Article

Hg Content in EU and Non-EU Processed Meat and Fish Foods

Vincenzo Nava [1], Giuseppa Di Bella [1], Francesco Fazio [2,*], Angela Giorgia Potortì [1], Vincenzo Lo Turco [1] and Patrizia Licata [2]

[1] BIOMORF Department, University of Messina, Polo SS Annunziata, 98168 Messina, Italy
[2] Department of Veterinary Sciences, University of Messina, Polo SS Annunziata, 98168 Messina, Italy
* Correspondence: ffazio@unime.it

Abstract: Mercury is one of the most dangerous toxic elements. Fresh food is the primary source of Hg exposure for humans. However, since processed foods are also a fundamental part of the food system, continuous monitoring of this contaminant in processed products is necessary to safeguard consumer health. The purpose of this study was to determine the Hg content using a direct mercury analyzer (DMA-80) in different processed food products (beef, pork, poultry, shellfish, and fish species) of EU and non-EU origin purchased in supermarkets and ethnic food shops in Messina (Italy). The results obtained were variable and correlated with the different types of food matrix. Low mercury levels were obtained for beef, pork, and poultry products, ranging from <LOQ for most samples to 3.727 µg/Kg. Higher concentrations were, however, obtained for fish species (9.249–290.211 µg/Kg). The mercury content was below the maximum levels specified in Regulation No 1881/2006 as amended. Percentage of Tolerable Weekly Intake (TWI) values of total Hg were evaluated in accordance with the European Food Safety Authority (EFSA). The results showed that some of the pelagic species of the genus Thunnus thynnus exceeded the TWI values.

Keywords: processed foods; mercury content; TWI

1. Introduction

Processed products are now an important part of the human diet. Food processing allows the state of raw materials to be altered to increase their shelf life or change their sensory characteristics [1]. The term "processing" refers to a series of operations (e.g., washing, grinding, heating, freezing, filtering, fermentation, extraction, centrifuging, cooking, drying, packaging) carried out on raw materials [1], i.e., all methods and techniques used by the food, beverage, and associated industries to transform whole fresh foods into food products [2].

These processes not only extend the shelf life of a product and maintain its quality but also allow most microorganisms to be eliminated [3].

Although there are many processed foods [4–7], meat and fish are certainly among the main food matrices subject to processing, given their great importance in the diet, being a source of protein and other nutrients.

However, as the consumption of processed food increases, their exposure to different types of hazardous contaminants is also of great concern. They, in fact, may contain more contaminants than natural foods. Such contamination can be of different types: chemical (e.g., from the addition of additives); process (contamination during cooking, heating, or storage of foods or during their transportation); or from the environment [8]. These, representing different sources of contamination, can consistently affect food quality and safety [3,9].

Among the many contaminants, toxic elements pose a serious threat to human health. They are nonbiodegradable xenobiotics and can accumulate in the environment, eventually reaching food [9–11]. In fact, it is the ingestion of contaminated food that is a major source of human exposure to toxic elements [9]. For this reason, knowing the degree of pollution

caused by toxic elements in processed samples is, therefore, crucial to be able to assess the possible risk to which consumers are exposed because of the overuse of such products [12].

Among the toxic elements regulated at the European level is mercury, a metal present in liquid form and silvery-white in color, which is highly toxic to the environment and living beings [13]. This element can occur in different forms: elemental, inorganic, and organic. Exposure to inorganic Hg can take place through occupation; exposure to organic Hg is mainly by ingestion of food and water [14]. Depending on its form, its characteristics vary in terms of circulation in the ecosystem, accumulation, exposure to the human body, biological effects, and toxicity [15].

However, the primary source of mercury exposure for humans is food. Forms most found in food, albeit at very low levels, are the cation Hg^{2+} and organic methylmercury, CH_3Hg [16]. Only fish products have higher mercury concentrations.

