

## MERCURY SPECIATION IN FISH TISSUE AND METHYLMERCURY RISK ASSESSMENT FOR HUMAN HEALTH: VALIDATION THROUGH HPLC-ICP-MS. A STUDY FROM THE SEAFOOD CENTRAL MARKET OF MEXICO CITY

Especiación de mercurio en tejido de pescado y evaluación del riesgo para la salud humana por metilmercurio: validación mediante HPLC-ICP-MS. El caso de la central de abasto de pescados y mariscos de la Ciudad de México

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Key words: bioaccumulation, seafood safety, Minamata Convention, Hazard Quotient (HQ), mercury speciation.

### ABSTRACT

Methylmercury (MeHg) is a potent neurotoxin that primarily targets the central nervous system. The population worldwide is exposed to MeHg mainly by ingesting seafood with high mercury (Hg) content. Regulatory frameworks and studies worldwide estimate the human health risk posed by MeHg using the total Hg concentration in fish tissue ( $Hg_{Tot}$ ) and consider an adjustment factor of 90% to estimate MeHg contribution, without any validation. In this study, we combine quadrupole inductively coupled plasma mass spectrometry (Q-ICP-MS) and high-performance liquid chromatography (HPLC) to quantify  $Hg_{Tot}$  and MeHg in three high-consumption fish species from the Seafood Central Market of Mexico City (SCMM), the second-largest seafood distributor in the world after the Toyosu market in Japan. SCMM serves 22 million people in Mexico City, millions across the entire country, and millions worldwide, such as the United States of America, Japan, Canada, and France. We pointed out that the MeHg proportion in fish tissue worldwide varies widely (from 19% to 100%), with a global median of 82.3% ( $n = 50$  published studies, including our results), significantly lower than the 90% adjustment factor commonly used. *Cathorops fuentii* shows a high mercury content, reaching up to  $0.70 \mu\text{g/g } Hg_{Tot}$ , with 78.9% as MeHg. Risk assessment reveals an important risk ( $HQ_{MEHg} > 1$ ) for children under six years old and pregnant women due to MeHg exposure in some localities of Mexico. Our findings highlight the need for species-specific monitoring of MeHg and updated daily safety intake guidelines. We recommend adopting HPLC-ICP-MS to replace  $Hg_{Tot}$ -based estimations, in line with the Minamata Convention's goals.

Palabras clave: bioacumulación, seguridad alimentaria en productos del mar, Convenio de Minamata, cociente de peligro (HQ), especiación de mercurio.

## RESUMEN

El metilmercurio (MeHg) es una neurotoxina altamente tóxica que afecta al sistema nervioso central. La población mundial está expuesta al MeHg a través de la ingesta de productos del mar con alto contenido de mercurio (Hg). Los marcos regulatorios y los estudios de riesgo para la salud estiman la exposición al MeHg determinando la concentración total de mercurio ( $Hg_{Tot}$ ) en tejido de pescado y considerando un factor de ajuste del 90 % para estimar la proporción de MeHg. Este estudio combina espectrometría de masas de cuadrupolo con plasma inductivamente acoplado (Q-ICP-MS) y cromatografía de líquidos de alta eficiencia (HPLC) para cuantificar simultáneamente  $Hg_{Tot}$  y MeHg en tres especies de pescado de alto consumo en México, provenientes de la Central de Abasto de Pescados y Mariscos de la Ciudad de México (CEDA), el segundo mayor distribuidor de productos del mar a nivel mundial. La SCMM abastece a 22 millones de personas en la Ciudad de México y a millones en Estados Unidos, Japón, Canadá y Francia. Nuestros resultados indican que la proporción de MeHg en tejido de pescado varía significativamente (19-100 %), con una mediana global de 82.3 % (basada en 50 estudios), significativamente menor que el factor de ajuste del 90 %. La especie *Cathorops fuertii* estudiada presentó un alto contenido de mercurio, con 0.70  $\mu g/g$  de  $Hg_{Tot}$ , de los cuales 78.9 % corresponde a MeHg. La evaluación de riesgo revela un valor considerable ( $HQ_{MeHg} > 1$ ) para niños menores de seis años y mujeres embarazadas, en algunas localidades de México. Nuestros hallazgos subrayan la necesidad de un monitoreo de MeHg por especie y de actualizar los marcos normativos sobre la ingesta diaria recomendable. Los autores recomiendan implementar HPLC-ICP-MS para reemplazar las estimaciones basadas en  $Hg_{Tot}$  y cumplir con los objetivos del Convenio de Minamata.

## INTRODUCTION

Mercury (Hg) originates from magmatic sources and is introduced into the lithosphere through geological processes. The principal mercury ore is the mineral cinnabar ( $HgS$ ), used from 4000 to 3000 years before the present, during the Neolithic period, as a human bone preservative in burials. Due to its vibrant red color,  $HgS$  was used by many cultures as a pigment, such as in Egyptian tombs, Roman cities (Parsons and Percival 2005), and the buildings of pre-Hispanic American cultures like the Mayans. Mercury poisoning is considered to be the cause of the sudden abandonment of their cities (Lentz et al. 2020). Mercury has been widely used in mining to extract economic elements such as gold, silver, platinum, copper, uranium, and lead. In places where artisanal gold mining still occurs, significant mercury emissions are released into the atmosphere. As a result of anthropogenic activities, mercury is dispersed in the environment, including soil, water, air, and biota, even several kilometers away from the mining zones (Parsons and Percival 2005).

Inorganic mercury ( $Hg_{In}$ ) enters the environment through atmospheric deposition and, under shallow conditions, is transformed into highly toxic organic forms by iron and sulfate-reducing bacteria (Polak-Juszczak 2018). Organic mercury tends to bioaccu-

mulate in living organisms and increase its concentration as it moves up the food chain (biomagnification). The most common type of organic mercury in foodstuffs is the mono-methylated species (MeHg), which is extremely toxic to humans and enters the organism through food products, especially seafood, that may come from areas affected by industrial or mining activities and from zones with volcanic and geothermal activity (Nogara et al. 2019).

Throughout history, there have been several cases of methylmercury (MeHg) poisoning, highlighting its high toxicity. For example, in Iraq (1956 and 1960) and Guatemala (1960), due to wheat flour and seeds contaminated by methylated mercury compounds used as fungicides (Bakir et al. 1973), and in Minamata and Niigata, Japan (1956 and 1965), through fish consumption with high levels of MeHg (McLaughlin et al. 2007). Due to the global concern generated by these events, in 2013, more than 140 countries worldwide (including Mexico) adopted the Minamata Convention, which aims not only to control the supply and trade of mercury but also to reduce its use and emissions to protect the environment and the population from its effects. The Minamata Convention entered into effect in August 2017, and although many countries have implemented policies to avoid the use of mercury and every government has adopted Maximum Permissible Limits (MPL)

for MeHg, governments and researchers remain concerned that some populations may be at risk from involuntary ingestion of seafood contaminated with mercury (Ruelas-Inzunza et al. 2008, Spanopoulos-Zarco et al. 2014, Zamora-Arellano et al. 2017, García-Hernández et al. 2018).

