CHAPTER I

INTRODUCTION

1.1 Problem Definition

Today's food cans come in a variety of can types, sizes, and designs. Most are covered with some kind of protective internal coating. This internal coating must be made stable during heating process and throughout the shelf life of canned food. In addition, the internal coating needs to be made resistant to rigorous conditions caused by various foodstuffs ranging from acidic to alkaline.

For over forty years, epoxy resin has been extensively used in various can coating applications. Epoxy resin is accepted to be used for both food and beverage cans, because of its exceptional combination of properties such as toughness, adhesion, and chemical resistance. Other than epoxy resins, PVC organosol lacquers are more frequently found in cans with "easy-open" lids or in deep-drawn two-piece cans because of their higher flexibility.

The epoxy resin contains bisphenol-A-diglycidyl ether (BADGE) as the cross-linking agent (Figure 1.1). In PVC organosols, BADGE and bisphenol-F-diglycidyl ether (BFDGE) are used as additives for the elimination of surplus hydrochloric acid apparently formed during heat treatment of the coating procedure.

Both coating types contain residual BADGE and BFDGE monomers and small oligomers that may migrate into the food. In organosols, BADGE and BFDGE are used to remove hydrochloric acid, which result in formation of chlorohydroxy compounds such as BADGE.HCl, BADGE.2HCl, BFDGE.HCl and BFDGE.2HCl. The remaining epoxy groups of BADGE and BFDGE may readily be hydrolyzed when contact with aqueous and acidic foods which may result in the formation of

mono- and di-hydrolyzed products such as BADGE.H_2O, BADGE.$2H_2O$, BADGE.HCl.H_2O, BFDGE.H_2O, BFDGE.$2H_2O$, and BFDGE.HCl.H_2O. Structures of the reaction products are shown in Figure 1.1.

