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Resin-based dental sealants as a source of human exposure to bisphenol analogues, bisphenol A diglycidyl ether, and its derivatives

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ABSTRACT

Although studies have examined leaching of bisphenol A (BPA) from dental sealants into saliva, occurrence of BPA, bisphenol A diglycidyl ether (BADGE), and their derivatives in dental sealants themselves has not been investigated. In this study, concentrations of eight bisphenol analogues (BPs), BADGE and its derivatives (BADGEs), including BADGE·H_2O, BADGE·HCl, BADGE·2H_2O, BADGE·2HCl, and BADGE·H_2O·HCl, were determined in 70 dental sealants collected from the U.S. market. Of the 70 dental sealants analyzed, 65 contained at least one of the target chemicals measured. BADGE·2H_2O was the most abundant compound, found at concentrations of up to 1780 μg/g. The geometric mean (GM) concentration of total BADGEs was 47.8 μg/g, which was two to three orders of magnitude higher than that of total BPs (GM: 539 ng/g). BPA was found in 46% of the sealants and BADGEs was found in 87% of the sealants analyzed. Majority of the dental sealants analyzed in this study were manufactured in the United States and Korea; no significant differences were observed in the concentrations of BPs and BADGEs between the two countries. An exposure assessment was made based on the concentrations of BPs and BADGEs measured in sealants and their application rates in dentistry. The worst-case exposure scenario with the highest measured concentration of total BPs and BADGEs and application on 8 teeth at 8 mg each yielded an estimated daily intake (EDI) of 1670 and 5850 ng/kg·bw/day for adults and children, respectively. Although the EDI is below the specific migration limit set by the European Food Safety Authority, dental sealants are a source of exposure to BPs and BADGEs, especially in children.

1. Introduction

Resin-based dental sealants, also known as pit and fissure sealants, are widely used in dentistry for the prevention of tooth decay. Pit and fissure sealants are applied to teeth that are vulnerable to decay by placing them within certain areas to create a smooth surface that is easy to clean. The effectiveness of these sealants in preventing and arresting the progression of dental caries has been demonstrated (Ahovuo-Saloranta et al., 2008; Gooch et al., 2009; Griffin et al., 2008). The use of dental sealants is more common among children due to their substantial risk for tooth decay. Over the last two decades, the use of pit and fissure sealants among children has steadily increased, following stimulation through federal programs, including those by the Centers for Disease Control and Prevention (CDC) and the Maternal and Child Health Bureau of the United States (Dye et al., 2007). From 1988–1994 to 1999–2004, the percentage of adolescents in the United States between 12 and 19 years of age who had at least one filling on permanent teeth increased from 18% to 38% (Dye et al., 2007).

The sealant and composite resin fillings are polymerized prior to use. A study showed, however, that these polymers are not chemically stable and can be released into the oral environment (Moharamzadeh et al., 2007). Incomplete polymerization of dental sealants and secondary decomposition of these composites under the influence of physical and chemical agents in the oral environment can contribute to human exposure to chemicals present in dental sealants (Leprince et al., 2012; Finer and Santerre, 2003; Malkiewicz et al., 2015; Olea et al.,

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1996). Bisphenol A diglycidyl ether (BADGE)-based epoxy resins are widely used in the manufacture of dental resins (Fleisch et al., 2010). Bisphenol A (BPA) is used in the production of BADGE and is present in dental sealants as impurities due to the incomplete polymerization. Nevertheless, studies about the occurrence of BPA and other bisphenol analogues (collectively referred to as BPs in this study), BADGE and its derivatives (collectively referred to as BADGEs in this study) in resin-based dental sealants and composites are very limited.

As an emerging class of endocrine-disrupting chemicals, BPs and BADGEs have been reported as reproductive and developmental toxicants (Hyoung et al., 2007; Richter et al., 2007; Kang et al., 2008). BPs, including BPA, BPF, and BPS, elicit estrogenic activities in in vitro bioassays (EHHI, 2008). Exposure to BADGE and its derivatives, BADGE·H_2O and BADGE·$2H_2O$, has been associated with reproductive failure in Spanish sows (Nerin et al., 2014). Although BADGE was classified as a Group 3 carcinogen (i.e., not classifiable as to its carcinogenicity in humans) by the International Agency for Research on Cancer (1999), a variety of in vitro assays have suggested genotoxic effects of this chemical (IARC, 1999; Suarez et al., 2000; Sueiro et al., 2001, 2006). Human exposure to BPA has been linked to endocrine disorders and obesity (Lang et al., 2008; EHHI, 2008). Thus, exposure of humans to BPs and BADGE is a matter of concern, and the assessment of sources of human exposures is important to the development of strategies to mitigate exposures. The objective of this study was to provide baseline information on the concentrations of BPs and BADGEs in dental sealants currently marketed in the United States. Exposure of adults and children to BPs and BADGEs through dental sealant application has also been assessed.

2. Materials and methods

2.1. Standards and reagents

Information regarding analytical standards and reagents used in this study is provided in the Supporting Information.

