



Research article

Zinc, aluminium, tin and Bis-phenol a in canned tuna fish commercialized in Lebanon and its human health risk assessment

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ARTICLE INFO

Keywords:

Food science
Food safety
Canned tuna
Heavy metals
Health risk
Lebanese market

ABSTRACT

One of the drawbacks of canning is the migration of various chemicals from the package into the food product. This work aimed at analyzing the concentrations of Bisphenol A (in 137 samples) and heavy metals (in 51 samples) of canned tuna commercialized in Lebanon while evaluating the variability across different brands, packing media, layer, and proximity to the expiry date. Accordingly, BPA was detected in 12 samples out of the 137 samples, run in duplicates. The estimated daily intake of BPA for the selected samples ($n = 274$) was lower than the tolerable daily intake of BPA, 0.004 mg/kg/day. Therefore, there is no health risk associated with BPA as a result of consuming canned tuna commercialized in the Lebanese market. Besides, the study has shown that 66 samples out of 102 were contaminated with Zn whereas 100% of the samples were contaminated with Aluminum and Tin. However, the calculated Health Risk Index of all the considered heavy metals are all within the safe limits as defined by EFSA (European Food Safety Authority) and Codex Alimentarius.

1. Introduction

The processing and packaging of food products have a great benefit for the end consumers, by easing handling and storage (Robertson, 2016). Besides, processing helps in retaining the nutritional value and the sensory characteristics of the food (Food Standards Australia New Zealand (FSANZ), 2017). However, this is sometimes associated with the potential migration of chemicals from the package to the food itself. Packaging materials like tin, glass, ceramics, and plastic, may release small amounts of chemicals when in contact with the food. This migration of chemicals from the packaging and other food contact materials to the food might be harmful to human health (Ardic et al., 2015).

Canning is an inexpensive food preservation method involving heat treatment of the canned food at temperatures reaching 121 °C (Robertson, 2013). Tinplate is one of the oldest packaging materials. It is a steel sheet covered with a protective coating, tin, to protect the steel from rust and corrosion (FAO, 2005). However, the disadvantage of using coated cans is the migration of tin and iron into the food resulting in a

potential alteration in the flavor. Another disadvantage of improper home canning of foods is foodborne botulism (Parkinson et al., 2017). Trace metal levels of different canned samples have been broadly reported in the literature (Tuzen and Soyak, 2007). Another chemical contamination due to package-product interaction which has been reported is the migration of Bisphenol A (BPA), an industrial chemical produced via the condensation of two moles of phenol and a mole of acetone (Geens et al., 2012). BPA is used in the production of epoxy resins, used as a protective can coating for food application (Beltifa et al., 2017). Its use is a debatable topic in food packaging (Schechter et al., 2010). According to the European Commission (2018), the specific migration limit (SML) for BPA is 0.05 mg/kg of food (European Commission, 2018).

In this study, three trace elements, Zinc, Aluminum, and Tin, were assessed. All three heavy metals can have detrimental health effects at high concentrations (Di Bella et al., 2015; Bella et al., 2017). Zinc, a major coating/component used to prevent corrosion of iron and steel, can migrate to the food (Popović et al., 2018; Noureddine El Moussawi et al.,

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Received 3 June 2020; Received in revised form 20 July 2020; Accepted 17 September 2020

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2019). According to the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), the permissible limit of zinc is 50 mg/kg body weight (FAO/WHO, 2017). On the other hand, Aluminum is used in canning with a provisional tolerable weekly intake (PTWI) of 2 mg/kg body weight (FAO/WHO, 2017). According to the Codex Alimentarius Commission (2009), tin, a protective coating for other materials due to its resistance to corrosion, is classified as a first triggering contaminant with a maximum permissible level in canned food of 250 mg/kg body weight (FAO/WHO, 2017; Codex Alimentarius Commission, 2009; Dikshith, 2014).

Many studies have assessed the toxicity of bisphenol A and heavy metals in canned food (Jaishankar et al., 2014; Di Bella et al., 2015; Suryabhan Shriram Dongre, 2019). According to the EFSA report in 2015, canned food presented higher BPA concentrations than non-canned food. The report showed that 7 out of 17 canned food categories contained an average BPA concentration above 30 $\mu\text{g}/\text{kg}$. The highest BPA concentrations were reported in meat, fish among other seafood categories with average BPA concentrations of 9.4 and 7.4 $\mu\text{g}/\text{kg}$, respectively (European Food Safety Authority (EFSA), 2015b). As for the heavy metals, Jaishankar et al. (2014) and Alissa and Ferns (2011) have shown that severe health implications are associated with heavy metals toxicity generally resulting in weakness, headaches, and fatigue (Alissa and Ferns, 2011; Jaishankar et al., 2014). However, each metal has its side effects that disturb human health (Suryabhan Shriram Dongre, 2019). To minimize the health risks of heavy metals, the WHO and the European Food Safety Authority (EFSA) have established guidelines and standards limits for BPA and heavy metals in foods (FAO/WHO, 2017; EFSA, 2015; European Commission, 2018).

A risk-benefit assessment of a given food balances the benefits of that particular item with any inherent risk associated with its consumption, taking into account the risks while recognizing the benefits of that particular food or food components for the health of the population. According to EFSA (2015a), the risk-benefit assessment provides evidence-based risk evaluation due to the exposure to certain contaminants associated with the consumption of a given food product. While fish consumption is recommended for a balanced diet, aquatic environments can be contaminated by anthropic substances that may end up in fish tissues raising food safety concerns (Di Bella et al., 2015).

