Food Additives & Contaminants: Part B
Surveillance

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tfab20

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To cite this article: Maria Alcala-Orozco, Prentiss H. Balcom, Elsie M. Sunderland, Jesus Olivero-Verbel & Karina Caballero-Gallardo (2021): Essential and toxic elements in sardines and tuna on the Colombian market, Food Additives & Contaminants: Part B, DOI: 10.1080/19393210.2021.1926547

To link to this article: https://doi.org/10.1080/19393210.2021.1926547

Published online: 08 Jun 2021.

FOOD ADDITIVES & CONTAMINANTS: PART B
https://doi.org/10.1080/19393210.2021.1926547

Essential and toxic elements in sardines and tuna on the Colombian market

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ABSTRACT
The presence of metals in canned fish has been associated with adverse effects on human health. The aim of this study was to evaluate risk-based fish consumption limits based on the concentrations of eight essential elements and four elements of toxicological concern in sardines and tuna brands commercially available in the Latin American canned goods market. One brand of canned sardines and six of canned tuna were collected and evaluated by ICP-MS and direct mercury analysis. The Hg content was much higher than that previously observed in scientific literature. According to the calculated hazard quotients, all brands may present some risk in terms of this element, especially brand F in which levels up to 3.1 µg/g were measured. Sardine samples surpassed the maximum limits of Mn and As. Stricter quality control in retail chains and industries should be implemented in order to guarantee safe levels in fishery products.

ARTICLE HISTORY
Received 15 January 2021
Accepted 2 May 2021

KEYWORDS
Essential elements; toxic elements; heavy metals; lead; cadmium; mercury; arsenic; Latin America; canned fish

Introduction

Fish are considered valuable organisms for human health as they are part of a balanced diet, providing proteins of high nutritional value, essential elements (Fe, Cu, Zn, Se), and vitamins (A, D, B2, and B12), which are highly recommended nutrients in terms of risk-benefit assessments for seafood ingestion (Gerstenberger et al. 2010; Calder et al. 2019). Fish are also an important dietary source of omega-3 (Di Lena et al. 2017). Thus, such consumption has been related to the prevention of cardiovascular diseases (Andayesh et al. 2015).

Fish intake provides essential nutrients, especially for special sectors of the population, such as children, older adults, and women of reproductive age or pregnant women. However, the safety of consuming this type of food is currently under debate, especially tuna, because these may accumulate elements of toxicological importance in their tissues (Andayesh et al. 2015). The presence of these elements in aquatic ecosystems results from multiple sources of contamination, which include the improper disposal of commercial and agricultural effluents, as well as atmospheric deposition and mining activities. Given the high capacity for bioaccumulation of heavy metals and other elements of toxicological concern, fish may constitute a major dietary source of such contaminants to humans (Murata et al. 2019). In addition, exposure to these compounds may reach adverse levels for human health based on high levels in the organisms and high rates of consumption.

Although essential elements are required to ensure normal growth, development, and maintenance of vital functions, they can generate negative effects when exposure to these is too high. Some elements (As, Cd, Hg, and Pb) might also generate a wide array of adverse consequences when daily consumption surpasses the maximum limits. For instance, exposure to As has been linked to several clinical manifestations, such as heart failure, skin problems, hepatological disorders, and diabetes. Oral exposure to Cd can produce injuries to the kidney, and skeletal and respiratory systems. In addition, this element has been classified as a human carcinogen. Medical manifestations of Hg-induced neurotoxicity includes paraesthesia, ataxia, impaired vision, sensorial disturbances, and lack of coordination (Alcala-Orozco et al. 2017). Lead exposure can affect the central nervous system in vulnerable populations and generate numerous dysfunctions at cardiovascular, haematological and reproductive levels (Lanphear et al. 2005; Alvarez-Ortega et al. 2017). Moreover, exposure to Hg and Pb, especially during early foetal development, has been associated with neurodevelopmental disorders and brain injury at low doses (Grandjean and Landrigan 2006; Landrigan et al. 2019).

Consumption of fishery products is increasing globally. In addition to the fact that tuna are caught and sold globally in the commercial seafood market, the rapid increase in the purchasing power of the consumers along with changes in the lifestyle pattern has led to

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a significant growth potential of the canned preserved food market in Latin America and Caribbean, especially in developing countries like Colombia. Moreover, Latin America accounts for approximately the 15% (250,000 tons) of world canned tuna consumption and Colombia, as a member of the CIAT/IATTC -Inter-American Tropical Tuna Commission, owns a total of 14 vessels with a cargo capacity that exceeds 1000 tons. Even though concentrations of metals in fish have been reported worldwide, the available information on their presence in canned products distributed in Colombia is limited. In fact, there is just one official report about Hg concentrations in tuna in the coastal city of Cartagena (Alcala-Orozco et al. 2017) and there are no studies addressing the levels of both essential and toxicological concern elements in fish commercialised in this territory. According to the National Aquaculture and Fisheries Authority (AUNAP), more than 30 years ago, per capita intake in Colombia was 1.7 kg; in 2018, this consumption reached between 8 and 10 kg/year, a value that is higher than those reported in Spain (3.7 kg/year), France (2.7 kg/year), and the United States (2.7 kg/year) (Zuleta and Becerra 2013). Purchase of fish and shellfish reaches about 350,000 tons nationwide in Colombia and a fraction of approximately 100,000 tons comes from imports (Herrera-Herrera et al. 2019). Additionally, tuna exports from Colombia are growing increasingly. The country experienced a growth of 10.4% compared to 2017. The main destinations of Colombian exports were the United States, where 25.4% was shipped; European Union (11.7%), China (9.7%), Panama (7.3%), Ecuador (4.4%), Mexico (3.9%), and Brazil (3.7%) (Eje-21 2019). Moreover, as a result of the joint efforts of the National Institute of Food and Drug Surveillance (Invima) and the Ministry of Agriculture and Livestock (MAG) of El Salvador, the sanitary conditions of the Colombian production plants were verified so that the fish sector could access the commercialisation of tuna in the Central American market (Radio 2020).

