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Lead exposure through eggs in Iran: health risk assessment

Sajad Faryabi[1], Mohsen Ghorbiani[1], Hadi Haghbin Nazarpak[2], Azadeh Rashidimehr[3,*]

[1] Shahid Chamran University of Ahvaz, Ahvaz, Iran

[2] Islamic Azad University of Garmsar, Garmsar, Iran

[3] Lorestan University, Khorramabad, Iran

* e-mail: azadehrm68@gmail.com

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Abstract:
Introduction. Contamination of food, including animal protein sources, with heavy metals is a major threat to humans. The aim of this research was to determine lead concentrations in eggs from different Iranian regions and assess risks to human health.
Study objects and methods. In this study, lead concentrations in eggs produced at laying hen farms in Qom, Isfahan (Kashan city), and Khorasan Razavi (Mashhad city) provinces were measured by an atomic absorption device. Health risk was estimated using the Human Health Risk Assessment (HHRA) model.
Results and discussion. The levels of lead in eggs were significantly different ($P \leq 0.05$) among the three regions. They were lower than the permissible limit (0.1 mg/kg) for Kashan (0.0756 mg/kg) and Mashhad (0.0633 mg/kg), but eggs from Qom contained 0.1163 mg/kg of lead. In all the three regions, the estimated daily intake (EDI) of lead was lower than the maximum tolerable daily intake (MTDI), indicating no health risk for lead through egg consumption among Iranian consumers. Also, no risks were detected for adults in terms of non-cancer risk, or target hazard quotients (THQ), and carcinogenic risk (CR) of lead (THQ < 1 and CR < 10^{-6}).
Conclusion. The results of this study indicated that lead health risk through egg consumption is within safe limits. However, the nutritional importance and high consumption of eggs among households necessitate a more careful monitoring of lead concentrations to meet public health requirements.

Keywords: Heavy metals, laying hen farm, estimated daily intake (EDI), carcinogenic risk (CR), lead, eggs

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INTRODUCTION

Environmental pollution caused by the development of livestock and poultry production has raised concerns about food safety, in particular about possible residues of heavy metals in feed additives or poultry feed and products, including eggs [1]. In addition to being non-biodegradable, heavy metals have a long biological half-life and can conceivably accumulate in different parts of the human and animal body due to the lack of sufficient mechanisms for their elimination [2].

Heavy metals can be transmitted from poultry to eggs through environmental pollution or via the food chain [3]. Thus, female poultry can absorb heavy metals from different sources in the environment and transmit them into their eggs [4].

Studies have shown that feed is one of the most important ways of absorbing heavy metals by laying hens [5, 6]. Several parameters influence the bioaccumulation of heavy metals by laying hens, including the chemical and physical properties of heavy metals, season, location, and chicken qualities (nutritional behaviors, metabolic cycle, and age) [5].

Since lead is one of the most common heavy metals in the environment, its low concentrations can be found in many organisms. Sources of lead pollution of soil, air, and water include lead gasoline, industrial effluents, lead pipes, lead-based paints, as well as alloy and oil processing plants [3, 7]. Exposure to lead can also result in the consumption of contaminated animal tissues or plants and seeds grown in contaminated soil [7].

Lead can enter the body through the skin, respiration, or, more importantly, via the digestive tract. In case of continued contamination, it can accumulate in the body, causing acute or chronic toxicity. Lead poisoning is a cause of disease in humans, animals, and birds. Nutrition and climate are the factors that influence this complication [8]. Symptoms of heavy metal poisoning include dizziness, nausea, vomiting, diarrhea, sleep disorders, loss of appetite, and decreased consciousness. Therefore, it is essential to monitor and estimate heavy metal levels due to their negative effects at different levels, from a biochemical response to population-level changes [9].

Currently, food safety is viewed as a significant worldwide concern, chiefly because more than 90% of human exposure to heavy metals is through food consumption [2]. In addition, food safety concerns are growing because of the accumulation of heavy metals in the environment and the consequent risks to human health [2]. One of the most important aspects of environmental quality control and food safety is monitoring the level of toxic and potentially toxic elements. Human health risk assessment is one of the most extensive systematic processes to estimate the potential impacts of health hazards on ecological systems or human populations within a specified timeframe.

