DAILY INTAKE OF ARSENIC AND MERCURY BY CONSUMPTION OF BULGARIAN BLACK SEA FISHES

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ABSTRACT

The consumption of marine fish as popular seafood has increased steadily over the past decades. Toxic effects evidenced in the environment are most often caused by a mixture of pollutants such as heavy metals. The daily intake of arsenic (As) and mercury (Hg) through the consumption of nine edible marine species by the general population of Bulgaria was estimated. Health risks derived from this intake were also assessed. The concentrations of As and Hg in nine edible marine fish species were determined by ETAAS and Milestone Direct Mercury Analyzer. The highest metal intake through fish consumption corresponds to As (7.837 *μg*/day) for male adults. The daily intake of these elements through fish consumption of these elements was compared with the provisional tolerable weekly intakes (PTWI). The data of this study showed that the metal concentrations in edible tissues of the sampled species were within the permissible safety levels for human consumption set by various health organizations.

Keywords: *Mercury, arsenic, daily intake, PTWI*

INTRODUCTION

Fish has been acknowledged as an integral component of a well balanced diet, providing a healthy source of energy, high-quality proteins, vitamins and a wide range of other important nutrients (14). Moreover, fish is a significant source of omega-3 polyunsaturated fatty acids (PUFAs) whose benefits in lowering the risk of coronary heart disease and contributing to normal neurodevelopment in children have been widely recognized (19).

In contrast to the potential health benefits of dietary fish intake, the chemical pollutants contained in these products have emerged as an issue of concern, particularly for frequent fish consumers (10). In this regard, heavy metals contamination is a worldwide-recognized public health hazard because these pollutants are widespread in the environment, including marine ecosystems, from either natural or anthropogenic sources (9). As a consequence, they can be accumulated by marine organisms through exposure to metals present in water and sediments or in the food chain. Thus, diet comprises the main route of exposure to these elements in the general population (7).

Many trace metals are micronutrients and represent essential dietary components of aquatic organisms. In natural marine environments and freshwaters, most trace elements are typically present in trace quantities (<10 nM) and are passively and/or actively assimilated by organisms to satisfy physiological requirements. Also trace metals can be classified as potentially toxic such as As, Cd, Pb, Hg, etc. Toxic elements can be very harmful even at low concentrations when ingested over a long time period.

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Received: February 27, 2014
Accepted: April 3, 2014

The essential metals are able to produce toxic effects when their intake is excessive. In metal contaminated systems, however, metals can accumulate within the cells and tissues of organisms, which could result in effects deleterious to cellular function.

Mercury is considered by the Environmental Protection Agency as a highly dangerous element because its accumulative and persistent character in the environment and biota. In 1973, calculations assessing the antropogenic atmospheric contamination by mercury estimated total mercury emissions to the atmosphere of around 10 000 tones. These emissions came from the calcination of sulphide ores, fossil fuels combustion and heating of other mercury-containing materials (8). Today, both inorganic and organic mercury compounds are produced in industrial processes in paper industry, agriculture, etc. and they are responsible for the vast majority of the present antropogenic contamination of our environment with this toxic metal.

Increasing concentrations of atmospheric Hg from anthropogenic sources have led to increased Hg deposition, which is the principal source of Hg in surface waters for example. In the aquatic environment, bacteria transform inorganic Hg into monomethylmercury (MMHg), which is highly toxic and readily biomagnified in aquatic food webs. It should be stressed that the most numerous cases of human poisoning by organometallics are those caused by the ingestion of methylmercury compounds. This is a particularly important issue for children, pregnant women and breast-feeding mothers (16). This organometallic species is neurotoxic, blocks binding sites of enzymes, interferes with proteine synthesis, impedes thymidine incorporation into DNA, etc. (46). Both inorganic and organic mercury tend to accumulate in sediments and biota, particularly fish and molluscs (10). This way, neurotoxic methylmercury would eventually reach our food chain from sea food (21). The high affinity of methylmercury to sulphydril groups and lipids of animals would explain its accumulation in living organisms, particularly in lipid tissue of mammals.

Nowadays it seems accepted that the rate and extent of methylation of Hg(II) in the waters and sediments depend upon different factors. It is interesting to note that levels of methylmercury in the waters are usually much lower than those of inorganic mercury due to the easy decomposition by solar UV light (15) of organomercury compounds in the aqueous phases on one hand, and the difficulty of methylation reactions in the same phase on the other. In general, the organic forms of metals (more hydrophobic) go through biological membranes quite easily as compared to inorganic forms. Thus, organomercuric compounds are much more toxic than inorganic mercury.

In sediments and biota the levels of methylmercury are higher than in waters because of accumulative phenomena (4). Inorganic mercury and methylmercury seem to be preconcentrated in sediments and are found at relatively high levels in fish (14).