Given that local fish production is decreasing in Colombia, access to low-cost imported products has become a primary tool to satisfy the needs of the family food basket. Relevant studies and surveillance systems on essential and toxicological concern elements in fishery products are required to identify the associated risks after human exposure. Thus, it may be useful to provide information for the creation of monitoring and evaluation programmes aimed at safeguarding food safety and minimising human exposure. Consumption advisories should be concentrated on fish contamination, but also on the co-occurrence of other elements that play an essential role in many biochemical processes to ensure human health. Given the above, the main objective of this study was to determine the levels of four metals of toxicological concern (As, Cd, Hg, and Pb), as well as eight essential elements (Cr, Mn, Fe, Co, Ni, Cu, Zn, and Se) in canned sardines and tuna commercially available in the Latin American market, in order to evaluate the exposure levels and the associated risks to consumers. In addition, given that public health advices for fish intake are required to decrease exposure to these metals in vulnerable and more sensitive populations, the estimation of the maximum weekly intakes of selected brands was carried out.

Materials and methods

Sample collection

Sample collection was performed as reported previously (Alcala-Orozco et al. 2017). Briefly, canned products were purchased in June 2019, in supermarkets situated in Cartagena (10°25′25″N 75°31′31″W), a city positioned on the shores of the Caribbean Sea. One brand of canned sardines (A) and six of canned tuna (B-G) packed in water (sample size: a net weight of 120 g) were selected from different suppliers. To ensure a collection of a representative samples based on availability, at least two production batches of each brand were chosen. The brands were selected taking into account several criteria, among which are their high worldwide distribution, especially in the Latin American, and Caribbean territory, including in addition to Colombia, countries such as Ecuador, Chile and Spain. For instances, brands D and F are leaders in the market for this type of product, with a market participation of 45% and 15%, respectively. In addition, these fish were chosen taking into account that they were certified by INVIMA (the National Regulatory Agency), a surveillance and control entity of a technical-scientific nature, in charge of applying health standards associated with the consumption and use of food, drugs, medical devices, and other products subject to health surveillance. For this, only those brands that had the respective sanitary registration were taken into account, through which the manufacture, packaging, and importation of products for human consumption is authorised.

Sample preparation

Initially, water was drained from the packages and the contents were transferred to a food processor to homogenise the samples. Once homogenised, the samples were stored at −80°C and then lyophilised to remove moisture using a benchtop freeze dryer (LABCONCO, Freezone 2.5 L, Kansas City, MO, USA).

Total mercury measurements

Total mercury levels (T-Hg) were assessed using a Direct Hg Analyser [Nippon MA-3000, Nippon Instruments Corporation (NIC), Osaka, Japan], following the USEPA method 7473 (USEPA 1998) at the Biogeochemistry of Global Contaminants Laboratory (Harvard University). Approximately 100 mg of dried material was used for analysis, employing external calibration curves whose R^2 was greater than 0.99 and analysing DORM-4 [National Research Council of Canada (NRC – CNRC), Ottawa, Ontario, Canada] fish protein certified reference material (CRM). At least one method blank (empty preheated combustion boat) and one CRM were tested on every 12 samples. Accuracy of the method was assessed as recovery [reference value: 0.410 ± 0.055 µg/g; obtained value: 0.405 ± 0.002 µg/g; average recovery: 98.7% (n = 6)]. Coefficients of variation, estimated by replicate analysis of DORM-4 and duplicate analyses of canned sardines and tuna samples, were below 1.4% and 1%, respectively. The limit of detection (LOD) was 0.15 µg/kg and the limit of quantitation (LOQ) was 0.48 µg/kg for mercury. These values were calculated as three and ten times the standard deviation of the blanks.

Analysis of toxic and essential elements

Approximately, a total of 100 mg of dried samples were digested at 95°C for 6 hours using a 4 mL nitric acid (JT Baker, Philipsburg, USA) and hydrogen peroxide (Sigma–Aldrich, Inc., St. Louis, MO, USA) mixture (HNO_3:H_2O_2 = 3:1, v/v) for trace elements measurement. Following digestion, samples were diluted to 30 mL with deionised (DI) water prior to analysis with an iCAP-Q ICP-MS (Thermo Fisher Scientific, Waltham, MA, USA) at the Biogeochemistry of Global Contaminants Laboratory (Harvard University). Analytical blanks were prepared in the same acid matrix as the samples and the concentrations of the elements were assessed using an external calibration with standard solutions prepared from the Multi-Element Solution 2 (Spex CertiPrep, Metuchen, NJ, USA). The CRMs DORM-4, DOLT-5 [Dogfish Liver, National Research Council of Canada (NRC – CNRC) Ottawa, Ontario, Canada] and NIST1643f CRM (trace elements in water, National Institute of Standards and Technology, Gaithersburg, MD, USA) were employed to validate the analytical procedure. The LOD and LOQ for each element are shown in Table 1. Analytical duplicate analysis of samples resulted in average relative percent differences (RPD) of 1 to 3 and analysis of CRM digestion duplicates resulted in RPDs of 2 to 5. Average recoveries for samples spiked with calibration standard before digestion ranged from 87% to 104% for all elements and average recovery for samples spiked prior to analysis ranged from 91% to 110% for all elements. Multiple concentrations of the calibration standard were analysed with samples for continuing calibration verification (CCV). The analysis was carried out using the internal standards Sc, Y, and Tb. It should be noted that low recoveries of Cr, Ni, and Pb were obtained for DORM-4 and DOLT-5, but not for the spiked digestions of these CRMs or the NIST1643f. Additionally, low average CCV recoveries of Cr and Pb (78% to 85%) were found at the lowest standard's concentrations (0.24 and 0.60 ng/mL). Therefore, Cr, Ni, and Pb data are presented for information purposes only (Table 1 and Figures S1-S3; S = supplementary material, which is available at the corresponding author). Uncertainty due to the traceability verification process was calculated according to the formula (Alcala-Orozco et al. 2017):

$$U_{Traceability} = \sqrt{\left(\frac{U_{standard}}{k}\right)^2} + \sqrt{\left(\frac{S_I}{\sqrt{n}}\right)^2}$$

Table 1. Average recoveries (%) obtained from CRM's, spiked digestions and calibration standards; LODs and LOQs.