There are several models for health risk assessment that require multiple contents to assess health risk. Modeling can provide a non-descriptive method for assessing exposure risks rather than damage to organisms [10–12]. The Provisional Tolerable Daily Intake (PTDI) rate is a reference value set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) that indicates a safe daily pollutant intake [13]. The amount of PTDI is based on the daily intake of such pollutants as heavy metals that should not accumulate in human body throughout lifetime. It is used as a primary health indicator for both food and non-food sources to determine the "total exposure level to a given contaminant" [5].

Since chickens and other birds are exposed to heavy metals, such as lead, they are highly suitable for monitoring the presence and effects of environmental pollutants. In addition, studies indicate that birds in many cases are more sensitive to environmental pollutants than other vertebrates [14, 15].

Lead has been identified in chickens and its public exposure is a potential health concern. The lack of clear clinical signs in chickens that cause lead exposure or toxicity makes early detection difficult [7]. Heavy metals accumulate in various bodily tissues and organs of chickens, hence the contamination of resultant products, depending on the type of feeding and breeding conditions, which has not yet been studied comprehensively in Iran [16]. The likely entry of heavy metals into the body of a chicken may result in the contamination of eggs from farms, in addition to tissue contamination. Heavy metals can be transferred to edible parts of the egg [17]. A global egg lead concentration of 0.01–0.97 mmol/kg has been reported considering seasonal effects, which highlights the importance of health and safety assessment of this product [18].

Eggs are highly nutritious foods that benefit human health [4]. They are a rich source of essential amino acids, vitamins (e.g., vitamin E), beneficial fats (e.g., ω3 fatty acids), and trace elements (e.g., Fe, Zn, and Se) [17, 19]. Over 80% of individuals believe that eating eggs is useful for their health [20]. According to recent research on egg consumption in adults, consuming one egg per day can diminish a risk of stroke by 12% [20]. The Food and Agriculture Organization (FAO) predicts that egg consumption will increase from 6.5 to 8.9 kg per year in developing countries and from 13.5 to 13.8 kg per year in industrialized countries between 1999 and 2030 [21].

Eggs may contain high levels of heavy metals, mainly derived from food and water consumed by laying hens, which are affected by the environment. Toxic heavy metals can affect the quality of consumable eggs. Lead accumulates in the shell, yolk, and albumin. Yet, higher lead deposits in the shell than in egg contents were reported in most studies, including [22]. There are a number of methods evaluate lead concentrations in birds and their products. Adapted from previous studies, the atomic absorption spectrophotometry with graphite oven is currently used as a rapid and accessible method for this experiment [23]. Exposure of hens to lead may be chronic, with variable individual clinical symptoms resulting from different concentrations of lead [24].

Currently, industrial and other wastes enter the environment without any treatment. It is, therefore, necessary to control the amounts of heavy metals in diets and ultimately in poultry products to ensure consumer health [25]. In connection with the above, our study aimed to estimate a lead exposure risk through eggs, determine lead concentrations in three provinces of Iran, and compare lead concentrations and exposure assessments between the three provinces of Iran.

STUDY OBJECTS AND METHODS

Egg preparation and analysis. For this study, 10 samples of consumable eggs were randomly collected from 25 industrial laying-hen farms (totally 250 samples) during 4 months and transferred to the laboratory. Since Qom province has a relatively higher production volume, 15 laying farms were selected from this province. In Khorasan Razavi and Isfahan provinces, five farms were sampled from their two industrial cities, Mashhad and Kashan, respectively. The samples from each farm were then homogenized completely for the experiment.

