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Original Article

Monitoring and Risk Assessment of Lead and Cadmium in Milks from East of Iran Using Monte Carlo Simulation Method

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ABSTRACT

Background and Objectives: Milk is an important component of the human diet. Exposure to heavy metals through dairy consumption of contaminated foods endangers human health. The aim of this study was to assess concentrations of lead and cadmium in raw milk samples from east of Iran and their safety risks.

Materials and Methods: In this study, 54 raw milk samples from five regions were selected by cluster sampling and 20 samples of various popular brands of pasteurized milks were randomly purchased from supermarkets in east of Iran. Samples were digested in laboratory using acid digestion method. Concentrations of elements were calculated using graphite furnace atomic absorption spectroscopy. Monte Carlo simulation (MCS) approach was used to assess safety risks and investigate noncarcinogenic effects of lead and cadmium.

Results: The mean concentrations of lead and cadmium in raw milks included 38.15 and 4.67 ±0.001 µg/kg, respectively. The mean concentrations of lead and cadmium in pasteurized milk included 48.49 ±0.001 and 6.84 ±0.001 µg/kg, respectively. Moreover, the target hazard quotient (THQ) for adults and children in all groups were reported within the safe limits. There were hence no possible risks of exposure to lead and cadmium as results of raw and pasteurized milk consumptions.

Conclusions: Lead and cadmium levels did not exceed maximum levels (MLs) in any samples of raw and pasteurized milks. Persistent monitoring is a critical action to protect consumers from contaminated foods. The health risk assessment pursuant to Monte Carlo simulation approach demonstrated that children and adults were not at impressive health risk (THQ < 1).

Keywords: Lead, Cadmium, Heavy metals, Raw milk, Pasteurized milk, East of Iran

Introduction

Milk is a high-value nutrient in human diets, reach in macronutrients and micronutrients. Milk, as a perfect food for humans, contains maximum levels of calcium, protein, magnesium and potassium. This explains why it is important to monitor milk for chemical contaminants. Heavy metal contamination frequently occurs in milk and its products (1, 2). Although development of technology has led to a higher quality of life, this has also resulted in increased contaminants and environmental problems (3). Some of these contaminants and toxic compounds such as dioxins, pesticides, heavy metals and metalloids enter human food chains directly and indirectly (4). The entrance and accumulation of heavy metals are mainly due to industrial activities, fertilizers, animal manures, municipal sewages, composts and pesticides and soil characteristics of agricultural fields (5). The most fundamental issue of heavy metals is that they are not

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metabolized in the body. After entering the body, heavy metals are not removed but are accumulated in tissues such as fats, muscles, bones and joints. This results in diseases and complications in the body. Of the contaminants entering the human food chain, lead and cadmium are accumulated in the body organs, especially lungs, liver, kidneys, thyroid gland and brain (6, 7). Poisoning by lead salts mainly occurs through environment and food intake. Due to solubility in lipids, these compounds are well absorbed through the skin and respiratory tract. The first symptoms of poisoning include tiredness, impaired sleeping and constipation. If the contact time increases, other side effects appear, including colic and anemia. The acute and rare side effect caused by oral absorption in children is encephalopathy (8–10). Cadmium is of metals that are widely dispersed in environment. The major sources of cadmium include industrial compounds and phosphate fertilizers. Cadmium poisoning symptoms include shortness of breath and renal complications. Dairy products, especially milks, are important components of a healthy diet. Studies have proven that consumption of foods containing heavy metals could endanger consumer health (2, 5, 11–14). Important sources of environmental contamination with cadmium include phosphate fertilizers, water, feed sources, industrial lime, weaving, ceramics and food factories (7). A comparison between these data and those reported in literatures is shown in Table 1.

In general, one of the most important health problems within the present society is food contamination with heavy metals. Of various foods, milk is further studied by researchers because of its wide consumption in all life ages, especially childhood (25, 26). Therefore, concentrations of lead and cadmium in raw milks produced in various regions of Shahroud, east of Iran, were assessed and compared to international standards. Then, the health risk of these metals in milk was assessed.

Materials and Methods

Reagents: Deionized water (DI) (resistance over 18 MΩ/cm; Millipore, Bedford, MA, USA) was used for the preparation of solutions and standards. The nitric acid (67%), hydrochloric acid (32%) and hydrogen peroxide (30%) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). To verify the accuracy of the lead and cadmium analyzes, certified reference materials of (CRM) BCR-063 and BCR-150 skim milk powders were used.

