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Assessing Dietary Exposure of Potentially Toxic Elements via Fish Consumption

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ABSTRACT

This study aims to assess the consumer health risk caused by metals in fish produced in Armenia. The collected fish samples were analyzed for copper, lead, molybdenum, and arsenic via the atomic-absorption spectrometry (AAS) method. Clusters of consumers were created after conducting public surveys and analyzing data. Risk assessment was done based on the Margin of Exposure (MOE). The results indicated that the consumer health risk is within allowable limits. Moreover, the study identified factors that influence the toxicity level of the product, pointing to a necessity for a more comprehensive approach to risk assessment.

Introduction

Food safety is a worldwide challenge significantly affecting trade and public health. Thus, countries need to implement effective food control systems to prevent food safety hazards and protect the population (WHO, 2022). Generally, surveillance programs are being conducted to assess the levels of various chemical hazards (e.g., potentially toxic trace elements) in consumed products to guarantee their conformity to national and international food safety requirements. The annual residue monitoring program currently being implemented in Armenia includes the detection of potentially toxic element contents in foods of animal origin. In 2019, in the frame of the monitoring program, fish samples were analyzed for four potentially toxic trace elements (PTEs): copper (*Cu*), molybdenum (*Mo*), lead (*Pb*), and arsenic (*As*). Among the studied PTEs, Mo and Pb were not detected (i.e., concentrations were below the LOD-limit of detection), meanwhile concentrations detected for *As* and *Cu* were reported.

In the Middle Ages, As was known as “the king of poisons” (Gupta, et al., 2017). This element has a toxic effect on living organisms and is included by the WHO in the list of 10 problematic and hazardous chemical elements (WHO, 2018).

The major sources of As exposure for humans are water and food. Plants take up *As* from soil, productive animals – from water and feed and particularly fish flour. Seafood build up significant amounts of As, some 90-95 % of which belongs to organic *As* (Berntssen, et al., 2021, Pei, et al., 2019). However, there are some exceptions: hijiki algae may contain up to 60 % of inorganic *As* (EFSA, 2009, Camurati and Salomone, 2020). Besides being highly toxic, inorganic *As* also has carcinogenic properties.

The compounds of inorganic arsenic pose no hazard but are recognized as potentially carcinogenic. (Berntssen, et al., 2021, EFSA, 2009, Camurati and Salomone, 2020).

Chronic exposure to *As* may cause skin lesions, neurotoxicity, anemia, diabetes, cancer (Berntssen, et al., 2021, IARC, 2004). Arsenic is also able to traverse placental barrier (Berntssen, et al., 2021, EFSA, 2009) and thus produce fetal malformations. Acute intoxication causes vomiting, diarrhea, convulsions, ataxia, in severe cases-lethal outcomes (IARC, 2004, Gupta, et al., 2021, Zhong, et al., 2019). A lethal dose of *As* for adults is estimated as 120-200 mg, for kids is 2 mg/kg (Jain and Chandramani, 2018).

Copper is an essential micro element, its presence in the organism supports the normal functioning of enzymes and systems of organs (Rehman, et al., 2018). Sixty-five percent of this element in the diet comes from marine food, cereals, potato and liver (CAC, 2016). Insufficient intake of *Cu* leads to its deficiency in the organism characterized by flabbiness of skin, muscle weakness, neurogenic symptoms, elevated risk of cardiovascular diseases (EFSA, 2015). Excessive *Cu* exposure also has a negative effect (Kim, et al., 2019). It can cause ictericity of mucous membrane, reproductive dysfunctions, hemoglobinuria, and apathy (Gupta, et al., 2021, CAC, 2016, Kim, et al., 2019). Since copper homeostasis is associated with other elements, its disturbance may be secondary, for instance, due to excess of zinc or deficiency of *Mo* (Gupta, et al., 2021, EFSA, 2015).

Mammals are known to have different mechanisms of *Cu* homeostasis; this element is easily excreted from the organism. In most cases, an excess or a deficiency of *Cu* is observed during genetic disturbances influencing *Cu* regulation or under specific conditions: pregnancy, physical loads, stress (Gupta, et al., 2021, EFSA, 2015, Kim, et al., 2019, Burkhead and Collins, 2022, Malhotra, et al., 2020). The lethal effect in adults occurs at a per oral dose of *Cu* 200 mg/kg (CAC, 2016).