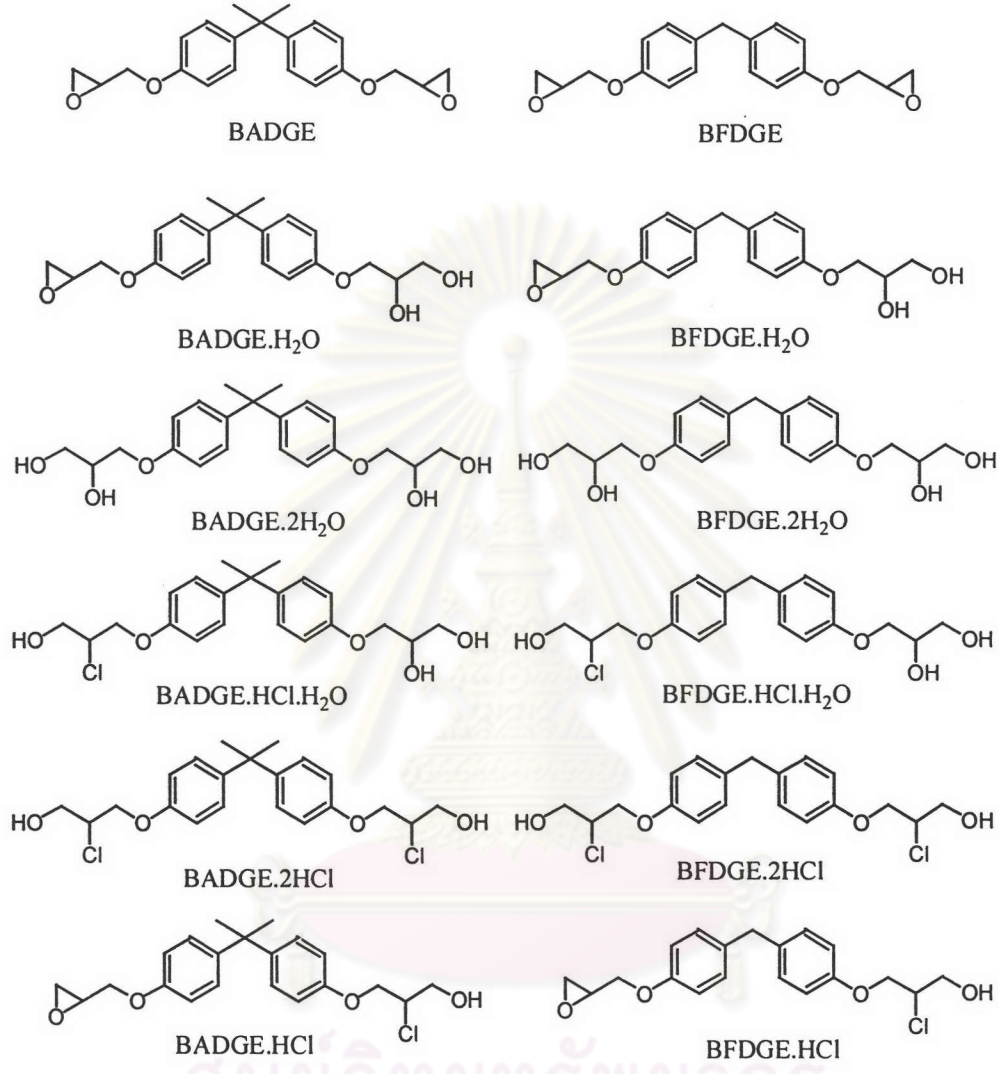

Figure 1.1. Structures of BADGE, BFDGE and their derivatives.

Key reasons for studying contaminations caused by chemicals leached from food packaging are potential adverse health effects on consumer exposed to these compounds. Recently, epoxy compounds are known as alkylating agents and may have specific cytotoxic actions in tissues affecting rates of cell division. This toxicity depends mainly on fractional concentration of unreacted epoxy groups. Moreover, the chlorohydroxy are also considered potentially toxic because of their structural analogy to the genotoxic monochloropropanediol and other chloropropanols (*1*).

1.2 Regulatory Standards in the European Union

The European Commission restricted extension of the legislation on plastics for food content to surface coatings on cans. In 1990, the Commission of the European Communities established a specific migration limit (SML) for bisphenol A at 3 mg/kg in food or food simulant, and for BADGE at 0.02 mg/kg in food or food simulant (*2*). Because of the lack of evidence concerning carcinogenic effect of BADGE in *in vivo* studies, in 1997, a legal limit was defined at 1.0 mg/kg for the sum of BADGE.H_2O, BADGE.HCl, BADGE.2HCl and BADGE.HCl.H_2O (*1*). So far the data of toxicological studies by the EU SCF indicated no carcinogenic activity for BADGE and BADGE.$2H_2O$. Thus, the EU SCF maintained the 1.0 mg/kg limit in 1999 (*3*) awaiting pending results of further toxicological data.

After the actions by some authorities, the can industry started to replace BADGE with similar compound of Novolac Glycidyl Ether (NOGE) that is made from BFDGE. BFDGE are not allowed to be used as additives because of insufficient in toxicity data. In 2001, the EU SCF set up a total SML of 1.0 mg/kg for BADGE, BFDGE and their derivatives in foods (*4*). The EU SCF is currently waiting for additional toxicity data on BADGE, BFDGE and their reaction products with HCl before posing new regulation.

1.3 Literature Review

a) Literature review on *The Migration of BADGE and BFDGE in Food Simulants:*

Migration testing is usually carried out with the starting point on food simulants rather than on the foods themselves. Foods are complex mixtures of variable compositions and their analyses can have many drawbacks in both analytical and practical aspects. Therefore, food simulants have been selected as models for various categories of foods (aqueous, acidic, alcoholic, and fatty) as simplified migration replica.

Paseiro-Losada and co-workers proposed HPLC method for determining the migration of bisphenol-A-diglycidyl ether in aqueous-base food simulants. In this work, RP-HPLC with fluorescence detection was used to study BADGE (*5*), and its hydrolysis products (*6;7*). They also presented the identification of bisphenol-F, BADGE, BFDGE, and their hydrolysis products by thermospray mass spectrometry (*8*) and gas chromatography/mass spectrometry (*9*).

The team also studied migration in fatty food simulants (olive oil) during processing and storage (*10*). The results indicated greater BADGE migration during the first 30 minutes of manufacturing process at 121 °C than during storage (accelerated study at 40 °C for 10 days).

Salafranca and co-workers developed a new method to analyze BPA and BADGE in aqueous base food simulants (*11*). The method used direct immersion of solid-phase microextraction (SPME) to extract the analytes from liquid matrix followed by subsequent chromatographic analyses by GC-MS. The SPME method showed good performance for simultaneous analyses of BPA and BADGE but showed poor performance for BADGE in aqueous alcohol solution because of reduced performance of the fiber in ethanol.

Simoneau and co-workers reported the influence of both processing and storage on the migration of BADGE into the can contents. Non-processing (filling of oil at room temperature) did not lead to a measurable migration even for long storage periods whereas elevated temperatures during sterilization led to migration of BADGE from the lacquer into the filling (*12*).

