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Exposure Assessment of Total Mercury: A Probabilistic-Approach Study Based on Consumption of Canned Fish

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ABSTRACT

Introduction: Exposure to mercury (Hg) by consumption of fish is a recent health concern. So, it is important to evaluate the health risks related to canned fish consumption. The purpose of this study was to investigate the potential health risk based on Hg concentration in people who consumed canned fish with a probabilistic approach in Isfahan City, the central province in Iran.

Materials and Methods: In this study, 20 popular brands of canned fish prepared in Iran and other countries were selected and analyzed for Hg concentration with atomic absorption spectrometer. The results were compared with the European Communities and JECFA guidelines. Then, a probabilistic method with Monte-Carlo simulation was used to assessment the Provisional Tolerable Daily Intake (PTDI) and the Hazard Quotient (HQ) for consumers in Isfahan City.

Results: The average Hg concentrations in samples were 0.251 ± 0.204 and 0.189 ± 0.152 µg/g in canned fish of Iran and other countries, respectively. The Hg level was found below the guideline limit for European Communities and JECFA. The estimated PTDI was 0.037 µg person/day and HQ was 0.074.

Conclusion: The results indicated that canned fish available in the markets of Iran did not have a health risk for adults. Moreover, canned fish consumption has a possible influence on the risk estimate and its risk should be assessed for vulnerable groups.

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Introduction

Humans usually consume fish in many parts of the world because it contains an appropriate source of protein and is considered as a necessary source of healthy nutrients. Based on the Food and Agriculture Organization of the United Nations (FAO), fish has consist of 16.6% of protein source in the world population's food and 6.5% of the total protein that consumed in the past years [1]. In comparison to the good health benefits, many cases of contamination were reported by fish due to the chemical pollutants in the environment [2]. Fish may be polluted by heavy metals through the commercial handling and canning process [3]. Exposure to toxic metals is important for children and pregnant women. The recent surveys found a correlation between fish consumption and hazardous effects on human health due to increased concentrations of mercury (Hg) and other pollutants [4-7].

Among the environmental contaminants, Hg is the most harmful to human health [8]. It is one of the most toxic components and a well-known global contaminant [9]. Human exposure to Hg leads to slow growth, cerebral palsy, blindness, and other birth defects. In addition, Hg is a neurological toxicant to humans and mainly affects organs such as brain and kidney [10]. The toxic limit of Hg for human fetus, determined by the US-EPA, is 0.1 µg/kg/day [11]. In 2003, more attention was paid to the risk attributed to Hg amounts in fish; then, canned fish has been considered as a main supply of Hg [12].

Adverse health impacts may occur if the fish is consumed too often or in large quantities. As a result, a perfect correlation was observed between Hg levels in humans and the amount of consumed fish [12, 13]. The incident of Hg in fish is a common issue for human health risk assessment and is of special concern to the United States Food and Drug Administration (US-FDA) [10]. Therefore, it is essential to know Hg concentrations in fish and ultimately advise the population about the health risks related to fish consumption [12].

Although some studies were conducted on the health risks of the canned fish consumption around the worlds [10, 13-17], limited data exist on the health risk assessment of Hg in canned fish in Iran. In other words, only a few studies attempted to deal with this in Tehran [18] and Mashhad [19], the two crowded cities in Iran. Moreover, all conducted studies focused on the deterministic risk assessment.

Therefore, it is necessary to determine the risks of Hg contained in canned fish consumed by the Iranians. Furthermore, the hazard levels of the pollution should be controlled in these sources.

Risk assessment is a systematic procedure that provides an assessment of the probability and severity of a specific risk related to consumption of contaminants in food sources [20]. Risk assessment studies consist of the deterministic and probabilistic methodologies. The deterministic or “point estimation” approach provides a single assessment of the risk to describe a variable in the model. However, the probabilistic or statistical method uses probability distribution functions (PDFs) to define uncertainty and variability of the model variables [21, 22]. Codex Committee on Food Additives and Contaminants (CCFAC) increasingly recommends application of the probabilistic technologies to determine the risk assessment of pollutants in the food sources [23]. Probabilistic methods conducted with Monte-Carlo simulation approach is a suitable computer-based method to reduce irresolvable calculations of the distribution densities by estimating an empirical distribution similar to the distribution of the risk across the population. It is based on statistical sampling methods for estimating a probabilistic approximation to the solution of a mathematical model. For each variable in the model, the probable values are calculated according to a probability distribution, which are determined by goodness-of-fit tests such as chi-square, Kolmogorov–Smirnov, and Anderson–Darling tests. If a Monte-Carlo simulation is run for 10,000 trials, 10,000 possible outcomes are anticipated and exposure and risk distributions of the population would be estimated using these simulated values [24, 25].