	CRM recoveries[c]			Spiked digestions		CCV	LOD	LOQ
Element	DORM-4[a]	DOLT-5[b]	NIST 1643 f [d]	DORM-4	DOLT-5	0.24–189.7 ng/mL*	(ng/mL)	(ng/mL)
Cr	97.6	105.7	94.3	58.2	23.5	76.1–105.9	0.075	0.249
Mn	-	76.7	106	-	121.6	95–113	0.175	0.582
Fe	86.6	89.6	90	-	-	101–118	0.700	2.333
Co	-	76.7	105	-	108.5	98–113	0.009	0.031
Ni	88.0	95.5	100.5	78.1	39.4	101.2–112.0	0.081	0.806
Cu	99.0	104.2	107	90.4	97.2	110–118	0.021	0.071
Zn	78.4	77.9	111	87.1	-	108–115	0.255	0.850
As	90.9	98.2	106	81.3	85.3	106–113	0.098	0.034
Se	94.5	90.4	117	83.0	98.9	117–122	0.039	0.131
Cd	90.3	74.7	103	85.0	115.1	106–116	0.011	0.037
Pb	96.1	96.2	92.8	37.7	71.0	76.2–100.1	0.009	0.031

[a]Fish muscle.
[b]Dogfish liver.
[c]DORM-4 and DOLT-5 recoveries corrected for digestion spike recoveries below 100%.
[d]Trace elements in water.
* Range of average recoveries for 5 Continuing Calibration Verification (CCV) standards.

where the first term refers to the square of the standard uncertainty of the reference material using coverage factor $k = 2.2$ and the second term is related to the uncertainty of the mean value (S_I), obtained when analysing the reference material (n = 6 for Hg, n = 5 for other elements).

Risk-based consumption limits

Within the framework of public health recommendations, the consumption of fishery products poses a risk factor through metals exposure. Thus, for children and the general population, standard hazard quotients (HQs) were considered as the ratio between E (potential exposure) and RfD (reference dose) of each element (Alcala-Orozco et al. 2017). If the resulting value of this ratio exceeds unity, potential adverse effects may be expected. The RfD values (μg/kg/day) used here were employed in accordance with previous studies: Cr: 3; Mn: 140; Fe: 700; Co: 0.3; Ni: 20; Cu: 40; Zn: 300; As: 0.3; Se: 5; Cd: 1; MeHg: 0.1; and Pb: 4 (USEPA 2014; Javed and Usmani 2016; Griboff et al. 2017). Exposure level (E) was estimated taking into account the concentration of the elements in the analysed samples (C), the standard portion size of canned products (MS) (170 g) and the body weight (W) of 70 kg for adults and 37.4 kg for children ($E = C \times MS/W$). The maximum acceptable fish intake rate in meals/week (CRmw) was calculated as $CRmw = (RfD \times W \times 7 \text{ days})/(C \times MS)$. Selenium:Hg molar ratios were considered to evaluate potential Hg toxicological hazards. Although the use of this criterion is currently being debated by industry and the scientific community, some reports have proposed it for risk assessment given that a molar ratio exceeding 1 could offer a protection against such toxicity (Alcala-Orozco et al. 2020).

Statistical data analysis

The results are shown as mean ± standard error. Normality of data was checked using the Kolmogorov–Smirnov test. Concentrations of the elements among brands were compared using the Kruskal–Wallis test followed by Dunn's multiple comparison test. In addition, such concentrations were compared with their corresponding maximum limit established by the environmental and health agencies, including international [Bulgarian Food Codex (BFC 2004), Brazilian Standard (Presidência-da-República 1965; Tarley et al. 2001), Food and Agriculture Organization/World Health Organization (FAO/WHO 1989), Food and Drug Administration (FDA), Mercado Común del Sur (MERCOSUR) (MERCOSUR 2011; ANVS 2013), Agency for Toxic Substances and Disease Registry (ATSDR) (ATSDR 2013) and European Commission (EC 2006)] and national agencies [Colombian Legislation (MSPS 2012)]. The criterion of significance was set at $p < .05$. The software GraphPad Prism 6.0 (San Diego, CA, USA) was utilised for statistical analysis.

Results

Elements in canned sardines and tuna

The mean concentrations (±SE) of elements in the canned sardines and tuna samples are shown in Figure 1(a) (essential elements), 1B (elements of toxicological concern), and figure S1 (Cr, Ni, and Pb), whereas main descriptive statistics are depicted in Table S1. Given the variety of elements evaluated, it was necessary to resort to regulations established by multiple agencies, in the absence of an institution that issued limits for all the elements taken into consideration in this study. With the exception of Cr, statistical analysis revealed significant differences in element concentrations among brands (Figure 1). Results indicated that for sardines (brand A), Fe (46 μg/g) was the highest mean concentration, followed by Zn (36 μg/g) and Mn (6.4 μg/g). Interestingly, when compared to the tuna samples, sardines had the highest levels of Mn, Co, Cu, As, and Pb, surpassing, in the case of Mn and As, the maximum limits set by various environmental and health agencies.

Among the tuna samples, brand B had the highest average levels of Co and Cu and brand C presented the highest concentrations of Fe, Ni, Se, and As, exceeding the limit of 2 μg/g set by the ATSDR for Se (ATSDR 2013) and the standard value of 1.0 μg/g of total As proposed by the Brazilian regulation and MERCOSUR (MERCOSUR 2011; ANVS 2013). The mean T-Hg level in C brand was also above the maximum limits set by the European commission (EC 2006) and the Colombian Legislation (MSPS 2012). Similarly, levels of As, Se, and T-Hg exceeded the corresponding limits in brand D. Brand E had the greatest content of Zn, Cd, and Pb among tuna samples, whereas the highest concentrations for Cr and Hg were observed in the brand F. It is important to note that T-Hg levels of up to 3.1 μg/g were found in this brand; however, this element was elevated in all brands with the exception of brands B and E. The concentrations of the elements were generally low in brand G; however, they did show the highest levels of Mn.