The samples were prepared for acid digestion and reading by an atomic absorption device (Varian SpectrAA-20 plus, Australia) according to the AOAC standard method [26]. It should be noted that a standard lead solution at a concentration of 1000 μg/mL was prepared from the stoke standard (Spex CertiPrep®,

Table1 Parameters for graphite atomic absorption spectrophotometry

Metal	Wave-length, (nm)	Temperature, °C				D2 Lamp
		Drying	Ashing	Atomi-zation	Cleaning	
Lead	283.3	130 10[a] 30[b]	650 5[a] 10[b]	1900 0[a] 2[b]	2500 2[a] 2[b]	on

a: Ramp; b: Hold

USA). Lead concentrations in egg samples were determined directly in the final prepared solution using an oven atomic absorption (Varian SpectrAA-20 plus, Australia) according to the AOAC standard method [26]. The temperature program of the oven is shown in Table 1. Nitric acid (10% v/v) (68%, Darmstadt, Germany) was used as the blank.

A homogenate sample (1 g) was weighed, transferred into a crucible, and placed on a hot plate (C-MAG HP, Germany) for the ashing process. After the end of smoke from the crucible, it was placed in an oven at 200–250°C, and the oven temperature was increased gradually to 500 ± 50°C with a rate of about 50°C/h within 8 h. The process of cooling the crucible, moistening with nitric acid, and re-incubating in the oven was repeated until the sample was completely transformed to ash (grayish-white). Five milliliters of 6 M chloridyric acid (37%, Darmstadt, Germany) was then added to the crucible so that all the ash was impregnated with the acid. The residual content in the crucible was dissolved with 5 mL of 0.1 M nitric acid, covered with a watch glass, and left for 30 min. The crucible content was passed through a Whatman filter paper (0.45 μm), transferred to a 100 mL volumetric flask, and made into volume [26].

Health risk assessment. Problem formulae. In this study, the human health risk assessment (HHRA) model was used to describe the potential risk of heavy metals through the consumption of poultry eggs collected from commercial strains of laying hens in three provinces of Iran. The HHRA model was proposed by the US Environmental Protection Agency (USEPA) to calculate health risk needs (estimated daily intake, target hazard quotient, and carcinogenic risks) [27, 28].

Daily intake assessment. Estimated daily intake (EDI) of heavy metal contaminants through egg consumption depends on heavy metal concentrations in the egg content, daily egg consumption, and consumer body weight [29, 30], which is obtained using the following formula:

$$\text{EDI} = \frac{C \times IR_d}{\text{BW}} \quad (1)$$

where C is a heavy metal concentration (mg/kg wet weight) in the egg content, IR_d is a daily egg intake (16.95 g per day), and BW is body weight (70 kg for an adult) [31, 32].

Non-carcinogenic and carcinogenic risks. In this study, non-carcinogenic health risks associated with egg consumption were investigated using the target hazard quotient (THQ). The calculations were based on the guidelines of the United States Environmental Protection Agency [33] as follows:

$$\text{THQ} = \frac{\text{EFr} \times \text{ED} \times FIR \times C}{\text{RfD} \times \text{BW} \times \text{AT}} \times 10^{-3} \quad (2)$$

where EFr represents exposure frequency (365 days/year), ED denotes exposure duration (70 years for adults), FIR indicates the egg consumption rate (g for person daily), AT symbolizes the average exposure time for non-carcinogenic risk (365 days/year×70 years), and RfD shows the reference food dose (1 mg/kg BW/day) for lead [34].

Carcinogenic risks (CR) are estimated as an incremental likelihood of developing cancer in an individual throughout lifetime as a result of exposure to potentially carcinogenic factors [28]. The cancer slope factor (CSF) provided by the USEPA's Integrated Risk Information System (IRIS) is 0.001 mg/kg/day^{-1} for lead. Acceptable levels of risk for carcinogens vary from 4^{-10} (1 out of 10 000 risk of cancer development during human lifetime) to 6^{-10} (1 out of 1 000 000 risk of cancer development in human lifetime). The following equation was used to estimate lifetime CR [27, 29]:

$$\text{CR} = \frac{\text{EFr} \times \text{ED} \times FIR \times C \times \text{CSF}}{\text{BW} \times \text{AT}} \times 10^{-3} \quad (3)$$

Statistical analysis. Data were analyzed by descriptive statistics using SPSS 17 software (SPSS, Chicago, IL).