Munguia-Lopez and Soto-Valdez determined the effect of heat processing and storage time on the migration of BPA and BADGE in aqueous food simulants in two types of cans used in Mexico: tuna and jalapeño peppers. The results showed that heat processing enhanced the rate of migration for both compounds in both types of cans. Storage time did not show any effect in BPA migration from tuna cans while the effect was detected in jalapeño peppers cans. Because BADGE was hydrolyzed in

aqueous simulants, its concentration decreased during storage in both types of cans (*13*).

b) Literature reviews on *The Stability of Starting Substances in Aqueous and Fatty Foodstuffs:*

Paseiro-Losada and co-workers characterized potentially toxic compounds and hydrolysis products of BADGE and BFDGE by studying BADGE degradation kinetics in three water-based food simulants, 3%(w/v) acetic acid, distilled water, and 15%(v/v) ethanol, recommended by the EU (*14*). They reported the half-life, Arrhenius constants, and activation energies of the reactions. First-orders rate coefficients for the hydrolysis reaction in each simulant at 40, 50, and 60 °C were estimated by nonlinear regression and the kinetic model was tested by comparing predictions obtained by using the Arrhenius equation with the experimental rate coefficients at 70 °C. The half-life for BADGE was the longest in the ethanolic simulants and shortest in acetic acid and ring-opening appeared to involve acid catalysis. The kinetics of degradation of BFDGE in the three water-based food simulants were studied at 40, 50, and 60 °C and found to have first-order kinetic as well (*15*). The half-life of BFDGE was also longest in ethanol and shortest in acetic acid. Hydrolysis of BFDGE was slightly slower than that of the BADGE.

Philo and co-workers studied the stability of BADGE and epoxide monomers in three aqueous food simulants and in fatty food simulants such as olive oil (*16*). The accelerated studies (175 °C) data indicated 90-100% loss of BADGE in all aqueous simulants but only 15-25% was lost in olive oil. The data confirmed the observations of Paseiro-Losada *et al. (14)*. Small quantity of BADGE diol was detected in olive oil samples because of partial hydrolysis of BADGE by trace of water in olive oil. In a related study, the BFDGE degradation products in water-based food simulants were identified using RPLC with detection by thermospray mass-spectrometry and GC-MS (*9*).

Biedermann and his group studied the migration from epoxy-based can coatings into edible oils and found these oils very efficiently extracted BADGE from the coatings and protected it from total hydrolysis. They showed that oil-preserved

BADGE survived for 3 hours in 3% hydrochloric acid implying that BADGE may pass through the stomach without being hydrolyzed (*17*).

c) Literature reviews on *The Contamination from Packaging Materials in Foods:*

Brotons *et al.* reported that up to 23 μg of BPA/can was found in the aqueous portion of canned vegetable but the total residue of BPA in food content was not measured (*18*). In addition, migrated BPA in infants formula liquid concentrates from epoxy can coatings was found at 13 ng/mL by Biles and co-workers (*19*).

Yoshida *et al.* developed analytical methods for the determination of BPA that migrated into canned fruits and vegetables. BPA was mainly detected in the solid portion of canned food and found at the maximum level of 11 μg/can (*20*).

The studies of BADGE contamination in food started at the beginning of 1996 during the authenticity control of olive oils in Switzerland by Biedermann *et al.* Incidentally, about 80 mg of BADGE/kg were found in the oil of canned sardines (*17*). They reported that the worst can released BADGE as much as 12 mg/dm^2 into food and the concentration in the can content could reach 600 mg/kg while the legal limit (SML) of BADGE in food in Switzerland was established at 0.02 mg/kg. Later, the team reported migrations of chlorohydroxy derivatives and oligomers of BADGE too (*21*). A total of 200 samples have been analyzed and 30 samples where BADGE existed at detectable concentrations were further analyzed for chlorohydroxy compounds. BADGE.HCl and BADGE.2HCl, formed by reactions of BADGE with hydrochloric acid can release from organosols during heat curing. Usually, chlorohydroxy compounds presented at 15-50% of the BADGE content, but in 6 samples the concentrations of BADGE.HCl and BADGE.2HCl far exceeded BADGE. The presence of chlorine in can coatings were confirmed by flame (Beilstein) method. The glycidoxy-terminated dimers and trimers of BADGE concentration are detected at higher concentration than for BADGE. In extreme sample, they found 80 μg of BADGE/kg along with 1,600 μg of dimer/kg and 1,800 μg of trimer/kg.

