

Fish and fish products as risk factors of mercury exposure

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Abstract

Introduction and objective. Mercury is ubiquitous in the biosphere, occurring in the air, water, land, and soil, as well as in living organisms. Excessive exposure to mercury is associated with a wide range of adverse health effects including damage to the central nervous system and the kidneys. Mercury exists in many different forms in the environment which produce various patterns of toxicity. Protection of the food chain from contamination by mercury is an important task in the protection of health of the human population.

Objective. The aim of the study was to monitor the concentrations of mercury in fish and fish products from food retail in Eastern Slovakia, and from the Ružín water reservoir, Košice district.

Materials and method. A total of 384 samples of fish and fish products were collected for the study. Atomic absorption spectrometry standard solutions for mercury were used at a wavelength of 254 nm.

Results. The majority of countries and global organizations now enforce a maximum concentration of mercury in fish of approximately 0.5 mg·kg⁻¹. All of the 184 samples (50.52 % of the total fish samples studied) were above the maximum level set by the European Commission Regulation for mercury in fish.

Conclusions. The systematic analytical control of contaminants in food is important. Mercury is concentrated in seafood, products of prey and marine fish, fish from rivers and lakes in the areas contaminated by mercury. According to the findings of this study with analyzer AMA 254, the consumption is not recommended of fish, especially seafood (meat of shark, swordfish and king mackerel), for selected groups of the population: children, women of childbearing age, pregnant women and nursing mothers.

Key words

fish, methylmercury, daily intake, atomic absorption spectrometry, seafood

INTRODUCTION

Mercury enters water as a natural process of off-gassing from the earth's crust, and also through industrial pollution. It is utilized in the electrical industry (batteries, fluorescent lamps, switches, thermostats), dental amalgams, and numerous industrial processes, including anti-fungal agents for wood processing, solvent for reactive and precious metals, or a preservative in pharmaceutical products. Methylmercury is the most frequently encountered compound of the organic form found in the environment. It is formed as a result of the methylation of inorganic forms of mercury by microorganisms found in soil and water. Because mercury is ubiquitous in the environment, humans, plants and animals are all unable to avoid exposure to some form of mercury [1–4].

Mercury was known to the Phoenicians, Ancient Greeks, Carthaginians and the Romans. Mercury can be found in the environment in various chemical and physical forms. One of the most toxic compounds of mercury is methylmercury. In the last decades of the 20th century, humans were poisoned by contaminated fish or food prepared from grain cereal [5–6].

Ružín is a seriously polluted water reservoir in Slovakia. Ružín receives waters from the Hornád and Hnilec Rivers, which drain the Spiš-Gemer Rudohorie Mountains, an area known for intensive mining and ore processing. The ichthyofauna of the reservoir is composed mainly of common carp (*Cyprinus carpio*), pike-perch (*Sander lucioperca*), European perch (*Perca fluviatilis*), northern pike (*Esox lucius*) and tench (*Tinca tinca*) [7].

Many papers stress the high content of chemicals in the bodies of fish, such as heavy metals, mercury, PCBs and others [1, 3, 4, 8–10]. Excessive exposure to mercury is associated with a wide range of adverse health effects, including damage to the central nervous system and the kidneys. The distribution of heavy metals in fish depends on the type of feed or the composition of the water. One of the possible reasons of the highest mercury level in the scales of fish is the high content of functional proteins in muscle, having a high affinity to this element [7, 10–12]. The death of aquatic animals can be caused by various compounds of mercury, cadmium and other biological poisons in lakes, ponds and rivers. Recently, we put emphasis to the high levels of chemicals in fish bodies such as heavy metals (particularly mercury), polychlorinated biphenyls and other elements [2, 5, 6, 13–20].