Materials: In total, 54 raw milk samples from five regions of Eastrn Iran were collected using cluster sampling and 20 samples of various popular brands of pasteurized milks were randomly purchased from supermarkets in east of Iran, 2015. Milk production in east of Iran included nearly 17,800 tons in 2015 (31).

Table 1. The mean lead and cadmium concentrations in milk samples from various countries

country	lead	N	cadmium	N	Origin/unit	Reference
Iran	0.018 ± 0.001	100	0.003 ± 0.001	100	Buffalo (mg kg−1)	(15)
Iran	0.010 ± 0.001	100	0.002 ± 0.001	100	Ewe (mg kg−1)	(15)
Italy	0.18 ± 0.069	8	0.07 ± 0.019	8	Ewe (mg kg−1)	(16)
China	35.01 ± 8.63	8	4.53 ± 3.01	8	Cow (µg kg−1)	(17)
Turkey	3.17 ± 0.86	4	0.13 ± 0.11	4	Ewe (mg kg−1)	(18)
Italy	0.06 ± 0.02	10	<0.03	37	Ewe (mg kg−1)	(19)
Turkey	3.01 ± 0.89	4	0.11 ± 0.07	4	Goat (mg kg−1)	(18)
Italy	0.06 ± 0.02	10	<0.03	37	Goat (mg kg−1)	(19)
Japan	12.95 ± 2.94	8	2.01 ± 1.06	8	Cow (µg kg−1)	(17)
Nigeria	5.50176	17	1.63176	17	Cow (µg kg−1)	(20)
India	0.060 ± 0.008	216	-	-	Buffalo (mg l−1)	(21)
California	92.1 ± 2.9	111	97.5 ± 2.5	111	Cow (µg kg−1)	(22)
Croatia	0.035 ± 0005	10	0.011 ± 0.003	10	Ewe (mg kg−1	(23)
Croatia	0.027 ± 0.06	60	0.037	60	Cow (mg kg−1)	(24)
Egypt	0.327 ± 0.124	21	0.007 ± 0.004	21	Cow (mg kg−1)	(7)

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Sample preparation: Each sample included 100 g of milk collected under sterile conditions. Samples were transferred to the laboratory using flasks containing ice. The screw-cap test tubes were used to prepare and transfer samples. To ensure that the tubes are free of lead, all tubes were washed with acid before sampling. First, tubes were soaked in 10% of hydrochloric acid for 24 h according to the Iranian National Standard (INS) No. 419. Then, tubes were rinsed with water and soaked in distilled water for 24 h. Finally, the tubes were rinsed with distilled water (DW) and dried. After drying, the tube caps were closed and used for the sampling.

Lead and cadmium assessments: Lead and cadmium were assessed using graphite furnace atomic absorption spectroscopy with longitudinal AC Zeeman (Perkin Elmer AAnalyst 600, USA), equipped with a transversely heated graphite atomizer. Preparation of samples for GFAAS was based on a method by Munoz et al. (27). Briefly, the milk sample was mixed with 2 ml of 65% HNO3 and 1 ml of 35% H_2O_2. After digesting and cooling, the solution was diluted with water to a final volume of 25 ml. Milk injected into the graphite furnace and atomized at 1600 °C for lead and 1,400 °C for cadmium. Triplicate analyses were carried out for each sample. Absorption of standards was record under the same conditions. An average of five consecutive absorptions and the standard curve were used to assess cadmium levels in samples. The limit of detection (LOD), limit of quantitation (LOQ) values included 0.001 and 0.002 for lead and 0.0001 and 0.0002 mg/kg for cadmium, respectively. Validation reports are presented in Tables 2 and 3. The repeatability of the measurements was reported excellent. Comparison of measured values and certified reference material values are shown in Table 3. ANOVA test was used for the statistical analysis.

Table 2. Validation parameters obtained for lead and cadmium by using GF AAS

Parameters	Cd	Pb
Limit of detection (LOD), mg/kg	0.0001	0.001
Limit of quantitation (LOQ),mg/kg	0.0002	0.002

Cd, cadmium; Pb, lead

Health risk estimation: Of distinctive strategies for approaching the risk assessment, Monte Carlo simulation (MCS) is the most practical strategy (Kentel and Aral, 2004). Model significant improvement in risk assessment is guaranteed using Monte Carlo uncertainty simulation (Qu et al., 2015). Crystal Ball Software v.7.2 (Oracle, Decisioneering, Denver, CO, USA) was used for the development of exposure and risk assessment models. Exposure assessment is important in epidemiology studies on risk assessments. In the current study, estimated daily intake (EDI) was used to characterize dietary exposure to heavy metals (lead and cadmium). The following Equation 1 was used to calculate EDI (27):

$$EDI = \frac{C_{metal} \times E_F\ eE_D \times F_{IR}}{W_{AB}\ BT_A} \quad (1)$$

