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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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 (Arctic 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 Soylak, 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 (Schecter 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., 2018).

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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 ìg/kg. The highest BPA concentrations were reported in meat, fish among other seafood categories with average BPA concentrations of 9.4 and 7.4 ìg/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 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 °C until further analysis (Garcia 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 50ppm. The calibration curve of BPA has various concentrations from 0.4 until 50 ppm. A linear line was illustrated between the AA signal and the various concentrations 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
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 taken 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, Potortì, 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. 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 BPA 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 isocratic elution at 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 × 250mm, 5μm). 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

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 (HNO3) and 1.5 ml of 30% hydrogen peroxide (H2O2). 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).

\[ \text{FIR} = \frac{\text{Imported canned tuna in Lebanon}}{\text{Lebanese population} \times 365} \]

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

| Variables | Water BPA | Heavy metals | Oil BPA | Heavy metals | Oil & Chili BPA | Heavy metals | Total 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) | 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          |
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 (μg/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):

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

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).

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

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

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

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

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 indicates 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 test. 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 μg/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 × 10⁻⁶ mg/kg/day, 8.93 × 10⁻⁶ mg/kg/day, and 5.93 × 10⁻⁶ mg/kg/day, respectively, which is below the tolerable daily intake of BPA (4 × 10⁻⁴ 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 μg/g in the collected samples. There is no
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 μg/g for water and oil and 0.198 μg/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 μg/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 μg/g and 0.198 μg/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%. The average level for Zn in the 66 positive samples was 7.490 μg/g, which is almost two times higher than the Al and Sn mean with values of 4.756 μg/g and 3.347 μg/g, respectively. 3.347 μg/g. However, our values compare to those reported by Korfalı and Abu Hamdan (2013) 6.57 μg/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 Tužen and Soylak (2007) ranging between 7.57 μg/g and 34.4 μg/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 μg/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 μg/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 μg/g) and higher than the mean concentrations found in canned tuna commercialized in Canada (1.806 μg/g) (Mahalakshmi et al., 2012). Furthermore, our reported values are higher than the ones obtained by Korfalı 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 Türkmenn et al. (2005) 0.02–5.41 μg/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 μg/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 Korfalı and Abu Hamdan (2013) (0.5 μg/g) and Sobhanardakani (2017) (0.18 μg/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 μg/g and 200 μg/g) as defined by

### Table 4. Probably daily intake of BPA.

| Countries | BPA Average (mg/kg) | US* | EU** | Lebanon |
|-----------|---------------------|-----|------|---------|
| n = 102   |                     |     |      |         |
| Average   | 0.15113              | 0.06163 | 0.06163 | 0.06163 |
| 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 | 0.0000093 |
| Tolerable Daily Intake (TDI) (μg/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) | P₁* | P₂* |
|----------------------------|---------------------|-----|-----|
| Brand                      |                     |     |     |
| A (n = 52)                 | 0.198               | 0.205 | 1   |
| B (n = 48)                 | 0.195               | 0.683 |     |
| C (n = 58)                 | 0.198               | 0.683 |     |
| D (n = 66)                 | 0.200               | 0.700 |     |
| E (n = 50)                 | 0.197               | 0.684 |     |
| Media                      |                     |     |     |
| Water (n = 74)             | 0.197               | 0.683 |     |
| Oil (n = 88)               | 0.197               | 0.683 |     |
| Oil & Chili (n = 112)      | 0.198               | 0.700 |     |
| Layer                      |                     |     |     |
| Center (n = 137)           | 0.198               | 0.684 |     |
| Outer (n = 137)            | 0.196               | 0.684 |     |
| Proximity to Production Date |                   |     |     |
| 0–6 (n = 68)               | 0.196               | 0.684 |     |
| 6–12 (n = 84)              | 0.198               | 0.684 |     |
| 12–18 (n = 66)             | 0.198               | 0.684 |     |
| 18–24 (n = 56)             | 0.197               | 0.684 |     |

*P₁*: P value for every variable.
*P₂*: P value across variables.
Table 6. Mean values (μg/g) of Zn, Al, and Sn in tuna samples according to different variables.

