JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Concentrations of Bisphenol A, Bisphenol A Diglycidyl Ether, and Their Derivatives in Canned Foods in Japanese Markets

JUN YONEKUBO,[†,§] KAZUICHI HAYAKAWA,[§] AND JUNKO SAJIKI*[,#]

Nihon Waters K.K., Katokichi Shin-Osaka Building, Osaka 532-0011, Japan; Graduate School of Natural Science and Engineering, Kanazawa University, Kanazawa 920-1192, Japan; and Chiba Prefectural Institute of Public Health, Chiba 260-8715, Japan

Bisphenol A (BPA), bisphenol A diglycidyl ether (BADGE), and their derivatives in 38 canned foods sold in Japan were measured using high-performance liquid chromatography–mass spectrometry (LC-MS) and LC−tandem mass spectrometry (LC-MS/MS). BPA, BADGE, BADGE·2H_2O, BADGE·HCl·H_2O, BADGE·HCl, and BADGE·2HCl were 0–235.4, 0–3.4, 0–247.2, 0.2–196.4, 0–3.0, and 0–25.7 ng/g, respectively, which did not exceed acceptable daily intake for BPA and specific migration limit for BADGEs. BADGE was degraded by 58, 100, 46, and 58% in water (pH 7), 0.01 N HCl (pH 2), 0.01 N NaCl (pH 6.8), and 0.01 N NaCl with acetic acid (pH 2.5), respectively, when it was allowed to stand at 120 °C for 30 min. The prominent derivatives formed were BADGE·2H_2O and BADGE·HCl·H_2O, which was formed not only in BADGE with added HCl but also in that with NaCl. Acetic acid accelerated the formation of both BADGE·2H_2O and BADGE·HCl·H_2O in NaCl. No BPA was detected in any simulation samples started from BADGE. The results suggest that BPA and BADGE are independently leached into canned foods and that BADGE is easily changed to more stable compounds such as BADGE·2H_2O and BADGE·HCl·H_2O by sterilization.

KEYWORDS: LC-MS; LC-MS/MS; BADGE degradation; can sterilization

INTRODUCTION

The endocrine effects of bisphenol A (BPA) on humans are controversial. Some researchers have concluded that these effects can be ignored, because the exposure of humans to BPA from food and wine as estimated by European Union scientific bodies (0.0005–0.009 mg/kg/day) is far lower than the reference dose (0.05 mg/kg/day) calculated by the U.S. Environmental Protection Agency as being a safe daily dose for humans over a lifetime of exposure (*1*). Furthermore, BPA is converted into a harmless metabolite, BPA-glucuronide, which has less estrogenicity, in the mammalian liver (*2, 3*). However, others have reported that BPA is transferred from the maternal body to the fetus (*4, 5*), causes abnormalities of reproductive organs (*6, 7*), advances female puberty (*8*), and changes behavior (*9*) in experiments using mammals. Thus, there is concern that BPA may influence human development throughout the fetal period. BPA is still being detected in human biological samples such as serum and urine (*10, 11*), indicating the continued presence of sources of BPA exposure.

Recently, Sajiki et al. (*12*) reported that humans could be exposed to BPA by the oral route due to its use in can linings as an additive or a contaminant; the high concentration of BPA in popular canned foods but not in plastic- or paper-packaged foods could result from its leaching into the canned foods at the high temperatures used for sterilization processes. In the study (*12*), bisphenol A diglycidyl ether (BADGE), a monomer of epoxy resin that is the main material used for can linings, leached into water in cans at a very low level (<0.5 ng/mL) when they were allowed to stand at sterilization temperatures. Pardo et al. (*13*) reported that no BADGE peak was detected in three canned fish and five baby foods. It has been reported that there is no problem in using epoxy resins for can lining due to the lower estrogenicity of BADGE compared with BPA (*14*). Whereas high levels of chlorohydrins of BADGE and BFDGE have been reported in canned foods and ready-to-drink coffee (*15*), there are few reports concerning the contents of BADGE and its derivatives in canned foods.

There are many reports of the physiological and pharmacological roles of BADGE in adipogenic cell lines, glioma cells, and gastric cancer cells, including induction of apoptosis by agonism or antagonism of the transcriptional activity of peroxisome proliferator-activated receptor (PPAR) γ (*16*). A cytotoxic effect of BADGE and BFDGE on Caco-2 cells, the mutagenic potential of BADGE and BADGE·H_2O on the Ames *Salmonella* test, and the genotoxicity of BADGE, BADGE·H_2O,

* Address correspondence to this author at The Public Health Laboratory of Chiba Prefecture, 666-2 Nitona-cho, Chuo-ku, Chiba City, Chiba 260-8715, Japan (e-mail j.sjk@ma.pref.chiba.lg.jp; fax +81432655544).

[†] Nihon Waters K.K.

[§] Kanazawa University.

[#] Chiba Prefectural Institute of Public Health.