Mercury intake by the human body. The development of human society is associated with technology and the

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processing of metals. The chemical form of mercury affects how it is absorbed and distributed in the body. Inhalation is the primary route of exposure to elemental mercury vapour or aerosols. Oral exposure is the primary route for inorganic mercury salts. Dermal penetration is usually not a significant route of exposure to inorganic mercury. Organic mercury compounds can accumulate in living organisms such as fish. Table 1 summarizes the absorption of these forms of mercury [1, 21].

Table 1. Extent of absorption of mercury by different routes [21]

Form of mercury	Ingestion	Dermal Contact	Inhalation
<i>Elemental</i> e. g. in thermometers	Very low for liquid form	Moderate for vaporized form	High for vaporized form
<i>Inorganic</i> e. g. in beauty products	Low or moderate (higher in infants)	Low or moderate	Low or moderate
<i>Organic</i> e. g. methylmercury in fish	High	Low or moderate	High

The estimated average daily intake of mercury in the general population are shown in Table 2. The values in parentheses represent the estimated amount retained in the body of an adult. Regarding the exposure to elemental mercury vapour from the air, the value in Table 2 is obtained by a concentration of 2–10 ng·m⁻³ and a daily respiratory volume of 20 m³. Exposure to methylmercury from fish is measured for 100 g of fish per week having 0.2 mg·kg⁻¹ mercury. Humans may be exposed to additional quantities of mercury occupationally and in heavily polluted areas, as well as to other forms of mercury, for example, to aryl and alkoxyaryl compounds widely used as fungicides [22, 23].

Table 2. Estimated average daily intake and retention (µg/day) of total mercury and mercury compounds in the general population not occupationally exposed to mercury [23]

Exposure	Elemental mercury vapor	Inorganic mercury compounds	Methylmercury
Air	0.03 (0.024)	0.002 (0.001)	0.008 (0.0064)
Dental amalgams	3.8–21 (3–17)	0	0
Food			
– Fish	0	0.6 (0.042)	2.4 (2.3)
– Non-fish	0	3.6 (0.25)	0
Drinking-water	0	0.05 (0.0035)	0
Total	3.9–21 (3.1–17)	4.3 (0.3)	2.41 (2.31)

Methylmercury is the predominant form of mercury in fish and other seafood, and is particularly toxic to the developing nervous system, including the brain. Whereas average exposure to methylmercury in food is unlikely to exceed the tolerable weekly intake, the likelihood of reaching such a level increases for high and frequent fish consumers. This group may include pregnant women, resulting in exposure of the foetus at a critical period in brain development. Inorganic mercury is less toxic and can also be found in fish and other seafood, as well as ready-made meals. Exposure to inorganic mercury through food is unlikely to exceed the tolerable weekly intake for most people, unless combined with other sources of exposure [24, 25].

OBJECTIVES

To determine levels of mercury in samples of fresh and frozen fish and fish products from food retail in Eastern Slovakia, and from the Ružín water reservoir (Košice district) by method of flameless atomic absorption spectrometry at a wavelength of 254 nm.

To compare the results obtained with those from other studies and with the maximum levels decreed by the Ministry of Agriculture of the Slovak Republic, and by the European Commission Regulation.

To describe the potential health risks and the recommendations for fish and seafood consumption for selected groups of the population.

MATERIALS AND METHOD

Preparation of fish and fish product samples. The study material comprised 384 samples of fish and fish products. The samples were selected randomly. European chub (*Squalius cephalus*), common roach (*Rutilus rutilus*), asp (*Leuciscus aspius*), common bream (*Abramis brama*), brown trout (*Salmo trutta fario*), northern pike (*Esox lucius*), common carp (*Cyprinus carpio*), pike-perch (*Sander lucioperca*), European perch (*Perca fluviatilis*), and tench (*Tinca tinca*) were caught in the Ružín water reservoir (an area of 3.9 km², a water volume of 59 million m³, located in Eastern Slovakia). Ružín water reservoir receives toxic elements through the draining of rivers of catchment areas polluted with the former extensive mining of ore-bearing deposits. The reservoir was created by damming the Hornád River in 1967 (Ružín I) and 1972 (Ružín II).