RESULTS AND DISCUSSION

Lead concentrations. Figure 1 compares lead levels in eggs sampled from 25 laying hen farms with the maximum permissible level (0.1 mg/kg) [35].

According to Fig. 1, the lead concentration in eggs from Qom exceeded the international standard, while eggs from Kashan and Mashhad demonstrated lower values.

Lead is a pollutant generally found in surface artifacts, but industrial emissions are a chief cause of lead exposure in animals and humans. In this research, high levels of lead in all the farms can be related to such factors as industrialization of the studied cities and high environmental contamination, including contaminated feed consumed by laying hens (Fig. 1). In addition, Islam *et al.* found high concentrations of lead in grains and vegetables, which are likely because of leads melting, heavy traffic, and other industrial activities near commercial farms [36].

After oral administration, lead or lead salts are merely partially absorbed (roughly 10% of the oral dose). Fortunately, absorption is limited due to the low solubility of many lead compounds, the deposition of lead ions with bile acids, and the formation of lead

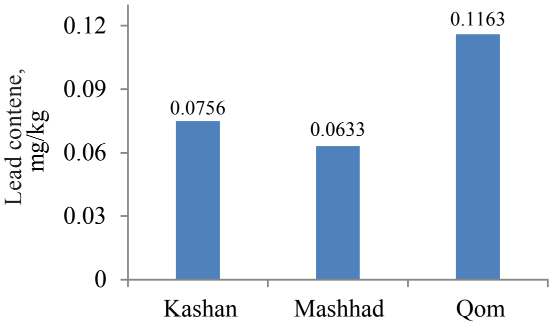

Figure 1 Comparison of international lead standard with lead contents in the egg samples under study (mean ± standard deviation)

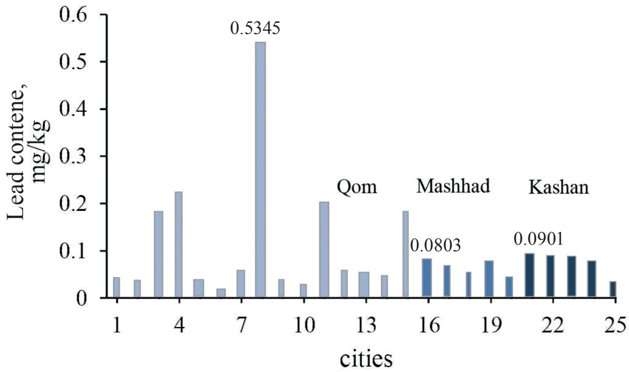

Figure 2 Comparison of lead concentration in eggs from studied regions

sulfide by Enterobacteria. It has previously been shown that interactions with other elements can affect the absorption of lead from the intestine [37].

An acute type of poisoning causes central neurological signs such as epileptic seizures or cramps, whereas chronic poisoning is often remarkably difficult to diagnose due to the lack of pathognomonic signs. Signs occur more in lead levels greater than 500 mg/kg feed. Therefore, this amount is several times higher than the maximum limit (10 mg/kg) for a separate feed of plant roots. Lead levels in laying hen feeds are higher than in broiler feeds, which may be due to the provision of calcium sources to increase the quality of eggshell, usually made from oyster or limestone shells.

It may well be contended that various kinds of calcium sources may be added to moderately higher measures of minor components in mixed feeds for laying hens, but a large scale calcium production seems to be negligible [37, 38]. Lead is naturally present in the environment as a pollutant derived from agricultural and industrial activities. Food is the principle source of human exposure to this element, which accumulates in the body and affects the central nervous system [39].

On the other hand, the number of pollutants can vary, depending on the type of industry (poultry versus livestock farming; freshwater or marine fisheries versus terrestrial animals; extensive system versus severe animal husbandry) and the geopedological attributes of soils where crops are cultivated. Also, the principal food source for laying hens in all poultry farms is grain or commercial layer pellets, and commercial feed exposure to heavy metals in storage is comparatively rare. The main route of exposure to heavy metals is actually food [37].