Research has been conducted worldwide where mercury concentrations in fish tissue have been related to human health risk (Li et al. 2010, Ruelas-Inzunza et al. 2012, Grosbois 2013, Basu et al. 2014, Delgado-Alvarez et al. 2017, Zamora-Arellano et al. 2017, Polak-Juszczak 2018, García-Hernández et al. 2018, Ramírez-Islas et al. 2018, Charette et al. 2021, Murillo-Cisneros et al. 2021, Santos et al. 2021, Vergara et al. 2024). Based on this knowledge, it has been determined that MeHg content in fish tissue may vary depending on species, size, position in the food chain, and geographic location. Most studies focus on measuring total mercury concentration ( $Hg_{Tot}$ ) and estimating the percentage of MeHg by assuming a 90% conversion factor for  $Hg_{Tot}$ , as recommended by the European Food Safety Authority since 2012 (EFSA 2012). This estimated MeHg concentration is then used as input to assess human health risk. Recently (Kennedy et al. 2025) estimated methylmercury contamination in Mediterranean seafood using this 90% factor. Based on 17 964  $Hg_{Tot}$  published data, they determined exposure to MeHg and the associated costs of illness. They point out that, when summed across all Mediterranean countries, those costs could exceed €10 billion. Nevertheless, when health assessment is the main objective, using a 90% adjustment factor leads to an inadequate MeHg risk assessment. In the present study, we determine simultaneously the inorganic ( $Hg_{in}$ ) and the methylated concentration of mercury (MeHg) via speciation by liquid chromatography coupled with mass spectrometry (HPLC-ICP-MS) in muscle samples of three highly consumed fish species in Mexico obtained from the Seafood Central Market of Mexico City (SCMM). Based on the methodology proposed by the Environmental Protection Agency (USEPA 1989) and using the results of the MeHg concentrations obtained here, we estimate the human health risk due to involuntary ingestion of MeHg in fish tissue ( $HQ_{MeHg}$ ) for the most vulnerable population groups: women of reproductive age (16 to 30 years) and children under or equal to 6 years in age, considering the recommendation of the European Food Safety Authority (EFSA 2012). Hazard quotient maps for involuntary MeHg exposure ( $HQ_{MeHg}$ ) were modeled for the Mexican territory with reported total mercury data, and the MeHg proportion found in this study was used. Given SCMM's national and

international commercial importance, we highlight that vulnerable groups may be at risk of MeHg exposure through seafood.

## MATERIALS AND METHODS

### Sample collection and preparation

To determine the content of inorganic and organic mercury in fish tissue, a pilot fish sampling was conducted in the SCMM in November 2018. Located in Mexico City and known as “La Viga”, the SCMM is the second-largest seafood distributor worldwide after the Toyosu market in Japan (CONAPESCA 2018). La Viga market has always supplied the Mexico City Metropolitan area, the Mexican states, and other countries like the United States of America (USA), Japan, Canada, and France (Amos et al. 2022). It handles 700 to 1500 t of different seafood species daily (Amos et al. 2022), serving 22 million people in Mexico City and millions in the country. According to the Annual Statistical Report from the National Aquaculture and Fisheries (CONAPESCA 2018), 82% of the fish managed in Mexico are wild-caught, while 18% come from aquaculture. In 2018, the total seafood volume caught for human consumption was 1 451 717 t, and 332 647 t were exported to countries like the USA, Japan, Spain, China, and others. In 2018, the main retail fish species consumed in Mexico after tuna (*Thunnus albacares*) and mojarra tilapia (*Oreochromis niloticus*) were horse mackerel or jurel (*Caranx caninus*) with 25 308 t, catfish or mexican bagre (*Cathorops fuerthii*) with 6464 t, and the Mexican barracuda (*Sphyranea ensis*) with 21 873 t (CONAPESCA 2018). Nine samples and three duplicates were collected from different sale points, each weighing 500 g (wet weight). Three species were evaluated: *Caranx caninus*, *Cathorops fuerthii*, and *Sphyranea ensis*. The samples were placed on ice in a cooler to maintain 4 °C during transportation and frozen in the laboratory at –18 °C. Each sample was cut into 50 – 100 g pieces for lyophilization, placed in individual beakers, and the wet weight (P1) was recorded, then covered with Parafilm® with miniature perforations to allow moisture exchange. The fish muscle samples were freeze-dried at the Laboratory of Experimental Soil Science, Institute of Geology, at the National Autonomous University of Mexico, under a pressure of 0.85 mBar and –47 °C for 48 h. At the end of the drying procedure, the weight of the dry sample (P2) was recorded, and the humidity percentage (% H) was calculated using the following equation:

$$\% H = \left( \frac{P1 - P2}{P1} \right) * 100 \quad (1)$$

The freeze-dried samples were ground using a Krups® seed mill, labeled, refrigerated, and stored until further analysis.

### Total mercury (Hg<sub>Tot</sub>) by Q-ICP-MS

To analyze the total mercury concentration, 200 mg of freeze-dried sample in dry weight (DW) were weighed in microwave oven liners, 2 mL of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were added, and allowed to react for 12 h in a fume hood. After this time, 6 mL of HNO<sub>3</sub> were added, and the mixture was digested in an Anton Paar Multiwave 3000 microwave platform system at the laboratories of the Instituto Nacional de Ecología y Cambio Climático. A 40-min program with three different ramps was used, the first at 160 °C for 10 min, followed by a ramp at 200 °C for 10 min, maintaining that temperature for 10 min, and then left to cool with a ramp of 10 min. At the end of the cycle, the rotor was kept in a cold room to cool overnight. The next day, the microwave liners were opened, and the solutions were transferred to 50 mL centrifuge tubes. The solutions were diluted to the mark with 2% HNO<sub>3</sub> containing 25 ppb of indium and rhenium (In-Re) as internal standards and 500 ppb of gold to chelate the mercury. The samples were analyzed in an Agilent Q-ICP-MS, model 7500ce, in the ICP-MS Laboratory at the Instituto de Geología de UNAM.

Quality control and quality assurance were conducted using analytical blanks, verification standard points with concentrations equivalent to the middle point of the calibration curve, and the Standard Reference Material Lake Superior Fish (LSF) from the National Institute of Standards and Technology (NIST® SRM® 1946) with Hg<sub>Tot</sub> certificate value of 0.433 ± 0.009 µg/g wet weight (WW). The average recovery of the verification standards with concentrations of 0.100 µg/g was 103.98% ± 10.3, while the average recovery for the NIST® SRM® 1946 was 93% ± 8.5. A duplicate sample was analyzed for each of the three samples, obtaining a relative standard deviation (RSD) < 20%.

