

Migration of BADGE (bisphenol A diglycidyl-ether) and BFDGE (bisphenol F diglycidyl-ether) in canned seafood

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Abstract

Migration of potentially toxic materials used for the lining of commercial can goods remains an important issue, especially with respect to certain types of processed foods. Seafood is one type where more information is needed with respect to other ingredients used for adding value to fishery products. Most cans are internally coated with starters of resins such as bisphenol A diglycidyl-ether (BADGE) and bisphenol F diglycidyl-ether (BFDGE), both considered as toxic compounds. Several seafood products, sardines, tuna fish, mackerel, mussels, cod and mackerel eggs, were manufactured in different conditions changing covering sauce, time and temperature of storage and heat-treated for sterilization in cans. Migration kinetics of BADGE and BFDGE from varnish into canned products were evaluated by HPLC in 70 samples after 6, 12 or 18 months of storage.

Results showed that there is no migration of BADGE in tuna fish, sardines, mussels or cod. However, migration of BFDGE occurs in all species, in a storage time-dependent way and content of fat, although migration of these compounds is not affected by sterilization conditions. All samples analyzed presented values lower than 9 mg BADGE/kg net product without exceeding European limits. However, concerning BFDGE migration, European legislation does not allow the use and/or the presence of BFDGE. Main migration takes place in mackerel reaching the highest values, 0.74 mg BFDGE/kg and 0.34 mg BADGE/kg net product, in red pepper sauce.

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1. Introduction

Migration of potentially toxic compounds in epoxy resins used for lining commercial cans is a very important food safety issue. These chemicals are potential endocrine disruptors although very recent data indicate lack of carcinogenicity and genotoxicity as it was first mentioned

(Commission, 2005). To avoid risks to human health a Commission Regulation was published to restrict the use of certain epoxy derivatives in material and articles intended to come into contact with food (Commission, 2005). The limits allowed for BADGE and derivatives are fully harmonized with European Community Commission Regulation. On the other hand, the presence of BFDGE is no longer permitted due to lack of toxicological data (Commission, 2005).

Several studies concerning hormonal disruptors migration were made in aqueous simulants or aqueous canned foods (Uematsu et al., 2001; Munguia-Lopez and Soto-Valdez, 2001). Migration of these compounds is affected by their instability due to hydrolyzation in contact with aqueous and acidic food (Hammarling et al., 2000; Paseiro et al., 1992, 1993). It was also reported that BADGE is

Abbreviations: BPA (bisphenol A), 4,4'-isopropylidene-2-diphenol; BADGE, bisphenol A diglycidyl-ether; BFDGE, bisphenol F diglycidyl-ether.

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more stable than BFDGE (Poustkova et al., 2004). In contrast, in the case of fat foodstuffs hydrolysis is not common, although some reports suggest that BADGE react with food components leading to a decrease of BADGE levels depending on the sterilization process and the storage time (Berger et al., 2001).

In world industry, major application areas for epoxy resins are protective coatings and civil engineering. Additional uses include printed circuit boards, composites, adhesives and tooling, while a relatively small amount of epoxy polymers are the most common varnishes used in food containing cans (Poole et al., 2004). Epoxy-phenolic resins are often polymerization products of bisphenol A diglycidyl-ether (BADGE) or NOGE (Novolac glycidyl ethers). The lowest molecular weight component of NOGE is bisphenol F diglycidyl-ether (BFDGE). These compounds are easily soluble in oil and food lipids and it is known that migration into oily food occurs (Rauter et al., 1999; Simoneau et al., 1999, 2002; Summerfield et al., 1998; Theobald et al., 2000; Uematsu et al., 2001, 2005; Nerin et al., 2002; Hammarling et al., 2000; Munguia-Lopez and Soto-Valdez, 2001).