CH_3Hg is the form of mercury of greatest concern because it readily crosses blood-brain and placental barriers and has an extremely high absorption efficiency (>90%) when ingested in food. This partly explains the higher relative risk associated with exposure to CH_3Hg compared to inorganic Hg species. Less than 10% of inorganic Hg in food is absorbed by the body, while the rest is rapidly excreted within 24 h [17].

The International Agency for Research on Cancer (IARC) does not list elemental mercury and inorganic Hg compounds as human carcinogens (Group 3). However, in 2010, the Panel on Contaminants in the Food Chain (CONTAM) of the European Food Safety Authority (EFSA) indicated a TWI value for inorganic mercury of 4 µg/kg body weight [18]. In contrast, methylmercury compounds are grouped in the category "possibly carcinogenic" (Group 2B) [19], with a TWI of 1.3 µg/kg body weight/week [16,20]. CH_3Hg binds to the sulfhydryl groups of proteins and accumulates in organisms [21].

There are several pathologies caused by mercury: lung damage because of acute Hg poisoning, neurological and psychological disorders (e.g., anxiety, sleep disorders and depression, irreversible kidney damage) due to chronic exposure to this element [14,22,23].

The largest number of studies in the literature concerns the determination of mercury content in fish products, both fresh and processed [24], while fewer studies are observed for processed meat products [10,12,14,25,26]. The common point of this research is the close correlation between the population's dietary exposure to mercury and different food habits.

For mercury, European legislation (EU Regulation 1881/2006 and its subsequent amendments) sets maximum permitted levels only for fish muscle meat, food supplements, and salt, indicating no further limits for the remaining foodstuffs [27,28].

There are various analytical techniques that allow the determination of Hg, which differ depending on the type of mercury compound to be determined and on the different types of sample processing. The most common methodologies for analyzing Hg include Cold vapor atomic absorption spectrometry (CV-AAS); Cold vapor atomic fluorescence spectrometry (CV-AFS); Inductively coupled plasma atomic emission spectrometry (ICP-AES) with hydride generation system; Inductively coupled plasma with mass spectrometry detector (ICP-MS) [29]. However, several analytical methods have now been developed that are characterized by their high versatility since they allow direct analysis of the sample without first treating it [29]. Among these analytical techniques is the Thermal decomposition amalgamation atomic absorption spectroscopy (TDA-AAS), the method on which the Direct Mercury Analyzer used in this study is based. Its use provides significant advantages compared with other analytical techniques: e.g., direct analysis of trace mercury; no sample mineralization required; rapid analysis; less loss of trace Hg; relatively low cost [29,30].

Starting from this assumption, the objective of the following work was to determine the mercury content via Direct Mercury Analyzer (DMA-80) in processed meat and fish products purchased in supermarkets and ethnic food shops in Messina (Italy). Furthermore, to assess the possible risks derived from the excessive consumption of such products, the total Tolerable Weekly Intake values of Hg were calculated for each species analyzed in accordance with the EFSA guidelines.

2. Materials and Methods

2.1. Samples

In this study, of all 72 samples processed of different brands and purchased between September and October 2022 in supermarkets and ethnic food shops in Messina, 30 were canned meat (beef, pork, chicken), 32 were canned, or dried fish (tuna, mackerel, sardines, salmon) and 10 were natural or dried shellfish (shrimp and crab). The characteristics and numbers of the samples investigated per type of foodstuff are shown in Table 1.

Table 1. Characteristics of processed foods investigated in this study.