### Mercury speciation and methylmercury content

This study represents the first documented research to accurately determine the concentration of MeHg in fish tissue through speciation analysis using HPLC-ICP-MS and to assess the human health risk associated with the MeHg obtained. The procedure proposed by Reyes et al. (2009) and the analytical methodology suggested by the United States Food

and Drug Administration (Cheng and Hight 2008) were adapted and validated using blank matrix and certified reference materials. The methodology was then used to determine the methylmercury content in commercial samples. 0.5 g of freeze-dried sample was weighed into a pre-cleaned 100 mL Teflon microwave vessel, 10 mL of 5 mol/L HCl and 0.25 mol/L NaCl were added to each vessel sample. A 15-min extraction program was performed in an Anton Paar Multiwave 3000 microwave platform system. The program used a 3-min ramping time to reach 60 °C and 12 min at a constant temperature. After extraction, the vessels were allowed to cool to room temperature for 3 h and centrifuged at 300 rpm for 20 min. The supernatants were filtered through a 0.22 µm glass fiber filter and transferred into pre-cleaned 50 mL polypropylene centrifuge tubes. The samples were kept at 4° C in a cold room for less than 48 h before analysis. Before the HPLC-ICP-MS analysis, the samples were diluted five times (1:5) and adjusted to pH 3 with ammonium hydroxide. Speciation analyses were conducted by coupling a Thermo Scientific Dionex Ultimate 3000 HPLC System with a Thermo Scientific, iCap ICP-MS. The HPLC was equipped with a six-port injection valve fitted with a 100-µL sample loop and an analytical column Hypersil Gold C18 (150 mm × 4.6 mm, 5µm Hypersil Gold™ Thermo Scientific, USA). The outlet of the chromatographic column was directly connected to the concentric nebulizer of the ICP-MS with perfluoroalkyl (PFA) tubing. 100 mL of the filtered diluted sample was injected into the HPLC. The mobile phase was composed of 30% methanol, ultrapure water (deionized, ≥18 MW-cm), 0.06 M of ammonium acetate, and 0.1% mercaptoethanol. The column temperature was set to 30 °C, and the mobile phase flow rate was 1 mg/min. After chromatographic separation, Hg was detected by ICP-MS at mass-to-charge ratios 200 and 202 (<sup>200</sup>Hg and <sup>202</sup>Hg), choosing <sup>202</sup>Hg to validate the methodology. Reference material NIST SRM 1946 Lake Superior fish tissue was prepared periodically and used for peak identification and separation in the HPLC, as well as for evaluating the precision and accuracy. The validation of the extraction method and the speciation analysis by the HPLC-ICP-MS were performed in collaboration with the INECC, under strict quality control and quality assurance established by the person responsible for the ICP-MS laboratory. The Eurachem guide was used to evaluate the method's performance (Cantwell 2025). The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using 10 replicate measurements of blank matrix containing no detectable Hg or MeHg. The following equations were used:

$$\text{LOD} = 3 \times \sigma'_0 \text{LOQ} = kQ \times \sigma'_0$$

$$\sigma = \sqrt{\frac{\sum (X_i - \bar{X})^2}{n-1}}; \sigma'_0 = \sigma \sqrt{\frac{1}{n}}$$

Where:  $\sigma$  is the standard deviation;  $\sigma'_0$  is the standard deviation for LOD and LOQ calculations;  $X_i$  is the  $i$  value,  $\bar{X}$  with bar is the average, and  $n$  is the number of observations. The method's trueness and precision were evaluated by measuring 10 independent spiked blank matrices.

Trueness was calculated using the following equation:

$$\%R = \left( \frac{\bar{X}}{X_{\text{spike}}} \right) \times 100$$

Where: % R is the relative per cent recovery,  $\bar{X}$  with bar is the average, and  $X_{\text{spike}}$  is the blank matrix spiked with the analyte at a concentration in the middle of the working range curve.

Precision was calculated using the relative standard deviation (RSD):

$$\text{RSD} = \left( \frac{\sigma}{\bar{X}} \right) \times 100$$

Where:  $\sigma$  is the standard deviation and  $\bar{X}$  with bar is the average.

Finally, standard reference material NIST SRM 1946 Lake Superior fish tissue was used to evaluate the accuracy by calculating the percentage recovery, and a t-test was used to assess whether a significant difference exists between the total concentration ( $\text{Hg}_{\text{Tot}}$ ) obtained from Q-ICP-MS and the speciated concentration ( $\text{Hg}_{\text{In}} + \text{MeHg}$ ) obtained by HPLC-ICP-MS. A null hypothesis was formulated, assuming no significant difference in the average total mercury concentrations for two fish species (*Cathorops fuerthii* and *Sphyraena ensis*) determined by the two methods (Q-ICP-MS and HPLC-ICP-MS).

### Mercury content in fish tissue worldwide

The MeHg :  $\text{Hg}_{\text{Tot}}$  proportion obtained in this study was compared with those reported in the literature for different fish species in Mexico and worldwide. We point out that the current percentage used worldwide for the indirect estimation of MeHg based on  $\text{Hg}_{\text{Tot}}$  concentrations is 90%. Reported values of  $\text{Hg}_{\text{Tot}}$  and MeHg around the world (Li et al. 2010, Polak-Juszczak 2018, Nogara et al. 2019, Charette et al. 2021, Santos et al. 2021, Vergara et al. 2024) and in Mexico (Ruelas-Inzunza et al. 2011, Grosbois 2013, Basu et al. 2014, Delgado-Alvarez et al. 2017, Zamora-Arellano et al.

2017, García-Hernández et al. 2018, Ramírez-Islas et al. 2018, Murillo-Cisneros et al. 2021) were reviewed. These values were compared with the  $\text{Hg}_{\text{Tot}}$  and MeHg values obtained in this study. Publications from 2010 to 2022 were used. The reported values and references are available in **Appendix 1** (<https://www.revistascca.unam.mx/rica/SupplementaryMaterial/55712-Hernandez-SupMat.xlsx>).

### Human health risk assessment

A health risk assessment was conducted using the methodology developed by the USA Environmental Protection Agency (USEPA 1989, USEPA 2011). This methodology evaluates the non-carcinogenic risks associated with MeHg exposure resulting from involuntary ingestion. The  $\text{HQ}_{\text{MeHg}}$  was calculated using the average daily dose for MeHg from fish ( $\text{ADD}_{\text{MeHg}}$ ) and the reference dose for MeHg ( $\text{RfD}_{\text{MeHg}}$ ), which indicates the tolerable daily intake of MeHg for non-cancer threshold.

The  $\text{HQ}_{\text{MeHg}}$  was obtained through the following equations:

$$\text{HQ} = \frac{\text{ADD}}{\text{RfD}} \quad (2)$$

According to IRIS (USEPA 2001) the  $\text{RfD}_{\text{MeHg}}$  is 0.0001 mg/kg-day and the  $\text{ADD}_{\text{MeHg}}$  was calculated using the following equation:

$$\text{ADD} = \frac{C \times \text{IR} \times \text{EF} \times \text{ED}}{\text{BW} \times \text{AT}} \quad (3)$$

Where  $C$  is the MeHg concentration in the sample of fish tissue obtained in this study ( $\mu\text{g/g}$ ) and for reviewed data MeHg was estimated as 82% of  $\text{Hg}_{\text{Tot}}$ ;  $\text{IR}$  is the ingestion rate based on the average consumption of fish for the Mexican population calculated by Murillo-Cisneros et al. (2021) for adult women of reproductive age (16 - 30 years old) and Ruelas-Inzunza et al. (2011) for children  $\leq 6$  years old (35.7 and 25.0 g of fish per day, respectively);  $\text{EF}$  is the exposure frequency (260 days per year);  $\text{ED}$  is the exposure duration (30 years for women and 6 years for children);  $\text{BW}$  is the body weight (60 kg for adult women, 15 kg for children), and  $\text{AT}$  represents the average exposure time (10 950 days for women and 2 190 days for children, USEPA 2011).