The objective of this study was to determine the concentrations of Hg in canned fish samples prepared in Iran and other countries consumed by Iranian people. In addition, this study represented the potential health risk assessment based on Hg concentrations in canned fish using a probabilistic approach.

Materials and Methods

Laboratory procedures

Twenty popular brands of canned fish samples including 14 samples prepared in Iran and 6 samples prepared in other countries (U.S, U.K and Australia) were purchased directly from markets in the city of Isfahan, the central province in Iran.

In this study, 1 g of each sample was mixed with 5 ml of nitric acid (HNO_3) (1 N), 3 ml H_2O_2, and 15 ml of $KMnO_4$ 5% in a Teflon vessel. The mixture was heated on a hot plate to 150˚C for 10 min for digestion. Then, the digested samples were diluted with 100 ml of double-deionized water. The Hg concentration in the samples was analyzed using a Perkin Elmer 4100 atomic absorption spectrometer equipped with cold vapor system [26]. The limit of detection (LOD) was 0.015 µg/g and the level of quantification (LOQ) was 0.005 µg/g. Analyses of each sample were performed in triplicate and no sample had an Hg concentration of less than LOD. In this study, all used reagents were of analytical grade (Merck, Germany).

Statistical analysis

The descriptive statistics (mean ± standard deviation, and median), Mann-Whitney test, and t-test analysis were directed using SPSS (Version 22, SPSS Inc., Chicago, IL, USA). A *P*-value of less than 0.05 was considered to indicate the statistical significance.

Human health risk assessment and model implementation

The health risk for human imposed by the intake of Hg caused by consumption of the canned fish was implemented by a probabilistic method in which variables were defined using with probability distributions and the risk estimate was obtained by Monte-Carlo simulation [20].

Exposure assessment

Probability distributions of Hg concentration, body weight, and canned fish consumption were fitted to normal, lognormal, uniform, exponential, logistic, beta, gamma, and Weibull distributions. The goodness of fit was measured using Chi-squared, Kolmogorov–Smirnov, Anderson–Darling statistics, and graphs (empirical and theoretical distributions plot and, P–P plot and Q–Q plot). Hg levels in canned fish and body weights (mean (μ_y) and standard deviation (δ_y)) were fitted with the generalized log normal distribution and canned fish consumption were fitted with the Weibull distribution as follows, respectively:

$$f(x) = (\frac{1}{x\delta_y\sqrt{2\pi}})exp\{[\ln(x) - \mu_y]^2/2\delta_y^2\} \quad (1)$$

$$f(x;\ \lambda, k) = \frac{k}{\lambda}(\frac{x}{\lambda})^{k-1}e^{-(x/\lambda)^k} \quad if\ x \geq 0 \quad (2)$$

Where, k > 0 is the shape parameter and λ > 0 is the scale parameter.

Canned fish consumption and average body weights (kg) were examined based on the questionnaires collected from 4763 adults in Isfahan City [27].

Risk characterization

Exposure has normalized for time and body weight and has typically expressed in units of µg Hg/kg body weight/day. The estimation of health risk was based on the PTDI through the following equations [28]:

NTMID = MI × F (3)

PTDI = NTMID / WAB (4)

Here, NTMID is the daily intake of Hg (µg/person/day), MI is concentration of Hg in canned fish (µg/g wet weight), F is the canned fish intake (g/person/day), WAB is the body weight (kg), and PTDI is the daily intake per body weight (µg/kg person body weight/day).

The HQ is calculated using the following equation:

HQ = EF × ED × PTDI / (RfD × TA) (5)

where, EF is the exposure frequency (365 days/year), ED is the exposure duration corresponding to the average lifetime (70 years), the Reference oral Dose (RfD) is an estimate of a safe daily oral exposure (0.5 µg/kg/day), and TA is the average exposure time for noncarcinogens (365 days/year x ED) [1, 12].

