subsequently contributes to inflated total BPA concentrations. High blood levels of aglycone BPA in some studies (Kosarac et al., 2012; Lee et al., 2008; Padmanabhan et al., 2008) was questioned because (i) deconjugation can happen during the sample collection, storage, or analysis, and (ii) measuring aglycone BPA but not its conjugated forms could reflect external contamination rather than blood levels (Ye et al., 2013). However, a recent review overrules the occurrence of external contamination in the current studies (vom Saal and Welshons, 2014). Unlike aglycone BPA, conjugates are not prone to external contamination (Fox et al., 2011).

Aglycone BPA was shown to passively cross the placenta in a bi-directional fashion between maternal and fetal compartments, while BPAG has limited permeability in either direction (Corbel et al., 2014). Moreover, the authors concluded that given the limited clearance of BPAG via placenta, it is likely that BPA conjugates form and accumulate

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