In this context, tuna fish, one of the most frequently consumed canned products due to its high content of essential nutrients – protein, omega-3 fatty acids, vitamin D, and selenium – represents an appropriate model for risk-benefit assessment (Ikem and Egiebor, 2005). Several studies have assessed heavy metals in canned tuna and associating this contamination with a polluted marine environment (Fakhri et al., 2018), municipal and agricultural wastewater (Domingo et al., 2007), and contamination occurring during the canning process (Domingo et al., 2007).

The main objective of this study was to assess the health risks associated with Bisphenol A, Zinc, Aluminum, and tin ingested via the consumption of canned tuna. For this purpose, BPA and the trace elements levels were evaluated and compared to other studies. Besides, a risk assessment for these contaminants was conducted to provide information on the associated human health risks linked to the consumption of canned tuna products in Lebanon.

2. Materials and methods

2.1. Sampling

The canned tuna samples were randomly purchased from retail stores in Beirut, Lebanon, in 2018–2019. As declared on the labels, all the collected samples originated from Thailand. 137 samples were bought for BPA analysis of BPA and 51 samples were acquired for the analysis of heavy metals. The sampling strategy took into consideration the different brands, packaging medium, and expiry/production dates. Random sampling was followed to select five brands. Three samples were selected

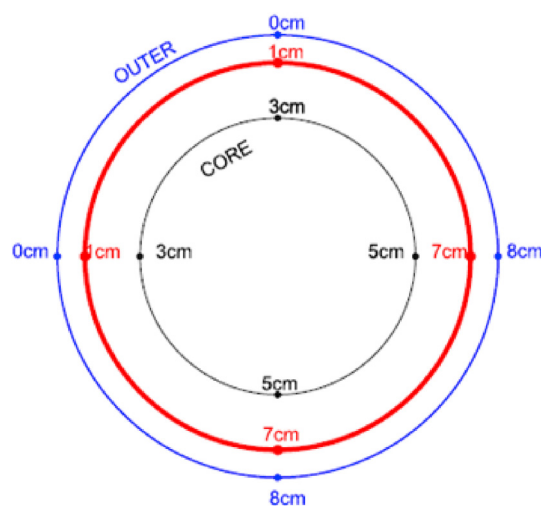


Figure 1. Scheme representing the layers of tuna.

from each brand to provide a representative dataset. To test the brine/solution effect, three different types of packing media were selected – water, oil, and oil with chili. Each collected sample was divided into two layers using a knife. The diameter of the core layer was 2 cm while the thickness of the outer layer was 1 cm. The two layers were mixed and homogenized separately. This allowed us to have two samples from each can, considering the center (core) and the outer layer (Figure 1) for a total of 274 samples used for BPA analysis and 102 samples used for heavy metal analysis. Accordingly, the generated number of samples ($n = 274$ and $n = 102$) were classified into four different categories corresponding to their nearness to the production dates (0–6, 6–12, 12–18, 18–24 months). The samples were then transferred into clean propylene bags (BPA free), coded with their brands, type of packing, layer (center/outer), and production date category, and stored at $-20\text{ }^{\circ}\text{C}$ until further analysis (García et al., 2016).

2.2. Chemical and reagents

2.2.1. Chemical and reagents for BPA

All reagents were of analytical grade. Bisphenol A and Bisphenol B were purchased from Sigma Fluka (Switzerland). All the standards were of high purity grades ($>99\%$).

An individual stock solution of BPA and BPB was prepared by dissolving 10.1 g of BPA/BPB powder with 5 ml acetonitrile to get 2000 ppm (mg/kg). The BPA and BPB stock solutions were made by diluting the standard solution with the mobile phase. The standard solutions for BPA were 0.2, 0.5, 1, 5, 10, 20, 30, 40 and 50 ppm whereas those for BPB were 10, 20, 30, 40, and 50 ppm. The calibration curve of BPA has various concentrations from 0.4 until 50 ppm. A linear line was illustrated between the HPLC signal and concentration with a high correlation coefficient ($R^2 = 0.9998$, $n = 9$).

2.2.2. Chemical and reagents for heavy metals

Deionized water was used for all dilutions. Extra pure quality (65% w/v) nitric acid (Merck, Germany) and (30% w/v) hydrogen peroxide (Spain). Standard stock solutions of zinc, aluminum, and tin (HIGH-PURITY) were prepared by diluting concentrated solutions to obtain a mixture of 1000 mg/l with deionized water.

The Zn calibration curve was constructed using stock solutions with concentrations of 0, 0.5, 1, 1.5, 2, 2.5, and 3 ppm; whereas for Al concentrations of 0, 20, 30, 40, 50, and 70 ppb were used and concentrations of 0, 20, 30, 40, 50, 60, 70, and 80 ppb were used for the calibration curve for Sn. For all the calibration curves, a linear fit was illustrated between the AA signal and the various concentrations with a high

Table 1. Descriptive table of BPA (n = 274) and heavy metal (n = 102) samples considering the variables media, brand, and proximity to production date.