Sample Code	Sample	Sample No	Constituents (%)			Species	Country or Origin
			Fat	Protein	Fiber		
F1	Canned tuna in olive oil	4	22	19	**	*Katsuwonus pelamis*	Spain
F2	Canned tuna in olive oil	3	33	18	0	*Thynnus albacares*	Italy
F3	Canned natural tuna	2	0.9	23	**	*Thynnus albacares*	Italy
F4	Canned tuna in olive oil	3	13	29	**	*Katsuwonus pelamis*	Italy
F5	Canned natural tuna	2	0.6	20	**	*Thynnus albacares*	Spain
F6	Natural shrimp	3	1	17	**	**	Italy
F7	Canned horse mackerel	3	4.9	24	**	*Trachurus murphyi*	Chile
F8	Canned sardines	3	42	16	**	*Sardina pilchardus*	Morocco
F9	Canned crab meat	3	0.5	12	**	**	Indonesia
F10	Canned pink Salmon	2	7	19	**	**	USA
F11	Canned tuna pate	3	25	13	**	*Euthynnus (Katsuwonus) pelamis*	Italy
F12	Canned mackerel fillets	4	22	22	**	**	Portugal
F13	Dried shrimp	4	2 *	19 *	0	**	Argentina
F14	Dried sardines	3	19 *	15 *	**	*Sardinella aurita*	Argentina
M1	Canned beef and pork pate	2	14	11	3.5	**	Italy
M2	Canned beef and pork pate	2	14	11	**	**	Italy
M3	Canned jelly with meat stock	4	1.2	11	<0.5	**	Italy
M4	Canned chicken	3	1	1.1	**	**	Italy
M5	Canned ham pate	3	26	9	**	**	Italy
M6	Canned beef	4	1.5	11	**	**	Italy
M7	Canned jellied chicken breast	3	1	11	**	**	Italy
M8	Canned chicken Luncheon Meat	4	10	12	**	**	Philippines
M9	Canned chopped Pork and Ham with real bacon	2	22	15	**	**	Denmark
M10	Canned chicken meat	3	14	13	**	**	Poland
Tot		72					

* not indicated on the label, but experimentally calculated. ** not indicated on the label.

For most of the samples, the fat, protein, and fiber percentages given on the label were indicated. The others, however, were determined experimentally (indicated in Table 1 with *), according to following methods: Folch method [31] for extraction and a gas chromatographic analysis with a flame ionization detector (GC-FID) for the determination of total fats according to the method developed by Tropea et al. [32] and Di Bella et al. [33].

To highlight a parameter that was neither indicated on the label nor experimentally calculated, the symbol ** was used (Table 1).

2.2. Material and Reagents

Hg solution (1000 mg/L in 3% hydrochloric acid) was obtained from Merck (Darmstadt, Germany). Pure HCl (37%), purchased from Merck (Darmstadt, Germany), was used to prepare a 3% HCl solution, useful for cleaning the instrument.

2.3. Mercury Analysis

A direct mercury analyzer (DMA-80, Milestone S.r.l., Milan, Italy) was used to determine the Hg content in each sample. This is a very versatile instrument that allows direct measurement of mercury content in solid, liquid, and gaseous samples without the need for pre-treatment. This not only ensures ease of use but also very low operating costs, as there is no use of chemical reagents or acids and no hazardous compounds to discard. Consequently, these features make the DMA-80 a "green" instrument. The analysis procedure is easy to apply. In fact, the sample is weighed into special cuvettes and automatically introduced into the furnace of DMA-80. The samples, once charged, are initially dried and, subsequently, thermally decomposed in an oxygen or air atmosphere. Under these conditions, mercury and other species present are released and carried by the gas stream into a catalyst, where interfering substances (halogens, sulfur oxides, and nitrogen) are removed. The mercury, then, is selectively trapped in a gold-containing amalgamation while the decomposition fumes are flushed away to avoid signal obscurations. When the amalgamator is heated, it rapidly releases the mercury, which is then transported to the measuring cell, and its content is determined by atomic absorption spectroscopy at the typical wavelength of mercury, i.e., 253.54 nm. The DMA-80 was used according to the US EPA 7473 method [34]. Briefly, ~0.1 g of each sample was subjected to a temperature ramp from 60 °C to 650 °C for 4–5 min. An external calibration of the instrument was performed by constructing a seven-point calibration curve. A Hg solution (1000 mg/L in 3% hydrochloric acid) was used for this purpose.