## RESULTS

### $\text{Hg}_{\text{Tot}}$ by Q-ICP-MS in fish tissue

**Table I** shows the values of  $\text{Hg}_{\text{Tot}}$  dry weight (DW), the percentage of humidity calculated per fish

species (% H), and the values of  $Hg_{Tot}$  wet weight (WW). Among the three fish species analyzed, *Sphyrana ensis* (common name Barracuda) presented the lowest average  $Hg_{Tot}$  concentration ( $0.25 \mu\text{g/g} \pm 0.05$ ). In contrast, the species with the highest average concentration of  $Hg_{Tot}$  were *Cathorops fuerthii* (common name catfish or bagre) and *Caranx caninus* (common name horse mackerel or jurel) with average  $Hg_{Tot}$  values of  $0.70 \pm 0.03$  and  $0.68 \pm 0.07 \mu\text{g/g}$ , respectively. When these values are compared with the Maximum Permissible Limits (MPL) of MeHg accepted in Mexico (SSA1 2009) for predatory species like tuna, marlin, mero, and bonito (MPL1 =  $1 \mu\text{g/g}$ ), and for the retail fish in general (MPL2 =  $0.5 \mu\text{g/g}$ ), both *C. fuerthii* and *C. caninus* were

above the MLP2. Although normativity does not specify how to measure MeHg concentration, the most used pathway is to measure  $Hg_{Tot}$  and consider the 90% as MeHg (EFSA 2012, Murillo-Cisneros et al. 2021). Nevertheless, even using that proportion, both species (*C. fuerthii* and *C. caninus*) remain above the MPL2 at 0.63 and  $0.61 \mu\text{g}_{MeHg}/\text{g}$ , respectively.

The fish species examined in this study are among the most highly consumed in Mexico.

**Figure 1** compares the  $Hg_{Tot}$  ( $\mu\text{g/g}$ ) results from this study on wet weight (WW) with values reported in the literature for Mexico. *Caranx caninus* and *Cathorops fuerthii* presented average total mercury concentrations of 0.68 and  $0.70 \mu\text{g/g}$  WW, respectively. In regions with high daily fish consumption, such as Alaska, MeHg exposure is typically assessed using the risk-based threshold MPL3 of  $0.2 \mu\text{g/g}$  MeHg (wet weight) for unrestricted fish consumption, as suggested by the Alaska Department of Health (Hamade 2014). This threshold is derived from the reference dose (RfD) established by the US Environmental Protection Agency, which represents the MeHg concentration at which unrestricted daily fish consumption is considered safe under standard exposure assumptions (USEPA 2001). In this context, *Sphyrana ensis* had an average  $Hg_{Tot}$  of  $0.25 \pm 0.05 \mu\text{g/g}$ , exceeding this threshold and suggesting the need for fish consumption guidance based on human health risk rather than on regulatory limits.

Generally, the  $Hg_{Tot}$  concentrations found in previous studies remain below the MPL1 and 2. However, values over MPL2 were found in Yucatán, Veracruz, Sinaloa, Sonora, and Mexico City. This study found that two species of retail fish from the SCMM exceeded the recommended MeHg level (MPL2) set by Mexico (SSA1 2009), which is also applied by Canada and the USA for total mercury in retail fish, highlighting international policy implications (HC 2023, USAFDA 2007, respectively).

It is important to note that Mexico City registered the highest values previously reported, from 0.63 to  $1.65 \mu\text{g/g}$   $Hg_{Tot}$  for retail fish and canned tuna (Basu et al. 2014), while studies in Sinaloa have reported a range of  $Hg_{Tot}$  values of 0.57 to  $1.43 \mu\text{g/g}$  in retail fish (Soto-Jiménez et al. 2010, Ruelas-Inzunza et al. 2011, Zamora-Arellano 2017, Ramírez-Islas et al. 2018). Sonora presented one value of  $0.68 \mu\text{g/g}$   $Hg_{Tot}$  (Grosbois 2013), and Veracruz state reported a value of  $1.89 \mu\text{g/g}$  of  $Hg_{Tot}$  for *Calamus brachysomus* species from the capital market (Grosbois 2013). **Figure 1** shows that the results obtained in this study were above the MPL3, as well as 50% of the values

**TABLE I.** TOTAL MERCURY CONCENTRATION IN THREE FISH SPECIES OBTAINED BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (DATA FROM TWELVE SAMPLES, INCLUDING DUPLICATES). Bg, Bc, AND J REPRESENT THE COMMON LATIN AMERICAN NAMES (BAGRE, BARRACUDA, AND JUREL, RESPECTIVELY). DW: DRY WEIGHT. WW: WET WEIGHT. THE NUMBERS 1, 2, AND 4 REPRESENT THE DIFFERENT SALE POINTS. MD REPRESENTS A DUPLICATE OF A SAMPLE IN THE SAME SALE POINT.

| ID                        | DW<br>μg/g | Humidity<br>(%) | WW<br>mg/g |
|---------------------------|------------|-----------------|------------|
| <i>Cathorops fuerthii</i> |            |                 |            |
| Bg-1                      | 2.65       | 74.55           | 0.67       |
| Bg-2                      | 2.44       |                 | 0.62       |
| MD Bg-1                   | 2.94       |                 | 0.75       |
| Bg-4                      | 2.95       |                 | 0.75       |
| X                         | 2.75       |                 | 0.70       |
| s                         | 0.25       |                 | 0.06       |
| <i>Sphyraena ensis</i>    |            |                 |            |
| Bc-1                      | 0.73       | 75.34           | 0.18       |
| Bc-2                      | 1.03       |                 | 0.18       |
| MD Bc-1                   | 0.73       |                 | 0.25       |
| Bc-4                      | 1.53       |                 | 0.38       |
| X                         | 1.01       |                 | 0.25       |
| s                         | 0.38       |                 | 0.09       |
| <i>Caranx caninus</i>     |            |                 |            |
| J-1                       | 2.25       | 74.29           | 0.58       |
| J-2                       | 2.53       |                 | 0.64       |
| MD J-1                    | 2.38       |                 | 0.61       |
| J-4                       | 3.38       |                 | 0.87       |
| X                         | 2.63       |                 | 0.68       |
| s                         | 0.51       |                 | 0.13       |