Simoneau *et al.* reported the results of a large European survey on the quantification of BADGE in 382 samples in canned fish from all Member States of the EU (*22, 23*). Although the results showed that the concentration that mostly exceeded the limit set by the European Commission (1999) often derived from the long storage cans and cans of smaller dimensions, it was found that the extractable amounts presented in the can were not related to the concentration found in the food itself (*24*). In another study on the BADGE contents in dairy products such as cream and vegetable oils and their cans, the results showed that no migration of BADGE occurred in sterilized milk and oil, but high amounts were present in the bodies of the vegetable oil cans (*25*). Furthermore, Summerfield et al. reported BADGE at level exceeding 1 mg/kg in 12 of 181 tested samples of fish-in-oil, meat and milk (*26*).

Biles and co-workers reported the migration of BADGE in canned foods, diet Cola beverages and infant formula concentrates (*27*). The level of BADGE in the foods surveyed in this study had a range from nondetectable (<0.3 ppb) to 50 mg/kg.

A later investigation found the reaction products of BADGE (BADGE.HCl and BADGE.2HCl) at high levels of in preserved foods in Swiss market (*21*). In addition, Cottier and Biedermann teams have been identified several other potential migrants from organosol lacquers (*28, 29*).

The levels of BADGE.H_2O and BADGE.$2H_2O$ in food samples were recently reported by Rauter *et al.* (*30*). BADGE.$2H_2O$ was found in concentration up to 0.5 mg/kg, whereas BADGE.H_2O was not found in samples from the Austrian market. The data indicated further derivatization of both diglycidyl ethers during hydrolysis.

Hammarling investigated the presence of BADGE and the chlorohydroxy compounds in 30 brands of canned foods. BADGE was founded at levels up to 5.1 mg/kg in the food and only from cans coated with chlorine containing lacquers. BADGE was found in both fish-in-oil and in fish-in-tomato-sauce, however, the highest amounts were found in the fatty foodstuffs. In aqueous and acidic foodstuffs BADGE readily hydrolyzed into mono- and di- hydrolyzed products, BADGE.H_2O and BADGE.$2H_2O$. The study detected only BADGE.$2H_2O$ at levels up to 2.6 mg/kg but did not find BADGE.H_2O (*31*).

After the EU regulations were introduced, the industry had substituted BADGE with resins of novolac glycidyl ethers containing BFDGE. Concentrations of NOGE in oily foods such as tuna in oil, rapidly rose to the level previously found for BADGE, i.e. up to 20 mg/kg (*21*). Bronz *et al.* was the first group to investigate BFDGE and NOGE in canned foods. From the investigation, they found that 20 samples out of 217 canned foods contained BFDGE at concentrations exceeding 20 μg/kg. For some of them, the three- and four-ring NOGE were analyzed. The chlorohydroxy derivatives, the hydrolysis products, and the oligomers of NOGE were in many of these samples, resulting in an exceedingly complex mixture (*32*).

Theobald *et al.* reported the content of BFDGE in cans, lids and fish collected from European Member States. The study showed that only low percentage of canned fish-in-oil contained BFDGE. Comparing the concentrations of BFDGE and BADGE in cans and lids, a tendency to release predominately only one of the components was observed (*33*).

Biedermann *et al.* analyzed for BADGE, BFDGE and their reaction products with water and hydrochloric acid in 270 products of canned food and found 103 products exceeded the temporary tolerance value of 200 μg/kg defined for the sum of all derivatives (*34*). Uematsu and co-workers surveyed the Japanese market and reported 46 samples of canned aqueous foods and beverages (containing ready-to-drink coffee and vegetables). The results focused on the reaction products of BADGE and BFDGE, primarily, chlorohydrins of the two components (*35*). BADGE.2HCl was found in one coffee sample and five samples of corn, while BFDGE.2HCl was detected in four samples of canned tomatoes and in one of canned corn. The Beilstein test confirmed that all food containing BADGE.2HCl or BFDGE.2HCl was packed in cans with at least one part coated with a PVC organosols. They concluded that the migration of chlorohydrin derivatives in aqueous foods were at lower level in the Japanese market (*34*).

d) Literature review of *The Analytical Procedures:*

Several methods were described in the literatures for the analysis of BADGE, BFDGE and their reaction products in food simulants by chromatographic techniques. Most published methods used reversed-phase HPLC on C18 or C8 silica gels with mixture of acetonitrile/water as mobile phase. Fluorescence detection (FD) was mostly used (*16, 36, 37, 38*). Mass spectrometry was also coupled with RPLC (*8, 39, 40*). RPLC-MS-MS was also proposed for the analysis of BADGE in food after freeze-drying of the product (*41*). When using RPLC determination, the fat or oil in samples has to be removed prior to injection, while normal phase HPLC enabled direct injection of organic extracts from oily foods (*17, 28, 42*).