Our study agrees with other researchers who have shown that high concentrations of heavy metals, such as lead, in commercial eggs can be associated with the use of dietary supplements and polluted water sources, as well as pesticides to discard insects [4, 39, 40].

In Qom eggs, sample 8 showed the highest concentration of lead (0.5345), with was much higher than the international limit (Fig. 2). This could result from the proximity of farms to industrial plants. Subsequently, the highest lead levels in eggs from Mashhad and Kashan were 0.0901 and 0.0803, respectively.

Lead concentrations in the eggs under study revealed no statistically significant differences between the studied regions (Table 2, $P \geq 0.05$). A significant difference ($P \leq 0.05$) was observed between the mean concentration (0.097 mg/kg) of the studied samples and the international standard lead level in eggs (0.1 mg/kg).

Zariff *et al.* studied lead uptake rates through eggs in children from one of southern Australian cities. The authors found that eggs produced from farms near industrial areas contained the highest lead concentrations, leading to increased lead levels in the blood of those children who consumed the products of these farms [41]. Their reported mean lead concentration (0.09) is in agreement with our study results.

Farahani *et al.* also reported a lead concentration of 0.75 mg/kg in 32 laying hen farms from Markazi province, which is significantly higher than the permissible level and our results [42]. Uluozlu *et al.* and Kirkpatrick and Coffin reported lead levels of 0.05 and 0.01 mg/kg in eggs, respectively, which are lower than those in our study [43, 44]. Further, egg lead concentrations of 0.51, 0.27, and 0.13 mg/kg were detected in the studies of Basha *et al.*, Abdulkhaliq *et al.*, and Khan *et al.*, respectively, which are higher than our results [45–47]. Basha *et al.* also found a mean lead of

Table 2 Concentration of lead in eggs from different cities ($P \leq 0.05$)

Metal	Lead permissible level, mg/kg	Samples	Mean lead concentration, mg/kg	Concentration of lead, mg/kg		
				Kashan	Mashhad	Qom
Lead	0.1	125	0.0970	0.0756	0.0633	0.1163

Table 3 EDI, THQ, and CR of lead for national consumers of eggs in different regions

	Region	EDI, μ/kg^{-1} BW/day	THQ	CR
	Kashan	0.018306000	0.004577	1.55601E-07
	Mashhad	0.015327643	0.003832	1.30285E-07
	Qom	0.028161214	0.007040	2.3937E-07
PTDI		3.57	3.57	3.57
TDI		3/1.5	3/1.5	3/1.5

0.02 mg/kg obtained by consuming one egg per day [45]. Grace and MacFarlane found that increased contents of heavy metals resulted from high uptake of these elements in birds through feed and water, along with possible effects of such factors as differences in their age, species, and laying cycle [14].

Contrary to our research, a study on eggs consumed in California and another study on eggs produced in New York local orchards reported lead concentrations above 0.97 and 0.167 mg/kg, respectively [16, 18]. In Saudi Arabia, feeds used in 74 poultry farms were reported to be heavily contaminated with heavy metals [46]. Relatively higher concentrations of lead in environmentally contaminated areas detected in the previous studies confirm our findings and also indicate that environmental concerns necessitate an increasing need to determine toxic metals in eggs [48].

In order to explain the effects of contamination on poultry products, many studies have been conducted on the fetal effects of metal contaminations. In a study by Surai, hens could control high metal deposition in eggs by avoiding mineral deposition [49]. However, protective layers that may be sufficient for such minerals as chromium and manganese may not be appropriate for other metals such as lead in eggs [50].

A matter of concern is that lead disrupts the bodily enzymatic reactions, particularly the synthesis of molecules that form an important part of hemoglobin and are an essential component of oxygen transfer in the body. An increased lead content was observed in blood groups of Arak city, which was attributed to elevated environmental pollution in the industrial areas [51].

