EVALUATION OF BISPHENOL A CONTENT IN FOOD FROM LACQUERED CANS

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ABSTRACT. This study describes the migration of bisphenol A from the cans to the food content through experimental tests using internal lacquered cans full of distilled water. Bisphenol A concentrations ranged between 3 and 320 µg.L^{-1} and increased with the increment of the storage duration, the temperature of storage and the temperature of sterilization. The highest concentrations were found in the set and sterilized cans. If such concentrations were found in canned foods, they could give rise to toxicological effects on the consumer.

KEY WORDS: Lacquered can, Bisphenol A, Migration, Epoxyphenolic resin

INTRODUCTION

Canned foods are taking increasingly importance in food packaging. Packaging is helpful in protecting, preserving and selling food products as well as in informing the consumer. In order to avoid migration issues, covering internal surface of the cans is widespread and manufacturers are continually looking for more efficient resins. A good resin is expected to cut down on corrosion and do away with interactions between cans and foods. Moreover, it must not only prevent the migration of its compounds to foods in amounts that may endanger consumers' health but also avoid altering the organoleptic qualities of canned foods.

The toxicity of the monomers of epoxyphenolic resins (bisphenol A, bisphenol F, bisphenol A diglycidyl ether), widely used as internal lacquers of cans, is now clearly established. The DL$_{50}$ of bisphenol A is estimated at 4.1 µg/kg of rat weight [1, 2] and 2.5 µg/kg of mouse weight [3]. Several studies on animals revealed the involvement of bisphenol A in the disturbance of the reproductive system causing sterility, genetic malformations and cancers of organs [4-13]. Brenman and collaborators associated bisphenol A to the alteration of DNA [14]. Other authors proved the cutaneous and allergens effects of bisphenol A on workers manipulating bisphenol A during the manufacture of epoxyphenolic resins [15]. The carcinogenicity effects to humans have been highlighted by Haighton [16] and Huff [17]. Some studies have even reported a modification in the morphology of plants [18]. Although results from studies on animals can not be generalized and extended to humans, it seems more cautious to avoid eating canned foods with high levels of bisphenol A. To this end, many studies throughout the world have been carried out to assess bisphenol A content in canned foods or bottled water. In 2007, the Environmental Working Group [19] conducted a survey of bisphenol A content in U.S. canned foods. This study considered 28 categories of canned foods (pasta, soup, beans, tuna, vegetables, milk, beverages, etc.) and found 7.9 µg.L^{-1} as the average concentration of bisphenol A in U.S. canned foods with a maximum of 385 µg.L^{-1}. Several other studies mentioned a possible migration of bisphenol A from cans or polycarbonate bottles to water or milk-like products [20-22].

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In view of the toxicity of bisphenol A, the European council fixed the specific migration limit of this compound in foods or water at 3 mg.kg^{-1} [23] and the European Community Commission set the Admissible Dose Intake at 50 µg.kg^{-1} of corporeal weight [24]. We undertook this study to assess the bisphenol A migration from epoxyphenolic resins to water through experimental simulations.

EXPERIMENTAL

Chemicals and reagents

Methanol (Fisher Bioblock Scientific, France), isooctane (Merk, France), dichloromethane (Carlo Erba, France), distilled water HPLC Grade, and a standard resin of 2.2-bis (4-hydroxy phenyl) propane (Merk, France) from European Certification Commission office were used.

Instrumentation

Bisphenol A concentrations were determined by High Performance Liquid Chromatography (HPLC). A Hewlett Packard HPLC chromatograph 1050 serial (USA) was used. It was equipped with an injection valve of 50 µL, a Hewlett Packard spectrophotometric UV detector serial 1050 and a Hewlett Packard integrator serial 3396. BADGE detections were made on 275 nm wavelength and compounds were separated with a column LiChrospher 100 RP-18 (Merck, 250 x 4 mm I.D., 5 µm) protected by a guard column LiChrospher 100 RP-18 (Merck, 5 µm). The mobile phase was a mixture of methanol, water, and dichloromethane (CH_3OH-H_2O-CH_2Cl_2) according to 50%-20%-30% proportions of solvents and the flow was 1 mL.min^{-1}.

Extraction of bisphenol A

A Supelco LC18 cartridge (USA) containing 1 g of resin was conditioned in accordance with the following sequence: methanol (CH_3OH, 20 mL), dichloromethane (CH_2Cl_2, 20 mL) and H_2O (2 mL). After that, the sample was first injected in the cartridge and then the cartridge was twice eluted with a mixture of CH_3OH-CH_2Cl_2 at different proportions (2 mL of 1:1 then 2 mL of 1:3). The extract obtained was evaporated under nitrogen gas and recovered in 1 mL of methanol and then stored at –20 °C until analysis. The extract was diluted in the mobile phase before HPLC analysis.