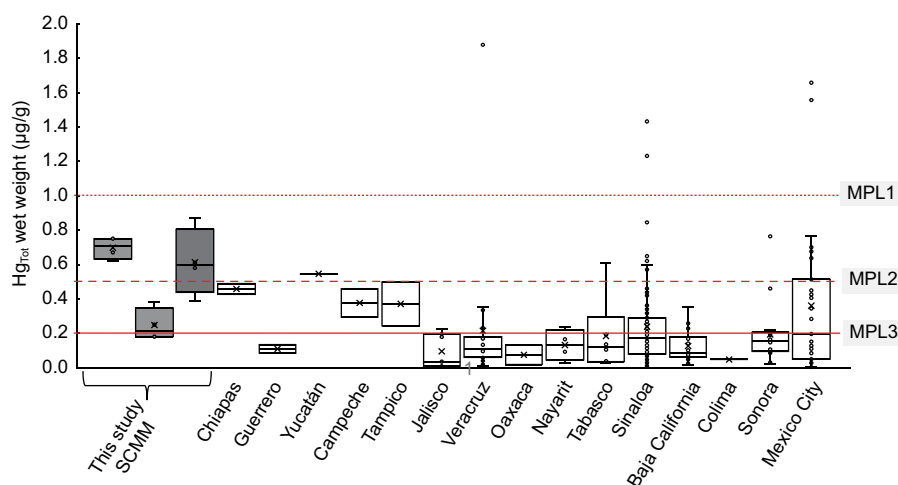


**Fig. 1.** Total mercury concentration ( $Hg_{Tot}$ ) in fish tissue obtained in this study and previously data reported in Mexican localities. Maximum Permissible Limit (MPL) 1 and 2 are the recommended values for MeHg in predatory and retail fish, respectively, from Mexico, Canada, and USA. MPL3 is the most conservative threshold concentration ( $0.200 \mu gMeHg/g$ ), recommended by the Alaska Department of Health (Hamade 2014). The box in the chart represents the interquartile range (25 to 75<sup>th</sup> percentile), the line inside the box represents the median value, and the bars represent the range of the data, minimum and maximum, excluding the outliers (points out of the range).

previously reported for Mexico City and Sinaloa, and 25% of the values reported for Sonora. However, it remains unclear what proportion of those retail fish represents a significant source of MeHg.

#### Mercury speciation in fish tissue via HPLC-ICP-MS HPLC-ICP-MS validation

**Figure 2A** displays the chromatograms obtained for reference material NIST SRM 1946 Lake Superior

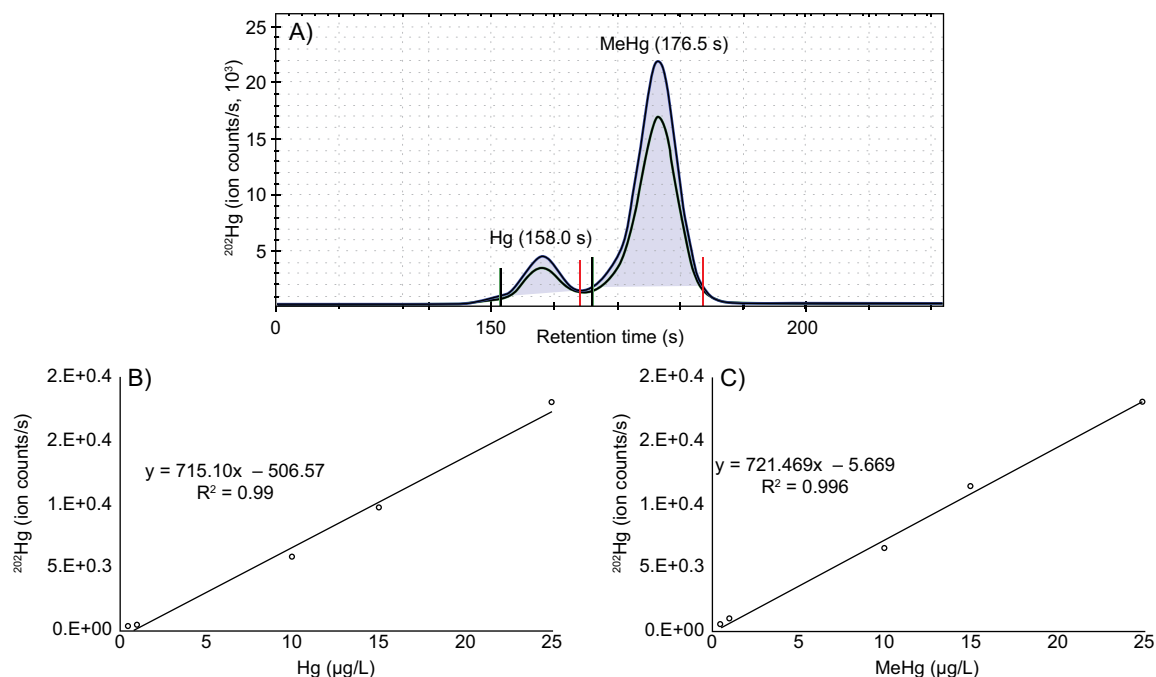


**Fig. 2.** A). Chromatograms for the Standard Reference Material NIST from the National Institute of Standards, Lake Superior Fish Tissue (NIST SRM 1946), showing the retention time for Hg and MeHg; B) and C) are the working curves for Hg and MeHg, respectively.

Fish Tissue, where the peak retention time for Hg was 158.0 s and 176.5 s for MeHg. Peak bases were delimited in the HPLC-ICP-MS program to set the area under the curve. As shown in **figures 2B** and **2C**, the working range for calculating Hg and MeHg concentrations was 1 to 25 µg/L, with an acceptance criterion of  $R^2 > 0.995$ . **Table II** shows the validation experimental parameters: limits of detection (LOD) and quantification (LOQ), working range, recovery percentages, and % RSD for spiked matrix for Hg and MeHg. After validation, the methodology was used to analyze MeHg concentrations in the NIST Standard Reference Material (SRM) 1946 and in muscle samples from three fish species. The accuracy of measurements for the NIST SRM® 1946 was  $103.0\% \pm 2.8$  for Hg<sub>Tot</sub> and  $101.6\% \pm 3.32$  for MeHg.

**Table III** shows the MeHg concentrations obtained by HPLC-ICP-MS for the fish species studied

**TABLE II.** VALIDATION PARAMETERS FOR MERCURY SPECIATION USING INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY COMBINED WITH HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY.

| Parameter     | (µg/L)   |            | (µg/g)     |            |
|---------------|----------|------------|------------|------------|
|               | Hg       | MeHg       | Hg         | MeHg       |
| LOD           | 0.05     | 0.06       | 0.01       | 0.01       |
| LOQ           | 0.48     | 0.79       | 0.10       | 0.05       |
| Working range | 1 – 25.0 | 0.8 – 25.0 | 0.10 – 3.0 | 0.05 – 3.0 |
| % R           | 105.76   | 112.06     | 88.93      | 109.17     |
| % RSD         | 5.47     | 4.93       | 3.39       | 6.24       |

LOD: limit of detection. LOQ: limit of quantification. % R: relative per cent recovery. % RSD: relative standard error.