Arsenic is a ubiquitous element, which occurs naturally in the earth's crust. More than 245 minerals contain arsenic and although the ultimate source of arsenic is geological, human activities such as mining, burning of fossil fuels, and pesticide application, also cause arsenic pollution (2,3). Arsenic exists in four oxidation states, +V (arsenate), +III (arsenite), 0 (arsenic), and –III (arsine). In addition to arsenite, arsenate, and their methylated derivatives, there are "fish arsenic" (arsenobetaine and arsenocholine) and arsenosugar compounds of environmental interest (12).

Arsenic is toxic to both plants and animals and inorganic arsenicals are proven carcinogens in humans (12). The toxicity of arsenic to human health ranges from skin lesions to cancer of the brain, liver, kidney, and stomach (18). Generally inorganic arsenic species are more toxic than organic forms to living organisms, including humans and other animals (13). Arsenite is usually more toxic than arsenate. The toxicity of trivalent arsenic is related to its high affinity for the sulfhydryl groups of biomolecules such as glutathione and lipoic acid and the cysteinyl residues of many enzymes (1). The formation of As(III)–sulfur bonds results in various harmful effects by inhibiting the activities of enzymes such as glutathione reductase, glutathione peroxidases, thioredoxin reductase, and thioredoxin peroxidase (8).

Both inorganic and organic forms of arsenic have been determined in water (6). The occurrence of organoarsenic compounds in fish and other aquatic fauna and flora has been shown in several studies

(5). It is noteworthy that arsenobetaine, which is the most commonly reported organoarsenical in marine animals, is virtually absent in the vetebrate and invertebrate freshwater organisms analyzed by Schaefer et al. (17). This represents the major difference in arsenic speciation between marine and freshwater organisms.

The aim of this study was to measure the levels of As and Hg in nine traditionally consumed species of fish among Bulgarian population and to determine the contribution of this food group to the dietary intake of As and Hg.

MATERIAL AND METHODS

Sampling and sample treatment

Nine fish species from two different locations were collected from the local fish market during 2010-2011. These fish species are goby (*Neogobius melanostomus*), sprat (*Sprattus sprattus*), Mediterranean horse mackerel (*Trachurus mediterraneus*), shad (*Alosa pontica*), gray mullet (*Mugil cephalus*), bluefish (*Pomatomus saltatrix*), turbot (*Pseta maxima*), red mullet (*Mullus barbatus ponticus*) and garfish (*Belone belone*). These sampling sites in the coastal waters of Bulgarian Black Sea were Kaliakra (North) and Nesebar (South). All the samples were immediately transported to the laboratory and frozen at -20 °C until analysis. All the equipments used for sample collection, transportation, and preparation were free from contamination.

All reagents used in this study were of analytical reagent grade unless otherwise stated. Double deionised water (Milli-Q Millipore 18.2 MΩ cm^{-1} resistively) was used for all dilutions. HNO_3 was purchased by Merck, Darmstadt, Germany.

The biometric data of the fish species are presented elsewhere. Samples were then dissected to separate muscle and stored in polyethylene plastic bags at -20 °C until chemical analysis.

Instrumental

To assess the total metal contents, microwave assisted acid digestion procedure was carried out. Microwave digestion system "Multiwave", "Anton Paar" delivering a maximum power and temperature of 1000 W and 300 °C, respectively, and internal temperature control, was used to assist the acid digestion process (Table 1).

Table 1. Microwave digestion system general parameters

Microwave digestion system "Multiwave", "Anton Paar" Acid mixture	
HNO_3	6.5 cm^3
Temperature (max)	300 °C
Pressure (max)	75 bar
Quartz vessels	HQ 50
Sample amount	1 g
Final volume	10 ml

Reactors were subjected to microwave energy at 800 W in five stages program described in Table 2.

Determination of As was performed using Electrothermal Atomic Absorption Spectrometry (EAAS) carried out on a Perkin Elmer Zeeman 3030 spectrometer with an HGA-600 atomizer. Pyrolytic graphite-coated graphite tubes with integrated platforms were used as atomizers (Table 3).

Determination of Hg was done by Milestone Direct Mercury Analyzer DMA-80.

Statistical analysis

The whole data were subjected to a statistical analysis. Student's-test was employed to estimate the significance of values.