Based on the aforementioned information, it's clear that both PTEs can have adverse effects on human health. Whether an element in food will harm the organism or not depends on a certain element and its daily intake (Nepovimova and Kuca, 2019). Hence, this study aims to assess the consumer health risk caused by two PTEs (*As* and *Cu*) in fish produced in Armenia.

Materials and methods

Sampling

Fish sampling was done on November 7th to 28th, 2019. In total, 8 fish composite samples were taken from artificial ponds run by local fish producers. This campaign is part of an annual program on monitoring residual substances in foods. Eight fish samples were tested for *Cu, Mo* and *Pb*, 7 out of 8 – for *As*. The sampled fish specimens belonged to the Cyprinidae, Salmonidae, Acipenseridae families.

Fish consumption

Fish consumption data were taken from the food consumption database formed using Food Frequency Questionnaire (FFQ) survey by the Informational-Analytical Center for Risk Assessment of Food Chain. Fish consumer clusters were produced after treating the data through MS Office Excel and IBM SPSS programs (Pipoyan, et al., 2020). Formation of homogenous clusters allows to assess the risk to different consumer groups more precisely, without averaging a fish consumption value.

Lab analysis

Lab work designated under this research has been done in "Republican Veterinary-Sanitary and Phytosanitary Center of Laboratory Services" SNCO. Tests were performed by the atomic-absorption spectrometry (AAS) method employing an iCE 3000 Series AA Spectrometer (Thermo Fisher Scientific, USA) in compliance with relevant normative documents (GOST R 51766-2001; GOST 30178-96). The analyzed fish samples were raw and did not undergo a culinary treatment. The samples were prepared by wet mineralization employing a Multiwave GO Microwave Digestion System (Anton Paar, USA).

Calculations. Risk assessment was done based on MOE calculations by Formula (1):

$$MOE = \frac{HBGV}{DI}, \qquad (1)$$

where $HBGV$ is a Health-Based Guidance Value (mg/kg body weight) of PTE, DI is the Daily Intake (mg/kg/day).

DI of the studied PTEs was calculated by Formula (2):

$$\mathrm{DI} = \frac{C \times IR}{BW}, \qquad (2)$$

where C is the mean content of PTE in a product (mg/kg); IR is the Ingestion Rate – the average daily intake of a product (kg/day), and BW is the Body Weight of a consumer. According to the FFQ, the average body weights of men and women are assumed as 74.7 kg and 59.8 kg, respectively.

The worst-case scenario

In the worst-case scenario, we assume the highest concentrations of arsenic and copper as mean concentrations in the fish samples. The average daily intake of fish will correspond to the cluster of consumers with the highest index.

Results and discussions

Fish consumption

The fish consumer clusters were constructed based on 867 questionnaires. Previous studies regarding risk assessment of other contaminants through fish consumption also include the values of these three clusters (Pipoyan, et al., 2020).

Concentration of PTEs

The highest concentration of Cu in the sampled fish was equal to 0.587 mg/kg, the lowest – 0.208 mg/kg, the average – 0.372 mg/kg. The concentration of As varied from 0.0025 to 0.423 mg/kg, with average concentration in seven samples being equal to 0.089 mg/kg.

The average daily intake amounts of *Cu* and As have been calculated based on the mean concentrations of these elements for each gender and cluster group (Table 1).

Table 1. The average daily intake of PTEs via fish consumption, mg/kg/day.

Consumers	Average daily intake for men		Average daily intake for women	
	Cu	***As***	***Cu***	***As***
Cluster 1	7.98E-05	1.91E-05	9.97E-05	2.38E-05
Cluster 2	3.14E-04	7.51E-05	3.92E-04	9.38E-05
Cluster 3	7.78E-04	1.86E-04	9.72E-04	2.32E-04

*Composed by the authors.

For freshwater fish, the maximum allowable concentration (MAC) of arsenic is 1 mg/kg, and for saltwater fish it can be up to 5 mg/kg (TR CU 021/2011). In 2021 in Poland during the customs inspection of frozen cod imported from Russia, the detected concentration of *As* (11.2 mg/kg) exceeded the allowable limit more than twice (RASFF Window). In our samples the highest concentration of *As* does not exceed the norm, and the mean value is 11 times lower than the MAC.