Other fish and fish products were obtained from food retailers in Eastern Slovakia. The analysis included perch, sardines, sprat, herrings, salmon, mackerel, fish pickle, fish salad, pickled herring, fish fingers, fish fillets, fish sticks, octopus, tuna, cod, seafood, anglerfish, Atlantic bonito (*Sarda sarda*), European eel (*Anguilla Anguilla*), King mackerel (*Scomberomorus cavalla*). The material collected was homogenized. The amounts of samples required for mercury analysis were very small.

Measurements of mercury content. The recommended method for determination of mercury in water, sediments, fish and shellfish is the flameless atomic absorption spectrophotometry (AAS). For the analysis of mercury in various fish species, a method of AAS at a wavelength of 254 nm (UV region) was applied using atomic absorption spectrophotometer AMA 254 (Altec, Prague, Czech Republic), specifically designed to determine total mercury content in various solids and liquids without sample pre-treatment or sample pre-concentration. Designed with a front-end combustion tube that is ideal for the decomposition of difficult matrices, e.g. coal, combustion residues, soils, and fish, the instrument's operation may be separated into three phases during any given analysis: Decomposition, Collection, and Detection [26].

A sample container with a nominal amount of the matrix is placed inside a pre-packed combustion tube (heated to 750 °C through an external coil) providing the necessary thermal decomposition of the sample into a gaseous form. The evolved gases are then transported to the other side of

the combustion tube. This portion of the tube, pre-packed with specific catalytic compounds, represents the area in the instrument where all interfering impurities are removed from the evolved gases. The cleaned, evolved gas is transported to the amalgamator, a small glass tube containing gold-plated ceramics, which collects all the mercury in the vapour. With a strong affinity for mercury and a significantly lower temperature than the decomposition phase, the amalgamator is capable of trapping all mercury for subsequent detection. When all the mercury has been collected from the evolved gases, the amalgamator is heated to 900 °C, essentially releasing all mercury vapour to the detection system. During the detection phase, all vapour passes through two sections of an apparatus known as a cuvette. AMA 254 uses an element-specific lamp that emits light at a wavelength of 253.7 nm, and a silicon UV diode detector for mercury quantitation [26].

Study quality control. The Test Laboratory of the EL Institute in Spišská Nová Ves carried out the analysis of the fish and fish product species of the current study. The laboratory performs chemical, physico-chemical, microbiological, biological and ecotoxicity testing, and the sampling of foodstuff, foods, beverages, and their individual components; biologic materials; drinkable, mineral, healing, waste, underground, surface, irrigation, industrial waters and bathing water, fodders and their individual components; pharmaceutical raw materials, drugs and auxiliary materials, chemicals, cosmetic articles, industrial products and toys, fertilizers, soil auxiliary materials and cultivating substrates, wastes and leaches, soils, geological materials, packaging, articles intended to come into contact with foodstuffs, and tests for health and hygienic centres. Moreover, the laboratory provides sampling and transportation of samples under controlled conditions to the testing laboratories.

The Test Laboratory earned accreditation certificates of the Quality Management Systems, Food Safety Management Systems and Hazard Analysis Critical Control Points. The EL Institute is a laboratory accredited by the Slovak National Accreditation Service. The first accreditation for testing and sampling was awarded in 1996. The EL Institute was also awarded the Good Laboratory Practice (GLP) certificate the first one in 1995 for assessment of conformity with GLP according to Law No. 163/2001 Coll. and to Commission Directive 2004/10/EC, of the European Parliament and of the Council (Statement of GLP Compliance No. G-017). According to the above-mentioned quality systems, the company has established, implemented and maintained a management system appropriate to the scope of its activities.