| Variables                  | Zn Average (μg/g) | P1* | P2* | Al Average (μg/g) | P1* | P2* | Sn Average (μg/g) | P1 | P2 |
|----------------------------|------------------|-----|-----|------------------|-----|-----|------------------|----|----|
| Brand                      |                  |     |     |                  |     |     |                  |    |    |
| A (a) (n = 12)             | 11.586±2.36d     | 0.000 | 1.000 | 5.781±39        | <0.01 | 1.000 | 3.385±47        | <0.01 | 1.000 |
| B (b) (n = 12)             | 2.989±1.59      |     |     | 2.291±4.05      |     |     | 3.041±8     |     |     |
| C (c) (n = 24)             | 4.122±4.25      |     |     | 6.438±5.86      |     |     | 2.965±5     |     |     |
| D (d) (n = 36)             | 10.332±5.23     |     |     | 4.286±4.82      |     |     | 2.211±9     |     |     |
| E (e) (n = 18)             | 6.443±6.87      |     |     | 4.559±5.85      |     |     | 5.557±9.56    |     |     |
| Media                      |                  |     |     |                  |     |     |                  |    |    |
| water (f) (n = 36)         | 5.773±0.888      |     |     | 3.125±3.69      | <0.01 | 1.000 | 3.068±0.43 |     |     |
| Oil (g) (n = 34)           | 10.037±0.792    |     |     | 5.181±1.45      |     |     | 3.106±9     |     |     |
| OilyChili (h) (n = 32)     | 6.409±0.475     |     |     | 5.841±1.59      |     |     | 3.865±1.9    |     |     |
| Center/Outer Layer         |                  |     |     |                  |     |     |                  |    |    |
| Center (n = 51)            | 6.576±0.729     |     |     | 4.417±0.412     |     |     | 3.303±0.742  |     |     |
| Outer (n = 51)             | 8.404±0.365     |     |     | 5.095±0.391     |     |     | 3.391±0.251  |     |     |
| Proximity to Production Date (months) |           |     |     |                  |     |     |                  |    |    |
| 0-6 (i) (n = 22)           | 4.423±0.265     |     |     | 2.304±0.183     | <0.01 | 1.000 | 2.901±0.251  |     |     |
| 6-12 (i) (n = 56)          | 8.274±0.345     |     |     | 4.682±0.348     |     |     | 3.485±0.251  |     |     |
| 12-18 (k) (n = 12)         | 7.075±0.345     |     |     | 7.693±0.345     |     |     | 3.528±0.251  |     |     |
| 18-24 (l) (n = 12)         | 10.446±0.345    |     |     | 5.155±0.345     |     |     | 3.321±0.251  |     |     |

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 (μg/g) in the 102 tuna samples is:

Zn (7.49 μg/g) > Al (4.756 μg/g) > Sn (3.347 μg/g)

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

Zn (9.31 μg/g) > Al (1.8 μg/g) > Sn (0.1 μg/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 μg/g), Al (4.757 μg/g) and Sn (3.348 μg/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 μg/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 μg/g and 10.037 μg/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 μg/g and 8.404 μg/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 nearest to production/expiry date vary between 4.423 μg/g and 10.446 μg/g. There is a significant difference between the nearest 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 μg/g and 6.438 μg/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 μg/g and 5.841 μg/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 μg/g and 5.095 μg/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 nearest to production date, the Al average concentration ranges between 2.304 μg/g and 7.693 μg/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 μg/g and 5.557 μg/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 μg/g and 3.865 μg/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 μg/g and 3.391 μg/g. As for the proximity to the production date, Sn average concentration ranges between 2.901 μg/g and 3.321 μg/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 (μg/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 Boufleur 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 (Boufleur, et al., 2013).

Analyzing the packing medium for Zn, Al, and Sn concentration averages (μg/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...
Sn, 5.841 and 3.865 μg/g, respectively, followed by oil (5.181 μg/g for Al and 3.106 μg/g for Sn) and water (3.125 μg/g for Al and 3.068 μg/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 Boufler et al. (2013). In concordance with our results, Boufler 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 (μg/g), there are no statistical differences for the different heavy metals (Zn, Al, and Sn), with P1-values of 0.421, 0.742 and 0.729, respectively. As for the nearness to the production date variable (P1-value of 0.888). A similar increase in Al concentration in canned tuna with regulations, the estimated weekly intake by individuals 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).

### 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 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 μg/g, which is higher than the permissible limit of 0.05 μg/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 all below the safe limits (HRI <1). Therefore, we conclude that there is no potential health risk associated with consuming canned tuna in Lebanon.
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. 

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Declarations
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