2.4. Method Validation

The DMA-80 method was validated in terms of linearity, sensitivity, accuracy, and precision according to criteria established by Eurachem [35]. The linear least-square regression method was used to determine linearity. Sensitivity was determined by calculating the limit of detection (LOD) and the limit of quantification (LOQ). To do this, the following experimental formulae were used: 3.3 σ/b and 10 σ/b, respectively, where σ is the standard deviation of the analytical blank ($n = 10$) and b is the slope of the relative calibration curve.

Accuracy was calculated by performing six replicates on the certified ERM-CE278k-Mussel Tissue matrix and reported as the percentage of recovery obtained from the ratio of the experimental value to the expected value. Repeatability was assessed in terms of precision and intermediate precision. For the first, the certified matrix and the spiked sample were analyzed on the same day; for the second, considering a longer period (1 week).

3. Results

3.1. Method Validation Results

The results obtained for the method validation proved to be efficient for the purposes of analysis. For the linearity, seven-point calibration curves were constructed using a standard solution of mercury in the range of 1–100 μg/L. The R^2 value obtained for Hg was 0.9998. The LOD and LOQ values were 1.000 μg /Kg and 3.000 μg /Kg, respectively. An average recovery of 98.59% was observed. Precision and intermediate precision percentages, expressed as relative standard deviation (RSD%) of 0.7% and 1.1%, respectively, were obtained.

3.2. Results

Table 2 and Figure 1 report the variable mean mercury concentrations (expressed in μg/Kg), determined in meat, fish, and shellfish processed by DMA-80. In general, processed fish and shellfish products showed a higher amount of Hg than meat. The

results were variable: the mercury concentration range in fish varied from 9.25 μg/Kg to 290.21 μg/Kg—minimum and maximum levels obtained for *T. thynnus* species. Among the aquatic species tested, the mean mercury content decreased in the following order: tuna > sardines > mackerel > salmon > shrimps > crab (Table 2).

Table 2. Mean concentration levels of mercury expressed as μg/Kg.

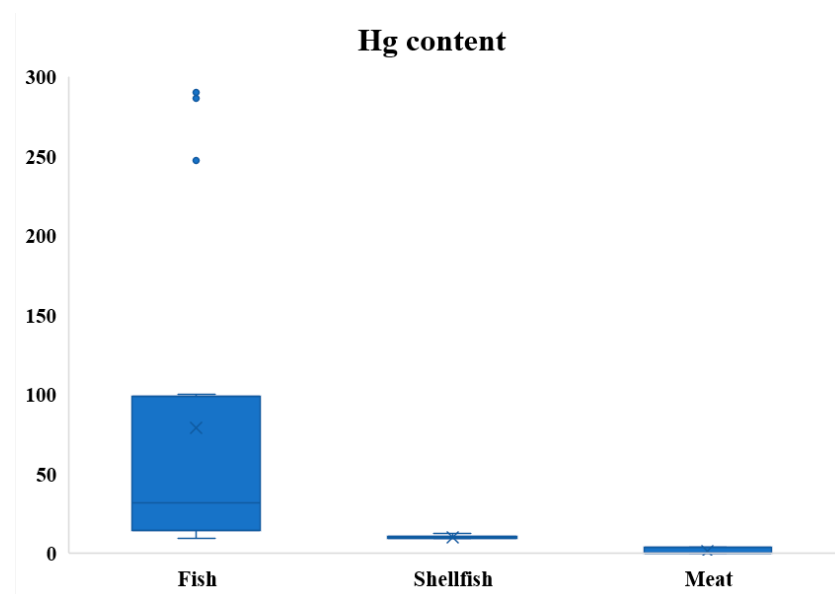| Sample	Mean Hg Concentration (μg/Kg)
F1	250.92 ± 3.68
F2	290.21 ± 4.64
F3	9.25 ± 0.85
F4	70.11 ± 4.22
F5	50.82 ± 3.58
F6	11.90 ± 0.88
F7	25.71 ± 1.40
F8	14.00 ± 0.18
F9	10.05 ± 1.74
F10	12.89 ± 2.55
F11	20.65 ± 3.52
F12	32.45 ± 5.68
F13	9.45 ± 0.30
F14	99.93 ± 1.43
M1	3.17 ± 0.12
M2	<LOQ
M3	<LOQ
M4	<LOQ
M5	<LOQ
M6	<LOQ
M7	<LOQ
M8	3.17 ± 0.11
M9	3.73 ± 0.18
M10	<LOQ