The special single-purpose atomic absorption spectrometer, the Advanced Mercury Analyzer AMA-254 (Altec, Prague, Czech Republic), is used in the majority of Czech and Slovak analytical laboratories. Measurement uncertainty depending on the type of material and working range are 13 % uncertainty for 0.002 – 0.05 mg·kg⁻¹; 9 % for 0.05 – 0.5 mg·kg⁻¹; 7 % for 0.5 – 5 mg·kg⁻¹; 5 % for 5 – 50 mg·kg⁻¹.

In the presented study, Hg single-element aqueous solution was analyzed as certified reference material (Hg092/12, Slovak Institute of Metrology, code: B15, certified value for the concentration of Hg: 0.9993 g·L⁻¹, expanded uncertainty 0.0027 g·L⁻¹, using a coverage factor k=2, which gives a levels of confidence of approximately 95 %; matrix 5 % HNO₃). Throughout the validation process, chosen parameters of the

method applied in this study were designed: limit of detection (LOD) 0.01 ng; recovery 98.2 %; limit of quantification (LOQ) 0.002 mg·kg⁻¹. The result of Z-score of proficiency test did not exceed the limit for satisfactory result, i.e. |Z|<2. The applicability of the AMA-254 in various fish and fish product species was confirmed.

The results were evaluated by Decree of the Ministry of Agriculture of the Slovak Republic and Slovak Ministry of Health, No. 608/3/2004–100 of 15 March 2004, which issued the Chapter of the Food Codex regulating food contaminants [27], and by European Commission Regulation No 1881/2006 setting maximum levels for certain contaminants in foodstuffs [28]. The majority of countries and global organizations now enforce maximum concentrations of mercury in fish at approximately 0.5 mg·kg⁻¹ (allowable mercury limit).

RESULTS

The current study investigated mercury, a contaminant and toxic substance which can affect human health by its presence in fish. The mercury concentrations in the species tested varied. The values of mercury concentrations were classified into three identical ranges regarding the allowable mercury limit. In the last range, the number of over-limit samples was indicated. The samples were statistically characterized by the values of minimum, maximum, mean, median, standard deviation and coefficient of variation. If the extreme values, the median was measured as a more relevant indicator, compared to the arithmetic mean. The coefficient of variation is the percentage of standard deviation and the arithmetic mean. A total of 384 fish samples were analysed. The results of measurements are shown in Table 3. As analyzer AMA 254 displays the concentration values with five decimal places, the results are shown as follows in Table 3.

Table 3. Concentration of mercury in fish samples

	Number of samples	First range [mg.kg ⁻¹] <0.166	Second range [mg.kg ⁻¹] 0.166 – 0.333	Third range [mg.kg ⁻¹] 0.333–0.5	Over-limit [mg.kg ⁻¹] > 0.5
Total	384	140	21	29	194
Minimum 0.08942			Average 1.17265		Standard deviation 1.22670
Maximum 6.55200			Median 0.57220		Coefficient of variation 104.60890

The mercury concentrations in fish samples measured by AMA 254 ranged from 0.08942 mg·kg⁻¹ – 6.552 mg·kg⁻¹. The over-the-limit group (more than 0.5 mg·kg⁻¹) contained the highest portion of the samples. The over-limit values of mercury concentration were determined in 194 samples of fish and fish products, i. e. 50.52 % of all samples (Fig. 1). The mean value of the results obtained was 1.2 ± 1.2 mg·kg⁻¹. The median of the samples was 0.6 mg·kg⁻¹. The arithmetic mean of samples exceeded the allowable mercury limit 2.3-times, median 1.1-times.

