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Widespread Occurrence and Accumulation of Bisphenol A Diglycidyl Ether (BADGE), Bisphenol F Diglycidyl Ether (BFDGE) and Their Derivatives in Human Blood and Adipose Fat

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Supporting Information

ABSTRACT: Despite the widespread use of bisphenol A diglycidyl ether (BADGE) and bisphenol F diglycidyl ether (BFDGE) in various consumer products, studies on human exposure to these compounds are scarce. In this study, BADGE, BFDGE, and seven of their derivatives were determined in human adipose fat and blood plasma samples collected from New York City, NY. Bisphenol A bis (2,3-dihydroxypropyl) ether [BADGE·2H_2O] was the major BADGE derivative found in 60% of the adipose samples and 70% of the plasma samples analyzed. High concentrations and detection frequencies of BFDGE were found in both adipose and plasma samples. BFDGE concentrations in adipose fat ranged from 19.1 to 4500 ng/g wet weight. A significant correlation between BADGE or BFDGE and their derivatives in adipose and plasma samples suggested hydration of these reactive compounds in humans. A significant positive correlation existed between BADGEs (i.e., the sum of BADGE and its five derivatives) and BFDGEs in adipose samples, which suggested similar exposure sources and pathways for these compounds in humans. Bisphenol A (BPA) also was analyzed in adipose fat and plasma, and its concentrations were positively correlated with those of BADGEs, which confirmed coexposure of BADGEs and BPA in humans.

INTRODUCTION

Bisphenol A diglycidyl ether (BADGE) and bisphenol F diglycidyl ether (BFDGE) are widely used in the protective coatings of food and beverage cans and in paints and adhesives[1,2]. These compounds also are used as monomers in the production of epoxy-based polymers and as additives for the elimination of surplus hydrochloric acid in polyvinyl chloride (PVC) production[1]. These are high production-volume chemicals. For instance, the annual global production of BADGE was estimated at 957 thousand metric tons in 2003[2].

BADGE is a reactive molecule and has been reported to bind to nucleic acids[3]. In vitro studies have shown mutagenic and teratogenic effects of BADGE and its derivatives (collectively referred to as BADGEs in this study)[4,5]. Bisphenol A bis (2,3-dihydroxypropyl) ether [BADGE·2H_2O], the stable hydrated product of BADGE, was reported to have endocrine disrupting potentials even greater than those of bisphenol A (BPA)[6]. The antagonistic activity of a chlorohydrin derivative of BADGE toward androgen has been reported[7]. In addition, genotoxicity and cytotoxicity as well as developmental and reproductive toxicity of BADGEs have been reported in laboratory animals[5,8−10]. A reduction in a follicle-stimulating hormone in male workers exposed to BADGE was reported[11]. The European Commission for food contact applications has set a migration limit for ten derivatives of BADGE from food cans at 1 mg/kg[12].

BADGE and its hydrated as well as chlorinated derivatives are present in many canned foods at concentrations on the order of several micrograms to milligrams per kilogram[13−17]. Studies on human exposure to BADGEs, however, are scarce. Limited human biomonitoring studies conducted in our laboratory have shown that BADGE·2H_2O and BADGE are widespread in urine specimens from Greece, China, India, and the U.S.[18−20]. Considering the moderately high lipophilicity (i.e., K_{ow}; see Table 1) of BADGE and its derivatives, there is a potential for these chemicals' accumulation in adipose tissue of humans. Because BADGE has been reported to elicit adipogenic gene expression in stromal stem cells[21], it is relevant to assess accumulation in human adipose fat. Nevertheless, no earlier studies have reported the occurrence of BADGEs in human fat.

BFDGE is also used as a coating material and has been detected in canned foods[22]. Cytotoxic, genotoxic, mutagenic,

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Table 1. Select Physicochemical Properties of Bisphenol A (BPA), Bisphenol A Diglycidyl Ether (BADGE), Bisphenol F Diglycidyl Ether (BFDGE) and their Derivatives