10.1021/jf073106n CCC: $40.75
Published on Web 02/20/2008

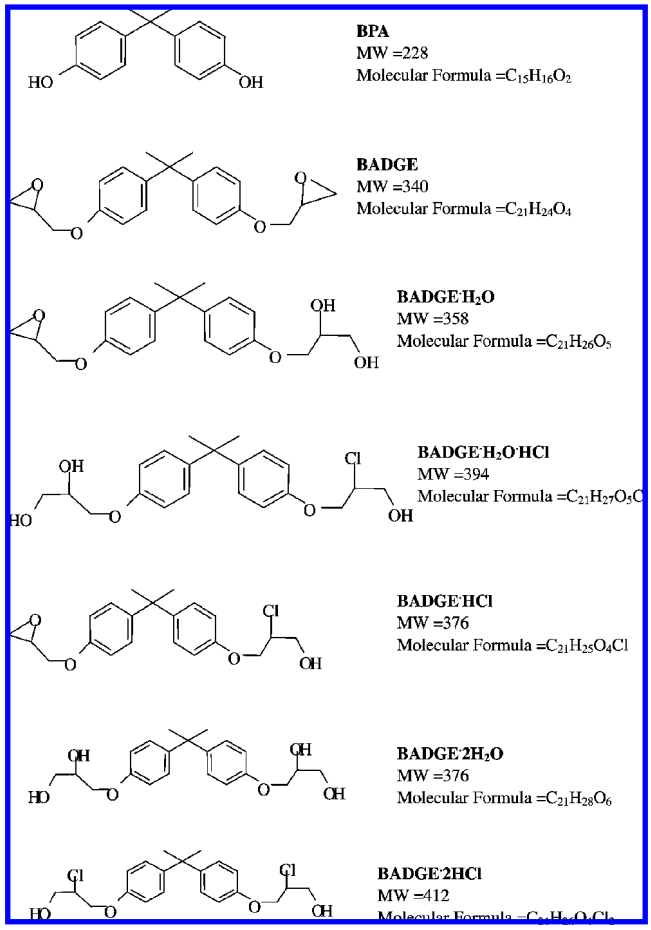

Figure 1. Structures of BPA and BADGEs used in the present study.

BADGE·$2H_2O$, and BADGE·2HCl on the micronucleus test have been reported in vitro (*17, 18*).

In addition, there are also studies on the endocrine effects of BADGE and its derivatives, including a report that there is no problem in using epoxy resins for can lining, due to the lower estrogenicity of BADGE compared with BPA (*14, 19*). Satoh et al. (*20*) observed androgen antagonistic activity of chlorohydrins of BADGE via high binding affinity for the androgen receptor, and Nakazawa et al. (*21*) reported the proliferation of breast cancer cells with BADGE derivatives at concentrations of $10^{-14}-10^{-4}$ M. Letcher et al. (*22*) reported an antiestrogenic effect of BADGE on vitellogenin production in male carp at $^1/_{10}$ of the potency of tamoxifen, a well-known estrogen receptor antagonist. Endocrine effects of chlorohydroxy compounds of BADGE as androgen antagonists (*20*) and estrogenic activity (*21*) have been reported. BADGE is also pharmacologically important in inducing apoptosis of various cells as an antagonist of the ligands that activate the transcriptional and adipogenetic action of PPARγ (*23*).

The aim of this study is to assess the risk of daily intake of BPA, BADGE, and their derivatives in canned foods sold in the Japanese market. A simulation of can sterilization was used to investigate what kinds of metabolites are formed from BADGE in canned foods.

MATERIALS AND METHODS

Samples and Chemicals. Thirty-eight canned foods were purchased from supermarkets in Chiba prefecture.

Authentic BPA and BADGE (**Figure 1**) were purchased from Wako Pure Chemicals (Tokyo, Japan) and Kanto Kagaku (Tokyo, Japan), respectively. Standards of BPA (2,3-dihydroxypropyl) glycidyl ether (BADGE·H_2O, ≥97%), BPA bis(2,3-dihydroxypropyl) ether (BADGE·$2H_2O$, ≥97%), BPA (3-chloro-2-hydroxypropyl) glycidyl ether (BADGE·HCl, ≥ 95%), BPA bis(3-chloro-2-hydroxypropyl) ether (BADGE·2HCl, ≥99%), and BPA (3-chloro-2-hydroxypropyl) (2,3-dihydroxypropyl) ether (BADGE·HC·H_2O, ≥95%) (**Figure 1**) were purchased from Fluka Chemi AG (Buchs, Switzerland). LC-grade ethanol (EtOH) and acetonitrile (Wako Pure Chemicals) were used. Other chemicals were of special grade (Wako Pure Chemicals). An Oasis HLB (Waters, Milford, MA) used for the solid phase extraction of BPA, and BADGE was washed with 3.5 mL of EtOH followed by 3.5 mL of water before use. BPA-free water prepared using ODS-silica Sep-Pak cartridges (Waters) was used throughout the experiments after deionization of tap water using Milli-RX12α (which uses a combination of reverse osmosis and electric deionization) (Nihon Millipore, Tokyo, Japan). Glass tubes for BPA analysis were prewashed with 99% ethanol. A multiblender mill (with stainless steel cups; Nihonseiki Kaisha Ltd., Tokyo, Japan) was used for homogenizing foods.

pH of Canned Foods. After homogenization of all contents of canned foods with a multiblender mill, 5 g of each food was dissolved in the same volume of BPA-free water. The pH of samples was measured using a pH-meter (Laboratory-F, Horiba, Tokyo, Japan).

Extraction of BPA and BADGE from Foods. BPA was extracted from canned foods immediately after the removal of the lid. After homogenization of all contents of canned foods with a multiblender mill, 2–5 g samples were taken. The samples were prepared according to the procedure described in the previous paper (*12*). Briefly, BPA and BADGE were extracted from 2−5 g food samples with acetonitrile and purified using Oasis-HLB with 15% ethanol and peritoneum ether. After evaporation of organic solvents under N_2 gas, the extract was dissolved in 1 mL of acetonitrile/water (40:60).

Degradation of BADGE to BPA during Simulation of Can Sterilization. Four hundred microliters of authentic BADGE (10 μg/mL in methanol) was put into four glass tubes (10 mm diameter and 10 cm length), each together with 3.6 mL of water (pH 7.2), 0.01 N HCl (pH 2), 0.01 N NaCl (pH 6.8), and 0.01 N NaCl with 0.1% acetic acid (pH 2.5). The tubes were allowed to stand at 120 °C for 30 min in a dry bath (Dry thermo unit TA-2H; Taitec Co.). After cooling at room temperature (20 °C), 0.1 mL of each sample was applied directly to liquid chromatography−tandem mass spectrometry (LC-MS/MS).

