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Original Article

Risk assessment of the total mercury in Golden gray mullet (*Liza aurata*) from Caspian Sea

Seyed Mehdi Hosseini *[1], Noorollah Mirghaffari[1], Nasrollah Mahbubi Sufiani[2], Seyed Vali Hosseini[3], Amir Faraz Ghasemi[4]

[1]Department of Environmental Science, Faculty of Natural Resources, Isfahan University of Technology, Isfahan, Iran, P.O. Box 8415683111, Isfahan, Iran.
[2]Department of Fisheries, Faculty of Natural Resources, Isfahan University of Technology, Isfahan, Iran, P.O. Box 8415683111, Isfahan, Iran.
[3]Department of Fisheries, Faculty of Natural Resources, University of Tehran, Karaj, Iran, P.O. Box: 31585-3314, Karaj, Iran.
[4]Department of Marine Biology, Faculty of Marine Science and Oceanography, Khorramshahr University of Marine Science and Technology, P.O. Box 64199-669, Khorramshahr, Khozestan, Iran.

Abstract: Mercury is the most toxic heavy metal in the aquatic ecosystems which originates both from natural and industrial resources and is ultimately deposited in sediments as methyl mercury. This metal is quickly transferred through the food chain and accumulated in organisms. In this study, the human health risk due to consumption of Golden grey mullet (*Liza aurata*) in the Caspian Sea, were evaluated by measuring the concentration of mercury in muscle samples using Atomic absorption spectrophotometer (Perkin Elmer FIAS-100) and cold vapor technique. A total of 60 fresh Mullet samples were collected by local fisherman from 12 stations on the southern coast of Caspian Sea in Mazandaran Province situated in the north of Iran. The average concentration of mercury in Mullet muscle was 0.137 µg/g of fresh weight (0.432 µg/g dry weight) which was less than the allowable amount for human consumption determined by the international organizations such as United States Environmental Protection Agency, World Health Organization, Food and Agriculture Organizations and the Food and Drug Administration. The calculations indicated that daily and weekly mercury uptake for Iranian consumers is lower than the guide values (Acceptable Daily Intake and Provisional Tolerable Weekly Intake) provided by international organizations. Also, Hazard Quotient Index was below 1 (0.35). Therefore, the consumption of the Mullet is not a serious threat to the consumer's health and a consumption permitted rate of 51 g per day is recommended.

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Introduction

Mercury is the most toxic heavy metal in the aquatic ecosystems which originates both from natural sources and human activities. The mercury cycle in aquatic environments has been receiving considerable attention because of the high toxicity of its compounds, accumulation of both the organic and inorganic forms of the element in organisms and their biotransformation and bio-magnification in the aquatic food chains (Houserova et al., 2006). Methylmercury is the main form of organic mercury found in the environment and due to its chemical persistence and lipophilicity, have a tendency to accumulate up the food chain. Therefore, human exposure to these pollutants occurs mainly from eating seafood. In this regard, fish consumption is often considered to be a major source of intake of mercury for humans (Ruelas-Inzunza et al., 2008). Communities that rely on fish intake for daily nutrient sustenance may be at risk from chronic, high exposure to methylmercury as well as other persistent organic pollutants (Burger et al., 2007).

United States Environmental Protection Agency (USEPA) classifies methylmercury as group "C" based on inadequate data in humans and increased incidence of kidney tumors in a single species and sex (USEPA, 2005). Methylmercury has also been shown to be a developmental toxicant, causing subtle

* Corresponding author: Seyed Mehdi Hosseini
E-mail address: Hosseini.Sayedmehdi@gmail.com

to severe neurological effects. EPA considers there is sufficient evidence for methylmercury to be considered a developmental toxicant, and to be of concern for potential human germ cell mutagenicity (USEPA, 2001).

The Caspian Sea is the biggest land-locked body of water and it has five major inlet rivers but no outlets and acts as a watershed reservoir for the region (Hosseini et al., 2008). The most widespread pollutants of surface waters are petroleum compounds, phenols, heavy metals and etc. from anthropogenic activities, including both land-based and offshore pollution (UNEP, 2008). Many potentially toxic contaminants such as heavy metals released into the Caspian Sea are lipophilic and insoluble in water.