Figure 1. Box plot reporting the variable mercury content in relation to different types of matrices. The "×" indicates the average value of mercury.

The processed meat samples, instead, showed a mercury content between <LOQ and 3.73 μg/Kg. Only nine of thirty samples reported a Hg concentration exceeding the limit of quantification (3.00 μg/Kg).

The TWI values calculated in this study were reported in Tables 3 and 4. In accordance with the EFSA guidelines, the Tolerable Weekly Intake (TWI) percentage values were calculated considering an average consumption of an amount of 200 g, considering FAOSTAT data [36] for both fish and meat products, of the processed products by 70 Kg adult body weight. To calculate the TWI percentage, the Formula (1) was used:

$$\text{TWI\%} = \left[\frac{\{[(C \times 0.2\,\text{Kg})/70\,\text{Kg}] \times 7\}}{\text{TWI}} \right] \times 100 \tag{1}$$

where C is the concentration of mercury (μg/Kg), 0.2 Kg is the food portion, 70 Kg is the body weight of an adult, TWI is the Tolerable Weekly Intake indicated by EFSA.

Table 3. Tolerable weekly intake (TWI) % values for Hg contents in analyzed processed fish and shellfish.

Samples	No Samples	Hg	
		µg/Kg	TWI%
		Range Min–Max	Range Min–Max
Canned tuna	17	9.25–290.21	4.63–145.11
Natural and dried shrimp	7	9.45–11.90	4.73–5.95
Canned sardines	3	13.89–14.21	6.95–7.11
Canned crab meat	3	8.07–11.30	4.03–5.65
Canned pink salmon	2	10.15–15.19	5.08–7.59
Dried sardines	3	98.34–101.08	49.17–50.54
Canned mackerel fillets	4	24.84–27.33	12.42–13.67
Canned horse mackerel	3	26.62–37.96	13.31–18.98

Table 4. Tolerable weekly intake (TWI) % values for Hg contents in analyzed processed meat.

Species	No Samples	Hg	
		µg/Kg	TWI%
		Range Min–Max	Range Min–Max
Canned beef and pork pate	4	<LOQ–3.27	n.d.–1.63
Canned beef	8	<LOQ	n.d.
Canned chicken	13	<LOQ–3.28	n.d.–1.64
Canned pork	5	<LOQ–3.92	n.d.–1.96

n.d. = not detected.

Table 3 reports the TWI values obtained for processed fish and shellfish. Only two samples of canned tuna (F1 and F2) exceeded TWI levels among the aquatic species tested. For the remainder, no risk was observed (Table 3). TWI values reported were lower for meat samples (Table 4), showing the security of these samples in terms of mercury content for the consumer.

In Table 5, tolerable weekly intakes in relation to different dietary habits were shown. TWI percentage values were calculated considering several average weekly consumptions correlated to the different world countries (Africa, America, Asia, Europe, and Oceania), considering FAOSTAT data [36] for both fish and meat products, by a 70 Kg adult body weight.

Table 5. Tolerable weekly intake (TWI) % values for Hg contents in relation to different dietary habits.