Determination of BPA. The high-performance liquid chromatography–mass spectrometry (LC-MS) system was a Waters Alliance 2690 equipped with a Waters ZQ2000 mass detector. Ionization conditions were as follows: ESI negative, capillary voltage, 3.5 kV; cone voltage, 39 V; source block temperature, 130 °C; dissolution temperature, 390 °C. Separation was carried out using a Symmetry C_{18} column (3.5 μm, 150 mm × 2.1 mm i.d.; Waters) at 40 °C under isocratic conditions with an acetonitrile/water (40:60) mobile phase. The flow rate and injection volume were 0.25 mL/min and 10 μL, respectively. Selected ion monitoring mode (*m/z* 227, $M - H^-$) was used for quantitative analysis of BPA.

The LC-MS/MS system consisted of a Waters Acquity UPLC system and a Waters Quattro Premier API MS/MS analyzer. Ionization conditions were as follows: ESI negative, cone voltage, 37 V; collision energy, 20 eV; source block temperature, 130 °C; dissolution temperature, 390 °C. Separation was carried out using an Acquity BEH C_{18} column (1.7 μm, 50 mm × 2.1 mm i.d.; Waters) at 40 °C, using a linear gradient of acetonitrile in water as the mobile phase: 0–1 min, 25% acetonitrile; 1–7 min, 25–75% acetonitrile; 7–9 min, 75% acetonitrile. The flow rate and injection volume were 0.35 mL/min and 5 μL, respectively. Selected reaction mode, with a precursor ion of 227 as $(M - H)^-$ and a product ion of 212 were used for quantitative analysis of BPA.

Determination of BADGE and Derivatives. BADGE and its derivatives were determined by LC-MS/MS. The LC-MS/MS system consisted of a Waters Acquity UPLC equipped with a Quattro Premier API MS/MS analyzer. Ionization conditions were as follows: ESI positive, source block temperature, 130 °C; dissolution temperature, 390 °C. Precursor and production ions, cone voltages, and collision energies for analysis of BADGE and its derivatives are shown in **Table 1**. Separation was carried out using an Acquity BEH C_{18} column (1.7

Table 1. Parameters of MS/MS for the Determination of BADGE and Its Derivatives

compound	precusor ion	cone voltage (V)	product ion for quantify	collision energy (eV)	product ion for confirmation	collision energy (eV)
BADGE	358 as $(M + NH_4)^+$	20	191	20	135	30
BADGE·H_2O	376 as $(M + NH_4)^+$	20	209	15	135	30
BADGE·$2H_2O$	394 as $(M + NH_4)^+$	20	209	20	135	30
BADGE·HCl·H_2O	412 as $(M + NH_4)^+$	20	227	15	135	30
BADGE·HCl	394 as $(M + NH_4)^+$	20	227	20	135	30
BADGE·2HCl	430 as $(M + NH_4)^+$	20	227	20	135	30

Table 2. Analytical Features of LC-MS/MS Methods for BADGE and Its Derivetives

	recovery (%)			
compound	authentic	sample	%RSD	LOD (ng/g)
BADGE	90	98	1.22	0.39
BADGE·H_2O	88	81	2.18	0.69
BADGE·$2H_2O$	101	69	1.74	0.57
BADGE·HCl·H_2O	103	90	1.85	0.60
BADGE·HCl	69	69	1.83	0.63
BADGE·2HCl	78	76	1.70	0.54

μm, 50 mm × 2.1 mm i.d.; Waters) at 40 °C using a linear gradient of methanol with 5 mM ammonium acetate as the mobile phase: 0–1 min, 25% methanol; 1–5 min, 25–75% methanol; 5–7 min, 75% methanol. The flow rate and injection volume were 0.25 mL/min and 1 μL, respectively.

RESULTS

Determination of BPA, BADGE, and Derivatives in Canned Foods. The limit of detection (LOD) and recovery of BPA by LC-MS were 0.3 ng/mL (%RSD = 1.8, $n = 7$) and 93%, respectively. **Table 2** shows the LODs and recoveries of BADGE and its derivatives (50 ng/mL) added to water and tomato juice sample. The concentrations of BPA, BADGE, and derivatives in canned foods are shown in **Table 3**. BPA levels were 0–30 ng/g for fish, 0–25 ng/g for vegetables, 1–235 ng/g for sauces, and 27–31 ng/g for others. BADGE levels were 0–3 ng/g for fish, 0–1 ng/g for vegetables, 0–3 ng/g for sauces, and 0–1 ng/g for others. BADGE·$2H_2O$ levels were 0–142 ng/g for fish, 1–106 ng/g for vegetables, 5–247 ng/g for sauces, and 22–220 ng/g for others. BADGE·$2H_2O$ was not detected in any analytical samples. BADGE·HCl·H_2O levels were 0–66 ng/g for fish, 1–27 ng/g for vegetables, 3–196 ng/g for sauces, and 27–125 ng/g for others. BADGE·HCl levels were 0–3 ng/g for fish, 0–1 ng/g for vegetables, 0–2 ng/g for sauces, and 0–0.1 ng/g for others. BADGE·2HCl levels were 0–25 ng/g for fish, 0–14 ng/g for vegetables, 0–26 ng/g for sauces, and 0–9 ng/g for others. The results show that BADGE·$2H_2O$ and BADGE·HCl·H_2O are the major derivatives of BADGE in canned foods. There were significant positive correlations between the levels of BADGE·$2H_2O$ and BADGE·HCl·H_2O ($r = 0.8209$, $p < 0.01$), between BADGE and BADGE·HCl ($r = 0.8170$, $p < 0.01$), between BADGE and BADGE·$2H_2O$ ($r = 0.5100$, $p < 0.01$), between BADGE·HCl·H_2O and BADGE·2HCl ($r = 0.3796$, $p < 0.05$), and between BADGE·HCl and BADGE·2HCl ($r = 0.5722$, $p < 0.01$). There were no significant correlations among other parameters, including BPA and the pH of the canned foods. **Table 4** shows the averages of the concentrations (nanograms per gram) of BPA, BADGE, and derivatives in various can contents as a function of type of can [classic or easy pull open (epo)]nd type of food (fishes, vegetables, sauces, and others). The concentrations of BPA, BADGE·$2H_2O$, and BADGE·HCl·H_2O were >2 times higher in epo cans than in classic cans. Both concentrations of BADGE·HCl and BADGE·2HCl were higher in epo cans than in classic cans.