Margin of Exposure (MOE)

As HBGV we employ the values derived from epidemiological investigations and observations of human responses, which excludes possible distortions as a result of different physiology and sensitivity of lab models. To assess risks of copper intake via fish we employed the Upper Intake Level (UL). The UL of *Cu* is estimated as 0.07 mg/kg bw/day (Pipoyan, et al., 2020). The derived MOEs are presented in Table 2.

Table 2. MOE for copper intake via fish*

HBGV	Clusters	MOE	
		Men	**Women**
UL	1	877.35	702.35
	2	222.82	178.37
	3	89.98	72.04

Note: HBGV – Health-Based Guidance Value, MOE – Margin of Exposure, UL – Upper Intake Level.

*Composed by the authors.

To assess the risk of As intake via fish consumption we employed the Benchmark Dose Level (BMDL). This point is identical for both men and women – 3 µg/kg bw. The accepted $BMDL_{05}$ (the lowest dose which with a 95 % probability will produce a ≤ 5 % frequency of the effect) is associated with a 5 % carcinogenic risk (EFSA, 2021). The derived MOEs are presented in Table 3.

Table 3. MOE for arsenic intake via fish consumption*

HBGV	Clusters	MOE	
		Men	**Women**
$BMDL_{05}$	1	157	126
	2	40	32
	3	16.1	12.9

Note: BMDL- benchmark dose (lower confidence limit).

*Composed by the authors.

Risk assessment

MOE≤10 denotes the presence of risk to consumer health. *Cu* intake via fish poses no threat to men and women in all three consumer clusters since MOE is greater than 10. In the case of *As* MOE is also > 10 for all the consumer clusters. However, in the case of women included in the 3rd cluster, MOE approximates to 10, which is indicative of a very low possibility to increase a serving size without carcinogenic risk.

The authors believe that there are factors influencing the toxicity level of *As* in the studied fish which however cannot be quantitatively accounted for. In this research, the sampled fish was analyzed for total *As*. The specificity of *As* bioaccumulation by fish is that on average inorganic

As does not exceed 9 % of total *As* (EFSA, 2021). For this cause real toxicity of the studied fish species is at least 11 times lower than the identified concentrations of total *As*. Another influencing factor is connected with the culinary treatment of fish. In Armenia, fish is cooked by various methods including boiling and stewing. However, water in some regions of the country may contain elevated levels of arsenic caused by mining production and the presence of ore deposits (Akopyan, et al., 2018, Tepanosyan, et al., 2021, Babayan, et al., 2019). Cooking foods in *As*-containing water suggests increased concentrations of *As* in cooked food (EFSA, 2009, Camurati and Salomone, 2020). Yet, it is difficult to quantify the effect of cooking the fish in the water of different regions on *As* concentrations.

The worst-case scenario

The highest concentrations of *Cu* and *As* in fish are equal to 0.587 and 0.423 mg/kg, respectively. In the worst-case scenario, these indices are assumed as mean concentrations of elements in fish. The average daily intake corresponds to the value of the 3rd cluster of consumers. The MOE values for the worst-case scenario are provided in Table 4.

Table 4. The worst-case scenario in respect of copper and arsenic concentrations*

PTEs	MOE	
	Men	Women
Cu	57.1	45.71
As	3.40	2.72

*Composed by the authors.

Copper intake via fish produces no harmful effect on consumer health even at the maximum values of variables. In the worst-case scenario for arsenic, the MOE values are <10. Assuming that the total *As* represents inorganic *As*, there can be a possibility of carcinogenic risk to consumers at the maximum levels of variables.

Conclusion

The results of this research indicate that the studied fish samples contain *Cu* and *As*. Three consumer clusters were identified through the analysis of FFQ data. MOE values calculated for both the mean and the highest concentrations (worst-case scenario) of *Cu* indicate that the consumers of all three clusters may safely increase the average daily intake of the studied fish species. As intake via fish for the 1st and 2nd clusters poses a risk within allowable limits. MOE for women included in the 3rd cluster points to the presence of an insignificant health risk. The worst-case scenario also points to the possibility of carcinogenic risk in the case of the increase in the average daily intake of fish containing the maximum concentrations of *As*.

This study is not exempt from several limitations. It should be noted that using the values of inorganic *As* instead of the total would lead to more objective results. Moreover, this study does not account for the role of culinary treatment that may potentially change *As* concentrations in the cooked food. Considering the above stated limitations as well as the need for a precise risk assessment, a more comprehensive approach is required.

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