	Variables	Water		Oil		Oil & Chili		Total	
		BPA	Heavy metals	BPA	Heavy metals	BPA	Heavy metals	BPA	Heavy metals
Brand	A	6	4	24	4	22	4	52	12
	B	18	4	6	4	24	4	48	12
	C	14	8	20	8	24	8	58	24
	D	18	12	24	12	24	12	66	36
	E	18	8	14	6	18	4	50	18
Proximity to Production Date (months)	Total	74	36	88	34	112	32	274	102
	0–6	18	8	20	6	30	8	68	22
	6–12	30	20	30	20	24	16	84	56
	12–18	14	4	24	4	28	4	66	12
	18–24	12	4	14	4	30	4	56	12
	Total	74	36	88	34	112	32	274	102

correlation coefficient R^2 for each metal, 0.9902, 0.9989, and 0.9826 for Zn, Al, and Sn, respectively.

2.2.3. Descriptive table of BPA and heavy metals samples

137 canned tuna samples (for heavy metal analysis) and an additional 51 samples (for BPA testing) were collected from the Lebanese market. While taking into consideration the layer variable, each sample was divided into 2 layers (center/outer). For this reason, two hundred and seventy-four samples and One hundred two of canned tuna were subjected to BPA and heavy metals analysis. Table 1 presents the list of samples taking into consideration the different variables; i.e. the packing media, brand, and production/expiry dates.

2.3. Determination of BPA

2.3.1. Sample preparation

A portion of 5g of each of the two layers of minced tuna was weighed and placed in a 50 ml capped, BPA free, propylene tubes (Di Bella, Potorti, Turco, Saitta and Dugo, 2014; Lo Turco et al., 2016). 5 ml of pure hexane was added and the mixture was shaken for 1 min. Then, 10 ml of acetonitrile was added and the mixture was vortexed again for 1 min. Afterward, the tubes were centrifuged at 5000 rpm, 15 °C for 15 min. The acetonitrile layer “lower layer” was transferred and filtered using PTFE 0.2 μm (Kinesis) before injecting the filtrate in the HPLC. 15 μl of BPB was added to the mixture as a control (Aristiawan et al., 2015). The HPLC unit was calibrated using BPA and BPB at different concentrations run in triplicates. The linearity shows acceptable performance for both bisphenols.

2.3.2. HPLC equipment and parameter

The High-Performance Liquid Chromatography unit used was HPLC (Agilent 1260, Agilent, CA, USA) equipped with UV Detector on wavelength 200 nm and autosampler system. 274 tuna samples were quantified by measuring the area of the BPA peaks and comparing them with the calibration curves. The sample analysis was performed using an Agilent ZORBAX ECLIPSE PLUS C18 (4.6 \times 250mm, 5 μm). 20 μl volume of sample was injected into the HPLC for analysis in isocratic elution at 1.2 ml/min at room temperature. The mobile phase used was water/ acetonitrile (60:40, v/v) for 17 min. The retention time was 10.2 min for BPA and 15.9 min for BPB.

2.3.3. Recovery

Recovery was conducted using BPA and BPB free samples. Before injecting the samples into the HPLC, they were spiked with 0.6 and 1.2 ppm BPA, and 30 ppm BPB stock solutions (Aristiawan et al., 2015). The calibration curve was plotted accordingly. Recovery was calculated by comparing the theoretical concentrations with the spiked concentrations.

The standard deviation was calculated based on three trials on 2 levels, 0.6 and 1.2 ppm, for BPA and on 1 level, 30 ppm, for BPB. The mean recoveries (65.00–111.64%) for the spiked samples were considered to be acceptable given that the acceptable %RSD value is between 80 and 110% for a concentration below 100 ppm (Aristiawan et al., 2015). The precision of this method was calculated at two different levels and expressed as %RSD. Precision for BPA was 4.19 while for BPB was 6.05. As an important step in the verification process, recovery for two spiked concentrations in a blank tuna matrix was determined with an acceptable percentage range between 88 and 90 % for BPA and 109–111% for BPB.

2.4. Determination of heavy metals

2.4.1. Sample preparation (microwave digestion)

For each canned tuna sample, 0.7g of wet tuna was weighed and put in a Teflon digestion vessel with 10 ml of 65% nitric acid (HNO_3) and 1.5 ml of 30% hydrogen peroxide (H_2O_2). The samples were digested in a microwave (Milestone Srl., Fatebenefratelli, BG, Italy) according to the following procedure: Stage 1, ramping from 25 to 200 °C at a power of 1800 W for 10 min; Stage2, constant temperature of 200 °C at a power of 1800 W for 10 min. The digested samples were diluted with deionized water to 10 ml (Korfali and Abu Hamdan, 2013).

2.4.2. Instrumentation

The Ethos Up high-performance microwave digestion system was obtained from Milestone Srl. (Fatebenefratelli, BG, Italy) and utilized to digest the canned tuna samples before running the metal analyses. An Analyst 700 atomic absorption spectrometer (Shimadzu, Kyoto, Japan) was utilized in this study. The element zinc was analyzed in an air-acetylene flame. While aluminum and tin were analyzed using a furnace with argon as the inert gas.

2.4.3. Calculation of the tuna consumption rate in Lebanon

As per our communication with the Ministry of Economy and Trade, Lebanon does not export or produce canned tuna. Therefore, Lebanese mainly rely on importing canned tuna for consumption. The ministry data about the imported canned tuna to Lebanon was an average of 6,862 tons/year. The Worldometer elaboration of the latest data from the United Nations has shown that the current population of Lebanon is 6.8 million. The calculation of the daily fish tuna consumption rate (FIR) in Lebanon was calculated using Eq. (1).

$$\text{FIR} = \frac{\text{Importedcannedtunainlebanon}}{\text{Lebanesepopulation} * 365} \quad \text{equation (1)}$$

Therefore, the daily fish tuna consumption rate in Lebanon is 2.75 g/person/day equivalent to 2.75×10^{-3} kg/person/day.