Degradation of BADGE in the Simulation of Can Sterilization. No BPA was detected in authentic BADGE solutions dissolved in any experimental solvents allowed to stand at 120 °C for 30 min followed by standing at room temperature (20 °C) for 180 min. Changes in the concentrations of BADGE and its derivatives are illustrated in **Figure 2**. In water (**Figure 2a**), BADGE decreased by almost half during incubation at 120 °C and to 3% after 30 min at room temperature. BADGE·$2H_2O$ and BADGE·H_2O were formed at levels of 313 and 62 ng/mL, respectively, during incubation at 120 °C. Thereafter, both BADGE derivatives remained at these levels to 180 min. No BADGE·HCl·H_2O and BADGE·2HCl was detected throughout the experimental period. In HCl solution (**Figure 2b**), BADGE decreased rapidly to zero during the heating period at 120 °C, whereas BADGE·$2H_2O$ and BADGE·HCl·H_2O decreased to 328 and 178 ng/mL, respectively, at 0 min (immediately after 30 min of heating at 120 °C). No BADGE·H_2O was formed during the experimental period except at 0 min (15 ng/mL formed). BADGE·2HCl was detected at levels of 40 and 39 ng/mL at 0 and 30 min, respectively. In NaCl (**Figure 2c**), BADGE decreased by almost half during the incubation at 120 °C and to 25 ng/mL in 30 min at room temperature, in a manner similar to that of the sample in water. In contrast, BADGE·$2H_2O$ and BADGE·H_2O were formed at levels of 200 and of 100 ng/mL within 30 min at 120 °C, and these levels were maintained for 180 min at room temperature. In the solvent, a low level of BADGE·HCl·H_2O was formed during incubation at 120 °C and maintained at 30 ng/mL until 180 min. BADGE·2HCl was detected at levels of 13 and 12 ng/mL at 0 and 30 min, respectively. In NaCl with 0.1% acetic acid (**Figure 2d**), BADGE decreased to 420 ng/mL during incubation at 120 °C and to 25 ng/mL in 30 min at room temperature, whereas both BADGE·$2H_2O$ and BADGE·HCl·H_2O increased to 306 and 73 ng/mL within 30 min at 120 °C, respectively. No BADGE·H_2O formed during the experimental period, as in the sample in HCl solution. BADGE·2HCl was detected at levels of 29 and 25 ng/mL at 0 and 30 min, respectively. In all samples under both acidic and neutral conditions, no BADGE·HCl was produced during the experimental period.

DISCUSSION

Despite studies confirming the estrogenicity of BPA with many kinds of biomaterials such as MCF-7 cells and mice (*24*), use of BPA has continued as it has been reported to be of low risk to humans (*25*). In Japan, the BPA supply of 576213 t and demand of 426674 t in 2003 were 1.35 and 1.11 times higher, respectively, than 5 years ago.

Fujimaki et al. (*26*) estimated the daily intake of BPA in Japanese pregnant women to be 0.3–7.9 μg/day (median < 2 μg/day), which is far below the acceptable daily intake (ADI).

Table 3. BPA, BADGE, and Its Metabolite Concentrations (Nanograms per Gram) in Various Can Contents[a]

	type of can	pH	BPA	BADGE	BADGE·2H$_2$O	BADGE·H$_2$O	BADGE·H$_2$O·HCl	BADGE·HCl	BADGE·2HCl
fishes									
tuna	classic	5.5	4.5	0.4	17.4	nd	8.0	nd	nd
shrimp	classic	6.5	30.5	nd	6.8	nd	4.6	nd	nd
Japanese sand lance	epo		0.7	nd	3.0	nd	4.0	nd	nd
squid	classic	6.5	tr	tr	53.6	nd	20.3	nd	nd
mackerel pike	epo	5.8	nd	nd	tr	nd	0.2	nd	nd
mackerel pike	epo	6.0	nd	nd	0.0	nd	0.4	nd	nd
salmon	epo	6.3	4.5	0.1	1.6	nd	12.0	nd	nd
salmon	epo	6.5	2.7	0.1	0.6	nd	3.5	nd	nd
mackarel	epo	5.6	4.0	0.1	1.0	nd	1.0	nd	2.6
mackarel	epo	6.3	2.0	0.1	64.0	nd	12.0	nd	nd
mackarel	epo	5.8	0.0	0.1	0.0	nd	2.0	nd	2.5
mackarel	epo	6.3	3.0	0.1	100.0	nd	20.0	nd	nd
mackarel	epo	6.0	3.0	1.0	113.6	nd	23.2	nd	1.2
sardine	epo	5.5	1.4	2.0	123.0	nd	30.0	3.0	17.6
sardine	epo	5.7	nd	1.1	142.0	nd	33.0	0.3	23.7
sardine	epo	5.4	1.0	2.0	126.0	nd	37.0	2.0	25.7
sardine	epo	5.9	0.0	3.0	114.0	nd	66.0	3.0	19.0
sardine	epo	5.2	4.0	1.0	112.0	nd	23.0	nd	nd
sardine	epo	5.5	3.0	1.4	73.0	nd	28.0	nd	nd
fish and vegetables	epo	6.3	3.0	0.1	94.0	nd	38.0	nd	3.9
fish and vegetables	epo	6.3	2.0	0.1	82.0	nd	24.0	nd	3.2
vegetables									
tomato	epo	4.3	14.2	1.0	31.6	nd	23.1	1.3	2.4
tomato	epo	4.3	6.3	tr	36.1	nd	22.9	nd	nd
asparagus	classic	5.6	3.3	nd	19.3	nd	14.5	nd	1.3
mushroom	classic	5.4	1.9	nd	106.4	nd	27.2	nd	nd
mount elephant	classic	5.6	24.8	nd	16.4	nd	5.4	nd	0.8
tomato juice	epo	4.3	tr	0.1	1.2	nd	0.8	nd	nd
carrot juice	epo	4.2	0.0	0.1	3.2	nd	18.0	nd	13.7
sauces									
tomato	epo	4.1	15.4	0.8	247.2	nd	196.4	0.6	12.1
meat	epo	4.3	4.4	0.1	5.1	nd	9.5	nd	2.8
demi-glace	epo	4.6	6.6	0.8	9.1	nd	34.1	nd	25.6
demi-glace	epo	5.1	34.8	0.2	242.0	nd	73.6	nd	2.8
demi-glace	classic	5.1	0.9	0.1	16.4	nd	6.5	nd	nd
fond de volaille	classic	5.6	1.6	nd	8.2	nd	2.7	nd	nd
white	epo	5.9	55.5	3.4	97.6	nd	48.0	1.5	3.9
gratin	epo	5.9	235.4	tr	66.4	nd	19.4	nd	3.7
others									
quail eggs	epo	6.6	31.0	0.0	219.6	nd	125.2	nd	9.2
coconut milk	epo	6.3	27.0	0.8	22.0	nd	26.7	0.1	nd