analyte	molecular formula	CAS	molecular weight	water solubility[a]	log K_{ow}[b]
BPA	$C_{15}H_{16}O_2$	80−05−7	228.29	172.7	3.64
BADGE	$C_{21}H_{24}O_4$	1675−54−3	340.41	3.7	3.84
BADGE·H_2O	$C_{21}H_{26}O_5$	76002−91−0	358.43	12.6	3.09
BADGE·HCl·H_2O	$C_{21}H_{27}ClO_5$	227947−06−0	394.89	5.5	3.25
BADGE·2H_2O	$C_{21}H_{28}O_6$	5581−32−8	376.44	96.2	1.93
BADGE·HCl	$C_{21}H_{25}ClO_4$	13836−48−1	376.87	5.25	4.00
BADGE·2HCl	$C_{21}H_{26}Cl_2O_4$	4809−35−2	413.33	−	−
BFDGE	$C_{19}H_{20}O_4$	2095−03−6	312.36	17.2	3.26
BFDGE·2H_2O	$C_{19}H_{24}O_6$	72406−26−9	348.39	−	−
BADGE·2HCl	$C_{19}H_{22}Cl_2O_4$	−	385.28	1.51	3.98

[a]Predicted water solubility at 25 °C (mg/L) cited from chemspider.com, which is estimated from Log K_{ow} WSKOW v1.41. [b]Predicted from chemspider.com, which is generated using the U.S. Environmental Protection Agency's EPISuite, (KOWWIN v1.67 estimate). −, Not available.

and endocrinal effects of BFDGEs have been reported in in vitro studies[7,10,23,24]. For instance, BFDGE was shown to elicit cytotoxic effects on human colorectal adenocarcinoma cell line (CACO-2), by inducing morphological changes, cell detachment from substratum, inhibition of cell proliferation and F-actin depolymerization[10]. BFDGE was also reported to induce mutagenic effects in prokaryotic cell bioassays, and to increase frequency of sister chromatid exchanges and micronuclei in human peripheral blood lymphocytes[23]. In addition, chlorohydrin derivative of BADGE was reported to be a strong antagonist for androgen receptor[7]. However, no earlier studies have reported the occurrence of BFDGEs in human tissues.

In this study, concentrations of six BADGEs, that is, BADGE, BADGE·H_2O, BADGE·2H_2O, bisphenol A (3-chloro-2-hydroxypropyl) (2,3-dihydroxypropyl) ether [BADGE·HCl·H_2O], bisphenol A bis (3-chloro-2-hydroxypropyl) ether [BADGE·2HCl], bisphenol A (3-chloro-2-hydroxypropyl) glycidyl ether [BADGE•HCl], and three BFDGEs, that is, BFDGE, bisphenol F bis (2,3-dihydroxypropyl) ether (BFDGE·2H_2O), and bisphenol F bis (2-chloro-1-propanol) ether (BFDGE·2HCl), were determined in 20 adipose fat tissue and 20 blood plasma samples collected from New York City. BPA also was analyzed in these samples to examine the association of this compound with BADGE/BFDGE. The objectives of this study were to (i) determine the occurrence, distribution, and bioaccumulation potentials of BADGEs/BFDGEs in human tissues; and (ii) elucidate the correlation between various BADGE/BFDGE derivatives and BPA in human tissues.

MATERIALS AND METHODS

Chemicals. Analytical standards of BADGE (≥95%) and its derivatives, BADGE·H_2O (≥95%), BADGE·2H_2O (≥97%), BADGE·HCl·H_2O (≥95%), BADGE·HCl (≥90%), BADGE·2HCl (≥97%), BFDGE·2H_2O (≥95%), and BFDGE·2HCl (≥90%), as well as BPA (>99%) and β-glucuronidase from *Helix pomatia* (145700 units/mL β-glucuronidase and 887 units/mL sulfatase), were purchased from Sigma-Aldrich (St. Louis, MO). BFDGE (98%) was purchased from LGC standards (Teddington, Middlesex, UK). ^{13}C-isotopically labeled 2-hydroxy-4-methoxybenzophenone ($^{13}C_{12}$−BP-3) (99%) and $^{13}C_{12}$−BPA (99%) were purchased from Cambridge Isotope Laboratories (Andover, MA). D_6-BADGE (99%) was purchased from Toronto Research Chemicals (North York, Ontario, Canada). The molecular structures and selected physicochemical properties of the target compounds are shown in Figure S1 (Supporting Information (SI)) and Table 1, respectively. Milli-Q water was prepared using an ultrapure water system (Barnstead International, Dubuque, IA). The stock solutions of target analytes and internal standards were prepared at 1 mg/mL in methanol and stored at −20 °C.