Table 2. Statistical tests for BPA and heavy metals.

	BPA	Zn	Al	Sn
Media	Kruskal-Wallis test	Kruskal-Wallis test	Anova and Post hoc LSD	Anova and Post hoc LSD
Center/Outer Layers	Wilcoxon Test	Wilcoxon Test	T-test	T-test
Brand	Kruskal-Wallis test	Kruskal-Wallis test and Wilcoxon	Anova and Post hoc LSD	Anova and Post hoc LSD
Proximity to Production Date	Kruskal-Wallis test	Kruskal-Wallis test and Wilcoxon	ANOVA and Post hoc LSD	ANOVA

2.4.4. Daily consumption of canned tuna

According to FAO/WHO, the consumption of canned tuna in the EU, and the USA was estimated to be 29 and 20 kg/person/week respectively (FAO/WHO, 2017). The probably daily intake of BPA by an adult consuming canned fish/day was calculated using Eq. (2) (Fattore et al., 2015).

$$PDI = \frac{C \times I}{BW} \quad \text{equation (2)}$$

where PDI is the probably daily intake of BPA (mg/kg/day); I is the Daily fish tuna consumption rate (kg/person/day); C is the average of BPA concentration in canned tuna taking into consideration only positives samples ($\mu\text{g/g}$), and BW is the average body weight assumed as 70 kg.

2.4.5. Estimated weekly intake

The consumption of canned fish will vary greatly from one person to another. The calculation of the estimated weekly intake (EWI) of heavy metals by an adult consuming canned fish/week was then calculated using Eq. (3) (Ikem and Egiebor, 2005):

$$EWI = \frac{\text{Mean concentration} \times \text{Amount of fish consumed weekly}}{\text{Average body weight of individual (70 kg)}} \quad \text{equation (3)}$$

2.4.6. Health risk assessment

The human health risk was assessed from persistent exposure to heavy metals. To assess the risk of canned tuna, the estimated daily intake (EDI) of metal was determined using Eq. (4) (Núñez et al., 2018).

$$EDI = \frac{FIR \times C}{BW} \quad \text{equation (4)}$$

where EDI is the estimated daily intake (mg/kg/day); FIR is the Daily fish tuna consumption rate (kg/person/day); C is the average of heavy metal concentrations in canned tuna ($\mu\text{g/g}$), and BW is the average body weight (70 kg).

The health risk index (HRI) was calculated using Eq. (5) (Sobhanardakani, 2017).

$$HRI = \frac{EDI}{RFD} \quad \text{equation (5)}$$

where EDI is the estimated daily intake and RFD is the reference dose of metal. The oral reference doses for Zn, Al, and Sn are 0.30, 1.0, and 0.20 mg/kg/day, respectively. A health risk index (HRI) less than one in-

icates the exposure to heavy metals from this specific product is considered to be safe (Antoine et al., 2017; Sobhanardakani, 2017).

2.5. Statistical analysis

The statistical analyses were conducted using SPSS version 24.0. A Shapiro-Wilk nonparametric test was used to check the normality of the data. The BPA and Zn data did not follow a normal distribution while Al and Sn followed a normal distribution. Kruskal-Wallis was conducted to analyze the media, brand, and nearness to production/expiry date, followed by an analysis with the Wilcoxon test. The layer (center/outer) variable was also assessed by the Wilcoxon test. ANOVA was used to study the media, brand, and nearness to production/expiry date, followed by an analysis with the Post hoc LSD. The T-test was used to analyze the layer (center/outer) variable (Table 2). P-values of less than 0.05 were considered statistically significant ($p < 0.05$).

3. Results and discussion

3.1. Summary of BPA occurrence in canned tuna samples

Table 3 presents a summary of the BPA results present in the 137 (in duplicate) samples of canned tuna. It shows that BPA is detected in 12 samples out of 274, with a percentage of positive samples of 4.38%. The average mean concentration of BPA in the canned tuna samples collected in this study ($n = 274$) is $0.197 \mu\text{g/g}$, which is lower than the 0.6 mg/kg permissible limit set by the European Commission (2011) (European Commission, 2011). However, a new regulation was published by the European Commission in 2018 defining the limit as 0.05 mg/kg which brings concerns about the detected concentration in the canned tuna products. According to the USA, EU, and Lebanese tuna consumption rates, the probably daily intake of BPA for our samples ($n = 274$) is $5.57 \times 10^{-6} \text{ mg/kg/day}$, $8.93 \times 10^{-6} \text{ mg/kg/day}$, and $5.93 \times 10^{-6} \text{ mg/kg/day}$, respectively, which is below the tolerable daily intake of BPA ($4 \times 10^{-4} \text{ mg/kg/day}$) (European Commission, 2018) (Table 4). Therefore, there are no health risks of BPA as a result of consuming canned tuna in Lebanon.