Golden mullets are an important and commercial fish in Caspian Sea particularly in Iran. Previous investigations have demonstrated the occurrence of Hg in fishes from the Caspian Sea. However, no studies have been conducted on contamination status of mercury in marine fish mid its risk assessment on human health in Caspian Sea. In the present study, contamination status of Hg was assessed in muscle of Golden gray mullet (*Liza aurata*) collected in the south coastal waters of the Caspian Sea (Mazandaran, Iran) and was compared with other studies and food guideline values to evaluate potential human hazard (Food safety and hygiene) from fish consumption.

Materials and methods

Study area: The investigated area (35°47′ - 36°35′N, 50°34′E) is located in the southern coastal of Caspian Sea, and stretch of sampling area is about 340 km. Twelve sampling sites were selected according to the localization of principal sources of pollution (waste from the main urban and sewage discharge points) (Fig. 1).

Sampling: *Liza aurata* were caught using beach seines from February through March 2010. After biometric measurements (weight determined 500-600 gr), the fish were immediately transported to the laboratory in ice box. Samples of muscle (the mid-

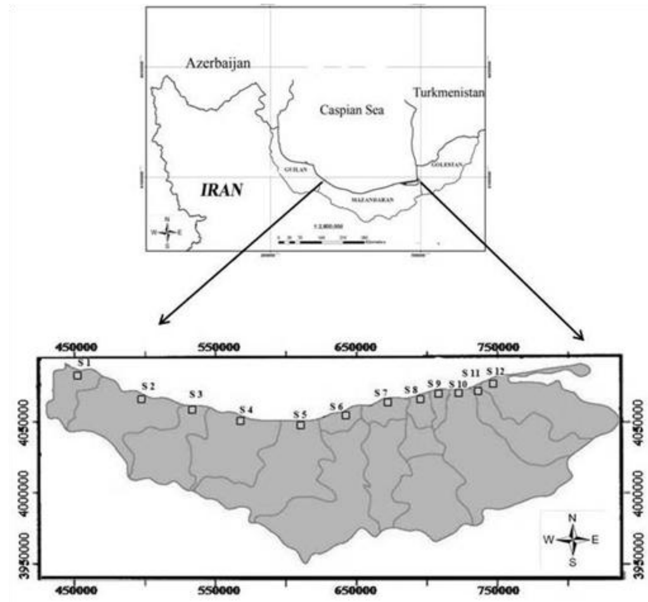

Figure 1. Sampling area and stations.

dorsal muscles) were dissected, washed with deionized water, packed in polyethylene bags and stored at -80 °C until chemical analysis.

Chemical analysis: The samples were thawed and a weighted sample (0.500 g) of homogenized tissue was taken from each specimen. Each sample was placed in a Teflon digestion vessel with 5 ml mixture of nitric acid and perchloric acid (3:1 v/v). The mixture was heated to 100-150 °C for 45 minutes until the tissue was dissolved, and was filtered through a membrane filter. After cooling, the solution was diluted to 50 ml with deionized water. Mercury was analyzed by the cold vapor technique using the Perkin Elmer FIMS-100 mercury analyzer (USEPA, 1998). Hg concentrations are expressed in μg/g (ppm or mg/kg) on a wet weight basis.

A DORM-2 certified dogfish tissue was used as the calibration verification standard. Recoveries between 90% and 110% were accepted to validate the calibration. The detection limit for Hg analysis was 0.005 μg/g.

Exposure assessment: Fish constitutes the main source of dietary exposure to mercury, which can cause adverse health effects in humans at sufficiently high exposures. The exposure assessment evaluates the potential exposure to methylmercury from the consumption of fish. Potential exposure is a function of (a) the amount of fish that is consumed on a

Table 1. Total Hg concentration (μg/g)[1] in muscles (wet weight[2] and dry weight) of *Liza aurata* from study area.

Station		Hg (w/w)	Hg (d/w)
Ramsar	Mean	0.205	0.645
	Min	0.098	0.309
	Max	0.316	0.997
Tonekabon	Mean	0.205	0.646
	Min	0.116	0.366
	Max	0.401	1.265
Chaloos	Mean	0.221	0.696
	Min	0.141	0.445
	Max	0.298	0.940
Noshahr	Mean	0.222	0.701
	Min	0.113	0.356
	Max	0.401	1.265
Noor	Mean	0.150	0.474
	Min	0.064	0.202
	Max	0.377	1.189
Mahmudabad	Mean	0.164	0.516
	Min	0.067	0.211
	Max	0.312	0.984
Fereidunkenar	Mean	0.112	0.353
	Min	0.014	0.044
	Max	0.319	1.006
Babolsar	Mean	0.129	0.408
	Min	0.025	0.079
	Max	0.331	1.044
Juibar	Mean	0.102	0.321
	Min	0.038	0.120
	Max	0.301	0.950
Sari	Mean	0.079	0.248
	Min	0.032	0.101
	Max	0.299	0.943
Neka	Mean	0.098	0.309
	Min	0.021	0.066
	Max	0.351	1.107
Behshahr	Mean	0.077	0.243
	Min	0.019	0.060
	Max	0.286	0.902
Total		**0.137**	**0.432**