Sample Collection. A total of 20 human adipose fat samples were originally collected in 2003−2004 from donors who underwent liposuction in New York City. The samples were stored in solvent-cleaned I-Chem jars at −20 °C, as recommended by the National Human Monitoring Program[25]. The samples were from 15 females (age: 18−58 years) and 5 males (age: 32−51 years). Additional demographic information on the donors is shown in SI Table S1. Further details on the sample collection have been provided elsewhere[26]. Plasma samples (n = 20) were collected in 2002−2003 from adult male donors (age: 24−58 years) in New York City. Further details of the plasma samples are shown in SI Table S1. Institutional Review Board approvals were obtained from the New York State Department of Health for the analysis of samples.

Sample Preparation. For adipose fat tissue samples, 200 to 300 mg of sample were accurately weighed and spiked with 50 μL of methanol containing $^{13}C_{12}$−BPA, D_6-BADGE, and $^{13}C_{12}$−BP-3 (200 ng/mL each). D_6-BADGE was used as the internal standard (IS) for the quantification of BADGE, BFDGE, BADGE·H_2O, and BADGE·HCl (analytes with epoxy moiety) (SI Table S2)[27], whereas $^{13}C_{12}$−BP-3 was used as the IS for BADGE·2H_2O, BADGE·HCl·H_2O, BADGE·2HCl, BFDGE·2H_2O, and BFDGE·2HCl (analytes without epoxy moiety). $^{13}C_{12}$−BPA was used as the IS in the quantification of BPA. After equilibration for 30 min at room temperature, 5 mL of acetone were added into the sample. The mixture was homogenized in a mortar, and the extract was then transferred to a 15 mL polypropylene (PP) tube by washing with 2 mL of methanol. The combined extracts were concentrated to ∼2 mL under a gentle nitrogen stream (to remove acetone). Then an ultralow temperature (−20 °C) incubation was used to separate the lipid from the solvent. After storage at −20 °C for 15 min, the extracts were centrifuged immediately at 4500g for 5 min (Eppendorf centrifuge 5804, Hamburg, Germany) for the separation of organic solvent and solidified lipid. The supernatant was transferred and concentrated to 0.5 mL under a gentle nitrogen stream and analyzed by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS).

Plasma samples were extracted using a liquid−liquid extraction (LLE) method. Five hundred microliters of plasma samples were transferred into a 15 mL PP tube, and 50 μL of

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Table 2. Concentrations (ng/g Wet Weight for Adipose Fat and ng/mL for Plasma/Urine) of BADGEs, BFDGEs and BPA in Human Adipose Fat[a] and Urine[b] from New York

adipose (n=20)	BADGE	BADGE·H_2O	BADGE·$2H_2O$	BADGE·H_2O·HCl	BADGE·HCl	BADGE·2HCl	BFDGE	BFDGE·$2H_2O$	BFDGE·2HCl	BPA
range	<LOQ~5.16	<LOQ~4.33	<LOQ~45.4	<LOQ~28.2	<LOQ~7.20	<LOQ~3.11	19.1−4500	<LOQ~12.6	<LOQ~5.20	<LOQ~20.9
median[a]	<LOQ	<LOQ	3.44	<LOQ	<LOQ	<LOQ	178	<LOQ	<LOQ	5.65
DF[c]	25%	15%	60%	20%	5%	5%	100%	20%	5%	90%

plasma (n=20)	BADGE	BADGE·H_2O	BADGE·$2H_2O$	BADGE·H_2O·HCl	BADGE·HCl	BADGE·2HCl	BFDGE	BFDGE·$2H_2O$	BFDGE·2HCl	BPA
range	<LOQ	<LOQ~9.54	<LOQ~65.1	<LOQ~1.41	<LOQ	<LOQ	23.3−180	<LOQ~6.65	<LOQ	0.784−4.97
median[a]	<LOQ	2.26	7.15	<LOQ	<LOQ	<LOQ	56.2	<LOQ	<LOQ	1.77
DF[c]	0	65%	70%	35%	0	0	100%	30%	0	100%

urine[a] (n=31)	BADGE	BADGE·H_2O	BADGE·$2H_2O$	BADGE·H_2O·HCl
range	0.105−2.321	0.121−1.361	0.150−4.604	<LOQ-3.412
median[a]	0.682	0.423	1.082	0.308
DF[c]	100	100	100	96.8%

[a]Samples of adipose (collected in 2003−2004) and plasma (collected in 2002−2003) are not paired. [b]Data for BADGEs in urine (collected from healthy volunteers in Albany, New York in 2011) were cited from our previous study[18]. [c]DF indicates detection frequency.