The Hazard quotient (HQ) indicates the health risk of the Isfahan population to Hg exposure and is obtained from the ratio between the canned fish ingestion rate and RfD. If HQ < 1, there will be no evident risk; however, if HQ = 1, the contamination itself seems to cause no risk; and if

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HQ > 1, we cannot exclude the possibility of the harmful effects to human health [29].

Sensitivity analysis

The sensitivity analysis was performed using the Spearman rank order correlation (ρ) between the Weibull distribution for canned fish consumption and the log normal distributions for Hg concentration, and body weight. The Monte-Carlo simulation analysis was done in 10,000 iterations by R version 3.4.3 software.

Results

Concentration of Hg in canned fish

The results of the present study showed the mean Hg concentration of samples prepared in Iran and in other countries (Table 1).

Risk assessment for human health

Dietary exposure (PTDI)

In this study, the PTDI values were calculated by taking into consideration the similar Hg concentrations for the canned fish samples prepared in Iran and other countries. Other parameters, used for PTDI calculation, were body weight and canned fish consumption rates based on the completed questionnaires in the study area [27]. In this study, mean ± SD of the daily consumption of the canned fish and the human body weight (with log normal and Weibull distributions, respectively) were earned 10.7 ± 11.8 g/person/day and 68.7 ± 12.8, respectively.

As the Hg concentrations were above the LOD, all data were used in the PTDI calculation. The calculated log normal distribution Hg concentration was 0.229 ± 0.1824 µg/g based on mean ± SD.

Finally, the 95% confidence interval (CI) for PTDI using a probabilistic approach by Monte Carlo simulation ranged from 0.0260 to 0.0525 µg/person/day (Mean = 0.037 µg/person/day) (Figure 1,a), which is below the respective guideline value recommended by European Communities and JECFA, 0.5 µg/g body weight [20].

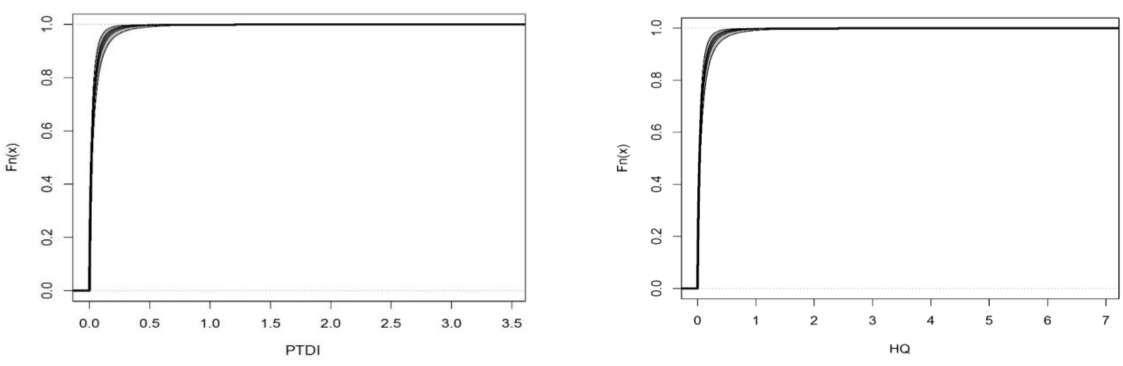
The 95% CI for HQ was 0.0521 to 0.1051 (Mean = 0.074) and showed that ingestion of the canned fish had no risk. Consequently, HQ lower than 1 is considered as a healthy and harmless food item with regard to Hg (Figure 1, b) and has no potential health impacts on consumers.

The results of the sensitivity analysis of the canned fish consumption through Hg concentration and average body weight are shown in Figure 2.

Table 1: Hg concentration (µg/g) in canned fish samples

Samples	No of samples	Median [Min-Max]	Mean ± SD	P-value
Iranian	14	0.138 [0.079-0.668]	0.251 ± 0.204	0.444
Other countries	6	0.102 [0.089-0.449]	0.189 ± 0.152	

P-value is based on Mann-Whitney test



(a) (b)

Figure 1: Variability cumulative distribution plots of the Hg PTDI and HQ. For each percentile of variability (y value), the corresponding x value is the point estimate of PTDI and HQ. The x value of the corresponding points on the light gray lines corresponds to the 95% CI.