Our interest is focused on the use of these compounds as internal coating materials for food cans, mainly seafood products. There are a few studies considering migration of BADGE, BFDGE and other derivatives from canned seafood products. Some of them were made with different epoxy resins or organosols. For instance, migration of BPA (bisphenol A) was evaluated in different foodstuffs taking into account several parameters, such as conditions of time and temperature of storage (Goodson et al., 2004). These authors indicated that migration of BPA occurs mainly during the can processing step and this level of migration was not increased during 9 months of storage (Goodson et al., 2004). Migration of BADGE was studied in different brands of canned fishes in oil, obtaining highest values of BADGE in canned sardine/sardelle in oil (Erkan et al., 2005).

On one hand, it is extremely important to study BADGE and BFDGE migration in canned seafood that use some kind of fatty stuff as covering sauce, since these compounds are lipophilic. On the other hand, there is a lack of information concerning BADGE or BFDGE migration from can coatings into fat food simulating normal conditions of a preserved food.

The purpose of this study was to determine effects of select seafood and various sauces used in commercial canning operations and the effect of time and temperature of storage on the migration of BADGE and BFDGE.

2. Materials and methods

2.1. Materials: Standards and reagents

Bisphenol A diglycidyl-ether and bisphenol F diglycidyl-ether were purchased from Sigma–Aldrich, St Louis, MO, USA. Stock solutions of BADGE or BFDGE were made in tetrahydrofuran at a concentration of 500 mg/l and stored at 4–8 °C. Intermediate solutions of BADGE and

BFDGE were prepared at a concentration of 5 mg/l in *n*-heptane. Acetonitrile was of HPLC supragrade and purchased from Panreac Química SA, Barcelona, Spain. Tetrahydrofuran and *n*-heptane were from HPLC grade and were purchased from Scharlab, Barcelona, Spain.

2.2. Samples

Seafood products consisted of: mussels, (*Mytilus galloprovincialis*), Atlantic mackerel (*Scomber scombrus*), sardine (*Sardina pilchardus*), cod (*Gadus morhua*) and tuna fish (*Thunnus albacares* and *Thunnus obesus*). Seafood was acquired from local markets and each sample consisted of the mixture of two cans elaborated under the same conditions.

Two-piece steel–tin cans with pull top lids were obtained from Mivisa Envases, S.A.U. The cans were lined with aluminized epoxy-phenolic base coats in the bodies, and gold epoxy-phenolic (1 layer) and organosol (2 layers) base coats in the ends, both having the standard composition provided by the producing company for aggressive products.

Tested food was manufactured in ANFACO's Pilot Plant. Cans were filled with about 85 g (3.0 ounces) of cooked fish or shellfish and covered with a sauce before sealing (see Table 1). Sauces were prepared following traditional recipes in Galician cooking. Filled cans were thermally processed at 115 °C for 45 min or 121 °C for 30 min as it is usual in the industry. We did two identical batches of samples, both stored in two different incubation chambers, one of them was stored at 20 °C and the other was stored at 30 °C up to 18 months, samples being analysed at 6, 12 and 18 months.

2.3. Extraction process

To perform BADGE and BFDGE extraction, 5 g of homogenized sample (net product) were mixed with 10 ml *n*-heptane and centrifuged. This operation was repeated three times as reported by Paseiro et al. (1999). The collected *n*-heptane fraction was evaporated under nitrogen stream up to approximately 10 ml. This solution was extracted with 10 ml acetonitrile 90%, which was filtered through a microfilter (PTFE membrane, diameter 13 mm, pore size 0.5 µm) and injected in the HPLC system.

2.4. Measurements of BADGE and BFDGE by HPLC

BADGE and BFDGE were analyzed by high performance liquid chromatography with fluorescence detection according to Paseiro et al. (1999) with light modifications. Calibration curve is constructed from samples without BADGE and BFDGE, in which standards are added in different concentrations to eliminate matrix interferences. Solutions of BADGE and BFDGE were made at concentrations of 20, 50, 100, 500, 1000, 1500 µg/l with *n*-heptane. Quantification of BFDGE results from the average of three isomers is shown in Fig. 1.

The analytical determination and quantification of BADGE and BFDGE was carried out on a HPLC Hewlett Packard 1100 series, equipped with a quaternary pump G1311A, a degasser G1322A, an autosampler G1313A, a thermostated column compartment G1316A, a fluorescence detector G1321A and a Kromasil reversed-phase column (5 µm, 150 × 4.0 mm I.D.) with column guard. For acquisition and processing spectral information, a Hewlett Packard "HP Chemstation" was used.