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methanol containing $^{13}C_{12}$–BPA, D_6-BADGE and $^{13}C_{12}$–BP-3 (200 ng/mL each) were spiked. The samples were then extracted three times each with 3 mL of ethyl acetate. The mixture was shaken in an oscillator shaker for 60 min (Eberbach Corp., Ann Arbor, MI) and then centrifuged at 4500*g* for 5 min (Eppendorf). The extracts were combined and washed with 1 mL of Milli-Q water. The supernatant was transferred into a glass tube and concentrated to near-dryness under a gentle nitrogen stream. Finally, 0.5 mL of methanol was added and vortex mixed for analysis by HPLC-MS/MS.

Instrumental Analysis. Chromatographic separation and detection of analytes were accomplished by the use of an Agilent 1100 Series HPLC (Agilent Technologies Inc., Santa Clara, CA) interfaced with an Applied Biosystems API 2000 electrospray triple quadrupole mass spectrometer (ESI-MS/MS; Applied Biosystems, Foster City, CA). Ten microliters of the sample were injected onto an analytical column (Betasil C18, 100 × 2.1 mm column; Thermo Electron Corporation, Waltham, MA), which was connected to a Javelin guard column (Betasil C18, 20 × 2.1 mm column). The mobile phase comprised 100% methanol (A) and 10% methanol in Milli-Q water that contained 2 mM of ammonium acetate (B). Two different gradient elutions at a flow rate of 200 μL/min were used for the analysis of BADGEs/BFDGEs and BPA (SI Table S3). The MS/MS was operated in the multiple reaction monitoring (MRM), positive and negative ionization modes for the analysis of BADGEs/BFDGEs and BPA, respectively, and the parameters were optimized by infusion of individual analytes (SI Table S4). Select MRM chromatograms of BFDGE in standard solution and in human specimens are shown in SI Figure S2.

Quality Assurance and Quality Control (QA/QC). Solvent-cleaned glass jars were used to store adipose fat samples. Contamination that arose from laboratory materials and solvents was evaluated by the analysis of procedural blanks with every batch of samples. Recoveries of native compounds spiked into procedural blanks ranged from 81% to 105%. Quantification of BADGEs and BFDGEs was performed by an isotope-dilution method based on the responses of D_6-BADGE (for BADGE, BFDGE, BADGE·H_2O, and BADGE·HCl), $^{13}C_{12}$–BP-3 (for BADGE·2H_2O, BADGE·2HCl, BADGE·HCl·H_2O, BFDGE·2H_2O, and BADGE·2HCl), and $^{13}C_{12}$–BPA (for BPA). Four adipose and four plasma samples were selected randomly; 6 BADGEs, 3 BFDGEs, and BPA were spiked along with $^{13}C_{12}$–BP-3, $^{13}C_{12}$–BPA, and D_6-BADGE (10 ng each) and passed through the entire analytical procedure. In spiked matrices, recoveries of all target compounds ranged from 98% to 125% for adipose tissue and from 94% to 107% for plasma (corrected for by the recoveries of D_6-BADGE or $^{13}C_{12}$–BP-3 or $^{13}C_{12}$–BPA). The recoveries of target chemicals through the analytical procedure are presented in SI Table S2.

Duplicate analysis of selected samples showed a coefficient of variation of <15% between determined concentrations. The limits of quantification (LOQs) for target analytes in adipose samples were 0.40 (BADGE), 4.0 (BADGE·H_2O), 4.0 (BADGE·H_2O·HCl), 0.16 (BADGE·2H_2O), 2.0 (BADGE·HCl), 4.0 (BADGE·2HCl), 0.40 (BFDGE), 2.0 (BFDGE·2HCl), 0.80 (BFDGE·2H_2O), and 0.80 ng/g (BPA), and the corresponding values for plasma samples were 0.25 (BADGE), 2.5 (BADGE·H_2O), 2.5 (BADGE·H_2O·HCl), 0.1 (BADGE·2H_2O), 1.25 (BADGE·HCl), 2.5 (BADGE·2HCl), 0.25 (BFDGE), 1.25 (BFDGE·2HCl), 0.50 (BFDGE·2H_2O), and 0.50 ng/mL (BPA). A calibration standard and methanol were injected after every 20 samples as a check for drift in instrumental sensitivity and carry-over of target compounds between samples, respectively. Instrumental calibration was verified by the injection of a 10-point calibration standard (ranging in concentrations from 0.01 to 10 ng/mL for BADGE, BADGE·2H_2O, BFDGE, and BFDGE·2H_2O and from 0.1 to 50 ng/mL for BADGE·H_2O, BADGE·H_2O·HCl, BADGE·HCl, BADGE·2HCl, BFDGE·2HCl, and BPA), and the regression coefficient (R) of the calibration curves was ≥0.99. For samples with concentrations above the upper limit of the calibration curve, extracts were diluted and reanalyzed. Concentrations are presented on a wet weight basis.