Where, C_{metal} (mg/kg) was the concentration of heavy metals in milk; E_F (365 days/year) was the exposure frequency; E_D (30 years for non-carcinogens (27) was the exposure duration; F_{IR} (239 ml/person/day for children and 175 ml/person/day for adults) was the average daily milk consumption; W_{AB} (kg) was the average body weight (15 kg for children and 70 kg for adults); and T_A was the average exposure time calculated by multiplying E_D to E_F (28–31).

Non-carcinogenic risk: In this study, a quantitative risk assessment technique was used for non-carcinogenic human health risk assessment using the following THQ Index Equation 2 (32):

$$THQ = \frac{EDI}{R_{fD}} \quad (2)$$

Where, THQ Index was the target hazard quotient index; EDI was the EDI (mg/kg/day); and R_{fD} was the oral reference dose (mg/kg/day). When THQ was lower than or equal to 1, no considerable risks were reported (32, 33). The RfD for lead and cadmium included 0.0035 and 0.001 mg/kg/day, respectively.

Statistical analysis: Data were analyzed using statistical analysis of variance (ANOVA) followed by Tukey multiple comparison test with a significance level of 5%. Data were analyzed using SPSS Software v.18.0 (SPSS, Inc., Chicago, IL, USA).

Results

Based on the results, Regions 4 and 2 showed a maximum of 67.84 µg/kg and a minimum of 15.77 µg/kg of lead concentrations in raw milk samples, respectively (Tables 3, 4, 5 and 6). The mean concentrations of lead and cadmium in samples included 38.15 and 4.67 µg/kg, respectively. Statistical analysis of the results showed a significant difference between lead and cadmium levels in raw milks produced in various regions of Eastern Iran. Furthermore, a significant difference was found between the cadmium levels in raw milks produced in

various regions of Eastern Iran. The average concentrations of lead and cadmium in raw milk samples included 38.15 and 4.67 µg/kg, respectively, which differ with concentrations of these heavy metals in other countries. Concentrations of lead and cadmium showed significant differences between the pasteurized milk samples (P<0.05). In all pasteurized milk samples, the level of cadmium was reported beyond the safe maximum permissible limit (MPl). The average concentrations of lead and cadmium in pasteurized milk were 6.48 µg/kg and 48.49 µg/kg, respectively (Tables 3, 4, 5 and 6). Milk brands D and A showed a maximum of 133.1 µg/kg and a minimum of 7.8 µg/kg of the mean lead concentrations, respectively. Milk Brands D and E showed a maximum of 12.5 µg/kg and a minimum of 1.2 µg/kg of the mean cadmium concentrations, respectively (Table 3–5). The EDI (µg) of cadmium through milk consumption included 1.58–3.49 µg/kg BW/day.

Table 3. Descriptive statistics levels of lead in raw milk samples (µg/kg)

		region 1	region 2	region 3	region 4	region 5
sample number		10	18	19	11	11
Standard Deviation		21.361	14.59	18.09	39.53	17.55
Mean		38.08	15.77	35.23	67.84	33.84
Maximum		83.5	44.9	60	133.1	54.2
Minimum		7.8	0.7	1.8	24.2	6.6
P-value				p≤0.001		
MRL* (µg/day)	Adults	75	75	75	75	75
	pregnant women,	25	25	25	25	25
	children	6	6	6	6	6

Table 4. Descriptive statistics levels of lead in pasteurized milk samples (µg/kg)

		Brand A	Brand B	Brand C	Brand D	Brand E
sample number		5	5	5	5	5
Standard Deviation		25.330	33.575	17.86	45.66	12.704
Mean		41.46	51.88	42.24	70.38	41.74
Maximum		83.5	91.5	60	133.1	54.2
Minimum		15.3	15	14.3	24.2	23.1
P-value		p≤0.001				
MRL* (µg/day)	Adults	75	75	75	75	75
	pregnant women,	25	25	25	25	25
	children	6	6	6	6	6