The HPLC system operated at 35 °C (column compartment). The injection volume was 50 µl and the flow rate was 1.0 ml/min. The mobile phase was acetonitrile and water. Binary gradient conditions were used: starting with 70% water and 30% acetonitrile, going to 80% of acetonitrile and 20% of water in 20 min, and finally up to 100% of acetonitrile. Detector conditions were 230 nm (excitation wavelength) and 305 nm (emission wavelength).

2.5. Confirmation methodology

The peak identification was confirmed by comparing excitation and emission spectra between chromatographic sample peak and standard. Emission spectra have been acquired by emission wavelength sweeps,

Table 1
Types of seafood products and covering sauces tested

	Pickled sauce (Escabeche)	Tomato sauce	“Cazuela” sauce	Ravigote sauce	Galician sauce	Biscayne sauce	Red pepper sauce	Olive oil	Sunflower oil	Sunflower + olive oil
Sardines	+	+						+	+	
Mackerel		+	+	+			+		+	+
Albacore								+		
Skipjack								+	+	
Yellowfin								+	+	
Mussels	+				+					
Cod fish						+				
Mackerel eggs								+		
<i>Sauce composition</i>										
Sunflower oil (%)	60	3	69	20	45	23			100	50
Olive oil (%)							97	100		50
Vinegar (%)	26		1	2			3			
Water (%)	13	59	19	47	4	24				
Salt (%)	0.5	1.5	0.6	0.7	0.7	0.7				
Spices (%)	0.5		0.4	0.3	0.6	0.3				
Tomato paste (%)		33.5	10	30	17	28				
Sugar (%)		3								
Wine (%)					0.7					
Pepper (minced) (%)						10				
Onion (minced) (%)					32	14				
Pepper			X							
Onion			X	X						
Carrot				X						

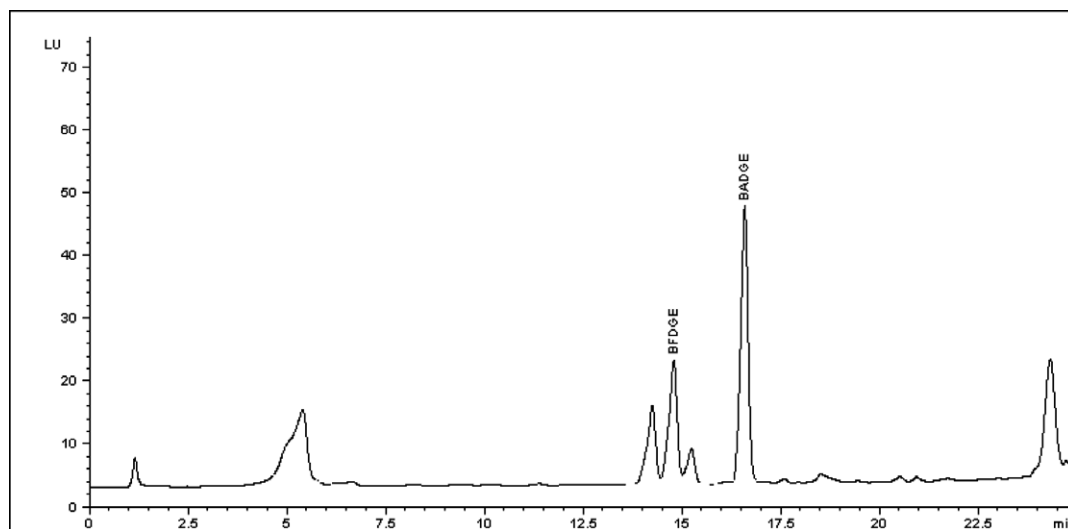


Fig. 1. Example of a chromatogram carried out by using BADGE and BFDGE standards (500 mg/kg net weight for both compounds). BFDGE quantification results from the sum of the three peaks observed in the chromatogram.

between 280 and 400 nm, to a fixed excitation wavelength of 230 nm. In a similar way, excitation spectra have been acquired by excitation wavelength sweeps, between 200 and 300 nm, to a fixed emission wavelength of 305 nm.