Data Analysis. Data were acquired using Analyst 1.4.1 software package (Applied Biosystems). Statistical analyses were performed with Origin 8.0 (OriginLab Corp., Northampton, MA). For data analysis, concentrations below the LOQ were assigned a value equal to the LOQ divided by the square root of 2 in the calculation of geometric mean (GM). Correlations between analytes were assessed by Pearson correlation analysis. A value of $p < 0.05$ was considered significant.

RESULTS

BADGEs, BFDGEs, and BPA in Adipose Fat. BADGE·2H_2O was found in 60% of adipose specimens, with a median and maximum concentration of 3.44 and 45.4 ng/g wet weight, respectively (Table 2). BADGE was found in 25% of the samples, at a maximum concentration of 5.16 ng/g. Chlorohydrin derivatives of BADGE, including BADGE·H_2O·HCl, BADGE·HCl, and BADGE·2HCl, were found in very few samples (<20%). Overall, at least one of the 6 BADGEs was found in 70% of the samples analyzed, and the sum of concentrations of the six BADGEs (i.e., $\sum_6$BADGEs) ranged from 0.78 to 64.6 ng/g, with a GM value of 4.80 ng/g.

BFDGE was detected in all adipose specimens, at a concentration range of 19.1–4500 ng/g (GM: 209 ng/g) (Table 2). BFDGE·2H_2O and BFDGE·2HCl were found less frequently (<20%) at concentrations below 15 ng/g. The GM value for the sum of concentrations of the three BFDGE derivatives (i.e., $\sum_3$BFDGEs) was 211 ng/g.

The sum concentrations of BADGEs and BFDGEs ($\sum_9$BADGEs&BFDGEs) in adipose fat ranged from 23.6 to 4560 ng/g, with a median concentration of 180 ng/g. No significant age- or gender-related differences were found for $\sum_9$BADGEs&BFDGEs in adipose tissue (SI Table S5). The median concentration of $\sum_9$BADGEs&BFDGEs in adipose tissue from African American donors was 342 ng/g, which was twice the concentration found for Caucasians (162 ng/g). Further studies with larger sample sizes are needed to elucidate demographic differences in BADGE/BFDGE accumulation. BPA was detected in 18 adipose samples (90%), at a median concentration of 5.65 ng/g (Table 2).

BADGEs, BFDGEs, and BPA in Plasma. BADGE was not found in plasma samples at concentrations above the LOQ (0.25 ng/mL) (Table 2). However, hydrated derivatives of BADGEs, BADGE·H_2O, and BADGE·2H_2O were frequently detected, with detection frequencies of 65% and 70%, respectively (Table 2). Concentrations of BADGE·H_2O in plasma samples ranged from <LOQ to 9.54 ng/mL, with a median concentration of 2.26 ng/mL. The median concentration of BADGE·2H_2O in plasma samples (7.15 ng/mL) was three times higher than that found for BADGE·H_2O (2.26 ng/

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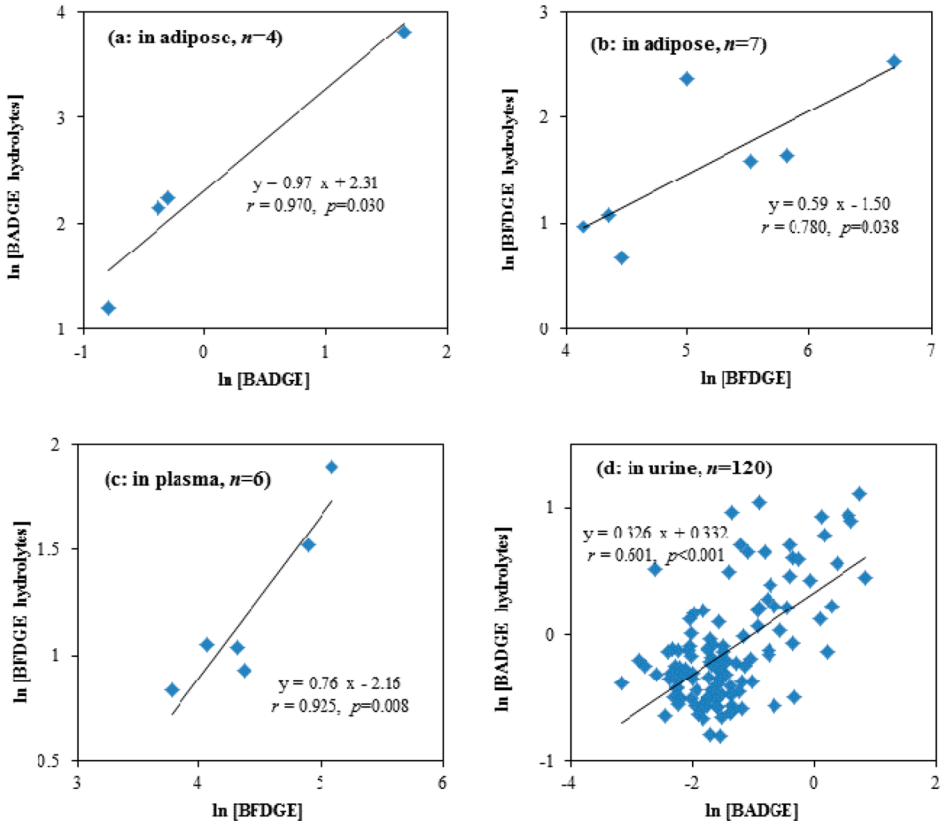