3.2. Effect of variables on BPA occurrence in canned tuna samples

The effect of four variables (brand, media, layer, and nearness to production/expiry date) on the BPA occurrence in canned tuna samples is summarized in Table 5. While assessing the brand, the BPA mean average ranges between 0.195 and $0.20 \mu\text{g/g}$ in the collected samples. There is no

Table 3. Summary of BPA ($n = 274$) and heavy metals ($n = 102$) results obtained for canned tuna. Percentage of positive samples, mean and median concentrations ($\mu\text{g/g}$) of BPA and heavy metals (Zn, Al, Sn) in canned tuna ($\mu\text{g/g}$) are reported.

Tuna samples	BPA ($n = 274$)	Zn ($n = 102$)	Al ($n = 102$)	Sn ($n = 102$)
Mean	0.197	7.490	4.756	3.347
Median	0.200	5.669	3.390	3.280
Max	0.205	121.120	23.890	8.960
Min	0.105	0.300	0.720	0.990
Standard deviation	0.029	13.471	4.369	1.423
n positive	12	66	102	102
% positive	4.38	64.71	100	100

Table 4. Probably daily intake of BPA.

n = 102	BPA			
	Countries	US*	EU**	Lebanon
Average ($\mu\text{g/g}$)		0.15113		
Rate consumption of canned fish (kg/person/day)		0.00258	0.00414000	0.00275
Probably Daily Intake (mg/day/70 kg body weigh)		0.00000557	0.00000893	0.00000593
Tolerable Daily Intake (TDI) ($\mu\text{g/g}$)		0.004		

US*(Joint [FAO/WHO, 2017](#)):
EU**(Joint [FAO/WHO, 2017](#)):

Table 5. Mean values of BPA for tuna samples (n = 274) according to different variables.

Variables		BPA Average (mg/kg)	P ₁ *	P ₂ *
Brand	A (n = 52)	0.198	0.205	1
	B (n = 48)	0.195		
	C (n = 58)	0.198		
	D (n = 66)	0.200		
	E (n = 50)	0.197		
Media	Water (n = 74)	0.197	0.683	
	Oil (n = 88)	0.197		
	Oil & Chili (n = 112)	0.198		
Layer	Center (n = 137)	0.198	0.700	
	Outer (n = 137)	0.196		
Proximity to Production Date	0-6 (n = 68)	0.196	0.684	
	6-12 (n = 84)	0.198		
	12-18 (n = 66)	0.198		
	18-24 (n = 56)	0.197		

*P₁: P value for every variable.

*P₂: P value across variables.

significant difference between the BPA means for brands A, B, C, D, and E (p-value = 0.205). The study of the media effect showed a BPA mean average of 0.197 $\mu\text{g/g}$ for water and oil and 0.198 $\mu\text{g/g}$ for oil with chili. There is no significant difference between the various packing media (p-value = 0.683). While evaluating the layer (center/outer), the BPA mean average ranges between 0.196 and 0.198 $\mu\text{g/g}$ with no significant difference between the center and outer layer in the canned tuna samples (p-value = 0.700). However, according to the nearness to production/expiry dates, the BPA concentration averaged between 0.197 $\mu\text{g/g}$ and 0.198 $\mu\text{g/g}$. There is no significant difference between the different production/expiry dates categories (0–6, 6–12, 12–18, and 18–24 months) (p-value = 0.684). Besides, there appears to be no significant difference between the BPA mean averages across the different variables – brand, media, layer, and nearness to production/expiry dates. [Table 5](#) summarizes the results of the statistical analysis for BPA.

3.3. Summary of heavy metals occurrence in canned tuna samples

[Table 3](#) presents the summary of the results on Zn, Al, and Sn occurrence in 51 (in duplicate) samples of canned tuna. 66 samples out of the 102 were contaminated with Zn, with a percentage of 64.7% positive samples. However, all the samples were contained Al and Sn, (100% of positive samples). The average level for Zn in the 66 positive samples was 7.490 $\mu\text{g/g}$, which is almost two times higher than the Al and Sn mean with values of 4.756 $\mu\text{g/g}$ and 3.347 $\mu\text{g/g}$, respectively. As can be seen in [Table 3](#), Zn has the highest mean level, although the percentage of positive samples was less than that of Al and Sn. The mean concentration of Zn in our samples is much higher than those reported by [Boadi et al. \(2011\)](#) for canned fish samples (n = 46) collected from Kumasi in the Ashanti Region of Ghana and ranging between 0.010 and 0.370 $\mu\text{g/g}$.

However, our values compare to those reported by [Korfali and Abu Hamdan \(2013\)](#) 6.57 $\mu\text{g/g}$ for 8 canned tuna samples which were collected from the Lebanese market. On the other hand, our reported values are lower than those reported by [Tuzen and Soylak \(2007\)](#) ranging between 7.57 $\mu\text{g/g}$ and 34.4 $\mu\text{g/g}$ for canned tuna samples commercialized in Turkey. The value reported in our study is lower than the maximum zinc level permitted (MPL) for fish which is 40–50 $\mu\text{g/g}$ according to the FAO (S. V. [Hosseini, Sobhanardakani, Miandare, Harsij and Regenstein, 2015](#)).