**TABLE III.** CONCENTRATIONS OF INORGANIC MERCURY (Hg<sub>in</sub>) AND METHYL-MERCURY (MeHg) IN THREE HIGHLY CONSUMED FISH SPECIES IN MEXICO. MAXIMUM PERMISSIBLE LIMIT (MPL) 1 AND 2 ARE THE RECOMMENDED VALUES FOR MeHg IN PREDATORY AND RETAIL FISH, RESPECTIVELY, FROM MEXICO, CANADA, AND USA.

| D                         |                  |      |                         | WW   |                         | MeHg (%) |
|---------------------------|------------------|------|-------------------------|------|-------------------------|----------|
| ID                        | Hg <sub>in</sub> | MeHg | Hg <sub>in</sub> + MeHg | MeHg | Hg <sub>in</sub> + MeHg |          |
|                           |                  | µg/g |                         |      | µg/g                    |          |
| <i>Cathorops fuerthii</i> |                  |      |                         |      |                         |          |
| Bg-1                      | 0.45             | 1.83 | 2.28                    | 0.46 | 0.57                    | 80.20    |
| Bg-2                      | 0.50             | 1.72 | 2.22                    | 0.43 | 0.56                    | 77.50    |
| X                         | 0.48             | 1.78 | 2.25                    | 0.45 | 0.57                    | 78.85    |
| s                         | 0.04             | 0.08 | 0.04                    | 0.02 | 0.01                    |          |
| <i>Sphyraena ensis</i>    |                  |      |                         |      |                         |          |
| Bc-1                      | 0.19             | 0.62 | 0.81                    | 0.16 | 0.20                    | 76.50    |
| Bc-2                      | 0.10             | 0.74 | 0.84                    | 0.19 | 0.21                    | 88.10    |
| X                         | 0.15             | 0.68 | 0.83                    | 0.18 | 0.21                    | 82.30    |
| s                         | 0.06             | 0.08 | 0.02                    | 0.02 | 0.01                    |          |
| <i>Caranx caninus</i>     |                  |      |                         |      |                         |          |
| J-3                       | 1.07             | 1.29 | 2.36                    | 0.32 | 0.59                    | 54.70    |
| J-2                       | 1.08             | 1.36 | 2.44                    | 0.34 | 0.61                    | 55.74    |
| X                         | 1.08             | 1.33 | 2.40                    | 0.33 | 0.60                    | 55.22    |
| s                         | 0.01             | 0.05 | 0.06                    | 0.01 | 0.01                    |          |
| MPL1                      |                  |      |                         |      |                         |          |
| Predatory fish            |                  |      |                         | 1    |                         |          |
| MPL2                      |                  |      |                         |      |                         |          |
| Retail fish               |                  |      |                         | 0.5  |                         |          |

DW: dry weight; WW: wet weight; X: mean; s: standard deviation. Bg, Bc, and J represent the common Latin American names (bagre, barracuda, and jurel, respectively). The numbers 1, 2, and 3 represent the different sale points.

(two previously  $Hg_{Tot}$ -analyzed tissue samples from each species were selected). Additionally, **table III** shows the inorganic mercury concentration ( $Hg_{In}$ ) and the sum of both mercury forms ( $Hg_{In} + MeHg$ ) in dry and wet weight. It can be observed that *Caranx caninus* had the highest total mercury average content by the sum of  $Hg_{In} + MeHg$  with a value of  $0.60 \pm 0.01 \mu g/g$  WW. However, it can be noticed that the MeHg percentage (% MeHg) obtained for this species had the lowest average value among all the species analyzed (55.2%). **Table III** also shows that *Sphyraena ensis* had the lowest  $Hg_{In} + MeHg$  average value ( $0.21 \pm 0.01 \mu g/g$  WW) but the highest average percentage of MeHg (82.3%). For *Cathorops fuerthii*, an average total mercury content of  $0.57 \pm 0.01 \mu g/g$  WW and a % MeHg average of 78.9% were obtained. MeHg WW content was below the MPL2.

On the other hand, **table IV** compares the average of  $Hg_{Tot}$  obtained by Q-ICP-MS with the average of the sum of organic and inorganic Hg obtained by HPLC-ICP-MS for two species: *Cathorops fuerthii* and *Sphyraena ensis*. The t-test was used to observe if there was a significant difference in the average of total Hg concentration obtained from both methods. The t-values from the t-test for both species were lower than the critical values. Additionally, the p-values are more significant than the significance levels when comparing the results of each species, indicating that no significant difference exists between the average total concentration of Hg obtained by Q-ICP-MS and the sum of inorganic and organic mercury obtained by HPLC-ICP-MS.

**TABLE IV.** T-TEST COMPARING  $Hg_{Tot}$  RESULTS OBTAINED BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS) VERSUS THE SUM OF  $Hg_{In} + MeHg$  CONCENTRATIONS MEASURED BY ICP-MS COUPLED TO HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY.

|                    | <i>Cathorops fuerthii</i> | <i>Sphyraena ensis</i> |
|--------------------|---------------------------|------------------------|
| Difference         | 0.025                     | -0.080                 |
| t-value            | 5                         | -4                     |
| Critical value     | 12.706                    | 12.706                 |
| Degrees of freedom | 1                         | 1                      |
| p-value            | 0.126                     | 0.156                  |
| Significance level | 0.05                      | 0.05                   |

**Figure 3A** compares the percentage of MeHg content (% MeHg) found in this study via HPLC-ICP-MS with those reported worldwide (Mexico included),

obtained by different analytical methods. It can be observed that the reported % MeHg varies from 19 to 100%. The lowest percentages were found in the species *Hilsa kelee* (19%), *Ilisha indica* (25%), and *Ilisha filigera* (27%) of anadromous fish from Bangladesh (Holsbeek et al. 1997) obtained by electron capture detection gas chromatography (ECD-GC), while the highest MeHg percentages reported (100%) were found in the species *Caranx caninus*, *Elops affinis*, *Thunnus albacares* from Sinaloa, Mexico, obtained by cold vapor atomic-absorption (CV-AA) and *Saurida tumbil*, from the Persian Gulf obtained by headspace gas chromatography (Agah et al. 2007, Ruelas-Inzunza et al. 2011, respectively). However, CV-AA is not a species-specific analytical method, and MeHg might be overestimated, suggesting that speciation analysis using HPLC-ICP-MS is a more accurate and reliable methodology, as supported by recent researchers (Ferreira et al. 2024).

The statistical distribution of the reported MeHg content worldwide, including the results obtained in this study (**Fig. 3B**), indicates that the average percentage of MeHg in fish muscle is 75.67% ( $n = 50$ ), the median value is 82.3%, and only the third quartile of fish reviewed has values  $\geq 91.7\%$ . It is important to note that standard techniques measure total mercury concentration and estimate MeHg content; however, this study shows that using a 90% factor to estimate the percentage of MeHg from total Hg leads to an inaccurate estimate of the true MeHg content. This observation emphasizes the importance of using an appropriate speciation methodology to accurately determine the concentration of MeHg in fish tissue consumed by the population.