Figure 1. Correlation between natural logarithmic concentration (ln) of BADGE/BFDGE and their corresponding hydrolytes in human specimens. (a) ln [BADGE] vs ln [BADGE hydrates] in adipose fat samples; (b) ln [BFDGE] vs ln [BFDGE hydrates] in adipose fat samples; (c) ln [BFDGE] vs ln [BFDGE hydrates] in plasma; and (d) ln [BADGE] vs ln [BADGE hydrates] in urine[18]. Note: [BADGE hydrates] is the sum concentration of BADGE·$2H_2O$ and BADGE·H_2O, [BFDGE hydrates] is the concentration of BFDGE·$2H_2O$. Only those samples with measurable levels of chemicals presented on x- and *y*-axes were used in the correlation.

mL). BADGE·H_2O·HCl was found at low concentrations in plasma (maximum: 1.41 ng/mL).

Similar to that found for adipose fat samples, high concentrations of BFDGE, at a range of 23.3−180 ng/mL, were found in all plasma specimens (Table 2). The median concentration of BFDGE in plasma was 58.3 ng/mL. BFDGE·$2H_2O$ was found in five plasma samples, ranging in concentrations from 2.31 to 6.65 ng/mL. BPA also was found in all plasma samples, at concentrations that ranged from 0.784 to 4.97 ng/mL.

Correlation between BADGE/BFDGE and their Derivatives. Among the 20 adipose samples analyzed, four samples contained detectable concentrations of BADGE and its derivatives. A positive linear correlation was found between the natural logarithm (ln) of BADGE and BADGE derivative concentrations (including BADGE•H_2O and BADGE•$2H_2O$) (r = 0.970, p = 0.030, Figure 1 (a)). Similarly, BFDGE and its hydrate were found in seven adipose samples, and a positive correlation was found between ln [BFDGE] and ln [BFDGE•$2H_2O$] (r = 0.780, p = 0.038, Figure 1 (b)). For the plasma samples, however, the association between BADGE and its derivatives was not examined due to the lack of BADGE in this matrix. In the six plasma samples that contained detectable concentrations of both BFDGE and BFDGE•$2H_2O$, a significant linear correlation was found between these two compounds (r = 0.925, p = 0.008, Figure 1 (c)). Based on the urinary concentrations reported for a population in New York State in our previous study, a positive linear correlation was also found between ln [BADGE] and ln [BADGE hydrates] in urine (r = 0.601, p < 0.001, Figure 1 (d)).

Correlation between BFDGEs and BADGEs. A total of 14 (70%) adipose fat samples had measurable concentrations for both BADGEs and BFDGEs. No correlation was found between the sum concentration of BADGEs ([$\sum_6$BADGEs]) and BFDGEs ([$\sum_3$BFDGEs]) in adipose fat (Figure 2). However, when the samples that contained high concentrations