Validation procedure of the HPLC method

The quality control of HPLC and bisphenol A extraction was determined according to French normalization method and included linearity, repeatability, reproducibility and extraction yield [25]. The linearity was tested from 0 to 40 mg.L^{-1} with 5 points of the standard curve: 0.5, 1, 10, 20 and 40 mg.L^{-1}. Five analyses were performed for each point. The repeatability and reproducibility tests were realized with standards of bisphenol A at 20 µg.L^{-1} and 40 µg.L^{-1}. Standard solutions were prepared in the mobile phase and 50 µL of these standard solutions were injected in the chromatograph. Five tests were done for the repeatability and fifteen for the reproducibility. The extraction yield was assessed by adding definite concentrations of bisphenol A (1, 10 and 100 mg.L^{-1}) to distilled water. After mixing, bisphenol A was extracted according to the extraction procedure previously described. Triplicate assays were done for each amount of bisphenol A added to distilled water. The detection and quantification limits were determined with a blank descended from extraction:

Limit of detection (LD) = $m_b + 3\sigma$

Limit of quantification (LQ) = $m_b + 10\sigma$

where m_b = average concentration with the blank; σ = standard deviation of blank values, n = 30.

The results of the validation tests are presented in Table 1. The variation coefficients for repeatability and reproducibility were similar and ranged between 2.3 and 4.1% for repeatability and 2.7 and 4.4% for reproducibility. The extraction yields were between 86.7 and 90.8%. The limit of detection was 0.5 $\mu g.L^{-1}$ and the limit of quantification was 0.8 $\mu g.L^{-1}$. These results account for the reliability of the technique we used for bisphenol A extraction and analysis.

Table 1. Results of validation procedure.

Linearity	
Standard range: 0.5, 1, 10, 20, 40 µg/L	
Standard equation: y = 200204x + 54808	
Variation coefficient: R^2 = 0.9992	
Repeatability (%) (n = 5)	*Variation coefficient*
20 mg.L^{-1}	4.10
40 mg.L^{-1}	2.30
Reproductibility (%) (n = 15)	*Variation coefficient*
20 mg.L^{-1}	4.40
40 mg.L^{-1}	2.70
Extraction yield (%) (n = 3) 1 mg.L^{-1}	86.7 ± 5.6
10 mg.L^{-1}	90.0 ± 4.3
100 mg.L^{-1}	90.8 ± 3.7
Limite of detection (LOD) (n = 30), µg/L	0.5
Limite of quantification (LOQ) (n = 30), µg/L	0.8

Migration tests of bisphenol A. Migration tests were realized with metallic sheets and lacquered cans. The cans were provided by a factory located in the region of Meurthe and Moselle in France and specialized in the manufacture of tin cans for foodstuffs packaging.

Characteristics of the metallic sheets. The circular metallic sheets used had a diameter of 1.7 dm and an area of 2.3 dm^2. The specifications of lacquers used were: epoxyphenolic lacquer = 5.5 g/m^2; organosol lacquer = 18 g/m^2.

Characteristics of the lacquered cans. The tin cans used were "3 pieces" (a cylindrical body, a bottom and a cover). Each can was of the following dimensions: 1.10 dm of height, 0.75 dm of radius and 3.5 dm^2 of area. The specifications of the resins were: body and covert: lacquer monocoat 5.5 g/m^2; bottom: lacquer monocoat 7 g/m^2; rechampi: acrylic lacquer 10 g/m^2. Three types of cans were used: non-set and non-sterilised cans (CNSeSt), set and non-sterilised cans (CSeNSt), set and sterilised cans (CSeSt). The migration tests were done according to the European directive 85/572/CEE [26] and we studied the influence of storage conditions (temperature and duration) and sterilization (temperature and number of sterilization) on bisphenol A migration.

Influence of storage temperature. The three types of cans filled with 375 mL of distilled water were stored for 10 days under the temperatures of 4, 20, 40 and 60 °C. After storage, the content of each metallic can was extracted in accordance with the extraction method previously described. Analyses were done in triplicate.

Influence of storage duration. The three types of cans filled with 375 mL of distilled water were stored for 1, 5 and 10 days under 20 and 40 °C. After storage, the content of each metallic can was extracted in accordance with the extraction method previously described. Analyses were done in triplicate.

Influence of sterilization temperature. Metallic cans lacquered with epoxyphenolic resins and filled with 375 mL of distilled water were set and sterilized at six different temperatures (100, 105, 110, 115, 121 and 130 °C) for 20 min. After cooling, the content of each metallic can was extracted in accordance with the extraction method previously described. Analyses were done in triplicate.