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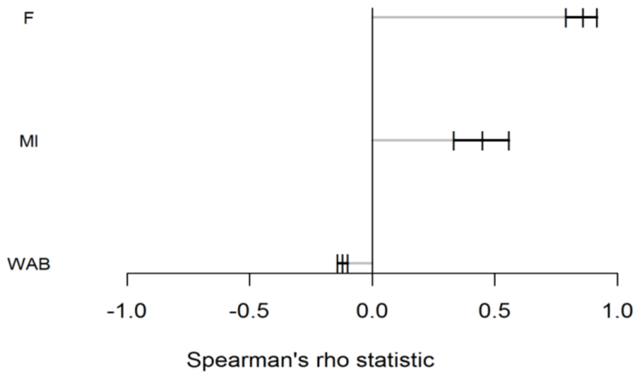

Figure 2: Tornado charts for the median estimates of the spearman's rank correlation between the input variables and the PTDI, bounded by the 95% uncertainty range, for adults that consumption of canned fish. MI is concentration of Hg in canned fish (µg/g wet weight), F is the canned fish intake (g/person/day) and WAB is the body weight (kg).

Discussion

Concentration of Hg in canned fish

We compared the mean Hg concentration obtained in this study with those of other studied (Table 2). According to our findings, Hg concentrations in the Iranian samples of canned fish were not much different from other areas of the world [2, 3, 13, 14, 33-37]. Burger and Gochfeld, Reported that the maximum Hg was 0.997 µg/g, but 25% of white tuna samples exceeded 0.5 µg/g [34].

The differences in accumulation patterns of Hg can be explained by their pathways of absorption and accumulation in the fish body [38]. The results of the present study demonstrated no significant difference between the Iranian and foreign canned fish with respect to Hg concentration (*P*-value = 0.44). In other words, the Hg contents of the canned fish samples prepared all over the worlds are similar.

Table 2: Mean concentration of Hg in Iranian canned fish samples compared to that in other literature

Country	Hg concentration (µg/g)	Reference Number
Iran	0.251	Present study
Iran	0.125	2
Iran	0.117	32
Iran	0.13	18
Turkey	0.14	3
Spain	0.222	16
Canada	0.6	30
India	0.62	30
Italy	0.41	13
Saudi Arabia	0.31	31
U.S	0.623	35
U.S	0.285	14
Australia	0.14	33
U.K	0.055	36
Lebanon	0.075	37
Libya	0.29	37

Risk assessment for human health

Since Hg is known as an environmental pollutant with high toxicity, even at low concentrations, and may develop neurological changes and some diseases, the European Communities and JECFA established a guideline level (0.5 µg/g wet weights) for its application [20]. In this study, we discussed the relationship

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between this guideline and intake of Hg through canned fish consumption in a risk assessment survey.

The deterministic model was usually used to estimate the dietary exposure. However, this method has a problem; it overestimates the risk, since it combines the single high-level consumption and single measured high contaminant concentration. In contrast to this method, probabilistic model calculates the exposure levels using distribution of the multiple input parameters. This approach estimates the human exposures compared to the acute Reference Dose (RfD) [23, 39]. Probabilistic dietary exposure to heavy metals throughout the food supply is concerned with the difference of the risk associated with different societies and differences in national and international levels related to local food consumption patterns [23]. The results of a study by Morales et al. showed that the deterministic approach could overestimate the risk in comparison with probabilistic approach [22]. Nevertheless, the deterministic approach is suitable to determine the ranges of the variables that contribute to the risk computation. It is important that fish consumption may vary considerably from one individual to another and the daily intake of a substance from food consumption is depending on the substance amount in consumed foods[10].