Among specialized techniques, it may be believed that HPLC-ICP-MS is expensive and time-consuming, or that it has a high detection limit for Hg speciation analysis. However, Ferreira et al. (2024) recently reviewed the best analytical techniques for quantifying chemical elements in fish tissue (including mercury). Ferreira et al. (2024) discuss the advantages and disadvantages of using several analytical techniques, such as inductively coupled plasma optical emission spectrometry (ICP-OES), atomic absorption spectrometry with electrothermal atomization (ETAAS), atomic fluorescence spectrometry (AFS), liquid chromatography and atomic fluorescence spectrometry (LC-AFS), and direct mercury analyzers for accurate mercury determinations. The authors emphasize that among the available techniques for determining organic mercury in fish samples, ICP-MS coupled with HPLC is the most recommended technique due to

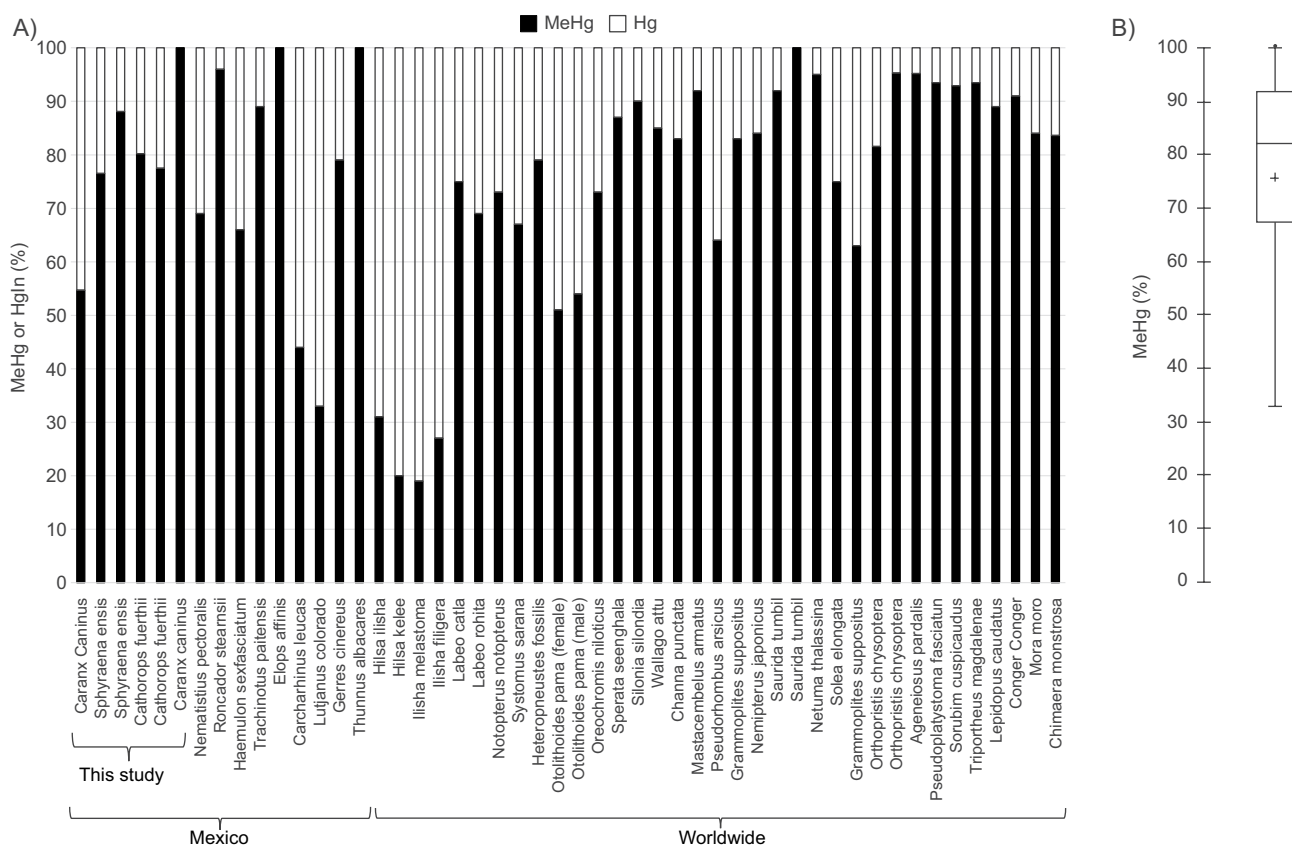


**Fig. 3** A), Percentage of MeHg and HgIn in fish tissue; B) Box plot of worldwide MeHg percentage; both in wet weight. The box plot in the chart represents the interquartile range (25 to 75<sup>th</sup> percentile), the line inside the box represents the median value, the mark represents the average value, and the bars represent the range of the data, minimum and maximum, excluding the outliers (point out of the range).

its sensitivity, very low detection limits, and multi-element capacity (Ferreira et al. 2024), which agrees with our observations.

### Assessment of human health risk

The Seafood Central Market of Mexico City supplies 120 million people across the entire country and exports large quantities of fish worldwide. The Secretaría de Agricultura y Desarrollo Rural (SADER 2024) reported the number of fish caught in Mexico by state. In 2024, the states with the highest fish production were: Sonora (656 552 t), Baja California (334 739 t), Sinaloa (320 944 t), Baja California Sur (197 105 t), Campeche (42 494 t), Veracruz (41 010 t), Yucatán (36 420 t), Colima (33 095 t), Tamaulipas (29 160 t), and Chiapas (20 407 t). Some of these states show a distribution of fish species with high mercury levels, likely containing a high proportion of MeHg, posing a health risk to the population that governments must address.

In the present work, the human health risk from a non-carcinogenic neurotoxic pollutant in fish tissue was determined using the accepted USEPA methodology. The risk of exposure to MeHg was estimated by assuming ingestion as the only route of exposure from consuming fish contaminated with mercury, based on reported values from scientific publications published from 2010 to 2024.

**Figure 4** shows the average hazard quotient for MeHg exposure ( $HQ_{MeHg}$ ) in Mexico by state, based on the median value of MeHg obtained in this study (82.3%), and the results of MeHg previously reported for two different vulnerable population groups: 1) children  $\leq 6$  years old with an average consumption of 175 g of fish tissue per week and a body weight  $\leq 15$  kg (**Fig. 4A**); and 2) women of reproductive age (16 – 30 years old) with an average consumption of 250 g of fish per week (**Fig. 4B**).

For the first case, an  $HQ_{MeHg} > 1$  was observed in 12 states. This suggests that in Sinaloa, Sonora,

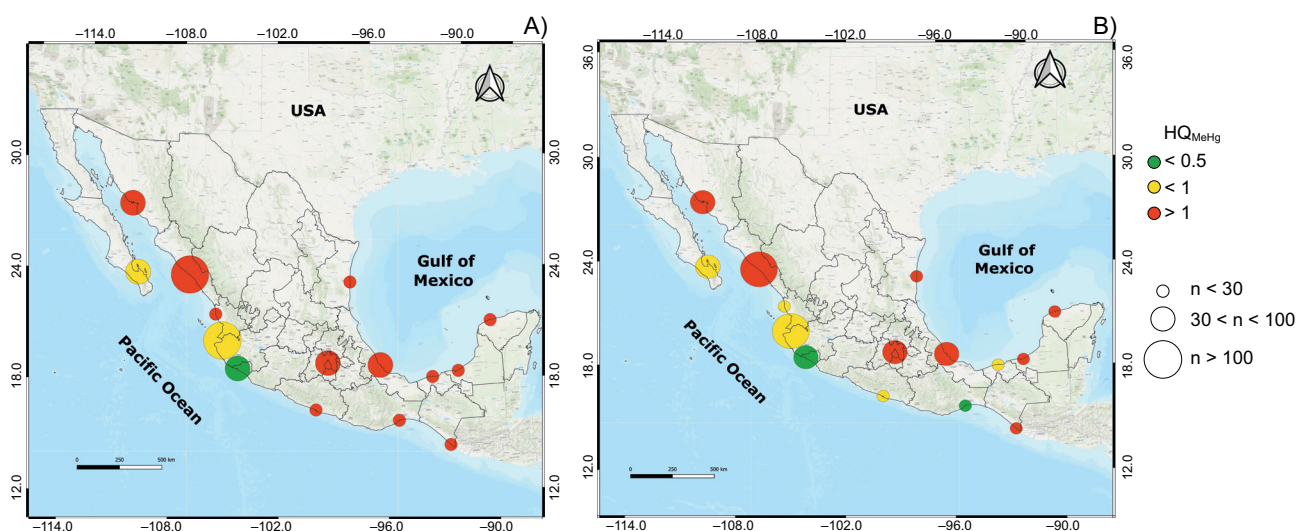


**Fig. 4.** Average human health risk values by exposure to MeHg through consumption of fish in Mexico. A)  $HQ_{MeHg}$  for children  $\leq 6$  years with consumption of 175 g of fish per week; B)  $HQ_{MeHg}$  for women between 16 – 30 years with consumption of 250 g of fish per week;  $n$  is the number of mercury data (reported values are in Appendix 1). The results of MeHg obtained in this study were used, and a percentage of 82.3% was considered to convert  $Hg_{Tot}$  to MeHg for previous reported data.