2.2. Sample collection and preparation

All dental sealants (n = 70) analyzed in this study were purchased from online vendors and distributors from June to August 2015, and these products originated from the United States, Korea, Japan, the Netherlands, Liechtenstein, and Greece. The dental sealants represented 15 manufacturers/distributors and 19 popular brands available in the U.S. market. Various shades of sealants (e.g., opaque, clear, ultra-clear, natural, off-white) and types of cure (e.g., light, self-cure) were included. Detailed information of the dental sealant samples is shown in Table S1.

The method for the extraction of BPs and BADGEs from the dental sealants was similar to that described elsewhere, with some modifications (Wang et al., 2016). Briefly, 100–200 mg of resin were accurately weighed and transferred into a 15-mL PP tube. Six milliliters of methanol were added, and samples were shaken in an oscillator shaker at 100 strokes per minute for 60 min. The mixture was then centrifuged at 5000 rpm for 5 min, and the supernatants were passed through an ENVI-Carb solid phase extraction (SPE) cartridge (Sigma-Aldrich, St. Louis, MO, USA), which was preconditioned with 6 mL of methanol. Analytes were eluted with 3 × 2 mL of methanol. Both elutes were combined and concentrated to 5 mL under a gentle nitrogen stream. An aliquot of the extract was vortex mixed and transferred into a glass vial for high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analysis. All procedural blanks and samples were spiked with 100 ng of ^{13}C-BPA and d_6-BADGE, as internal standards, prior to extraction.

2.3. Instrumental analysis

Instrumental analyses of BPs and BADGEs have been described in detail elsewhere (Xue et al., 2016, 2017). Briefly, chromatographic separation of BPs was carried out using a Shimadzu Prominence Modular HPLC system (Shimadzu Corporation, Kyoto, Japan), consisting of a system controller, a binary pump, and an auto sampler. Identification and quantification of BPs were performed with an Applied Biosystems API 3200 electrospray triple quadruple mass spectrometer (ESI-MS/MS; AB SCIEX, Framingham, MA, USA). Further details of instrumental methods are provided in the Supporting Information.

Chromatographic separation of BADGEs was carried out using an Agilent 1100 Series HPLC system (Agilent Technologies Inc., Santa Clara, CA, USA). Identification and quantification of the target chemicals were performed with an Applied Biosystems API 2000 electrospray triple quadrupole mass spectrometer (ESI-MS/MS; Applied Biosystems, Foster City, CA, USA). Further details of the instrumental analysis of BADGEs are provided in the Supporting Information.

2.4. Quality assurance/quality control

Quantification was performed by an isotope-dilution method based on the responses of ^{13}C-BPA (for BPs) and d_6-BADGE (for BADGEs). Instrument calibration standards were injected at concentrations that ranged from 0.1 to 1000 ng/mL. For BADGE·$2H_2O$ and BADGE·HCl·H_2O, that were found at elevated concentrations in dental sealants, the highest concentration used for instrumental calibration was 10,000 ng/mL. Both linear and polynomial curves were applied in the calculation of concentrations. The regression coefficients (r) were ≥ 0.99 for all calibration curves. The consistency and appearance of the dental sealant matrix varied, depending on the brand, and, therefore, the method limits of quantification (MLOQs) were determined based on the minimum concentration of analytes in sample extracts that provided a signal-to-noise ratio (S/N) ≥ 10. The MLOQs of BPF, BPA, BPB, BPS, BPZ, BPAP, BPAF, and BPP were 21.9, 175, 21.9, 8.77, 21.9, 13.2, 4.39, and 132 ng/g, respectively, and those of BADGE·$2H_2O$, BADGE·H_2O, BADGE, BADGE·HCl·H_2O, BADGE·HCl, and BADGE·2HCl were 1.31, 2.19, 0.44, 0.44, 0.44, and 0.88 μg/g, respectively. For the calculation of the MLOQ, an average weight (114 mg) of dental sealants used for extraction was applied. BPA and BADGE·$2H_2O$ were found in procedural blanks at respective concentrations of 15.8 and 5.33 ng/mL, and these values were subtracted from the measured concentrations in samples. Five samples were selected randomly for pre-extraction matrix spike (MS) by fortification of 100 ng of target analytes and by passing them through the analytical procedure. Post-extraction matrix matches (MM) of the five samples were followed for the calculation of relative recoveries, which ranged from 84% to 105% and 73% to 84% for BPs and BADGEs, respectively.

2.5. Data analysis

Data were acquired by Analyst software version 1.4.1 (Applied Biosystems, Foster City, CA, USA). Statistical analyses were performed with statistics software package R v.3.1.0 and Microsoft Excel 2007. For the calculation of geometric mean (GM) and arithmetic mean, we substituted values below the MLOQ with a value equal to half the MLOQ. A Shapiro-Wilk test and quantile-quantile (Q-Q) plot were used to determine the normality of the data. To examine the relationship between chemicals, Spearman (when data did not follow a normal distribution after logarithmic transformation) or Pearson (when data followed a normal distribution after logarithmic transformation) correlation analyses was used. Only those samples with detectable concentrations of the target analytes were used in performing correlation analysis. To assess the difference between means, Student's t-test (when data followed a normal distribution after logarithmic transformation) or Mann-Whitney U test (when data did not follow a normal