			Hg				
Samples	No Samples	µg/Kg	TWI%				
			Africa	America	Asia	Europe	Oceania
		Range Min–Max	Range Min–Max	Range Min–Max	Range Min–Max	Range Min–Max	Range Min–Max
Canned tuna	17	9.25–290.21	1.55–48.61	1.36–42.81	1.11–34.83	2.54–79.81	3.47–108.83
Natural and dried shrimp	7	9.45–11.90	1.58–1.99	1.39–1.76	1.13–1.43	2.60–3.27	3.54–4.46
Canned sardines	3	13.89–14.21	2.33–2.38	2.05–2.10	1.67–1.71	3.82–3.91	5.21–5.33
Canned crab meat	3	8.07–11.30	1.35–1.89	1.19–1.67	0.97–1.36	2.22–3.11	3.03–4.24
Canned pink salmon	2	10.15–15.19	1.70–2.54	1.50–2.24	1.22–1.82	2.79–4.18	3.81–5.70
Dried sardines	3	98.34–101.08	16.47–16.93	14.50–14.91	11.80–12.13	27.04–27.80	36.88–37.91
Canned mackerel fillets	4	24.84–27.33	4.16–4.58	3.66–4.03	2.98–3.28	6.83–7.52	9.32–10.25
Canned horse mackerel	3	26.62–37.96	4.46–6.36	3.93–5.60	3.19–4.56	7.32–10.44	9.98–14.24
Canned beef and pork pate	4	<LOQ–3.27	n.d.–0.71	n.d.–4.82	n.d.–1.85	n.d.–4.50	n.d.–4.99
Canned beef	8	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
Canned chicken	13	<LOQ–3.28	n.d.–0.71	n.d.–4.83	n.d.–1.56	n.d.–3.77	n.d.–4.18
Canned pork	5	<LOQ–3.92	n.d.–0.85	n.d.–5.78	n.d.–1.86	n.d.–4.51	n.d.–5.00

n.d. = not detected.

4. Discussion

The mercury content was determined by DMA-80, a rapid technique that permitted a direct analysis of processed foods investigated.

Hg levels were variable. In general, aquatic products showed a higher content than the other samples. This trend was expected, given the higher mercury accumulation capacity of aquatic species [21,37], and is comparable to other studies in the literature [3]. Furthermore, the different mercury content observed in fish and shellfish samples is related to the different accumulation capacities of the various species. This capacity is strongly correlated with the age and size of the product [37]. Several studies exist in the literature explaining the occurrence of a strong correlation between the mercury content and the size of fish and shellfish, especially in big predators, such as tuna fish and sharks [37,38]. This is coherent with our study, where the highest mercury concentration was obtained for several types of canned tuna. In addition, the mercury content in the investigated fish samples was lower than in one study in the literature [39].

The data obtained shows a higher mercury content in the samples of canned tuna in olive oil, which had a higher fat content than natural canned tuna. In general, this trend seemed to be present in most of the fish and shellfish samples analyzed in our study. Species containing less than 10 percent fat showed the lowest mercury levels. This trend could depend on several factors, including the different accumulation capacities of the species under study. In addition, consideration must also be given to the types of samples examined, namely processed foods which are characterized by a different composition than the corresponding raw materials. In other studies reported in the literature, fat content and mercury concentration followed the opposite trend. In fact, in one such research, the highest percentage of fat (11.49%) was shown by the herring sample, which nevertheless contained low mercury concentrations (0.021 mg/Kg) [40]. Further specific studies will therefore be necessary to demonstrate a possible correlation between fat content and mercury content and to obtain more significant data to confirm our hypothesis.

Relative to the processed meat samples, mercury contents were remarkably low. The range was from <LOQ to 3.73 ± 0.18 µg/Kg. These levels were either comparable to some studies [16,25] in literature or even lower than others [14]. This demonstrates the safety and quality of the meat products analyzed.

European Regulation No 1881/2006 and subsequent amendments (Regulation (EU) No 2022/617) [27,28] specify maximum mercury levels in three food classes: fish, salt, and food supplements. There are no guidelines for the other food types. Comparison with the maximum levels set by European regulations is of fundamental importance to be able to assess the safety of products. In turn, these could provide further information and be used as sentinel organisms for assessing the degree of environmental contamination [41,42].