Influence of the number of sterilization. A metallic sheet lacquered with organosol resin was immersed in an Erlenmeyer flask filled with 500 mL of distilled water. The Erlenmeyer flask covered with an aluminium film was four times successively sterilized in an autoclave LX Lequeux for 20 min at 121 °C. After each sterilization, distilled water was collected and the metallic sheet was rinsed, then immersed again in an Erlenmeyer flask containing 500 mL of distilled water for another sterilization in the autoclave. Five different measurements of bisphenol A concentration in distilled water were done after each sterilization.

RESULTS AND DISCUSSION

Influence of storage temperature on bisphenol A migration

The results of the influence of storage temperature on bisphenol A migration are shown in Figure 1. The concentrations of bisphenol A found in the non-set and non-sterilized cans (CNSeSt) and the set and non-sterilised cans (CSeNSt) were significantly inferior to the concentrations of bisphenol A in set and sterilised cans (CSeSt). The concentrations ranged from 12 to 33 μg.L^{-1} for CNSeSt, 3.8 to 24 μg.L^{-1} for CSeNSt and were about 300 μg.L^{-1} for CSeSt. Bisphenol A migration was more marked with the non-sterilized cans (CNSeSt and CSeNSt) and increased with the increment of their storage temperature. Although concentrations in sterilized cans were the most significant, they were only slightly affected by an increase in temperature. Concentrations of bisphenol A in sterilized cans were at least ten times inferior to the value of the specific migration limit (3 mg/kg of food or water) fixed by the European Council [24]. However, this limit seems overvalued given that Brotons and collaborators [27] observed a significant oestrogenic response for bisphenol A content at 4 μg per can or 12 μg.kg^{-1} of food. Nagel and collaborators [28] administered bisphenol A to compound mice at concentrations ranging from 2 to 20 μg.kg^{-1} of corporal weight between the eleventh and the seventeenth day of pregnancy. They observed, in male progeny of 6 months old, an increase in the prostate weight ranged from 30 to 35%.
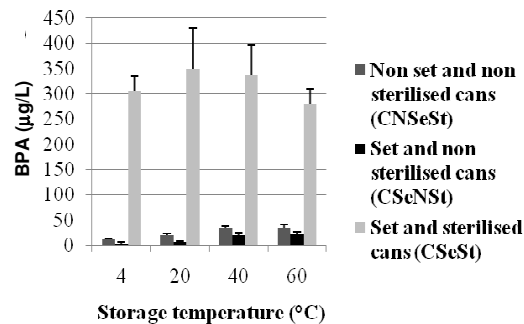
Figure 1. Influence of cans storage temperature on bisphenol A migration.

Influence of storage duration on bisphenol A migration

The results of the influence of storage duration on bisphenol A migration are shown in Figure 2 (2a, 2b and 2c). The bisphenol A concentrations in sterilized cans were about 300 µg.L^{-1} whatever the storage duration. Concentrations of bisphenol A in sterilized cans were at least ten times higher than the concentrations of bisphenol A in the non-sterilized cans. Concentrations of bisphenol A in the non-sterilized cans increased with the increment of storage duration but were always lower than the concentrations in the sterilized cans. For storage at 4 °C the concentrations of bisphenol A in the non-sterilized cans ranged between 3 and 12 µg.L^{-1} (CNSeSt) and were about 3 µg.L^{-1} (CSeNSt). For storage at 20 °C the concentrations of bisphenol A in the non-sterilized cans ranged between 4 and 20 µg.L^{-1} (CNSeSt) and between 5 and 10 µg.L^{-1} (CSeNSt). For storage at 40 °C the concentrations of bisphenol A in the non-sterilized cans ranged between 9 and 35 µg.L^{-1} (CNSeSt) and between 6 and 21 µg.L^{-1} (CSeNSt).
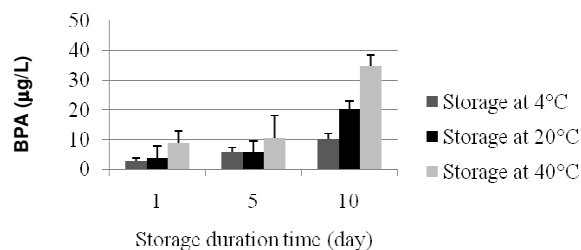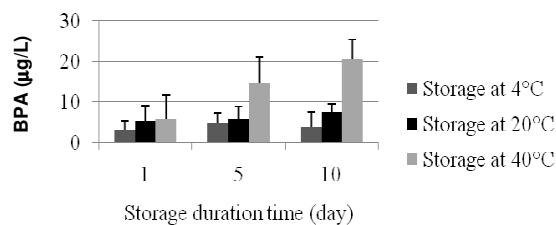

Figure 2a. Non-set and non-sterilised cans (CNSeSt).