In a study on metal contents in eggs in Nigeria, an average total concentration of 0.59 μg/g was found for lead, with a strong positive correlation between metal contents in feed and the corresponding levels in eggs [52]. In Pakistan, a research on the elemental composition of eggs revealed an average lead concentration of 0.52–0.62 mg/kg [22]. Hui detected high concentrations of lead in eggs similar to those reported in eggs collected from California cities. Although the author did not specify the exact concentrations, the highest concentration was shown to exceed the estimated safe concentrations [50]. However, the need for monitoring lead concentrations has been emphasized in all of these studies.

Health risk assessment. In the present study (Table 3), lead EDI (mg/kg body weight per day) was estimated based on per capita egg consumption (16.95 g/day) in adults (70 kg body weight) [28]. Our study also compared the EDI values with those of the Provisional Tolerable Daily Intake (PTDI) and the respective Tolerable Daily Intake (TDI). The EDI values were lower than the PTDI and the TDI, indicating no lead-related health risks through egg consumption. Qom accounted for the highest value among the three provinces in this study.

Risk assessment determines the potential health effects of doses that a human receives from a contaminant through one or more exposure routes. Non-cancer risk (THQ) and CR of egg consumption for Iranians are shown in Table 3. The THQ is a ratio of the dose determined from a contaminant to the level of a reference dose. If the ratio is greater than one, the exposed population is likely to experience conspicuous adverse effects [53]. We also recorded a THQ value lower than one for lead in eggs, suggesting that lead uptake through eggs does not pose a significant non-carcinogenic risk, in line with Hashemi *et al.* [5]. Finally, the level of non-carcinogenic risk by egg consumption did not exceed the USEPA risk management criterion.

For people who consume eggs exposed to lead, there is a potential risk to their health. Although most families consume eggs three times per week, continuous exposure to high concentrations of lead can prompt adverse health impacts in children. These impacts include attention-deficit hyperactivity disorder, behavioral disorders, IQ deficiencies, and diminished brain volume [54, 55]. In fact, there are no clearly defined thresholds for lead adverse health impacts [55]. Subsequently, laying hen owners should know about the risk of repeated consumption of lead-polluted eggs, even at concentrations that they see to be inconsequential.

Carcinogenic risks. Based on animal studies, lead has been classified among carcinogenic agents [29]. In general, cancer risk at the lead concentration of less than 10^{-6} is insignificant, above 10^{-4} is considered unacceptable, and between 10^{-4} and 10^{-6} is regarded as acceptable [34]. In our study, carcinogenic risks from lead were in negligible ranges in all the three regions, indicating no carcinogenic risk for Iranian consumers through the consumption of egg-borne lead, which is consistent with other studies in Iran [32, 56]. Ki *et al.* examined cancer development through the consumption of eggs containing heavy metals in Tehran, indicating safe metal concentrations for Iranian consumers [56].

CONCLUSION

Our study investigated the presence of lead, whose potential toxicity is properly known, in eggs produced in three Iranian regions. We found that lead health risks through egg consumption are within a safe range. Despite the relatively low lead concentrations in eggs, except for Qom province, and also the acceptable range of health risk for consumers determined here, the eggs produced in these regions contribute to a portion of daily lead consumption sources. Regular consumption of contaminated eggs in these areas is a source of lead, particularly for children and pregnant women. Given the importance of eggs in the daily diet, tolerable human intake increases economically and nutritionally, along with the availability of other food and environmental sources of this metal. Thus, more attention should be paid to this issue to consider strategies for the control and reduction of lead intake by humans. Also, control programs are recommended to reduce lead concentrations.

Therefore, a safer option is to raise awareness among poultry proprietors, public health offices, and veterinarians of the problem and potential resources. In higher risk environments (e.g., farms in industrial cities), testing environmental samples before placing poultry is recommended, and monitoring poultry and eggs after placing them ought to be considered.

CONTRIBUTION

The authors were equally involved in writing the manuscript and are equally responsible for plagiarism.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ORCID IDs

Azadeh Rashidimehr https://orcid.org/0000-0001-8935-8321