The peak was confirmed when the match of spectra between standards and samples was higher than 800 per 1000. Besides, the difference between maxima wavelength (of both emission and excitation spectra) between standards and sample has to be ± 5 nm. An example of sample chromatogram is shown in Fig. 2.

2.6. Validation of method

The method has been validated inoculating samples with different concentration levels of BADGE or BFDGE. Three different fishery can-

ned products were fortified with concentrations ranging from 20 to 1500 $\mu\text{g}/\text{kg}$ for each compound. Recoveries and relative standard deviations (RSD) were in the range 84–92% and 1.8–4.9%, respectively for BFDGE and 89–100% and 1.6–2.3% for BADGE. The detection limit corresponding to a signal-to-noise ratio of three, of BFDGE and BADGE was 2.4 and 3.4 $\mu\text{g}/\text{kg}$, respectively.

2.7. Crude lipid determination

Fat is extracted from dried samples of canned seafood by 2-step treatment with diethyl ether solvent. Solvent is recovered by condensation in Det-Gras 4000847, leaving extracted soluble material. Fat is determined by weight after drying as described (AOAC, 2002).

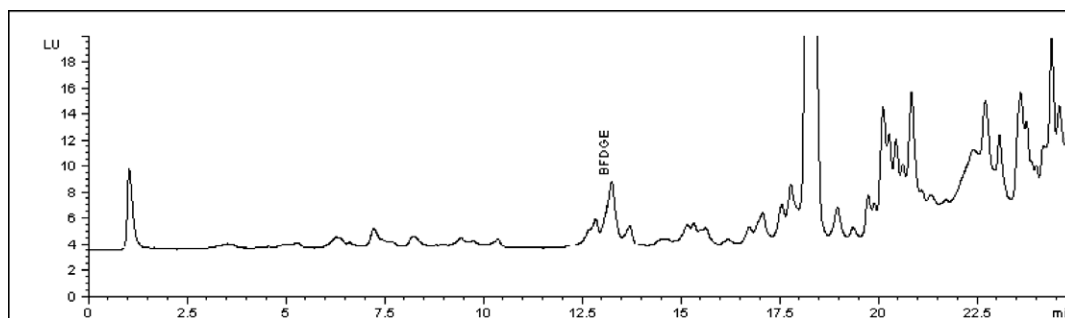


Fig. 2. Example of a chromatogram obtained after extraction and HPLC analysis of a sample. The presence of BFDGE is detected and assessed with spectra, as mentioned in methods.

2.8. Statistics

Data were statistically analysed using SPSS software (release 12.0). One-way Analysis of Variance (ANOVA) was used to detect significant differences between BADGE and BFDGE levels depending on species, time and temperature of storage, sterilization conditions, and sauces composition.

Student–Newman–Keuls post-hoc analysis was used in the classification of different groups when ANOVA test is significant ($p < 0.05$).

3. Results

3.1. BADGE and BFDGE migration depending on the type of seafood

One-way ANOVA was used to study BADGE and BFDGE levels in each species. Results of ANOVA show that there are significant differences depending on the sample ($p < 0.05$); suggesting that BADGE and BFDGE migration is higher in some species than in others as observed in Tables 2 and 3. Mackerel has the highest levels

of BADGE and BFDGE migration; on the contrary, tuna, cod, mussels and sardines present very low BFDGE and BADGE levels of migration, as illustrated in Figs. 3 and 4.

3.2. BADGE and BFDGE migration depending on sterilization conditions

Data were statistically treated to evaluate if levels of contaminants were influenced by different time/temperature binomials of sterilization (115 °C/45 min and 121 °C/30 min). One-way ANOVA analysis shows that there are no significant differences among binomials of sterilization ($p > 0.05$) (data not shown). This means that, in our conditions, BADGE and BFDGE migration does not depend on time and temperature of sterilization.