None of the samples surpassed the Recommended Dietary Allowances for Fe (FAO/WHO 1989) and Zn (FAO/WHO 1989); the maximum limits established for Ni (USFDA 1993), Cu (FAO/WHO 1983), and Pb (EC 2006; MSPS 2012); or the maximum level for Cr (0.3 μg/g)

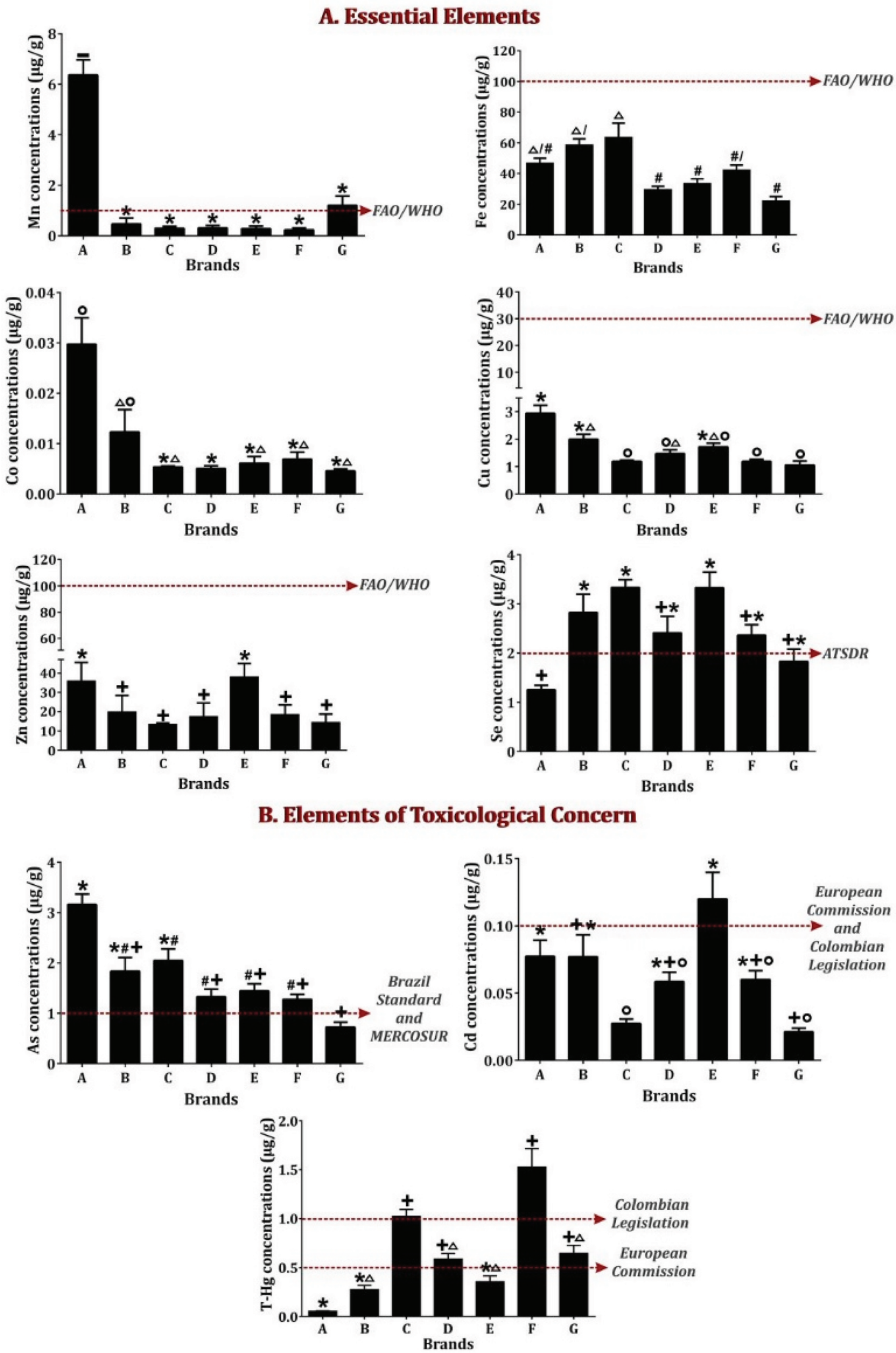

Figure 1. Average concentrations of both essential and toxic elements (µg/g, w/w) in canned sardines and tuna marketed in Cartagena, Colombia. Dotted lines correspond to the maximum limits. Different symbols represent statistically significant differences among target brands (Dunn's multiple comparisons test).

Table 2. Percentage of samples that exceeded the limits set by environmental or health authorities.

Sample matrix	Brazil Standard (Cr, 0.1 μg/g)	FAO/WHO (Mn, 1 μg/g)	Regulation of Brazil/MERCOSUR (As, 1 μg/g)	ATSDR (Se, 2 μg/g)	EC* and MSPS** (Cd, 0.1 μg/g)	EC (Hg, 0.5 μg/g)	MSPS (Hg, 1 μg/g)
Canned sardines	-	100	100	-	-	-	-
Canned tuna	5.9	6.7	77	67	18	65	33
References	(Presidência-da-República 1965, Tarley et al. 2001)	(FAO/WHO 1989)	(ANVS 2013, MERCOSUR 2011)	(ATSDR 2013)	(EC 2006, MSPS 2012)	(EC 2006)	(MSPS 2012)

*EC: European Commission.
**MSPS: Colombian Ministry of Health and Social Protection (Colombian legislation)

set by the Bulgarian Food Codex (BFC 2004). However, all sardines samples surpassed the reference values of Mn and As and at least 18% of the tuna was above those corresponding to As, Se, Cd, and Hg (Table 2). Even though mean levels of Cr were generally below the recommended value, some samples of brand F exceeded this value (Table S2).

Risk-based consumption limits

Calculated HQs and CRmw in canned sardines and tuna commercially available in the Latin American market are depicted in Figure 2(a) (essential elements), 2B (elements of toxicological concern) and figure S2 (Cr, Ni, and Pb) in the case of adults and in Figure 3(a,b) and figure S3 for children. For both adults and children, the highest HQs were those for Se and Hg. Given that the resulting values exceeded 1, potential health effects may be expected. In the case of Hg, the CRmw indicated that most brands should be consumed no more than once a week. When contrasting the observed values with the Se:Hg molar ratios, it is possible to observe that for the analysed brands, this relation was above 1: the calculated values were 59.5, 39.8, 9.1, 16.6, 35.5, 4.4, and 7.2 for brands A, B, C, D, E, and F, respectively (Table S3).