[1]geomean
[2]the mean moisture content of tissue in *Liza aurata* was 68.3.

regular basis, and (b) the amount of methylmercury that is present in fish (Bureau of Nutritional Sciences of Canada, 2007).

Ratio of methylmercury to total mercury and mercury toxicity assessment: From a human health perspective, it is the amount of methylmercury rather than total mercury that is of most interest, since methylmercury is much more readily absorbed into the human bloodstream. As a result, in the absence of detailed information on mercury speciation, it is simply assumed for the purposes of health risk assessments, that 100% of total mercury is in the methylated form as methylmercury (Bureau of Nutritional Sciences of Canada, 2007). Several studies have measured the actual portion of total mercury that is present in fish as methylmercury (Bloom, 1991; Lansens et al., 1991; Bureau of Nutritional Sciences of Canada, 2007).

When making quantitative estimates of non-cancer hazards from mercury exposure, the methylmercury Reference Dose (RfD) (developed by USEPA is used (RfD = 0.0001 mg/kg day or μg/g/day). Specifically, the RfD for methylmercury is used because the sampling program was not designed to differentiate between elemental, organic, and inorganic mercury. This approach is consistent with observations that most (>95%) of the total mercury content of fresh and saltwater fish is methylmercury (USEPA, 1997). Therefore it was assumed that all mercury present was methylmercury. This assumption will tend to overestimate the toxicity of mercury. By using the RfD for methylmercury, the toxicity assessment takes a conservative approach to estimating the potential health hazard from exposure.

Risk characterization (Estimation of human exposure to methylmercury in fish) daily and weekly intake: To evaluate the potential health risk to people through consumption of Golden grey mullets, Hg intake rates (Estimated daily intake (EDI) and Estimated weekly intake (EWI)) were estimated for the general adult population (μg/day/adult) on the basis of the mean Hg levels in fish muscle (wet weight basis) multiply daily and weekly fish consumption (Kojadinovic et al., 2006; Hajeb et al., 2009).

Hazard Quotient (HQ): A hazard quotient (HQ) is the ratio of the estimated exposure dose of a contaminant (a single substance exposure level) to its RfD or MRL. The HQ can be calculated with the following formula:

$$HQ = ((MCC \times CR) / BW) / RfD$$

Where:

MCC: Mean contaminant concentrations in fish

CR: Consumption Rate

RfD = Reference Dose (Hg = 0.0001 mg/kg/day)

BW = Body weight (70 kg for adults)

Table 2. Results of risk assessment of mercury in *Liza aurata* from study area[1].

HQ	DI (μg Hg day^{-1} adult^{-1})	WI (μg Hg week^{-1} adult^{-1})	CR (g/day)	number of meals per week based on USEPA[2]	number of meals per week based on JECFA[2]
0.35	2.402	16.814	51	1	3

[1]These estimations do not apply for pregnant woman and children.
[2]Based on an adult standard portion size of 230 g.

An HQ exceeding one, suggests the potential of health effects (Castilhos et al., 2006).

Consumption Limits (CR_{lim}): The maximum allowable fish consumption rate for a non-carcinogen can be calculated with the following formula (USEPA, 2000):

$$CR_{lim} = (RfD \text{ or } MRL \times BW) / MCC$$

Where:

CR_{lim} = Maximum allowable fish consumption (kg/day)

RfD = Reference Dose (Hg = 0.0001 mg/kg/day)

BW = Body weight (70 kg for adults)

MCC: Mean contaminant concentrations in fish

Statistical analysis: Analysis of variance and means comparison (Duncan's multiple range test) were performed using SPSS (Chicago, IL) software.

Results and discussion

Mercury level: Levels of the total mercury in the muscle of *L. aurata* from coastal waters of Caspian Sea are shown in Table 1. No significant difference (P>0.05) in Hg concentration between various stations was found.