For fish, different contents of this element are indicated depending on the species: *Thunnus species* (1.0 mg/Kg); Shellfish (0.5 mg/Kg); *Scomber species*, *Salmo species*, and *Sardina species* (0.30 mg/Kg). In general, however, all samples were below the maximum levels reported. This is an important result, considering that the accumulation of mercury in processed fish can occur due to the natural presence of this element in the aquatic environment and the different processing steps they undergo [37].

As already mentioned, no maximum mercury levels are specified for meat and processed meat products. However, the content shown by the analyzed samples was very low and did not cause any concern. Furthermore, the results obtained from our study were comparable with those found in the literature, where mercury concentrations in processed meat samples were minimal [3,14,16,25].

The following study, in addition, included the assessment of mercury exposure because of excessive consumption of the products analyzed. In this regard, in fact, tolerable weekly intake values of Hg were calculated for each species investigated. An initial assessment was made considering an average consumption of 200 g of each processed product by a 70 Kg adult. No other factors, such as age or gender, were considered because the Panel on Contaminants in the Food Chain (CONTAM) of the European Food Safety Authority (EFSA) indicates a TWI value for inorganic mercury of 4 µg/kg body weight [18] for all age groups and not differentiating by gender. Given the results obtained and shown in Tables 3 and 4, only two fish samples (F1 and F2) exceeded TWI levels, while the remaining products analyzed were within the normative levels. A second assessment was conducted by considering the average consumption and food habits of different world countries. In this regard, Table 5 shows that seafood products exhibited a wide range of TWI percentages, ranging from n.d. and 108.83%. As a result, only sample F1 showed a TWI percentage level above the norm but relative to the average consumption reported for Oceania.

The TWI percentage levels we obtained were compared with other studies in the literature concerning both raw materials and processed products. In two studies [21,40], the first concerning mercury accumulation in fish from the Portuguese coast and the second on post-consumption risk assessment of certain types of freshwater and seawater fish, TWI percentage values were, in some cases, lower (1.845–41.056%) than those found in our study. This could be due to the greater possibility of contamination caused by all those processes to which raw materials are subjected to be "processed". In addition, the different mercury intake is closely related to the size of the fish [21].

The range of mercury TWI percentage (n.d.–1.96%) obtained for meat products was far lower than those shown by fish samples. This demonstrates the higher Hg accumulation capacity of fish products [21,37]. Slightly higher, but still within the threshold levels, are the TWI percentage calculated considering the different world food habits. Some pork samples showed the highest TWI percentages, followed by canned beef and pork, canned chicken, and canned beef. Moreover, TWI rates have been shown to be comparable to those reported by other studies [3,16].

5. Conclusions

In this study, mercury content in processed foods was determined by DMA-80 analysis. It was shown that this method is a convenient, fast, and reliable process for measuring mercury. This was demonstrated by the linearity, LOD, LOQ, and accuracy values obtained, which were found to be acceptable in accordance with EURACHEM guidelines.

The results showed a variable mercury content depending on the type of food analyzed. Aquatic products had higher Hg levels than land-based products, in agreement with other studies in the literature. However, all samples were within European legislative limits. Furthermore, from the TWI assessment for mercury, only two types of canned tuna exceeded the stated intake values., considering an average consumption of 200 g. For TWI percentages calculated relative to food habits, these varied according to the average consumption in the area of interest. Only in the case of fish consumption in Oceania did one sample exceed the normative TWI.

In addition, the present study showed a possible direct correlation between mercury and fat content for some of the analyzed species. However, further studies will be needed, given the lack of statistical data to support this hypothesis.

However, given the high consumption of these processed foods and their higher contamination than raw materials, continuous monitoring of this food category is essential to safeguard consumer health.

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