The average mean content of Al in the canned tuna samples collected in our study (n = 102) is 4.756 $\mu\text{g/g}$. Al in our canned tuna is probably due to leaching of Al from the metal can or from the lacquer, which contains aluminum-based additives ([Kontominas et al., 2006](#)). This value is slightly higher than the aluminum content found in canned tuna commercialized in India (3.161 $\mu\text{g/g}$) and higher than the mean concentrations found in canned tuna commercialized in Canada (1.806 $\mu\text{g/g}$) ([Mahalakshmi et al., 2012](#)). Furthermore, our reported values are higher than the ones obtained by [Korfali and Abu Hamdan \(2013\)](#) 0.81 mg/kg for 8 canned tuna samples collected from the Lebanese market. However, our values were within the range reported by [Türkmen et al. \(2005\)](#) 0.02–5.41 $\mu\text{g/g}$. It is worth noting that our values are within the permissible limits for Al set by FAO/WHO as 60 mg/day.

The average mean content of Sn in the canned tuna samples collected in our study (n = 102) is 3.347 $\mu\text{g/g}$. This could be due to the leaching of tin from the cans since it is preserved in liquid media. Our Sn values are higher than those obtained by [Korfali and Abu Hamdan \(2013\)](#) (0.5 $\mu\text{g/g}$) and [Sobhanardakani \(2017\)](#) (0.18 $\mu\text{g/g}$). The higher values obtained in our study could be due to poor lacquering/coating (S. V. [Hosseini et al., 2015](#)). On the other hand, our values are below the maximum permitted levels (MPL) of Sn in canned food (250 $\mu\text{g/g}$ and 200 $\mu\text{g/g}$) as defined by

Table 6. Mean values ($\mu\text{g/g}$) of Zn, Al, and Sn in tuna samples according to different variables.

Variables		Zn Average ($\mu\text{g/g}$)	P ₁ *	P ₂ *	Al Average ($\mu\text{g/g}$)	P ₁ *	P ₂ *	Sn Average ($\mu\text{g/g}$)	P ₁	P ₂
Brand	A (a) (n = 12)	11.586 ^{b,c,d}	0.000	1.000	5.781 ^b	<0.01	1.000	3.385 ^{d,e}	<0.01	1.000
	B (b) (n = 12)	2.98 ^a			2.291 ^{a,c}			3.04 ^e		
	C (c) (n = 24)	4.122 ^a			6.438 ^b			2.965 ^e		
	D (d) (n = 36)	10.332 ^a			4.286			2.211 ^{a,e}		
	E (e) (n = 18)	6.443			4.559			5.557 ^{a,b,c,d}		
Media	water (f) (n = 36)	5.773	0.888		3.125 ^{g,h}	<0.01		3.068 ^h	<0.01	
	Oil (g) (n = 34)	10.037			5.181 ^{f,h}			3.106 ^h		
	Oil&Chili (h) (n = 32)	6.409			5.841 ^{f,g}			3.865 ^{f,g}		
Center/Outer Layer	Center (n = 51)	6.576	0.729		4.417	0.421		3.303	0.742	
	Outer (n = 51)	8.404			5.095			3.391		
Proximity to Production Date (months)	0-6 (i) (n = 22)	4.423 ^l	0.026		2.304 ^{j,k}	<0.01		2.901	0.251	
	6-12 (j) (n = 56)	8.274 ^l			4.682 ^{i,k}			3.485		
	12-18 (k) (n = 12)	7.075			7.693 ^{i,j}			3.528		
	18-24 (l) (n = 12)	10.446 ^{i,j}			5.155			3.321		

In the same line, different letters (a → l) represent the statistical differences ($p < 0.05$).

No letters represent no significance.

*P₁: P value for every variable.

*P₂: P value across variables.

WHO (JECFA, 2006) and (EU, 2006), respectively. The ranking order of heavy metals mean concentrations ($\mu\text{g/g}$) in the 102 tuna samples is:

Zn (7.49 $\mu\text{g/g}$) > Al (4.756 $\mu\text{g/g}$) > Sn (3.347 $\mu\text{g/g}$)

The same order was obtained by Rahmani et al. (2018) while assessing heavy metals occurrence in 1295 canned tuna samples:

Zn (9.31 $\mu\text{g/g}$) > Al (1.8 $\mu\text{g/g}$) > Sn (0.1 $\mu\text{g/g}$)

Comparing the heavy metal concentrations in canned tuna with permissible limits according to EU, ATSDR, and FAO/WHO (50 mg/kg body weight per day for Zn, 60 mg per day for Al and 250 mg/kg body weight per day for Sn), the mean concentrations of Zn (7.508 $\mu\text{g/g}$), Al (4.757 $\mu\text{g/g}$) and Sn (3.348 $\mu\text{g/g}$) were lower than the permissible limit.

3.4. Effect of variables on heavy metals occurrence in canned tuna samples

The effect of four variables (brand, media, layer, and proximity to production/expiry date) on the heavy metals (Zn, Al, and Sn) occurrence in canned tuna samples is presented in Table 6. While assessing the brands, Zn ranges between 2.98–11.586 $\mu\text{g/g}$. There is a highly significant difference between brands with a P₁ value <0.001: (A, B, C, and D), (B and A), (C and A), and (D and A). While taking into consideration the packing medium, Zn average concentrations range between 5.773 $\mu\text{g/g}$ and 10.037 $\mu\text{g/g}$. There is no significant difference between water, oil, and oil and chili (P₁ value = 0.880). As for the layer effect (center versus outer), the average Zn concentration ranges between 6.576 $\mu\text{g/g}$ and 8.404 $\mu\text{g/g}$. Besides, there is no significant difference between the center and the outer layer (P₁-value = 0.729) when examining Zn mean concentrations in the samples. However, the Zn average ranges for the nearness to production/expiry dates vary between 4.423 $\mu\text{g/g}$ and 10.446 $\mu\text{g/g}$. There is a significant difference between the nearness to the production date for the categories 0–6, 6–12, and 18–24 (P₁-value = 0.026).