The total mercury levels in the samples of this study were comparable with mercury concentrations of fish muscle in similar or related studies of the Caspian Sea fishes. Average of mercury concentrations have been reported 0.190 µg/g dry weight in muscle of *Rutillus frisii kutum* (Anan et al., 2005). Yazdaninasab et al. (2004) reported the mercury concentrations of 0.259 and 0.262 µg/g dry weight in abdominal muscles and tail muscles of *L. aurata* (Yazdaninasab et al., 2004).

Our study indicates that accumulation of mercury in the muscle of mullet is higher comparing to the before mentioned studies. In a study carried out by Agusa et al. (2004), the accumulation of mercury in muscle of five species of Caspian Sea sturgeon (*Huso huso*, *Acipenser persicus*, *Acipenser guldenstadti*, *Acipenser nudiventris* and *Acipenser stellatus*), was reported as 0.33, 0.07, 0.08, 0.16 and 0.015 ɥg/g wet weight, respectively, (or 1.40, 0.330, 0.320, 0.670 and 0.06 µg/g dry weight, respectively) (Agusa et al., 2008). Nozari (2011) reported the average concentration of mercury in muscle of Pike (*Esox lucius*) 0/322 µg/g dry weight (Nozari et al., 2011).

Comparison of mean concentration of mercury in muscle of Golden grey mullet with the studies listed above indicate that although these fishes are bottom feeders and almost are at the top of trophic chains in the Caspian Sea ecosystem, but the accumulation of mercury in their muscles has been relatively lower than those of reported by United States Environmental Protection Agency, the World Health Organization, Food and Agriculture Organization and the Food and Drug Administration which is 0.3, 0.5, 0.5 and 1 µg/g wet weight of fish, respectively. These higher levels of mercury are considered as dangerous levels for human body (Shi et al., 2005).

Risk Assessment for Fish Consumption: In general, mercury levels increase with the size and age of the fish. However, this is not always the case (Stafford and Haines, 2001). We have used marketable and equality sized fish (553 ± 56 g) to elimination of the effect of size on mercury bioaccumulation. On the other hand, because of popularity of marketable size for consumption, investigation of risk assessment was carried out for this size.

Risk to the food chain: Accumulation of mercury (Methylmercury) in the fish poses a risk both to the fishes themselves, and to their predators. In the fishes themselves, levels of 5–20 µg/g in the muscle are associated with toxicity (Wiener et al., 2003).

The mean mercury level of 0.137 μg/g in *L. aurata* in this study was well below these levels. Mercury accumulates in larger fish, so it magnifies as it moves up the food chain (to humans or other top- level predators). However, mercury concentrations in muscle are available to predators.

The critical effects levels for consumption by piscivorous mammals are 0.1 μg/g, and for birds are 0.02 μg/g (Yeardley et al., 1998), although seabirds are generally less sensitive (Furness, 1996). The mean mercury levels in the *L. aurata* from Caspian Sea (0.137 μg/g in muscle) are clearly higher than the levels known to pose a problem for sensitive birds or mammals that scavenge them along the shore, or for sensitive marine mammals especially Caspian seal (*Phoca caspica*).

The Caspian seal, which is endemic to the Caspian Sea, is the only mammal within the aquatic fauna of the region. It is an ichthyophagous predator and is at the top of trophic chains in the Caspian Sea ecosystem. Crab, shrimps and mullet are consumed by Caspian seal to a different extent (Badamshin, 1966).

However, the ability to detoxify (demethylate) and store mercury in the form of less toxic (divalent) may not be present in newborn and young seals following exposure to the mother's burden in utero and while nursing, thus, these young and developing seals may be at risk for mercury-related neurotoxicity and other effects (Wagemann et al., 2000).

Risk to Human: The average of Iranian fish consumption is 6400 g (6.4 kg/year) per capita (FAO, 2009); therefore, the 17.5 g/day (122.5 g/week) of consumption rate is used in our health-risk assessment. Based on the mean of Hg concentrations in *L. aurata* (Table 1) and seafood consumption rate of an Iranian, dietary exposure to Hg via fish consumption was estimated for Iranian people. EDI and EWI were obtained 2.402 and 16.814 μg day^{-1}, respectively.