In this study, the mean ± SD of the daily consumption of the canned fish and the human body weight were determined as 10.7 ± 11.8 g/person/day and 68.7 ± 12.8 kg, respectively based on statistical distribution. Although other studies were reported these parameters without considering the statistical distribution and they had considered with one point estimate; for example, the amounts of canned tuna fish that it had consumed by adult with bodyweight is equal 60 kg were 3.92, 1.9, and 17.2 g/person/day in Mexico, Spain, and Italy, respectively [12, 13]. However, the Iranian studies assumed that the Iranian people with 60 and 70 kg of body weight consumed 20.5 and 3.5 g/ person/day canned fish, respectively [18, 19]. In our study, the Hg levels were calculated as 0.229 ± 0.1824 µg/g based on log normal distribution. In addition, the PTDI was calculated as 0.037 µg/person/day by the probabilistic approach, which is below the respective guideline value of 0.5 µg/g body weight suggested by the European Communities and JECFA [20]. The results of a research by Hajeb et al. indicated that the EWI (Estimated Weekly Intake) of total Hg by a 50 kg adult was below the guideline value of 0.5 µg/g body weight [10]. In another study, the metal contamination was analyzed including levels of Hg in canned fish in Andalusia. The researchers reported that the calculated Hg intake was 63.63 µg/person/week (9.1 µg/person/day) below the regulatory maximum levels [15]. Assessment of trace elements in canned fish, including mackerel, salmon, tuna, sardines, and herrings purchased from Georgia and Alabama was conducted by Ikem et al. They found that the EWI of Hg for a 60 kg adult consuming 350 g of fish/person/week were was the guideline value in mg/kg body weight [14]. This value was lower than the one found by Rubio et al. in the Canary Islands (5.69 µg/person/day) and Falcó et al. in the Spain (9.89 µg/person/day) [40, 41]. So, it can be said that the results of the present study are in line with the previous studies.

In our study, the HQ was calculated to determine the health risk caused by intake of Hg through canned fish consumption. The calculated (HQ = 0.074) showed no concern for potential health impacts on consumers. The study by Jorge Ruelas-Inzunza et al. indicated that none of the HQ values of tuna canned in water and oil was higher than 1 [12]. Another study showed that the calculated HQ values of Hg were within the safe limits. Therefore, no potential significant health risk was observed in consuming canned fish in Tehran City [18].

Sensitivity analysis and Recommendation

Spearman's rank correlation showed that canned fish consumption, Hg concentration, and average body weight, had a possible influence on the risk estimate based on PTDI (Figure 2). Jiang et al. investigated the sensitivity analysis to determine the effective variables of the dietary Hg exposure for Taiwanese mothers [42]. Their results indicated

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that concentration of Hg in fish was an important parameter and the rate of fish ingestion influenced the risk of Hg exposure [42]. Usually, the food habits are important parameters that influence the Hg exposure caused by fish [43]. In this study, we considered the Hg exposure caused by canned fish in adults, but the distribution of Hg concentrations in fish and the individuals' rate of consumption are important factors in assessing the probabilistic exposure.

Consequently, Hg exposure caused by fish or canned fish sources should be investigated among pregnant women, women in childbearing age, nursing mothers, and young children. However, the results of this study showed that consumption of the canned fish was a more influencing parameter and both high risk (children and pregnant women) and low risk (adults) populations are required to observe the health risk of this source [19]. Moreover, they should consume moderate amounts of fish, because large consumption patterns, especially for tuna and mackerel, may lead to increased health risks [10].

Since fish products contain high-quality protein and essential nutrients, it is necessary to examine other hazardous substances such as other toxic metals, especially arsenic [22], dioxin/furans (PCDD/PCDF), polychlorinated biphenyls (PCB), and pesticides in the canned fish Since no fish advisories have been established in Iran, a necessity is felt to provide the Iranians with information on the comparative distribution of the nutrients and chemicals in canned fish to formulate dietary recommendations [11].

Conclusion

In this research, the concentration of Hg was studied in canned fish samples. The findings showed that the level of Hg in the studied samples did not exceed the recommended level established by the European Communities and JECFA. The comparative of Hg concentration between canned fish prepared in Iran and other countries showed no significant difference ($p > 0.05$). The risk assessment revealed a low risk of Hg intake from canned fish for adults. The HQ was estimated as 0.074 for adults and the recommended threshold of 1 was recommended as the acceptable level. Moreover, the sensitivity analyses exhibited that the canned fish consumption and Hg content had a main effect on increasing the risk of Hg intake from the canned fish.

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Conflicts of interest

The authors declare no conflict of interest.

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