The highest concentration of mercury was found in the muscle of asp (*Leuciscus aspius*) from the Ružin water reservoir. Concentration of mercury in muscle (6.552 mg·kg⁻¹) exceeded more than 13 times the maximum mercury level admitted in foodstuffs in European countries. Regarding fish

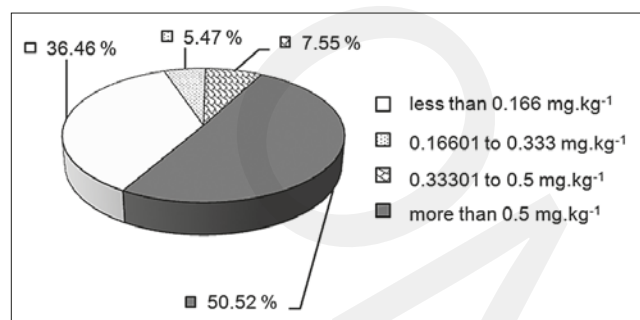


Figure 1. Classification of fish samples by percentages

and fish samples from food retailers, the highest concentration was measured in King mackerel (3.722 mg.kg^{-1}) (Fig. 2).

King mackerel, European eel and Atlantic bonito were fish samples obtained from food retailers, the rest were obtained from the Ružín water reservoir.

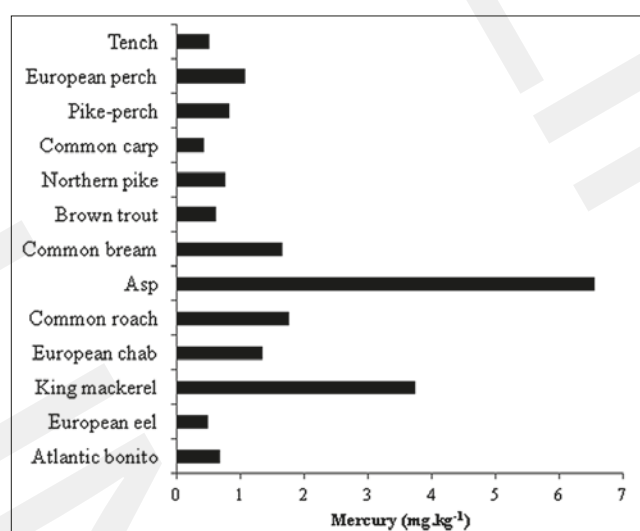


Figure 2. Maximal contents of mercury in various fish species

To summarise, 33.3 % of the allowable mercury limit did not exceed 36.46 % of the samples and 66.7 % of the allowable mercury limit was not exceeded by 41.93 % of samples. The minimal level of mercury in fish and fish products was 17.88 % of allowable mercury limit.

DISCUSSION

Mercury is ubiquitous in the biosphere, occurring in the air, water, land, and soil, as well as in living organisms. The impact of mercury on health is the subject of recently published papers by the authors of the presented study [13–20]. The transfer of mercury to the food chain, to the fish and fish products, is also present in non-contaminated areas.

The results of the current study indicate high levels of mercury in fish and fish products. Historically, mining for gold and mercury leads to high Hg concentrations in mining areas, and the latter may be the reason for the high Hg concentrations in the samples obtained from Eastern Slovakia for this study. The Ružín water reservoir branches of the Hornád and Hnilec Rivers drained a former mining area, and for a long time has been polluted by heavy metals [29, 30]. In samples of sediment load from the water reservoir Ružín

Table 4. Results of fish monitoring in Slovakia [48–51]

Year	Number of fish samples	Number of over-limits	Proportion of over-limits
2010	42	9	21.4 %
2011	51	4	7.8 %
2012	55	12	21.8 %
2013	51	3	5.9 %
2014	40	5	12.5 %
2015	41	7	17.1 %

I (main reservoir) and Ružín II (compensatory reservoir) in 2010–2014 indicated significant contamination with various heavy metals. The asp (*Leuciscus aspius*), the sample with the highest concentration of mercury, occurred in the open waters of rivers and large lakes. Juveniles are gregarious predators, while adults hunt in small groups.

Since 2003, the monitoring of fish contaminants in Eastern Slovakia (Třebišov, Michalovce) has been carried out, and included the rivers Laborec, Uh, Latorica and Ondava were included. Since 2005, dioxin and persistent organic pollutants have also been observed in fish samples from these sources. Table 4 indicates the number and proportion of mercury over-limit samples in fish taken in Slovakia in 2010–2015 [31–34]. In the presented study, the number of over-limit samples was 50.52 %.