Different Acceptable Daily Intake (ADI) limits have been established by national and international instances. The ADI set by the World Health Organization (WHO) for T-Hg is 0.71 μg day^{-1} kg^{-1} body weight, and restricted to 0.35 μg day^{-1} kg^{-1} body weight for pregnant women because foetus are more sensitive to Hg toxicity, as well as nursing mothers and children less than 10 years (DHHS and EPA, 2004). The French (French Agency for Food Safety (AFSSA)) and the Canadian health agencies follow the same guidelines as the WHO, whereas the US FDA and US EPA have set more restrictive ADI limits for MeHg (0.4 and 0.1 μg day^{-1} kg^{-1} body weight, respectively for all the population) (Hirsch, 2002).

Considering an average adult body weight of 70 kg (USEPA, 1994), the T-Hg WHO ADI, MeHg US FDA ADI and MeHg USEPA ADI can be approximated as 50 (hence 350 μg Hg $week^{-1}$), 28 (196 μg Hg $week^{-1}$) and 7 (49 μg Hg $week^{-1}$) μg day^{-1} $adult^{-1}$, respectively (Kojadinovic et al., 2006; Goldblum et al., 2006). On the other hand, a provisional tolerable weekly intake (PTWI) of 1.6 mg MeHg/kg body weight/ week was established in the 61st meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 2003). Considering an average adult body weight of 70 kg, the guideline value calculated from PTWI of JECFA was 16 μg /day and 112 μg/week, respectively.

These health risk limit were compared to the estimated daily and weekly intake of Hg in this study. The results indicated that the EDI and EWI of total mercury by a 70 kg adult consuming 17.5 g fish/day and 122.5 g fish/week is below the respective ADI and PTWI.

The resulting HQ is a unitless number that represents the ratio of the estimated exposure dose from Hg at the site to its RfD, which is assumed to be without adverse health impacts. In this study HQ Index was below 1 (0.35). Since the HQ is < 1, adverse health effects are not expected from the exposure described in the assessment. Therefore, the consumption of Golden gray mullet from the given location is not a serious threat.

Based on the above mentioned levels, consumption of *L. aurata* is safe, although pregnant women and infants should take into account some considerations for consumption of these fishes. Because fetuses, infants and children under 10 years old are the most sensitive group to mercury toxicity (UNEP, 1999).

In addition, according to World Health Organization, every levels of mercury can be harmful, and no specific level for the health effects of mercury can be identified (WHO, 1990) and guidance or standards for Hg in fish tissues are not always uniform (Burger and Gochfeld, 2005), It is recommended that the population restrains from consuming, on a regular basis, species exceeding these values.

Therefore, it is essential to determine the allowable fish consumption (daily or weekly). Based on the measured concentration and body weight, a consumption permitted rate of 51 g/day (357 g/week) is recommended. The CR_{lim} is the maximum consumption rate allowable without human health effects.

In order to better appreciate the safe amounts of fish for consumption, exposure limits can be expressed as the number of meals that an adult can eat per day, or per week. In risk assessment, the standard portion size of uncooked fish eaten by an average adult is estimated to be 230 g (USEPA, 1994). Safety limits, expressed as the frequency of meals for which fish is the main element.

Because almost all Hg are present as MeHg in the edible portions of fish (Bloom, 1991), we assumed that concentration of total Hg is equal to that of MeHg. Also, the body weight of an Iranian was assumed to be 70 kg. Based on these assumptions, the number of meals for safe consumption calculated from ADI of US EPA and PTWI of JECFA for MeHg was 1 and 3 meals per week, respectively (Table 2).

For individuals weighing more or less than 70 kg, it is assumed that their consumption rates and number of meals will be proportionally higher or lower, respectively.

Conclusion

Major source of methylmercury for fish is from mercury that has been methylated after atmospheric transport and precipitation or runoff, followed by food chain bio-magnification. Fish consumption is the only significant source of methylmercury exposure for the public. Therefore, fish consumption is a matter of risk balancing.

The average concentration of mercury in Mullet muscle from southern coast of Caspian Sea in the Mazandaran Province in Iran, was 0.137 µg/g of fresh weight (0.432 µg/g dry weight) which was less than the allowable amount for human consumption but more than the allowable amount for piscivorous mammals and birds determined by the international organizations. The calculations indicated that daily and weekly mercury uptake for Iranian consumers, according to FAO (the amount consumed per capita) is lower than the guide values (ADI and PTWI). Also, HQ Index was below 1 (0.35). Therefore, the consumption of the Golden grey mullet is not a serious threat to the consumer’s health and a consumption permitted rate of 51g is recommended.

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