Discussion

Essential elements

As shown in Table 3, the concentrations of the essential elements for the countries with published values confirm the potential global exposure. For both sardines and

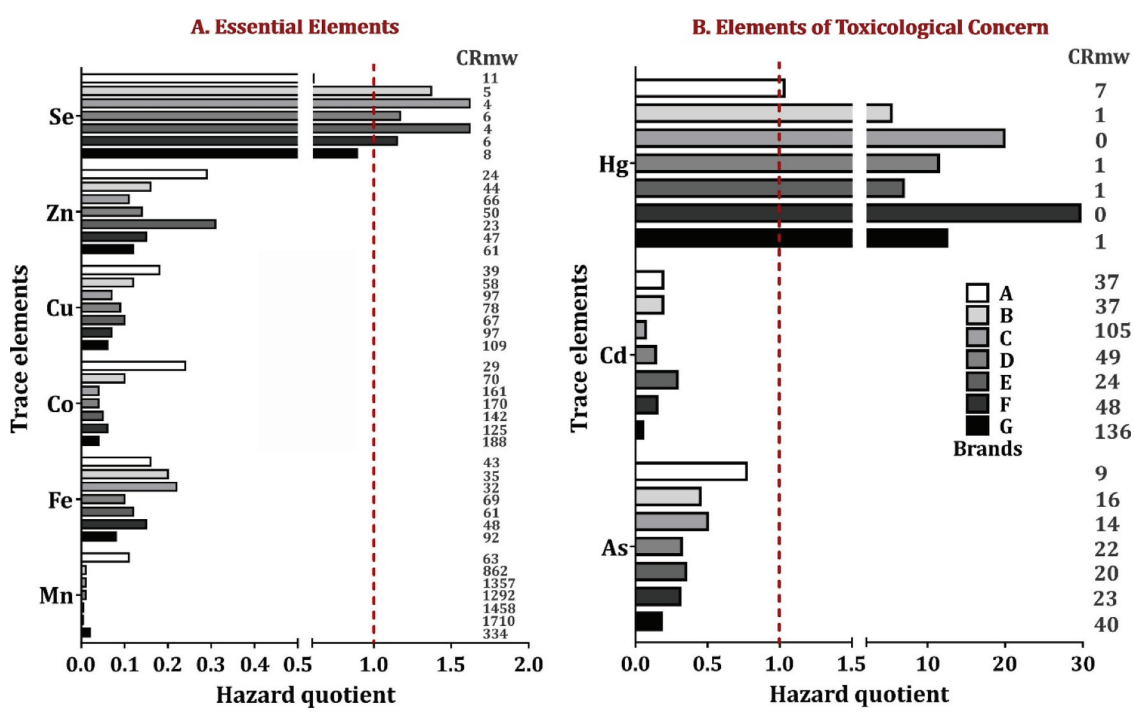

Figure 2. Hazard quotients (HQ) for adults and maximum allowable fish consumption rate in meals per week (CRmw) according to the element concentrations in canned sardines and tuna samples (a: essential elements; b: elements of toxicological concern). Arsenic calculations were made by assuming that inorganic As was 3% of the total concentration found in the analysed samples (Varol et al. 2017; Núñez et al. 2018).

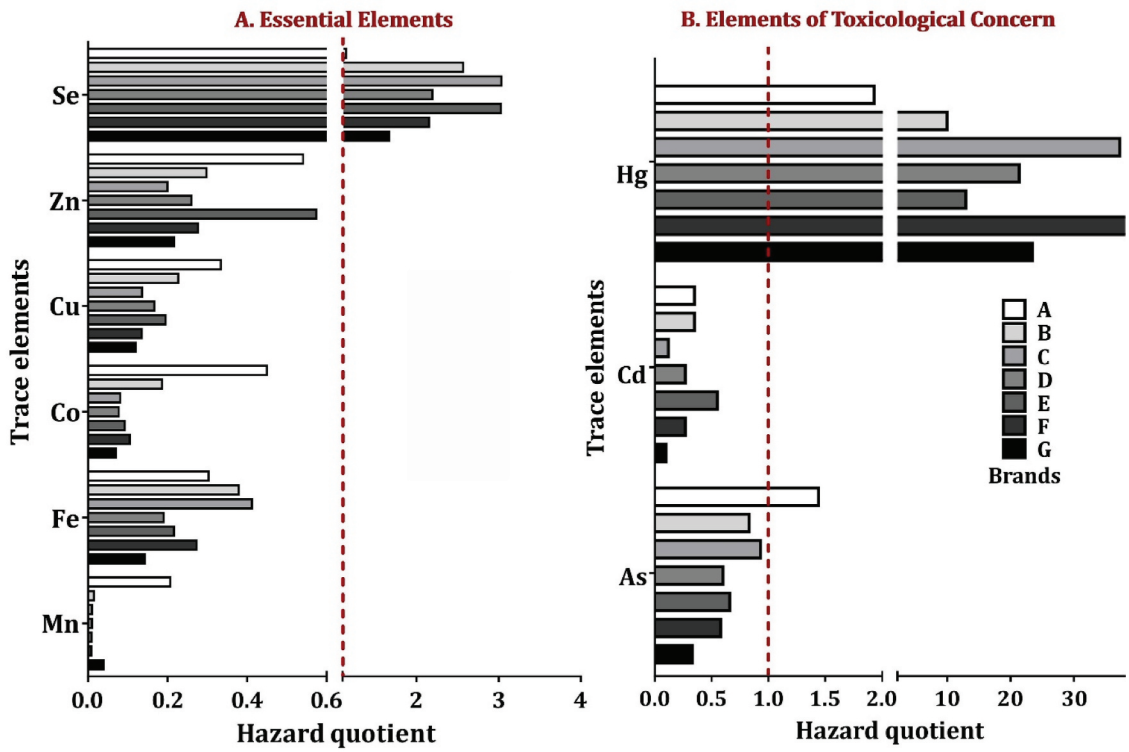

Figure 3. Hazard quotients (HQ) for children according to the element concentrations in canned sardines and tuna samples (a: essential elements; b: elements of toxicological concern). Arsenic calculations were made by assuming that inorganic As was 3% of the total concentration found in the analysed samples (Núñez et al. 2018).

tuna, the mean Cr value found in this study was much lower than that obtained for samples from Jordan (Massadeh et al. 2018), Saudi Arabia (Ashraf et al. 2006), South Korea (Park et al. 2019), and Turkey (Tuzen and Soylak 2007). Chromium is considered an essential element in humans and animals and has an important role in the metabolism of insulin, as a glucose tolerance factor. Its deficiency causes a deterioration of glucose metabolism due to the poor efficiency of insulin. When this element is deficient, symptoms similar to those caused by diabetes and cardiovascular diseases appear, including impaired glucose tolerance (Tuzen and Soylak 2007). The general population is mainly exposed to this element (generally Cr [III]) by fish consumption. Intense industrialisation and other anthropogenic activities, including manufacturing processes, have led to the global occurrence and exposure to soluble Cr(VI), which has been categorised as a human carcinogen (Velma et al. 2009). However, with the exception of the brand F, samples measured in the current study did not surpass the maximum limits.