[a] epo, easy pull open; nd, not detected; tr, trace.

Table 4. Averages of BPA, BADGE, and Its Derivative Concentrations (Nanograms per Gram) in Various Can Contents as a Function of Type of Can and Food[a]

type of can	no. of cans	BPA	BADGE	BADGE·2H$_2$O	BADGE·HCl·H$_2$O	BADGE·HCl	BADGE·2HCl
epo	30.0	15.6	0.7	71.1	31.9	0.4	6.0
classic	8.0	8.4	0.1	30.6	11.2	0.0	0.3

[a] epo, easy pull open.

They also calculated that the maximal level reached was $^1/_{10}$ that of the lowest level at which adverse effects of BPA (2 μg/kg/day) were reported in pregnant mice (*6*). The daily intake of BPA estimated from the BPA concentration in hospital meals, with which care is taken by using cooking implements that avoid BPA exposure and using wrapping films that do not contain BPA, was 0.42 μg/day, and this probably originated from tuna meat in cans (*27*). In our previous paper, three canned foods with the same brand name and manufacturer but obtained in different places at different dates showed the highest BPA levels (canned brown sauce at 428.4, 547.2, and 842.3 ng/g). If an adult weighing 50 kg ingested such canned food every day, the estimated BPA exposure would be 4.89 μg/kg/day. This value corresponds to around $^1/_{10}$ of the ADI (0.05 mg/kg/day) proposed by the European Scientific Committee on Food, which indicated that the main source of human exposure to BPA is foods from cans with linings that contain high percentages of BPA as an additive. In the present analysis, BPA levels in foods from cans were lower than those reported in the previous study. Six of the eight sauce cans had the same manufacturer, and the BPA concentrations in the sauces from varied widely (1−235 ng/g), which suggests that different BPA levels are caused by differences in the materials used for can linings.

No BPA was detected in authentic BADGE solutions dissolved in water, 0.01 N HCl (pH 2), 0.01 M NaCl (pH 6.8), or 0.01 M NaCl with 0.02–0.1% acetic acid allowed to stand at 120 °C for 30 min, although BADGE immediately decomposed to derivatives such as BADGE·H$_2$O, BADGE·2H$_2$O, or