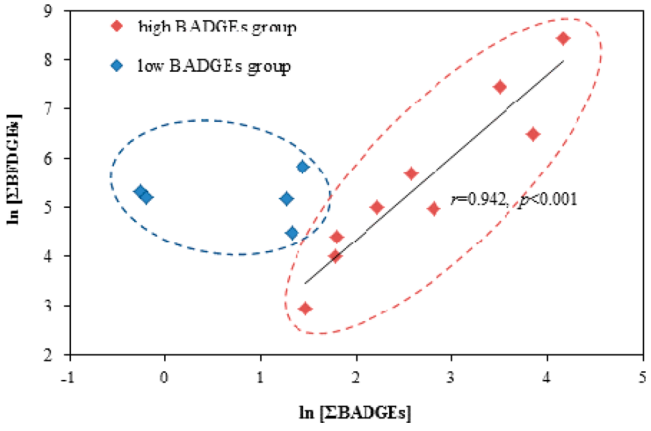

Figure 2. Correlation between natural logarithmic concentration (ln) of ∑BADGEs and ∑BFDGEs in human adipose fat samples. Note: Only those samples with measurable levels of chemicals are presented on *x*- and *y*-axes.

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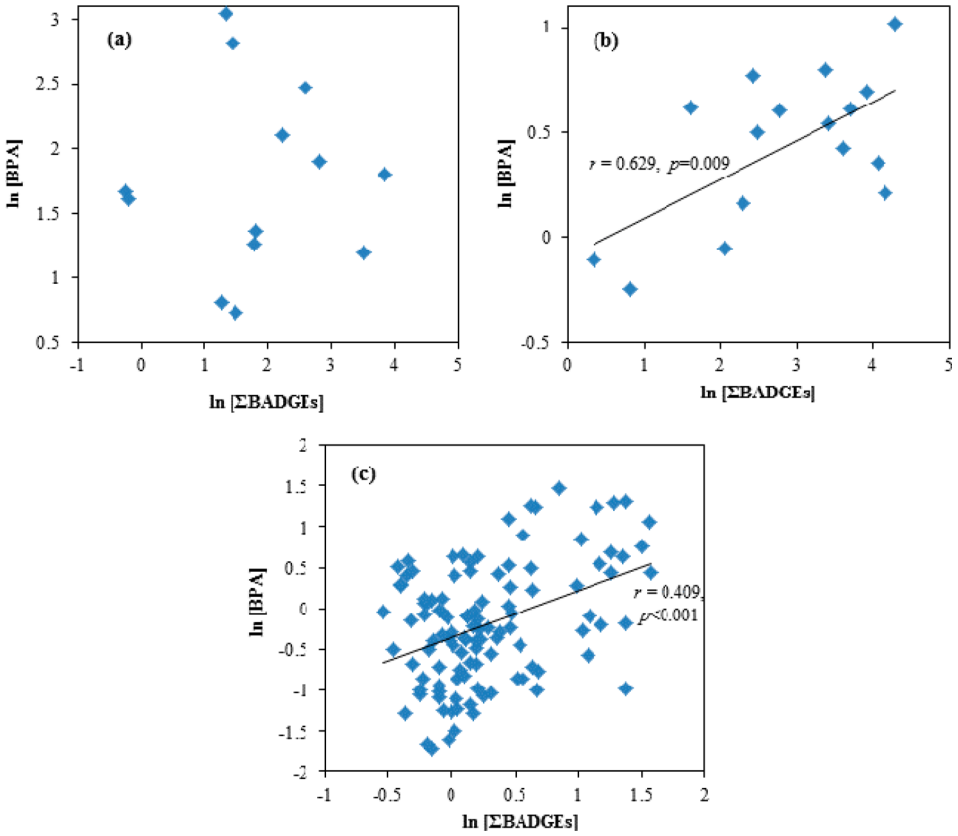

Figure 3. Correlation between the natural logarithmic concentration (ln) of BADGEs and bisphenol A (BPA) in human specimens. (a) adipose fat, (b) plasma, (c) urine.[18]

of BADGEs (ranged 4.41−64.6 ng/g) were examined, a significant positive correlation was found between ln [$\sum_6$BADGEs] and ln [$\sum_3$BFDGEs] (r = 0.942, p < 0.001, Figure 2). In plasma samples that contained quantifiable concentrations of both BADGEs and BFDGEs (n = 16), no obvious correlation was found between these two classes of compounds (SI Figure S3).

Correlation between BADGEs and BPA. A total of 13 adipose fat samples contained measurable concentrations of both BADGEs and BPA; no significant correlation was found between ln [BPA] and ln [$\sum_6$BADGEs] in adipose fat (Figure 3(a)). However, in plasma samples that contained detectable concentrations of both BADGEs and BPA (n = 16), a significant correlation between ln [BPA] and ln [$\sum_6$BADGEs] was found (r = 0.629, p = 0.009, Figure 3(b)). Similarly, in urine samples from New York State, concentrations of BPA were significantly correlated with [$\sum_4$BADGEs] (r = 0.409, p < 0.001, Figure 3(c)).