Similarly, higher levels of mercury were found in the muscles of chub and roach in areas of the rivers Hornád and Hnilec, near Spišské Vlachy in Eastern Slovakia [35, 36]. Mercury levels exceeding the European limit were noted in nine species of seafood available on the Turkish market. This is mainly because of the greater variety of products available [37, 38].

In a similar study in Poland, concentrations of mercury in fish and fish products varied but were rather low (5.9–10.5 % of maximum value 0.5 mg.kg^{-1}), excluding oilfish [37, 39]. The results on undesirable substances in the studied fishes revealed that their levels were low, only in the oilfish (*Ruvettus pretiosus*) was an elevated level of mercury found – 0.3 mg.kg^{-1} on average, with the maximum allowable concentration being 0.5 mg.kg^{-1} . Mercury concentrations in three flatfish species – flounder (*Platichthys flesus*), plaice (*Pleuronectes platessa*), and Baltic turbot (*Scophthalmus maximus*), netted in the southern Baltic Sea, were assessed and compared to concentrations of this metal in sediments, sea water, and flatfish food – bivalve *Macoma balthica*, isopod *Saduria entomon*, and sprat (*Sprattus sprattus*). The different concentrations of mercury depending on species, tissue or organ, gender, individual length, kind of food and region, were determined in [40].

According to the data collected by Szprengier-Juszkiewicz [41], the products of animal origin (meat, milk, eggs) in Poland amounted to 27.4 % of the daily consumption of mercury. The fish provided 11.5 % of the mercury. The high intake of fish may occur that the tolerable weekly intake of methylmercury (limit of 1.3 µg.kg^{-1} launched in 2012) was exceeded several times. The general recommendations include reducing consumption of predator fish, variation in diet, and reduction in the consumption of fish by children and pregnant women [24, 25, 42].

The hygienic risk of mercury in the waters of rivers, lakes and seas is based on the biotransformation of inorganic mercury to methyl compounds which are very toxic. The mercury is especially present, in fish, which become a risk factor for humans, animals and birds. Specifically, the anaerobic microorganisms, which occur naturally in sediments of the seabed and of the bottoms of lakes, have the ability to methylate inorganic mercury compounds, a process that has a key role in the mercury cycle of the food chain of aquatic organisms [43].

The occurrence of mercury in fish, and thus in their products, is significant. Fish are the final link in the food chain in the aquatic environment. Fish are the major source of food in several countries, and consequently, the major source of mercury in the diet. Inorganic mercury can convert to organic in the digestive tract of fish. Mercury is fixed in almost all the organs and tissues of fish, whereby the mercury is practically not released from the body of the fish. The main form of mercury in fish is methylmercury [44]. Accumulation of mercury in fish muscles depends on its concentration in the environment. Particularly, it depends on the concentration in the sediment, the physical-chemical properties of the water, the temperature, and oxygen concentration. At elevated temperatures, particularly in standing water, the degree of accumulation of mercury increases. For this reason, the subject of mercury in fish has been studied thoroughly. Methylmercury in the human body is rapidly demethylated and remains in various organs and tissues in the form of inorganically bound mercury. Excretion of inorganically bound mercury is very slow. Inorganic mercury is excreted by the kidneys. Methylmercury is transported in the blood by breast milk and hair [44].

The concentrations of mercury in the various samples of fish (33 species of fish, 159 samples) were analyzed in [45]. Over-limits were found for 10 species of fish, the highest concentration of mercury (up to 10.42 mg·kg⁻¹) observed in frozen seafood products from predators. The product was withdrawn from the food market in the Czech Republic, and a warning was sent to the Public Health Office in Slovakia. Excessive values of mercury have been identified in the frozen products of swordfish and sharks. A total of 485 samples of the 43 most frequently consumed fish and shellfish species in Andalusia (Southern Spain) were analyzed for their toxic elements content. High mercury concentrations were found in some predatory species (blue shark, cat shark, swordfish and tuna), although they were below the regulatory maximum levels [46].