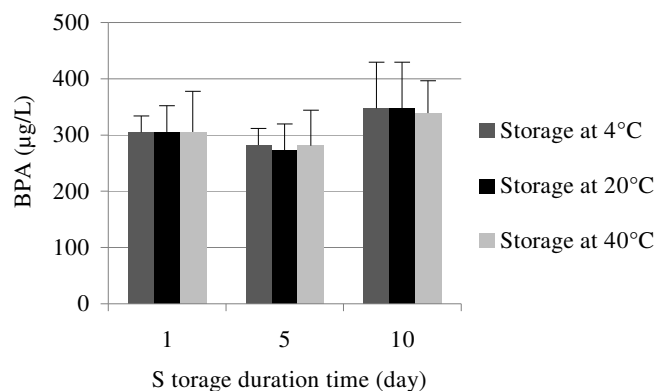Figure 2b. Set and non-sterilised cans (CSeNSt).

Figure 2c. Set and sterilsed cans (CSeSt).

Figure 2. Influence of cans storage duration bisphenol A migration.

Influence of sterilisation temperature on bisphenol A migration

The results of the influence of sterilisation temperature on bisphenol A migration are shown in Figure 3. Concentrations of bisphenol A were between 72 µg.L^{-1} at 110 °C and 320 µg.L^{-1} at 130 °C. Two landings of concentrations (100 to 110 °C and 115 to 130 °C) were observed. The concentrations of bisphenol A ranged, respectively from 72 to 86 µg.L^{-1} for the first landing and from 275 to 320 µg.L^{-1} for the second landing. High temperatures break the resin down and are responsible for the release of such significant amounts of bisphenol A. Bretons and collaborators [27] conducted a similar study. After thirty minutes of sterilization at 125 °C, they found bisphenol A in lacquered cans at concentrations lower or equal to 33 µg per can. Concentrations of bisphenol A in our study were between 28 and 128 µg per can. The maximum concentration in our study was approximately four times higher than the maximum concentration in their study. The migration was more intense when temperatures were superior or equal to 115 °C. This may correspond to the required temperature for the mobilisation of free molecules of bisphenol A that did not partake in the different condensation and addition reactions leading to epoxyphenolic resins.
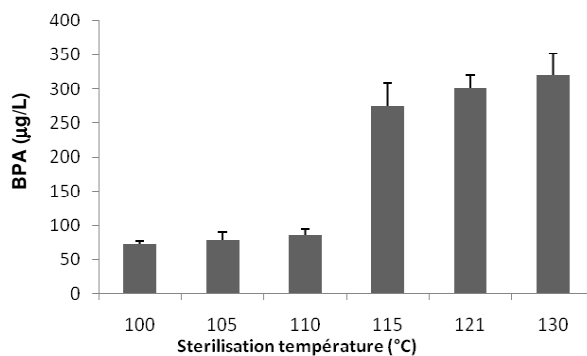

Figure 3. Influence of the sterilisation temperature on bisphenol A migration.

Influence of the number of sterilization on bisphenol A migration

The influence of the number of sterilization on bisphenol A migration is presented in Table 2. The quantity of bisphenol A released in distilled water by the metallic sheet was 329 µg.L^{-1} after the first sterilization and 4 µg.L^{-1} after the fourth sterilization. After the second sterilization, the sample only contained 19 µg.L^{-1} of bisphenol A that is 94% percentage of reduction in comparison with the sample from the first sterilization. The percentage of reduction was, respectively 97% and 99% after the third and the fourth sterilization. It appeared clearly that more the metallic sheet was sterilized more the migration of bisphenol A was substantial.

Table 2. Influence of the number of sterilization on bisphenol A migration (concentration: µg.L^{-1}, n = 5).

Sterilisation number	S1	S2	S3	S4
Bisphenol A concentration	329 ± 102	19 ± 10	9 ± 2	4 ± 4

CONCLUSIONS

Migration of bisphenol A in non-sterilized cans was influenced by storage temperature and duration. Concentrations of bisphenol A in sterilized cans were the most significant (about 300 µg.L^{-1}) and were not influenced by storage temperature and duration. The levels of bisphenol A in sterilized cans were ten times inferior to the specific migration limit fixed at 3 mg/kg of food. The temperature of sterilization proved to be critically important for bisphenol A migration which was significant depending whether the cans were sterilized at high temperatures. Finally, the number of sterilization was also affected the level of bisphenol A migration. The more the metallic sheet was sterilized, the more the migration of bisphenol A was substantial. This toxic constituent of resins (bisphenol A) can be reduced, even more, could be eliminated after numbers of sterilization at high temperatures (115-120 °C) before food conditioning.

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