Table 2. Microwave digestion system operational parameters

Step	Initial power (W)	Time (min)	Final power (W)	Fan
1	100	5	600	1
2	600	5	600	1
3	600	5	800	1
4	800	15	800	1
5	0	15	0	3

Scripta Scientifica Pharmaceutica, vol. 1, 2014, pp. 38-43

Table 3. Instrumental parameters for HGA 600

Parameter	Drying	Ashing	Atomization	Cleaning
Temperature, °C	150	var	var	var
Ramp time, sec	10	20	0	1
Hold time, sec	10	60		2
Read			on	
Ar flow, ml min^{-1}	300	300	0	300

Element	Ashing	Optimal atomization	Cleaning
As*	1000	2200	2300

• *modifier Pd as $(NH_4)_2PdCl_4$*

RESULTS AND DISCUSSION

In recent years, a notable number of surveys carried out in different countries have determined the concentration of metals in various edible marine species and estimated human exposure by their consumption. However, comparison among studies is not always easy, as the dietary habits depend on each specific region or country. Moreover, fish and seafood species in the different surveys are not generally the same.

For health risk assessment, it must be noted that the European Union legislation law (European commission Decision 78/2005) on Hg concentration in edible marine species sets a limit of 0.5 mg/kg of wet weight for fishery products and muscle meat of fish. In this study, all the levels of total Hg found in fish samples do not exceed these limits. On the other hand, the current intakes concerning As and Hg were compared with the respective values of the Reference Daily Intake or Recommended Daily Intake (RDI) and Provisional Tolerable Weekly Intakes (PTWI) established by the FAO/WHO. RDI is calculated based on the concentration of the heavy metal in certain fish species multiplied by the grams of daily intake divided by the body weight. For inorganic As, the PTWI is 0.015 mg/kg of body weight per week, or 0.129 mg/day for a 60 kg female subject and 0.171 mg/day for of a 80 kg male a subject. As indicated above, in the present study all analyses were carried out for total (inorganic and organic) arsenic. However, it is well-known that most As found in fish and seafood is in the form of organic As, which is the less toxic form (Table 4).

Daily intake calculations were performed based on the following: for females it is set a 30g intake of fish per day for 60 kg body weight and for males - 50g intake of fish per day for 80 kg body weight (according to The results are shown in Table 5.

In the literature, the percentage of inorganic As in fish and sea food has been reported to be between 0.02 and 11% (11), whereas the maximum acceptable daily load for As, set by the WHO in 1967 and unrevised in 1989, is 3000 µg for a subject of 60 kg (22). Taking this into account, in the current study As intake would not be of concern for any sex group. This intake, 6.274-7.837 µg/day, is quite similar to other literature studies.

In the current study, all Hg intakes were under the established PTWI, 3 µg/kg/week. Assuming that in fish and seafood most Hg is in the methylmercury form, the intakes for most age groups would be under the limit if safety, 1.6 µg/ kg of body weight/week of methyl mercury.

Table 4. Mean concentration with standard error of As and Hg (expressed as mg/kg wet weight)

	Goby	Sprat	Horse Mackerel	Shad	Grey Mullet	Bluefish	Turbot	Red mullet	Garfish
Hg	0.05±0.01	0.11±0.014	0.125±0.049	0.09± 0.01	0.085±0.007	0.1±0.014	0.085±0.01	0.065±0.064	0.14±0.01
As	1.56±1.27	1.33±0.847	1.075±0.488	0.925± 0.771	1.07±0.240	0.895±0.177	2.845±0.023	2.525±0.247	0.32±0.11

Table 5. Daily intake ($\mu g\ kg^{-1}\ d^{-1}$) for females and males

	Female		Male	
Fish	As	Hg	As	Hg
Sprattus spratus	0.665	0.055	0.831	0.069
Trachurus mediterrneus	0.538	0.063	0.672	0.078
Neogobius melanostomus	0.78	0.025	0.975	0.031
Alosa pontica	0.463	0.045	0.578	0.056
Mugil cephalus	0.535	0.043	0.669	0.053
Pomatomus saltatri	0.448	0.062	0.559	0.063
Psetta maxima	1.423	0.043	1.778	0.053
Mullus barbatus ponticus	1.262	0.033	1.578	0.041
Belone belone	0.16	0.07	0.2	0.088
Total	6.274	0.439	7.837	0.532
WHO/FAO, 2004	2.14	0.23	2.14	0.23

CONCLUSION

The daily arsenic and mercury diet intake through fish of general population in Bulgaria is much lower than the PTWI values proposed by WHO. Moreover, this study was conducted based on the total concentration of toxic elements but biological availability (hence toxicity) of metals in aquatic systems is strongly dependent on the nature of metal species present. Generating and understanding of the chemical form (or speciation) of metal in the environment is fundamental to predicting its impacts on aquatic biota.

Also a possible hazard and carcinogenic risk derived for As and Hg ingestion using non-carcinogenic target hazard quotient (THQ) should be performed.

Consequently, it would be recommendable that monitoring studies are periodically performed to assess the temporal trends in human exposure to these toxic elements through fish consumption.

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