3.3. Crude lipid content of seafood

Results obtained after analysis of lipid contents in canned seafood are shown in Table 4. Seafood products

Table 2
BADGE migration data depending on type of seafood

BADGE Species	Mean (mg/kg net weight)	Median (mg/kg net weight)	RSD (%)	P50 (mg/kg net weight)	P90 (mg/kg net weight)
Sardine	<0.020	<0.020	0.022	<0.020	<0.020
Tuna	<0.020	<0.020	0	<0.020	<0.020
Mackerel	0.10	0.034	0.106	0.031	0.177
Mussel	0.021	<0.020	0.032	<0.020	<0.020
Cod	<0.020	<0.020	0	–	–
Mackerel eggs	<0.020	<0.020	0.01	–	–

Table 3
BFDGE migration data depending on type of seafood

BFDGE Species	Mean (mg/kg net weight)	Median (mg/kg net weight)	RSD (%)	P50 (mg/kg net weight)	P90 (mg/kg net weight)
Sardine	0.108	0.13	0.071	0.13	0.20
Tuna	0.068	0.071	0.03	0.12	0.10
Mackerel	0.248	0.160	0.242	0.16	0.60
Mussel	0.115	0.110	0.077	0.06	0.13
Cod	0.040	0.040	0.042	–	–
Mackerel eggs	0.032	0.032	0.031	–	–

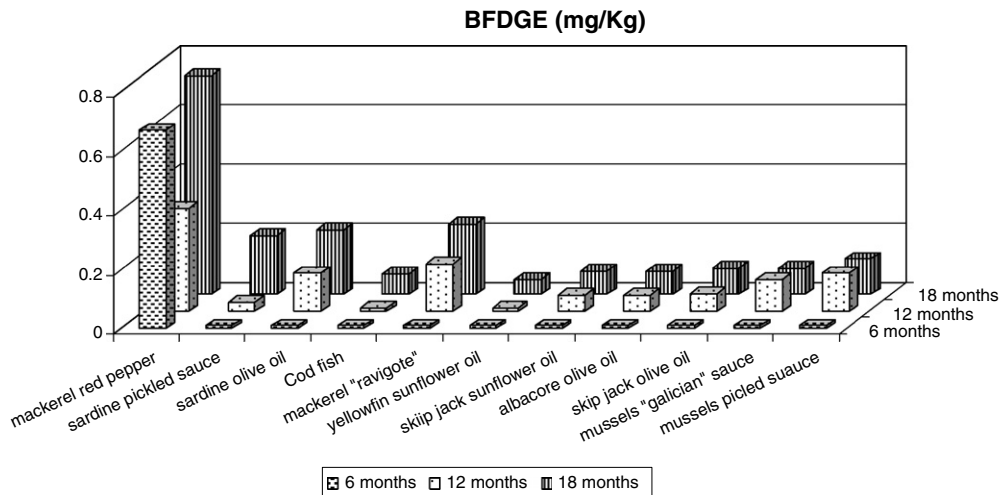


Fig. 3. BFDGE migration (mg/kg net weight) in several seafood products analyzed after 6, 12 or 18 months. Figure only represents positive results.

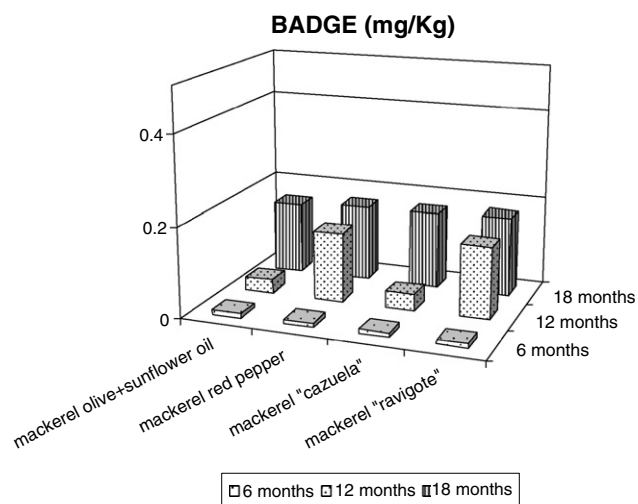


Fig. 4. BADGE migration (mg/kg net weight) in several seafood products analyzed after 6, 12 or 18 months. Figure only represents positive results.

that contain the highest amount of fats are mackerel in red pepper sauce and sardine in olive oil. Fat results refer to original products.