Veracruz, and Mexico City, there may be a risk of adverse effects in children aged 6 years and younger from consuming fish containing MeHg. These findings are consistent with those of Zamora-Arellano et al. (2017), who reported a high risk of Hg exposure ( $HQ_{Hg} = 10.9$ ) among children from Sinaloa who frequently consumed canned tuna and retail fish. On the other hand, **figure 4B** shows the risk for the second case, women of reproductive age. It can be observed that eight locations present  $HQ_{MeHg} > 1$ . The locations with higher  $HQ_{MeHg}$  were Veracruz (max  $HQ_{MeHg} = 8.6 \pm 0.42$ ), Mexico City (max  $HQ_{MeHg} = 7.6 \pm 0.63$ ), Sinaloa (max  $HQ_{MeHg} = 5.7 \pm 0.30$ ), and Sonora (max  $HQ_{MeHg} = 3.5 \pm 0.39$ ). During pregnancy, the most significant concern is the direct transmission of MeHg to the fetus. Our observations agree with other authors. For example, Zamora-Arellano et al. (2017) found that females from the fishing-related population in Sinaloa present an average  $HQ_{MeHg}$  of 1.89; García-Hernández et al. (2018) reported that females of reproductive age from coastal communities of Sonora presented an average  $HQ_{MeHg}$  of 3 up to 16.98.

Two studies contrast with our observations: Ruelas-Inzunza et al. (2011) and Spanopoulos-Zarco et al. (2014), which reported that exposure to a variety of retail fish from Sinaloa and the coast of Guerrero (respectively) resulted in lower  $HQ_{Hg}$  values ( $< 1$ ). However, it's important to note that those studies assumed an average daily fish consumption of 25 grams

(9 kilograms per year). The actual annual average fish consumption in Mexico is 12 to 13 kilograms, according to the Centro de Investigación en Alimentación y Desarrollo (CIAD 2024).

The key factors influencing the magnitude of HQ are the MeHg concentration in fish tissue, the ingestion rate, exposure frequency, body weight, and exposure duration. In this study, we calculate the HQ for two vulnerable groups: women of reproductive age (30 years old) with a body weight of 60 kg, and an ingestion rate of 35.7 g of fish per day; and children aged 6 years or younger, with a body weight of 15 kg and an ingestion rate of 25.0 g of fish per day. The magnitude and variability of reported HQs depend mainly on the MeHg concentration used as input and on the exposure parameters such as the body weight. We point out that fishermen and their families in coastal communities who base their diet on fish consumption ( $> 12$  kg per year) could face an actual threshold for MeHg exposure.

### Limitations

Although it might appear that this study uses an insufficient number of samples to assess the human health risk from seafood products, our research was designed in two stages. First, we made an initial exploration to highlight the assessment inaccuracies of using a 90% adjustment factor to estimate MeHg concentration in fish consumed worldwide. The second stage involves a large-scale sampling

campaign to assess MeHg levels in the most consumed fish species. The present study is the first to validate a methodology for accurately quantifying MeHg in fish tissue using HPLC-MS-ICP-MS in Mexico. We examined three highly consumed fish species (nine samples and three duplicates) collected from the Seafood Central Market of Mexico City (the second-largest seafood distributor in the world). Despite the number of samples, our results align with previous studies worldwide and highlight the need to use more robust Hg speciation analytical techniques, such as HPLC-ICP-MS. We demonstrate that accurately determining MeHg content requires rigorous methodology and validated techniques rather than simply relying on a large number of samples, as we contrast with the study published by Kennedy et al. (2025), who estimated methylmercury contamination in Mediterranean seafood using the 90% factor, based on 17 964 Hg<sub>Tot</sub> previously published data.

Furthermore, this study marks the beginning of a future modification to the environmental regulations regarding the methods used to determine MeHg concentrations in fish tissue. This initiative represents a collaboration between environmental authorities and scientific experts to fulfill an international commitment to mitigate the harmful effects on human health caused by mercury exposure, addressing the Minamata Convention. Our approach and findings will be the basis for future studies.

## CONCLUSIONS

This work has validated the levels of total mercury and methylmercury in three species of retail fish from the international Seafood Central Market of Mexico City using HPLC-ICP-MS. We compare Hg<sub>Tot</sub> obtained by Q-ICP-MS with the sum of Hg<sub>in</sub> and MeHg concentrations determined by HPLC-ICP-MS, analyzed in two different laboratories and using two different mass spectrometers. We observed no significant difference between the total mercury concentration and the combined amounts of Hg<sub>in</sub> and MeHg in fish tissue, indicating that MeHg is the main organic compound in fish tissue affecting human health.

The percentage of MeHg reported in this study is consistent with previously reported values. Furthermore, based on the results of this study and previously reported data, we point out that the median value of the MeHg content in fish muscle worldwide is 82.3%. Therefore, assuming an adjustment factor of 90% of Hg<sub>Tot</sub> leads to an incorrect assessment of the risk posed by MeHg from consuming fish. Although

some MeHg concentrations in the fish species studied here remain below the MPL1 and MPL2, certain coastal regions of Mexico with high fish consumption may exceed the MeHg dose recommended by EPA ( $RfD_{MeHg} = 1 \times 10^{-4}$  mg/kg-day). Because fishermen and their families regularly consume fish, some coastal states in Mexico (such as Sinaloa, Sonora, and Veracruz) and the areas where this production is delivered (such as Mexico City) may face a high risk of exposure to methylmercury.

Considering the global commercial significance of the SCMM and recent international research, we emphasize that millions of people worldwide may be at risk of involuntarily consuming MeHg through seafood. Based on the findings here, the authors recommended that pregnant women and children aged  $\leq 6$  years limit consumption of fish species with high Hg<sub>Tot</sub> concentrations ( $> 0.200$   $\mu\text{g/g}$ ) to one portion of  $\leq 175$  g WW per week. The second stage of this study will launch a retail fish monitoring campaign that involves testing various marine and freshwater fish species to determine the accurate MeHg concentration using this speciation methodology (HPLC-ICP-MS) and raise awareness among authorities to establish new Maximum Permissible Limits for MeHg in fish tissue, as well as recommend daily intake based on fish species.

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