Interestingly, when compared to tuna, sardines samples had higher concentrations of Mn, exceeding the dietary allowance set by FAO/WHO (1989). This finding is in good agreement with Tuzen and Soylak (2007) and Ikem and Egiebor (2005), who also reported elevated Mn levels in these species. A deficiency of this essential element may lead to the appearance of conditions that may affect the skeletal system, as well as changes in the growth process.

Similarly, Fe, Co, Ni, Cu, and Zn play important physiological roles related to cellular homoeostasis and survival, acting as functional components and activators in various metalloenzymes. In this study, neither the sardines nor tuna brands measured showed levels of these elements above the maximum limits. Mean Fe concentrations were much higher than those found in Saudi Arabia (canned tuna: 2.9 vs 43; canned sardines: 6.8 vs 46 µg/g) (Ashraf et al. 2006) and the United States (canned tuna: 0.02; canned sardines: 0.01 µg/g) (Ikem and Egiebor 2005). There are no guidelines for Co in fish; however, exposure to this element may produce heart effects and dermatitis (Nfon et al. 2009). Moreover, international food safety standards do not recommend Co salts to be employed as foam stabilisers in beverages or in foods (United-States-National-Toxicology-Programme 2004) and previous studies recommend a dietary Co restriction to reduce dyshidrotic eczema flares (Stuckert and Nedorost 2008). Although Ni is widespread among living organisms, the main importance of its determination in food and diet relies on its potential migration during food

Table 3. Comparison of the average element concentrations in the current study (μg/g, w/w) with literature data.

Location	Product	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Cd	Hg	Pb	Reference
Colombia, Cartagena	Canned tuna	0.038	0.39	43	0.0068	0.045	1.5	20	1.5	2.7	0.063	0.77	0.015	This study
	Canned sardine	0.020	6.4	46	0.029	0.056	2.9	36	3.2	1.2	0.077	0.053	0.031	
Brazil, Campinas	Canned sardine in tomato sauce	Up to 0.07	-	15.0–42.0	-	-	-	-	0.66–4.18	-	Up to 0.43	-	< 0.01–0.09	de Mello Lazarini et al. (2019)
	Canned sardine in oil conserve	Up to 0.13	-	13.3–41.4	-	-	-	-	0.77–6.44	-	Up to 0.13	-	< 0.01–0.08	
Colombia, Barranquilla	Water-packed Tuna	-	-	-	-	-	-	236.6	-	-	0.03	-	0.10	Herrera-Herrera et al. (2019)
	Oil-packed- Tuna	-	-	-	-	-	-	238.9	-	-	0.03	-	0.19	
	Canned sardine	-	-	-	-	-	-	141.6	-	-	0.11	-	0.12	
Ghana, Kumasi	Canned tuna	-	-	-	-	-	-	-	-	-	<0.01–0.45	0.12–0.20	<0.01–0.3	Okyere et al. (2015)
	Canned sardine	-	-	-	-	-	-	-	-	-	<0.01	0.01–0.04	<0.01–1.44	
Iran, Persian gulf	Canned tuna	-	-	-	-	-	-	-	0.13	-	0.02	0.0117	0.04	Khansari et al. (2005)
Iran, Tehran	Canned tuna	-	-	-	-	-	-	-	0.64	-	0.01	0.11	0.05	Andayesh et al. (2015)
	Canned tuna	-	-	-	-	-	-	12.6	-	2.04	0.10	0.13	0.75	Sobhanardakani (2017)
Jordan, Irbid	Canned tuna	0.53	-	-	-	2.8	0.73	1.63	3.48	-	0.47	-	2.8	Massadeh et al. (2018)
	Canned sardine	0.47	-	-	-	1.74	0.80	0.43	1.85	-	0.42	-	2.5	
Morocco, Croatia, Russia	Oil-packed- Sardine	-	-	22.0	-	-	2.49	18.2	1.20	-	0.02	0.13	0.15	Novakov et al. (2017)
Saudi Arabia	Oil/broth-packed Tuna	0.18	-	2.9	-	0.41	1.02	10.4	-	-	0.22	-	0.23	Ashraf et al. (2006)
	Oil/broth-packed- Sardine	0.31	-	6.8	-	1.33	2.26	16.15	-	-	0.18	-	0.84	
Serbia, Belgrade	Oil-packed- Tuna	-	-	-	-	-	-	-	0.62	-	0.01	0.068	0.05	Popovic et al. (2018)
	Oil-packed- Sardine	-	-	-	-	-	-	-	1.70	-	0.026	0.043	0.05	
South Korea	Canned tuna	0.29	0.11	7.2	0.01	0.03	0.48	5.6	-	0.463	-	-	-	Park et al. (2019)
Thailand, Vietnam, Indonesia, Spain	Oil-packed- Tuna			20.4	-	-	2.60	22.0	1.68	-	0.03	0.18	0.15	Novakov et al. (2017)
Turkey	Canned tuna	1.08	0.90	14.9	-	0.85	2.50	17.8	-	2.98	0.08	-	0.10	Tuzen and Soylak (2007)
	Canned sardine	0.97	2.02	17.4	-	0.72	1.96	7.57	-	2.77	0.19	-	0.09	
Turkey, Istanbul	Canned sardine	-	-	22.2	-	-	1.02	23.3	-	-	0.01	0.046	0.28	Mol (2011)