GC-MS can be applied to the determination of these compounds as well (*11, 13, 35*). GC-MS of NPLC fractions was mostly used for peak identification or confirmation (*29, 43, 44*). Bronz et al. used size exclusion chromatography for the determination of the oligomers (*45*).

Biedermann and co-workers presented a set of three methods for the analysis of these compounds starting with RPLC-FD. The positive results were further confirmed by acetylation and analysis by NPLC-FD. When results disagreed, NPLC fractions were collected and analyzed by GC-MS (*44*). In this study, they achieved adequate separations with ethanol as organic modifier, but this condition resulted in co-elution of other compounds.

Lintschinger and Rauter separated all of BADGE/BFDGE and their derivatives using binary systems consisting of methanol/water and acetonitrile/water (*46*). To achieve sufficient resolution of BADGE.H_2O and BADGE.HCl.H_2O, they used a system of methanol/water under isocratic conditions.

1.4 Hypothesis

At first, oily and fatty canned foods were considered as high risk products badly contaminated by can coatings because oil swells and extracts coating constituents out.

In addition, oils and fats tend to protect the epoxy groups from further hydrolysis. However, results from many studies indicated that aqueous foods, regardless of the missing of BADGE and BFDGE, did not imply contamination free. The released epoxides (BADGE and BFDGE) can be readily converted to many hydrolysis products and contaminated the food content. When used as scavengers in organosol coatings, BADGE and BFDGE are converted to chlorohydrins (*29*). Thus, in this study, we tried to determine the contamination of BADGE, BFDGE and all their major derivatives in aqueous-based canned foods and oil-in-water canned foods. Because of the difference in polarity between the BADGE/BFDGE and their polar reaction products, gradient reverse-phase HPLC is selected for simultaneous determination method.

Since sample preparation is a crucial part of quantitative analyses. Two separate methods were developed for oil-in-water-based foods and for aqueous-based foods.

1.5 Purpose of the Study

From literature review, many authors have studied the migration and stability of BADGE and BFDGE in both aqueous and fatty food simulants. There are also many reports on contamination of BADGE, BFDGE and some derivatives from can coatings and suggestion of possible health risks. However, reported analysis methods are rather cumbersome including many steps using more than one instrument. Up to now, there is no analytical method developed for simultaneous determination of BADGE and BFDGE along with their key hydrolysis and chlorohydroxy products. Since canned foods are very popular worldwide including Thailand where its popularity peaks and sets out to be a new staple. There should be a safety evaluation of canned food in local market where variety of unique food types palatable to the Thais are available such as sardines-in-tomato-sauce, Tom-Yum sardines, tuna steak, fruit in syrup, special beverages, etc. Since the matrixes of Thai foods are different from the international ones, new sample preparation method and analysis method must be developed.

We intended to develop a method for simultaneous determination of BADGE, BFDGE and 10 of their derivatives namely BADGE.H_2O, BADGE.$2H_2O$, BADGE.HCl.H_2O, BADGE.HCl, BADGE.2HCl, BFDGE.H_2O, BFDGE.$2H_2O$, BFDGE.HCl.H_2O, BFDGE.HCl, BFDGE.2HCl. Two sample preparation procedures were developed for oil-in-water and aqueous-based foods since the matrixes as well as the derivatives presence were different. In this research, pre-cooked tuna was chosen as representative for the matrix of oil-in-water-base samples because canned tuna earns the highest export values for Thailand. For the aqueous-base foods, canned lychee in syrup was obtained as the matrix. All procedures were validated and used to test various canned foods made by the local manufacturers.

The work also covered the syntheses of 3 nonavailable standard reference materials: Bisphenol F (2,3-dihydroxypropyl) glycidyl ether (BFDGE.H_2O), Bisphenol F (3-chloro-2-hydroxypropyl) glycidyl ether (BFDGE.HCl), and Bisphenol F (3-chloro-2-hydroxypropyl) (2,3-dihydroxypropyl) ether (BFDGE.HCl.H_2O).