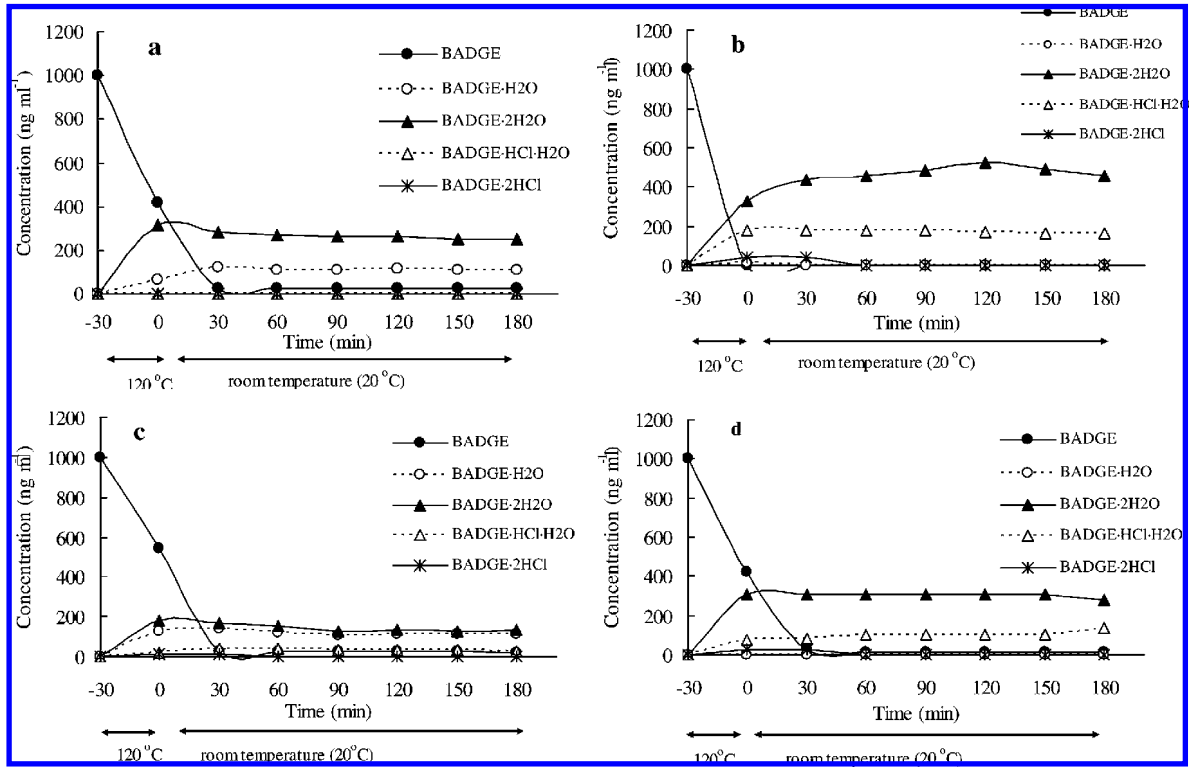

Figure 2. Change in the concentrations of BADGE and its derivatives when authentic BADGE was allowed to stand in water (**a**), 0.01 N HCl (**b**), 0.01 N NaCl (**c**), or 0.01 N NaCl with 0.1% acetic acid (**d**) at 120 °C for 30 min followed by standing at room temperature (20 °C) for 180 min.

BADGE·HCl·H_2O. The data support the suggestion that BPA detected in can contents could originate from contamination of can lining materials with BPA but not from the degradation of BADGE. The lack of correlations between BPA and the BADGE derivatives in canned foods and the absence of degradation of BADGE to BPA in the present study support our previous suggestion that BPA could come from additives and contaminants in can linings (*12*).

Among BADGE derivatives, BADGE·2H_2O and BADGE·HCl·H_2O were abundantly leached into can contents, whereas BADGE, BADGE·HCl, and BADGE·H_2O were present at very low or zero levels in the samples examined. In the simulation of the sterilization process using neutral solutions of water and NaCl, BADGE·2H_2O and BADGE·H_2O (at a similar level to BADGE·HCl·H_2O in the sample with NaCl) were predominantly produced and their levels maintained to 180 min, although BADGE decreased drastically. In acidic solutions of HCl and NaCl with acetic acid, BADGE·HCl·H_2O was abundantly produced, after BADGE·2H_2O. These results suggest that products such as BADGE·2H_2O and BADGE·HCl·H_2O could be formed by the sterilization of canned foods containing BADGE accompanied with and without acidic degradation of BADGE. The most abundant derivatives leached into canned foods were BADGE·2H_2O followed by BADGE·HCl·H_2O, which agreed with the results of other studies (*28*). It was not clarified whether BADGE was present in the epoxy resin as a contaminant or was released from the resin through the sterilization of canned foods. The fact that no significant correlations were observed between the pH of the can contents and BADGE or its derivatives suggests that BADGE could have been present due to the use of additives to remove HCl or as a contaminant, but was not formed by hydrolysis in the foods.

In the present study, the average BPA level in epo-canned foods was twice that in classic cans, having lid linings that were shown to be epoxy resin by IR analysis (data not shown). In addition, many cans used in the present study were of the epo type, and they had significantly higher levels of both BADGE·2H_2O and BADGE·HCl·H_2O besides BADGE·2HCl compared with classic cans. Particularly, BADGE·2HCl was also 20 times higher in epo cans than classic cans. Munguia-Lopez et al. (*29*) reported that leaching of BPA into fatty food simulated from cans coated with polyvinyl chloride (PVC) resins was much greater than that from cans coated with epoxy resins. BADGE·HCl·H_2O detected in canned food is usually thought to be formed as a byproduct of the reaction of BADGE with HCl, which is produced spontaneously by the degradation of organosol PVC, in which BADGE is usually used as a stabilizer to scavenge HCl (*30*). The high BPA and BADGEs levels of epo-canned foods might be due to the use of PVC resins in the lining of the can lids, as mentioned by Uematsu et al. (*15*). However, these compounds were detected even in classic cans having lining materials in the side, lid, and bottom that were all epoxy resin. Judging from the production of these compounds in the sterilization simulation study with BADGE in both NaCl and NaCl with acetic acid, BADGE·HCl·H_2O might be formed by the sterilization process in foods containing chlorine as an added ingredient or during the sterilization of tap water whenever BADGE is presented in can lining materials, even if PVC is not used for the lining material.

Although it was produced during sterilization, BADGE·H_2O had decreased to a very low level on day 3 at room temperature (data not shown), suggesting that it is fragile and therefore could not be detected in any canned foods. The fact that no BADGE·H_2O was detected in acidic samples with HCl or NaCl with acetic acid would support this assumption. This may be why no BADGE·H_2O was detected in canned foods from supermarkets, although experiments concerning time and temperature of cans were not carried out in the present study. In the simulation with BADGE in NaCl with acetic acid, the level

of BADGE·2H_2O was almost twice that of BADGE·HCl·H_2O even at 180 min, which agrees with the fact that 26 of 35 cans in which both compounds were detected showed higher levels of BADGE·2H_2O than of BADGE·HCl·H_2O. Nine cans showing the opposite (BADGE·HCl·H_2O > BADGE·2H_2O) had relatively low values of BADGE·2H_2O, suggesting a low concentration of BADGE in the lining materials compared with chlorine in foods or lining materials.

EC 1895/2005 (*31*) states that the specific migration limit (SML) of the sum of BADGE·H_2O and BADGE·2H_2O and of chlorohydroxy compounds of BADGE should be restricted to below 9 and 1 mg/kg in food, respectively. As the sum of BADGE and its derivatives in canned foods in this survey was far lower than the SML, we can conclude that it is probably safe to use the materials currently used for can linings with regard to the carcinogenicity and toxicological effects of BADGE and its derivatives, as reviewed by Poole at al (*19*). In previous papers by other researchers (*32*), very high BADGE levels in fish in oil (exceeding 1 mg/kg) were due to the use of organosol PVC lacquers for can linings. Very low levels of BADGE in canned fish may be due to the efforts of can-makers to avoid the HCl formation caused by using organosol PVC lacquers for can linings. Pardo et al. (*13*) reported that no BADGE peak was detected in three canned fish and five baby foods. High levels of BADGEs in some kinds of sauces might be due to a characteristic of oil facilitating BADGE migration to sauces.