processing or from food packaging, which is an issue of great concern (Olivares Arias et al. 2015). In fish and shellfish, ship waste and anticorrosive paints that are applied to ships are the main sources of contamination (Dhaneesh et al. 2012). Cobalt and Ni concentrations in tuna samples from Cartagena were similar to those reported for South Korea (Park et al. (2019). Adverse effects, including liver and kidney damage, have also been linked to high exposure to Cu and Zn (Nfon et al. 2009). Previous studies have highlighted the importance of food, including seafood, as the main source of Cu in daily life. This is how adult foods, including water, may account for 90% or more of Cu intake. Although the main signs of Cu toxicity are manifested as vagal stimulation, eliciting a reflex response of nausea and vomit, these are rare. Clinical manifestations are much more common in the case of its deficiency, which are characterised by bone malformation during development, risk of developing osteoporosis in later life, impaired melanin synthesis, poor cardiovascular health, and alterations in cholesterol metabolism. As observed in the present study, none of the brands evaluated exceeded the limit established by FAO/WHO (De Romaña et al. 2011). Canned sardines samples from this survey had a higher mean Cu (2.9 μg/g) and Zn (36 μg/g) level than that presented by Mol (2011) for Istanbul, Turkey. However, according to Table 3, Zn concentrations were comparable to those presented for both sardines and tuna from other countries, with the exception of Herrera-Herrera et al. (2019), whose levels were approximately 10-fold greater than those observed in this study.

It is vital to note that the occurrence of these elements, especially Mn, Ni, Cu, and Zn, could be due to contamination during food processing/packaging (Ashraf et al. 2006). Metal levels in this study could be linked to environmental pollution in the capture zones, cross-contamination during the canning process and the equipment employed during food processing. In this sense, packaging has gained an extensive importance in food safety due to diffusion of chemicals from packaging to the foodstuff. One of the most widely used methods in can manufacturing to preserve metallic surfaces from external damage and corrosion and prevent possible reactions with the can content, is sheet varnishing. Tin, Pb, Cr, Mn, and Ni are considered as the elements of most concern since these are often used in the packaging material. Moreover, Cr treatment is usually employed to make the Sn layer of cans less vulnerable to environmental injury and increase the coating adherence (Petropoulos et al. 2018). Thus, some studies have evaluated factors in the migration of heavy metals to canned products, including varnish type, width and porosity (Buculei et al. 2014). Likewise, since canned fish muscle is usually boiled, cooking processes may increase the risk of contamination with metals, such as the case of cooking in Ni-containing vessels (Ashraf et al. 2006).

Selenium has been documented as an essential oligo element for humans, playing important roles for proper physiological function. It has anticancer and antioxidant properties and can act as a regulator of the proper functioning of thyroid hormone. However, when Se concentrations are low, anomalies in organisms may be produced and high levels are known to be toxic (Tuzen and Soylak 2007). As observed in Figure 1(a), canned tuna samples displayed high concentrations of Se, exceeding the maximum limit. The mean Se concentrations evaluated here were similar to those reported for Iran (2.7 vs 2.04 μg/g; Sobhanardakani 2017) and much higher than those corresponding to South Korea (2.7 vs 0.463 μg/g; Park et al. 2019).

Elements of toxicological concern

The average As concentrations in most samples analysed were found to be above the maximum limit. Sardines samples showed the highest content of this element and among tuna, brand C had the highest concentration. The mean As concentration in canned tuna commercially available in Cartagena (1.5 μg/g) was slightly lower than that found in Thailand, Vietnam, Indonesia, and Spain (1.68 μg/g; Novakov et al. 2017), but much higher than that reported by Khansari et al. (2005) for the Persian Gulf (0.13 μg/g), Andayesh et al. (2015) for Tehran (0.64 μg/g) and Popovic et al. (2018) for Belgrade (0.62 μg/g).

Given the importance of fish in the human diet, it is necessary to establish its benefits versus risks, but very uncommon studies have taken As as an element of high concern. To date, estimations of toxicological effects of As have focused on inorganic species, which is the major form of this element in drinking water and cereals such as rice and wheat. However, As species in fishery products are mainly detected as arsenobetaine (Næss et al. 2020) and as a result of the limitations of toxicity data for organo arsenicals and the deficiencies in the toxicological characterisation, the assessment of the actual risk of exposure to As through diet has been difficult (Núñez et al. 2018). Further research is necessary to evaluate the toxicokinetics processes, including the bioavailability, transformation and elimination of these organic species, in order to perform comparisons between these parameters and those of inorganic As compounds. Recent studies have shown that some of these compounds are bioaccessible and cytotoxic (Luvonga et al. 2020).

Cadmium is recognised as a non-essential and toxic heavy metal. Its use in the food industry as well as for agricultural purposes has been documented as key sources of its occurrence in the aquatic ecosystems and food. In this study, brand E surpassed the maximum limit of 0.1 μg/g established by both national and international authorities (EC 2006; MSPS 2012) and the same was true for about 18% of the tuna samples (Brands B-G). Cadmium levels in the tuna brands analysed showed a mean concentration (0.063 μg/g) similar to those found for samples from Turkey (0.08 μg/g; Tuzen and Soylak 2007), but higher than 0.017 μg/g as reported by Jinadasa et al. (2019) for yellowfin tuna commercialised in Sri Lanka and by de Paiva et al. (2017) for canned tuna conserves in water (mean: 0.012–0.026 μg/g) consumed in São Paulo, Brazil. In contrast, the mean sardine concentration (0.077 μg/g) was higher than that observed in Morocco, Croatia, Russia (Novakov et al. 2017) and Belgrade (Popovic et al. 2018), but below that found in Jordan, Irbid (Massadeh et al. 2018).