3.4. BADGE and BFDGE migration depending on the type of covering sauce

One-way analysis of variance (ANOVA) was used to study BADGE and BFDGE migration depending on sauces composition. ANOVA results show that there are significant differences ($p < 0.05$) in BADGE and BFDGE concentrations according to sauce composition. Post-hoc

test (Student–Newman–Keuls) establishes groups comparing the mean values in each condition. Then, three or two main groups were created by applying this test for samples related to BADGE and BFDGE migration, respectively. This test shows that “cazuela”, “ravigote”, olive + sunflower oil and red pepper sauces have BADGE migration levels significantly higher than other sauces such as oil, tomato, pickled, Galician and Biscayne sauce, as illustrated in Table 5. Furthermore, BFDGE levels are significantly higher in cans containing red pepper sauce than cans with other sauces, as observed in Table 6. Similar to results obtained with BADGE migration, “cazuela”, “ravigote” and red pepper sauces are again those sauces with higher levels of BFDGE migration (Tables 5 and 6).

3.5. BADGE and BFDGE migration depending on storage time

The influence of storage time on BADGE and BFDGE migrations in each sample was studied using one-way analysis of variance (ANOVA). Samples were stored for 18 months, and analyzed at months 6, 12 and 18. Table 7 shows that there are significant differences ($p < 0.05$) in sardine, mussels and tuna with respect to BFDGE migration. Student–Newman–Keuls post-hoc analysis shows that BFDGE levels at 12 and 18 months, are significantly different than that at 6 months, indicating that BFDGE migration is very dependent on storage time as illustrated in Fig. 3. This fact occurs in several species such as sardines, mussels and tuna. Nevertheless, there are no significant dif-

Table 4
Lipid content depending on type of seafood

SAMPLE	Sardine pickled sauce	Mackerel "cazuela" sauce	Mussels "galician" sauce	Mackerel sunflower oil	Mackerel red pepper sauce	Mackerel tomato sauce	Sardine olive oil	Sardine tomato sauce	Mackerel "ravigote" sauce	Skipjack olive oil
% Lipid	14.89	6.08	7.88	22.50	38.62	3.87	37.50	9.20	10.69	27.41

Table 5
Post-hoc SNK test obtained with BADGE migration data depending on covering sauce

BADGE Sauce	1st group (mg/kg)	2nd group (mg/kg)	3rd group (mg/kg)
Sunflower oil	0.0100		
Pickled sauce	0.0100		
Tomato sauce	0.0100		
Galician sauce	0.0100		
Biscayne sauce	0.0100		
Olive oil	0.0100		
Olive + sunflower oil		0.0442	
Cazuela sauce		0.0573	
Ravigote sauce		0.1178	
Red pepper sauce			0.1678

Table 6
Post-hoc SNK test obtained with BFDGE migration data depending on covering sauce

BFDGE Sauce	1st group (mg/kg)	2nd group (mg/kg)
Sunflower oil	0.0606	
Pickled sauce	0.1119	
Tomato sauce	0.0767	
Galician sauce	0.0680	
Biscayne sauce	0.0400	
Olive oil	0.0806	
Olive + sunflower oil	0.1266	
Cazuela sauce	0.2148	
Ravigote sauce	0.1925	
Red pepper sauce		0.4075

Table 7
ANOVA statistic test obtained with migration data depending on storage time

Species	BADGE significance (<i>p</i>)	BFDGE significance (<i>p</i>)
Sardine	1.000	0.002*
Tuna	1.000	0.002*
Mackerel	0.364	0.757
Mussel	1.000	0.029*
Cod	— ^a	— ^a
Mackerel eggs	— ^a	— ^a

* Significant values (*p* < 0.05).

^a ANOVA could not be performed. There were not enough cases.

ferences, related to storage time, with respect to BFDGE data in mackerel.