In the USA, the Environmental Protection Agency (EPA) has called on representatives of industry and government to radically reduce air pollutants and to allow the consumption of fish as important part of the diet. The EPA refers to pregnant women and infants. Mercury is toxic to the human nervous system and studies have demonstrated the associations between prenatal mercury exposure and the neurological development of children [8, 47, 48].

RECOMMENDATIONS

The primary source of mercury for humans is the consumption of fish, which can be consumed in a reasonable amount from verified water sources. Nevertheless, the consumption of fish should be reduced in the risk groups. Consumption of the

meat of shark, swordfish and king mackerel is not appropriate for children, women of childbearing age, pregnant women, nursing mothers, and workers with the analyzer AMA 254. Dietary recommendations include reduction of meat consumption of tuna and other fish, or an alternative dietary composition.

In the USA, the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) have issued advice regarding fish consumption. This advice is geared toward helping women who are pregnant or may become pregnant – as well as breast feeding mothers and parents of young children – to make informed choices when it comes to which fish are healthy and safe to eat. To help these consumers more easily understand the types of fish to select, the agencies have created an easy-to-use reference chart that lists 62 types of fish into three categories: best choices (two – three servings a week: anchovy, herring, sardine, and others), good choices (one serving a week: bluefish, carp, monkfish, and others), and also lists fish to avoid (king mackerel, marlin, orange roughy, shark, swordfish, tuna, and tilefish from Gulf of Mexico).

For adults, a typical serving is four ounces of fish, measured before cooking. Serving sizes for children should be smaller and adjusted for their age and total calorie needs. It is recommended that children eat fish once or twice a week, selected from a variety of fish types [8, 47, 48].

Patients with hepatic parenchymal disease of the liver, kidneys, spleen should avoid the consumption of fish due to the natural accumulation of mercury in these organs. Mercury has an affinity for the central nervous system; therefore, the recommendations are also applied to patients with neurological diseases, such as Parkinson's and Alzheimer's disease among others.

Due to the high protein content of fish, it is becoming an ever-more popular choice for health conscious consumers wishing to avoid the health drawbacks of meat. Despite fish and seafood containing key nutrients, including omega-3 polyunsaturated fatty acids, essential amino acids, trace elements and vitamins, they are also accumulators of mercury, posing a potential threat to human health [49]. If fish is removed from the diet, an adequate intake of the essential unsaturated fatty acids (omega-3 and omega-6) needs to be ensured by replacing the fish by cold pressed oils from seeds of fruit. The seeds of flax contain high levels of these fatty acids, and consumers are also interested in the seeds of *Salvia hispanica*, commonly known as chia seeds.

The debate about the risk posed by and the benefits of fish consumption is an important issue in public health. Disseminating information regarding fish consumption can help consumers to choose fish with good nutritional values and low level of harmful substances [37]. Long-term consumption of contaminated fish meat from the Ružín water reservoir, mainly by fishermen and their families, could cause hydrargyris, which can cause alterations of the nervous system, brain damage, induction of reproductive diseases, and many other disorders [7].

CONCLUSIONS

Human activities mobilized greater amounts of mercury during the Industrial Revolution. Organisms, ecosystems, and human beings are being exposed to a particular toxic form, methylmercury, from contaminated fish.

Scientists have established the limit levels for concentrations for the mercury. The systematic analytical control of contaminants in food is important in the process of the production and distribution of food. The potential elements with higher levels of mercury are included in seafood, products of prey and marine fish, fish from rivers and lakes in the areas contaminated by mercury. The risks can be also eliminated by the proper removal of broken mercury thermometers, energy saving lamps and other mercury-containing products.

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