Besides, as summarized in Table 6, there is no significant difference across the various variables (brand, media, center/outer, and proximity to production/expiry date) with a P₂-value = 1.000.

Observing the various brands, the Al average concentration ranges between 2.291 $\mu\text{g/g}$ and 6.438 $\mu\text{g/g}$ for the samples. There is a significant difference between brands A, B, and C (P₁-value < 0.01). As for the packing medium, Al average concentration ranges between 3.125 $\mu\text{g/g}$ and 5.841 $\mu\text{g/g}$. The difference between water, oil, and oil & chili is

shown to be significant (P₁ value <0.01). The effect of the layer variable on Al concentration shows that the average concentration of Al ranges between 4.417 $\mu\text{g/g}$ and 5.095 $\mu\text{g/g}$. There is no significant difference between the inner core and outer layer within a can (P₁-value = 0.421) when examining Al concentration. Examining the nearness to production date, the Al average concentration ranges between 2.304 $\mu\text{g/g}$ and 7.693 $\mu\text{g/g}$. Based on our analysis, there is a significant difference between the proximity to the production date categories 0–6, 6–12, and 12–18 months (P₁ value <0.01). As summarized in Table 6, there is no significant difference across the different variables brand, packing medium, layer, and proximity to the production date for Al concentrations (P₂ value = 1.000).

Examining the effect of brand on Sn concentrations, the average ranges between 2.211 $\mu\text{g/g}$ and 5.557 $\mu\text{g/g}$ with a significant difference between brands (P₁ value <0.01) (A, D and E), (B and E), (C and E), (D, A and E), and (E, A, B, C, and D). Similarly, looking at the effect of packing medium on Sn, the average concentrations range between 3.068 $\mu\text{g/g}$ and 3.865 $\mu\text{g/g}$ with a significant difference between the packing medium (P₁ value <0.01) (water and oil & chili), (oil and oil & chili), and (oil & chili, water, and oil). Whereas the layer variable shows no significant difference between the center and the outer layer (P₁-value = 0.742) with average Sn concentrations varying between 3.303 $\mu\text{g/g}$ and 3.391 $\mu\text{g/g}$. As for the proximity to the production date, Sn average concentration ranges between 2.901 $\mu\text{g/g}$ and 3.321 $\mu\text{g/g}$ with no significant difference between 0–6, 6–12, 12–18, and 18–24 months (P₁-value = 0.251). Besides, there is no significant difference across the different variables brand, media, layer, and proximity to production date when evaluating Sn (P₂ value = 1.000).

While assessing the brand variable for Zn, Al, and Sn averages together ($\mu\text{g/g}$), the brands A, B, C, D, and E showed a significant difference, (P₁ values <0.01). This finding is in agreement with the study conducted by Bouffleur et al. (2013) who reported significant variation in the concentrations of Mg, P, K, and Zn across three brands of canned tuna (p-value < 0.05). This difference may be attributed to several factors such as the fish species used by the manufacturers, type of cans, processing steps, and storage conditions (Bouffleur et al., 2013).

Analyzing the packing medium for Zn, Al, and Sn concentration averages ($\mu\text{g/g}$), there is a significant difference for all elements except for Zn (p-value < 0.05). A significant difference was noted between water, oil, and oil & chili for Al (p-value < 0.01) and Sn (p-value < 0.01) with oil & chili medium presenting the highest concentration values for Al and

Table 7. Estimated weekly intake by individuals consuming canned tuna.

n = 102	Zn			Al			Sn		
	US	EU	Leb	US	EU	Leb	US	EU	Leb
Average ($\mu\text{g/g}$)	7.50800			4.75660			3.34770		
Rate consumption of canned fish (kg/person/week)	0.02000	0.02900	0.01925	0.02000	0.02900	0.01925	0.02000	0.02900	0.01925
Estimated weekly intake (mg/week/70 kg body weight)	0.00214	0.00311	0.00206	0.00135	0.00197	0.00130	0.00095	0.00138	0.00092
Provisional permissible tolerable weekly intake (PTWI) (mg/kg body weight)	7			2			14		

Table 8. Estimated Daily Intake (EDI, mg/kg body weight/day) and health risk index (HRI) for individuals consuming canned tuna.

Tuna samples (n = 102)	Zn			Al			Sn		
	US	EU	Leb	US	EU	Leb	US	EU	Leb
Average ($\mu\text{g/g}$)	7.50800			4.75660			3.34770		
Fish tuna Consumption Rate (kg/person/day)	0.00258	0.00414	0.00275	0.00258	0.00414	0.00275	0.00258	0.00414	0.00275
Estimated Daily Intake (EDI) (mg/70 kg body weight/day)	0.00027	0.00044	0.00029	0.00017	0.00028	0.00018	0.00012	0.00019	0.00013
Oral reference doses (mg/kg/day)	0.3			1			0.2		
Health Risk Index (HRI)	0.00090	0.00146	0.00096	0.00017	0.00028	0.00018	0.00060	0.00095	0.00065
HRI < 1 safe	Safe			Safe			Safe		

Sn, 5.841 and 3.865 $\mu\text{g/g}$, respectively, followed by oil (5.181 $\mu\text{g/g}$ for Al and 3.106 $\mu\text{g/g}$ for Sn) and water (3.125 $\mu\text{g/g}$ for Al and 3.068 $\mu\text{g/g}$ for Sn). On the other hand, the different mediums had no significant difference in the average concentration of Zn (p-value = 0.888). A similar result was reported by Bouffleur et al. (2013). In concordance with our results, Bouffleur et al. (2013) has also reported higher levels for most elements in oil-packed than in brine-packed tuna.