Hanaoka et al. (*33*) reported that the concentrations of urinary BPA were higher but those of plasma follicle-stimulating hormone were lower in epoxy resin sprayers who deal with BADGE occupationally. They speculated that BPA might be generated endogenously in workers. The high concentrations of BADGE derivatives in canned foods detected in the present study do not exclude the possibility of endogenous BPA formation, although BADGE levels were very low in the foods. Because BFDGE is also used in the manufacture of epoxy resins and BFDGE derivatives were found at levels similar to those of BADGE and its derivatives in foods stored in cans (*34*), assessment of the safety of these compounds in terms of their toxicity and endocrine effects is necessary for their use in can linings.

In conclusion, the data for BPA, BADGE, and derivatives in 38 canned foods showed that BPA and BADGE were independently leached from can lining materials. As BADGE was easily degraded to mainly BADGE·2H_2O or BADGE·H_2O in water and to BADGE·2H_2O or BADGE·HCl·H_2O in chlorine-containing solutions even at neutral pH at the high temperature (120 °C) used for sterilization of canned foods, it is reasonable that BADGE was detected in the canned foods at very low or zero levels. If abundant BADGE is leached from can linings, a significant amount of BADGE·HCl·H_2O could be formed and remain in acidic canned foods containing chlorine. It is important to control can properties to maintain low levels of BPA and BADGE from the viewpoint of their estrogenicity. Further studies on the endocrine effects of BADGE derivatives, particularly chlorohydrins, at environmentally relevant doses are necessary for risk assessment.

LITERATURE CITED

(1) Gray, G.; Cohen, J. Weight of the evidence evaluation of low-dose reproductive and developmental effects of bisphenol A. Risk in perspective. *Harvard Center Risk Anal.* **2004**, *12* (3)1–4; http://www.hsph.harvard.edu/ccpe/programs/RISK.shtml.

(2) Volkel, W.; Colnot, T.; Csanady, G. A.; Filser, J. G.; Dekant, W. Metabolism and kinetics of bisphenol a in humans at low doses following oral administration. *Chem. Res. Toxicol.* **2002**, *15*, 1281–1287.

(3) Tsai, W. T. Human health risk on environmental exposure to Bisphenol-A: a review. *J. Environ. Sci. Health C: Environ. Carcinog. Ecotoxicol. Rev.* **2006**, *24*, 225–55.

(4) Miyakoda, H.; Tabata, M.; Onodera, S.; Takeda, K. Passage of bisphenol A into the fetus of the pregnant rat. *J. Health Sci.* **1999**, *45*, 318–323.

(5) Zalko, D.; Soto, A. M.; Dolo, L.; Dorio, C.; Rathahao, E.; Debrauwer, L.; Faure, R.; Cravedi, J. P. Biotransformations of bisphenol A in a mammalian model: answers and new questions raised by low-dose metabolic fate studies in pregnant CD1 mice. *Environ. Health Perspect.* **2003**, *111*, 309–319.

(6) Vom Saal, F. S.; Cooke, P. S.; Buchanon, D. L.; Palanza, P.; Thayer, K. A.; Nagel, S. C.; Stefano, P.; Welshons, W. V. A physiologically based approach to the study of bisphenol A and other estrogenic chemicals on the size of reproductive organs, daily sperm production, and behavior. *Toxicol. Ind. Health* **1998**, *14*, 239–260.

(7) Gupta, C. Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals. *Proc. Soc. Exp. Biol. Med.* **2000**, *224*, 61–68.

(8) Howdeshell,; K, L.; Hotchkiss, A. K.; Thayer, K. A.; Vandenbergh, J. G.; vom Saal, F. S. Exposure to bisphenol A advances puberty. *Nature* **1999**, *401*, 763—764.

(9) Kubo, K.; Arai, O.; Omura, M.; Watanabe, R.; Ogata, R.; Aou, S. Low dose effects of bisphenol A on sexual differentiation of the brain and behavior in rats. *Neurosci. Res.* **2003**, *45*, 345–356.

(10) Matsumoto, A.; Kunugita, N.; Kitagawa, K.; Isse, T.; Oyama, T.; Foureman, G. L.; Morita, M.; Kawamoto, T. Bisphenol A levels in human urine. *Environ. Health Perspect.* **2003**, *111*, 101–104.

(11) Calafat, A. M.; Kuklenyik, Z.; Reidy, J. A.; Caudill, S. P.; Ekong, J.; Needham, L. L. Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. *Environ. Health Perspect.* **2005**, *113*, 391–395.

(12) Sajiki, J.; Miyamoto, F.; Fukata, H.; Mori, C.; Yonekubo, J.; Hayakawa, K. Bisphenol A (BPA) and its source in foods in Japanese markets. *Food Addit. Contam.* **2007**, *24*, 103–112.

(13) Pardo, O.; Yusa, V.; Leon, N.; Pastor, A. Determination of bisphenol diglycidyl ether residues in canned foods by pressurized liquid extraction and liquid chromatography−tandem mass spectrometry. *J. Chromatogr., A* **2006**, *1107*, 70–78.

(14) Stroheker, T.; Picard, K.; Lhuguenot, J. C.; Canivenc-Lavier, M. C.; Chagnon, M. C. Steroid activities comparison of natural and food wrap compounds in human breast cancer cell lines. *Food Chem. Toxicol.* **2004**, *42*, 887–897.

(15) Uematsu, Y.; Hirata, K.; Suzuki, K.; Iida, K.; Saito, K. Chlorohydrins of bisphenol A diglycidyl ether (BADGE) and of bisphenol F diglycidyl ether (BFDGE) in canned foods and ready-to drink coffees from the Japanese market. *Food Addit. Contam.* **2001**, *18*, 177–185.