DISCUSSION

Despite their widespread use in food can coatings[13−17,22], the occurrence of BADGEs and BFDGEs in human specimens have been reported in very few studies[18−20]. This is the first study to report the occurrence of BFDGEs in human tissues. The concentrations of BFDGEs were higher than those of BADGEs in human adipose fat. An earlier study showed that migration of BFDGE from epoxy coatings into canned food products was faster than that of BADGE[28].

It should be noted that adipose fat and plasma were collected from different individuals, and, therefore, direct comparison of measured concentrations between these two matrices is not appropriate. However, it is clear that adipose tissues contained higher concentrations of BFDGE than did plasma samples. The GM concentration of BFDGE in adipose fat (209 ng/g) was nearly four times higher than that in plasma (58.3 ng/g). High concentrations of BFDGE (as high as 4500 ng/g) were found in the adipose fat of some donors, which suggests considerable accumulation of this compound. The measured concentration of BFDGE in adipose tissue of several individuals was higher than the tested concentrations of BADGE that induced adipogenesis in stromal stem cells[21]. Although the obesogenic effect of BFDGE is still unknown, considering the similarity in molecular structure with BADGE, our results suggest the significance of monitoring of BADGE/BFDGE in adipose tissues in populations to examine the potential association between exposures and obesity-related health outcomes. In comparison with the reported concentration of BADGEs in urine from the USA[18], concentrations in plasma are lower.

Hydration of BADGE has been reported in laboratory animal studies[29−31]. More frequent detection of BADGE·2H_2O than that of BADGE in human specimens (adipose, blood, urine) supports the notion that BADGE is hydrated quickly in human bodies[18−20]. A positive correlation between oxirane-containing precursors and their hydrated products in human specimens supports the biotransformation of BADGE/BFDGE. However, in contrast to BADGE, hydrolysis of BFDGE seems to be less efficient, as evidenced by less frequent detection of BFDGE·2H_2O. Further studies are needed to examine the persistence of BFDGE and BADGE in humans.

In adipose fat samples that contained high concentrations of BADGEs, a significant positive correlation between BFDGEs and BADGEs suggested similar exposure sources and pathways

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for these compounds. Information regarding usage, environmental release and stability of BADGE and BFDGE is limited. However, as mentioned above, faster migration of BFDGE from epoxy coatings into canned food than that of BADGE has been reported[28]. A recent study also reported high concentrations of BFDGE in canned meat and seafood from Spain (mean: 240 ng/g in meatballs, 314 ng/g in tripe, 251 ng/g in mussels), while BADGE was not detected in those samples[22]. These results suggest that human dietary exposure to BFDGE can be higher than that of BADGE.

Occurrence of high concentrations of BPA was reported in urine samples of individuals who were occupationally exposed to BADGE[11]. Nevertheless, studies have shown that BADGE was not biotransformed into BPA by the mammalian metabolic system[29,32,33]. BADGE is a reaction product of one mole of BPA with two moles of epichlorohydin[1]. Therefore, free BPA commonly present in many BADGE-containing technical formulations[34] can be released into canned food[35]. Positive correlations between BADGEs and BPA in various human specimens (blood and urine) provide further confirmation for concurrent exposure of BADGE and BPA in humans. However, the relationship between BADGE and BPA was not significant in adipose, which might suggest differences in toxicokinetics and bioaccumulation potentials of these two classes of chemicals[36,37]. The measured concentrations of BPA in adipose and plasma samples in this study were similar to those reported in other biomonitoring studies[36−38]. Overall, in comparison with BPA, concentrations of BFDGEs and BADGEs (e.g., BFDGE, BADGE·2H_2O) in human tissues are higher, which suggests the need for inclusion of BADGEs and BFDGEs in future biomonitoring studies. It should be noted that the donors of adipose tissue underwent liposuction and therefore they may not represent the population in general. However, since studies have related exposure of BADGE to obesity[21], further studies are needed to assess exposures to these chemicals and health outcomes in populations. Furthermore, it is not known whether BFDGE is used as an alternative for BADGE in the production of "BPA-free" products. However, the relatively high concentrations of BFDGE in human tissues found in this study suggest the need for further studies to understand sources, environmental transport and fate of BPA-related compounds[39].

■ ASSOCIATED CONTENT

Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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