On the other hand, there are no significant differences concerning the kinetics of BADGE migration and storage time, as observed in Table 7. Moreover, there is a non significant, but slight trend to increase BADGE levels with storage time mainly in mackerel, as illustrated in Fig. 4. These results show that BADGE migration levels are lower than BFDGE migration levels.

4. Discussion

In this paper, we manufactured several seafood products in our pilot plant in conditions similar to those used in the industry. Then, we simulated the can shelf life in the mar-

ket, meaning the temperature of storage (20 or 30 °C) and storage time (6–18 months), both at home or at the super-market. Several findings have been obtained regarding the migration of BADGE, and other derivatives from can coatings into aqueous or oily foods, however, there is a lack of information concerning BFDGE migration. In addition to that, most of the studies reflect simulations or probabilistic approaches of migration of some of these compounds (Uematsu et al., 2003, 2005; Poustkova et al., 2004; Holmes et al., 2005). There are no exhaustive and complete findings reflecting a real situation that usually occurs in the case of canned seafood in the market.

4.1. Effect of time and storage temperature

In general, our results indicate that there is an increase of BFDGE levels at longer storage time. On the contrary, there are no significant differences concerning the kinetics of BADGE migration and storage time (12–18 months). In addition to that, in this paper, we showed that BADGE migration levels are lower than BFDGE migration levels, the latter increasing in a significant manner after 6 months of storage. Several studies have shown that there is a low migration of BADGE and BFDGE in canned seafood. However, these studies were carried out without the knowledge of storage time due to the fact that most of these studies were made with cans acquired from the market (Biedermann and Grob, 1998). On the other hand, results related to storage temperature indicate that in some species a higher BADGE and BFDGE migration takes place at 30 °C (data not shown). However, this fact does not occur in all studied products.

4.2. Effect of sauce composition and fat content

Concerning the covering sauces, one of these studies showed that BADGE was found both in fish in oil and in fish in tomato sauce and the highest migration was found in the fatty foodstuffs (Hammarling et al., 2000). In Hammarling et al. (2000) the authors only studied BADGE migration, however, in our paper we evaluated BFDGE levels obtaining similar results, demonstrating that BFDGE migration occurs mainly in canned seafood with elevated lipid content. In this context, among all analyzed products, mackerel manufactured in red pepper sauce showed the highest levels of BFDGE and BADGE. It is worth mentioning that this product contains the highest levels of fat. Then, these results agree with several authors who found the highest migration of BADGE in products with elevated levels of fat content (Hammarling et al., 2000; Munguia-Lopez and Soto-Valdez, 2001).

4.3. Effect of time and temperature of processing

Relating to time and temperature of processing, it was reported that the migration of BADGE in tuna fish was higher when heat process was applied to the cans, a situa-

tion that reflect reality since cans are mostly sterilized (Munguia-Lopez and Soto-Valdez, 2001). Some studies showed an effect of the temperature of processing on BADGE migration that used 115 °C for 30 or 60 min (Simoneau et al., 2002) although these parameters are not usually applied in can industry. Sterilization features, normally applied in the industry for the formats used in our study are 115 °C for 45 min or 121 °C for 30 min. In our hands, i.e. industry conditions, we did not find significant differences with respect to BADGE or BFDGE migration.

In summary, these results show that BFDGE migration from varnish into seafood mainly depends on storage time and the content of fat. Canned products containing “cazuela”, “ravigote” and red pepper sauces are more prone to BADGE and BFDGE migration than those with oil, tomato, pickled, Galician and Biscayne sauces. BADGE and BFDGE migration is different depending on the species of seafood; in mackerel, concentrations of these compounds are especially high. Furthermore, in our conditions, BADGE and BFDGE levels are not influenced by sterilization parameters such as time and temperature. Migration levels of BADGE did not exceed the European legal limits in any condition. Finally, European legislation does not allow the use and/or the presence of BFDGE, however, we detected BFDGE migration in several conditions although in low levels.

Conflict of interest statement

All the authors are employees of the (Spanish) National Association of Fish and Seafood Canning Manufacturers, which includes canning industries, raw product dealers, can manufacturers and other related industries.

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