When analyzing the effect of the layer (center/outer) variable on Zn, Al, and Sn average concentrations ($\mu\text{g/g}$), there are no statistical differences for the different heavy metals (Zn, Al, and Sn), with P_1 -values of 0.421, 0.742 and 0.729, respectively. As for the nearness to the production date, there is a significant difference for the Al concentration for the proximity to production date categories of 0–6, 6–12, and 12–18 months, whereas there is no significant difference between the category 18–24 months and the others. The increase in the Al values between categories 0–6 months and 12–18 months, passing from 2.304 $\mu\text{g/g}$ to 7.693 $\mu\text{g/g}$, is in concordance with the study conducted by Dantas et al. (2008) who reported a significant increase in Al concentration in canned tuna over 180 days. The category 18–24 months has shown no significant difference in the heavy metal concentrations with a drop in the average concentration to 5.155 $\mu\text{g/g}$ which can mainly be attributed to the small number of samples in this category (n = 12) since products nearby expiry date are usually recalled from the market.

For Zn, a significant difference was noted among the proximity to production date variable (P_1 -value = 0.026) with category 18–24 months from production date showing the highest average concentration value for Zn (10.446 $\mu\text{g/g}$). This is in-line with the study conducted by Bouffleur et al. (2013), who reported a positive correlation between storage time and trace metal content. Conversely, for Sn, no significant difference was noted for any of the proximity to production date categories.

To compare the mean average of heavy metals occurring in canned tuna with regulations, the estimated weekly intake by individuals consuming canned tuna was calculated and summarized in Table 7.

The estimated weekly intake (EWI) of Zn, Al, and Sn by an individual with a bodyweight of 70 kg consuming 0.02 kg of fish per week according to the US in 2014 (FAO/WHO, 2017), 0.029 kg of fish per week according to EU between 2000–2005 (FAO/WHO, 2017) and 0.01925 kg of fish per week for the Lebanese consumption were all below the provisional tolerable weekly intake (PTWI). The PTWIs of the heavy metals are 7 $\mu\text{g/g}$ for Zn (equivalent to 1 mg/kg/day), 2 $\mu\text{g/g}$ for Al, and 14 $\mu\text{g/g}$ for Sn (FAO/WHO, 2017).

3.5. Risk assessment of heavy metals occurrence in canned tuna samples

The average concentration values for BPA, Zn, Al, and Sn obtained were used to perform a risk assessment for heavy metal occurrence in canned tuna. Table 8 summarizes the estimated daily intake and health risk index for individuals (70 kg average body weight) consuming canned tuna in Lebanon. The calculated Health Risk Index values for Zn are 0.00090, 0.00146, and 0.00096 (in the USA, EU, Lebanon, respectively), Al are 0.00017, 0.00028, and 0.00018 (in the USA, EU, Lebanon, respectively), and Sn are 0.00060, 0.00095, and 0.00065 (in the USA, EU, Lebanon, respectively). This indicates that all the calculated HRI values of heavy metals were within safe limits (HRI < 1). Consequently, there is no potential health risk associated with the consumption of canned tuna in the US, EU, and Lebanon. This in concordance with the findings of Sobhanardakani (2017) showing no potential health risk for adults via the consumption of canned fish. A similar study conducted in Egypt by Hussein and Khaled (2014) showed that Zn occurrence in tuna fish does not pose a risk for consumers with HRI < 1. Similar results were also found for heavy metals (Cr, Cu, Fe, Mn, Ni) in canned fish in Iran, where the Health Risk Index (HRI) values were within the safe limits (Sobhanardakani et al., 2018).

4. Conclusion

This work has been conducted to study the effect of different variables including brand, packing media, layer (center versus outer), and the proximity to the production date on the presence of BPA and heavy metals (Zn, Al, and Sn) in canned tuna samples commercialized in the Lebanese market. The results of this study have shown that the mean concentration of BPA was 0.197 $\mu\text{g/g}$, which is higher than the permissible limit of 0.05 $\mu\text{g/g}$. However, the probably daily intake of BPA for our samples (n = 274) is below the tolerable daily intake of BPA (0.004 mg/kg/day). Therefore, there is no health risk of BPA toxicity from canned tuna consumption in Lebanon. Besides, the results of this study have shown as well that the mean concentrations of Al, Zn, and Sn were lower than the permissible limit with tuna in water presenting the lowest concentrations. Additionally, it is recommended to consume tuna during the first six months of its production date. The calculated Health Risk Index values for Zn, Al, and Sn were within the safe limits (HRI < 1). Therefore, we conclude that there is no potential health risk associated with consuming canned tuna in Lebanon.

Declarations

Author contribution statement

Lara Al Ghoul: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Adla Jammoul, Mohamad G. Abiad, Joseph Matta: Contributed reagents, materials, analysis tools or data.

Nada El Darra: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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