It is important to highlight that the data presented here in relation to T-Hg support the latent risks related to canned tuna marketed in Latin America. Concentrations of up to 3.1 μg/g (brand F) were measured. In general, we would expect to find low Hg levels in this type of product as these tuna will be destined for industrial canning processes, in which rigorous monitoring must be done before the products are released to the market. However, the concentrations found here were above the limits established by the regulatory agencies and anomalous in the international context, since low-to moderate concentrations have been reported in these food items, for instance, samples from the American (0.3–0.59 μg/g; Karimi et al. 2012; Sunderland et al. 2018), Sri Lankan (0.48 μg/g; Jinadasa et al. 2019), Brazilian tuna samples conserved in water 0.05–0.46 μg/g and for samples packed in oil 0.044–0.402 μg/g; de Paiva et al. 2017) and Iranian seafood (0.024 and 0.0394 μg/g; Mansouri et al. 2020). Moreover, the mean concentrations of this element found in the current study were greater than those measured in canned tuna samples from the same brands, also marketed in Cartagena (Alcala-Orozco et al. 2017), which demonstrates that the presence of this element in concentrations that exceed national and international regulations is an issue that persists over time. Thus, adequate actions need to be taken by the National Institute of Food and Drug Monitoring, such as the performance of more frequent monitoring in the production and distribution chains and if it is the case, establish the pertinent sanctions to the manufacturers.

Several studies have shown that predatory species of high trophic level are essential sources of toxic pollutants, including methylmercury (Sunderland et al. 2018). Environmental and anthropogenic issues, such as discharges of Hg from industrial or rural developments, could accelerate and support the bioaccumulation of this toxic element in such organisms (Ashraf et al. 2006). Geographic location of capture is a key factor for determining concentrations of Hg in tuna. It is essential to mention that the precise source of these fish is often unknown, as the available information on labels about zones where they were obtained is restricted. In addition, the information provided on can labels does not stipulate whether handling site corresponds to the same fish capture region (Alcala-Orozco et al. 2017). Therefore, molecular identification of the tuna or sardines species in these products through amplification of specific genetic markers is highly recommended for an efficient fishery administration and defence of consumers' rights (Chapela et al. 2007; Chuang et al. 2012; Yao et al. 2020). These processed fishery products usually do not retain adequate distinctive morphological features for species documentation due to filleting or processing in the industry.

Regarding Pb concentrations, both sardines and tuna brands showed (Fig. S1) levels below the limit of 0.3 μg/g (EC 2006; MSPS 2012). However, the mean level found here for canned tuna (0.015 μg/g) was in agreement with that reported for fresh *Thunnus albacares* (0.0183 μg/g), *Thunnus* sp. (0.0271), and canned tuna (0.0128 μg/g) in compliance with the European Commission Regulation No. 1881/2006, during 2014–2019 (Miedico et al. 2020).

Risk-based consumption limits

Human health risk calculation was performed in order to evaluate the risk that the evaluated elements may represent as a result of exposure to fishery products. Risk-based consumption limits were assessed by calculating the HQ. This ratio exceeded unity for all tuna brands in terms of Hg. In the case of the Se HQ for both adults and children, it was above 1 for brands B, C, D, E, and F, which represent a potential risk even if it is an essential element. However, when these results are contrasted with those obtained for the molar ratios, it is possible to observe that the brands displayed a favourable (>1) Se:Hg ratio. At present, Se:Hg ratios are not part of risk advisories and the use of this single criterion to evaluate the health risk resulting from Hg exposure through fish intake, is being debated in the scientific community, since in addition to underestimating the adverse effects, there exist interspecies variations product of differences in Hg concentrations in fish capture areas. Furthermore, this ratio is very sensitive to changes since Se is physiologically regulated, whereas Hg bioaccumulates in the food web and its concentrations can fluctuate substantially in different waterbodies as a result of biogeochemical processes and anthropogenic activities. Moreover, for consumers of large predatory fish of high Hg levels such as tuna, any protection against accumulation of this element or toxicity as a result of co-exposure to Se is limited because of a more marked bioaccumulation and biomagnification of this toxic element through the trophic chain compared with the essential nutrient (Karimi et al. 2013). Recent studies have argued that there still exist noticeable uncertainties and gaps with regard to Se mediation of Hg behaviour and toxicity in both abiotic and biotic compartments. Therefore, scientific evidence is needed to provide adequate tools to develop stricter fish consumption advisories based on this ratio (Gerson et al. 2020; Gochfeld and Burger 2021). On the other hand, it has been stated that assessing Hg concentrations in fish alone may be incomplete, given the significant correlations observed between Se and Hg molar concentrations in tuna fish (Okati et al. 2020).

Although As calculations were carried out by taking the inorganic species as 3% of the total levels, sardines were very close to the limit (HQ = 0.77) for adults and above 1 in the case of the potential risk for children (HQ = 1.4). The HQ data obtained here were consistent with the concentrations measured for each element, as observed in other studies (Popovic et al. 2018; Herrera-Herrera et al. 2019). For each brand, taking into consideration the risk of their intake, a CRmw is recommended (Figure 2 and S2). These results are concerning because this type of canned product is extensively distributed given its accessibility and easy preparation, flexible price, and health benefits. A high ingestion rate may pose important side consequences on consumers due to the bioaccumulation of these metals. Thus, in order to perform a more precise assessment, it is required to have specific data regarding patterns of intake in particular sectors of the population, such as children, older adults, and women of reproductive age or pregnancy.

Conclusions

Canning of fish is one of the most accepted preservation techniques in the world. However, the presence of metals in these products as a result of aquatic environment contamination has been associated with adverse effects on human health. Additionally, there may be contamination during handling and processing. The present study demonstrated that canned sardines and tuna marketed in Latin America, may represent a threat to consumers, especially in terms of Se, Cd, and Hg. Importantly, Hg levels were clearly above ranges as described in the literature. However, the analysed brands depicted a favourable (>1) Se:Hg molar ratio. Since these metals exceeded legislative limits, regular monitoring is necessary to safeguard human health. Given that the body burden of these elements relies not only on their levels in fish, but also on the amount and frequency of fish consumption, further research should be carried out to estimate the risk according to these factors.

Acknowledgments

The authors thank Minciencias – National Program for Doctoral Formation (Grants 757-2016); the U.S. Fulbright Foreign Student Program, Colombian doctoral student fellowship; the initiatives to Support Research groups (2018–2020); the Doctoral Programs (2018–2020); and Vice-Presidency for Research of the University of Cartagena (Grant N153/2019).

Funding

This work was supported by the Initiatives to Support Research groups – University of Cartagena [2018–2020]; Vice-Presidency for Research of the University of Cartagena [153/2019]; Minciencias – National Program for Doctoral Formation [757-2016]; Doctoral Programs – University of Cartagena [2018–2020].

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