(16) Fehlberg, S.; Gregel, C. M.; Goke, A.; Goke, R. Bisphenol A diglycidyl ether-induced apoptosis involves Bax/Bid-dependent mitochondrial release of apoptosis-inducing factor (AIF), cytochrome *c* and Smac/DIABLO. *Br. J. Pharmacol.* **2003**, *139*, 495–500.

(17) Sueiro, R. A.; Araujo, M.; Suárez, S.; Garrido, M. J. Mutagenic potential of bisphenol A diglycidylether (BADGE) and its hydrolysis-derived products in the Ames *Salmonella* assay. *Mutagenesis* **2001**, *16*, 303–307.

(18) Ramilo, G.; Valverde, I.; Lago, J.; Vieites, J. M.; Cabado, A.G. Cytotoxic effects of BADGE (bisphenol A diglycidyl ether) and BFDGE (bisphenol F diglycidyl ether) on Caco-2 cells in vitro. *Arch. Toxicol.* **2006**, *80*, 748–755.

(19) Poole, A.; van Herwijnen, P.; Weideli, H.; Thomas, M. C.; Ransbotyn, G.; Vance, C. Review of the toxicology, human exposure and safety assessment for bisphenol A diglycidylether (BADGE). *Food Addit. Contam.* **2004**, *21*, 905–919.

(20) Satoh, K.; Ohyama, K.; Aoki, N.; Iida, M.; Nagai, F. Study on anti-androgenic effects of bisphenol a diglycidyl ether (BADGE), Bisphenol F diglycidyl ether (BFDGE) and their derivatives using cells stably transfected with human androgen receptor, AR-EcoScreen. *Food Chem. Toxicol.* **2004**, *42*, 983–993.
(21) Nakazawa, H.; Yamaguchi, A.; Inoue, K.; Yamazaki, T.; Kato, K.; Yoshimura, Y.; Makino, T. In vitro assay of hydrolysis and chlorohydroxy derivatives of Bisphenol A diglycidyl ether for estrogenic activity. *Food Chem. Toxicol.* **2002**, *40*, 1827–1832.
(22) Letcher, R. J.; Sanderson, J. T.; Bokkers, A.; Giesy, J. P.; van den Berg, M. Effects of bisphenol A-related diphenylalkanes on vitellogenin production in male carp (*Cyprinus carpio*) hepatocytes and aromatase (CYP 19) activity in human H295R adrenocortical carcinoma cells. *Toxicol. Appl. Pharmacol.* **2005**, *209*, 95–104.
(23) Wright, H. M.; Clish, C. B.; Mikami, T.; Hauser, S.; Yanagi, K.; Hiramatsu, R.; Serhan, C. N.; Spiegelman, B. M. A synthetic antagonist for the peroxisome proliferator-activated receptor γ inhibits adipocyte differentiation. *J. Biol. Chem.* **2000**, *275*, 1873–1877.
(24) Krishnan, A. V.; Stathis, P.; Permuth, S. F.; Tokes, L.; Feldman, D. Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinology* **1993**, *132*, 2279–2286.
(25) EU RAR, 2003. *European Union Risk Assessment Report*. http://ecb.jrc.it/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/bisphenolareport325.pdf.
(26) Fujimaki, K.; Arakawa, C.; Yoshinaga, J.; Watanabe, C.; Serizawa, S.; Imai, H.; Shiraishi, H.; Mizumoto, Y. Estimation of intake level of bisphenol A in Japanese pregnant women based on measurement of urinary excretion level of the metabolite. *Shokuhin Eiseigaku Zassi* **2004**, *59*, 403–408 (in Japanese).
(27) Higuchi, M.; Miyata, D.; Kawamura, S.; Ueda, E.; Imanaka, M.; Tonogai, Y. Estimation of daily intake of phenols in hospital meal samples. *Shokuhin Eiseigaku Zasshi* **2004**, *45*, 339–343 (in Japanese).
(28) Rauter, W.; Dickinger, G.; Zihlarz, R.; Lintschinger, J. Determination of Bisphenol A diglycidyl ether (BADGE) and its hydrolysis products in canned oily foods from the Austrian market. *Eur. Food Res. Technol.* **1999**, *208*, 208–211.
(29) Munguia-Lopez, E. M.; Gerardo-Lugo, S.; Peralta, E.; Bolumen, S.; Soto-Valdez, H. Migration of bisphenol A (BPA) from can coatings into a fatty-food simulant and tuna fish. *Food Addit. Contam.* **2005**, *22*, 892–898.
(30) Berger, U.; Oehme, M. Identification of derivatives of bisphenol A diglycidyl ether (BADGE) and Novolac glycidyl ether (NOGE) in can coatings by HPLC/ion trap mass spectrometry. *J AOAC Int.* **2000**, *83*, 1367–1376.
(31) Commission regulation (EC) N0. 1895/ On the restriction of use of certain epoxy derivatives in materials and 33 articles intended to come into contact with food. *Off. J. Eur. Union* **2005**, *L302*, 28–32.
(32) Summerfield, W.; Goodson, A.; Cooper, I. Survey of bisphenol a diglycidyl ether (BADGE) in canned foods. *Food Addit. Contam.* **1998**, *15*, 818–830.
(33) Hanaoka, H.; Kawamura, N.; Hara, K.; Tsugane, S. Urinary bisphenol A and plasma hormone concentrations in male workers exposed to bisphenol A diglycidyl ether and mixed organic solvents. *Occup. Environ. Med.* **2002**, *59*, 625–628.
(34) Theobald, A.; Simoneau, C.; Hannaert, P.; Roncari, P.; Roncari, A.; Rudolph, T.; Anklam, E. Occurrence of bisphenol-F diglycidyl ether (BFDGE) in fish canned in oil. *Food Addit. Contam.* **2000**, *17*, 881–887.

Received for review October 23, 2007. Revised manuscript received January 16, 2008. Accepted January 16, 2008.

JF073106N