Table 5. Descriptive statistics levels of cadmium in raw milk samples **(**µg/kg)

	region 1	region 2	region 3	region 4	region 5
sample number	10	18	19	11	11
Standard Deviation	1.741	3.99	4.21	3.65	4.72
Mean	3.16	9.17	6.44	6.97	5.07
Maximum	5.6	15.8	15.8	12.5	11.6
Minimum	0.5	4.8	0.4	1.5	0.5
P-value			p≤0.001		
average daily intake (µg)	0.44	1.28	0.9	0.97	0.70
MRL (µg/day)	14	14	14	14	14

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Table 6. Descriptive statistics levels of cadmium in pasteurized milk samples (µg/kg)

		Brand A	Brand B	Brand C	Brand D	Brand E
sample number		5	5	5	5	5
Standard Deviation		1.72	1.954	3.75	4.04	5.2
Mean		3.92	7.5	7.46	7.88	5.78
Maximum		6.1	9.9	10.9	12.5	11.6
Minimum		1.8	5.3	1.2	1.5	1.2
P-value		p≤0.001				
MRL* (µg/day)	Adults	75	75	75	75	75
	pregnant women,	25	25	25	25	25
	children	6	6	6	6	6

Monte Carlo simulation is an effective technique to calculate uncertainties of each factor. In this approach, each estimation of parameter distribution is incorporated in the risk assessment equation of results, which covers uncertainty of the input factors (34). In order to assess non-carcinogenic risks of the highlighted elements, THQ was estimated for children and adults. Results demonstrated that values of THQ assessed for lead and cadmium through consumption of raw cow milk included 0.86 and 0.366 for children and 0.0116 and 0.0049 for adults, respectively. The THQ for lead and cadmium through consumption of pasteurized cow milk included 0.910 and 0.54 for children and 0.0146 and 0.0072 for adults, respectively.

Discussion

In recent years, lead and cadmium in milk and dairy products have appealed much public attentions because of legal food safety issues and consumer concerns. The aims of the current study included assessment of lead and cadmium levels in milk samples based on individual milk consumption frequency of people in Shahroud, east of Iran. Results showed that lead and cadmium levels in the study area were lower than the national levels (Table 4) (35). Bilandz *et al.* assessed levels of metals in raw milks produced in northern and southern regions of Croatia. The average concentrations of lead in the northern and southern regions included 58.7 and 36.2 µg/ml, respectively. The average concentrations of cadmium in northern and southern regions of Croatia respectively included 1.76 and 3.4 µg/ml, which were higher than those from the current study (14). In another study, Patra *et al.* investigated heavy metals in milks of dairy cows near the industrial zones (13). The highest level of cadmium in milk samples included 0.23 µg/ml. Tajkarimi *et al* assessed lead residues in in 97 milks produced in various areas of Iran. The average lead concentration included 7.9 ng/ml with a range of 1 to 46 ng/ml (4). Rahimi *et al.* investigated concentrations of lead and cadmium in 137 milk samples from goat, cow, sheep and buffalo from various regions of Iran using graphite furnace atomic absorption spectrometry. The average lead and cadmium respectively included 1.93 (0.18–6.11) and 9.51 (1.84–30.50) ng/ml. Lead concentrations in 8.1% of the sheep milk samples and 1.9% of cow milk samples near the industrial cities were higher than Codex Standards (36). Maas *et al.* assessed levels of trace metals in milk and cheese samples. Concentrations of lead and cadmium were reported as 0.009–0.126 µg/g and 0.34–1.01 ng/g, respectively. They found that metal contents significantly increased when milks turned into cheeses. Relatively, concentrations of lead and cadmium in cheese samples included 0.68–11.37 µg/g and 0.020–0.925 ng/g, respectively (2). Kim studied lead contents in milks and dairy products in Korea. He found that lead concentrations in raw cow milks from nine districts and in imported butter, cheese, cream and milk from 15 countries were lower than allowable limits (37). Similarly, Najarnezhad reported that milk cadmium contents from West Azerbaijan Province, Iran, included 0.003 ±0.001 mg/kg for buffalo milks; 0.001 ±0.001 mg/kg for raw cow milks; and 0.002 ±0.001 mg/kg for raw ewe milks (15). A comparison of data reviewed from literatures is presented in Table 6.

The provisional tolerable weekly intakes (PTWI) of 25 µg/kg BW (equivalent to 3.6 µg/kg BW/day) of lead are established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for all human groups, based on the fact that lead is a cumulative poison. The provisional tolerable monthly intakes (PTMI) of cadmium is reported as 7 µg/kg BW (or 0.83

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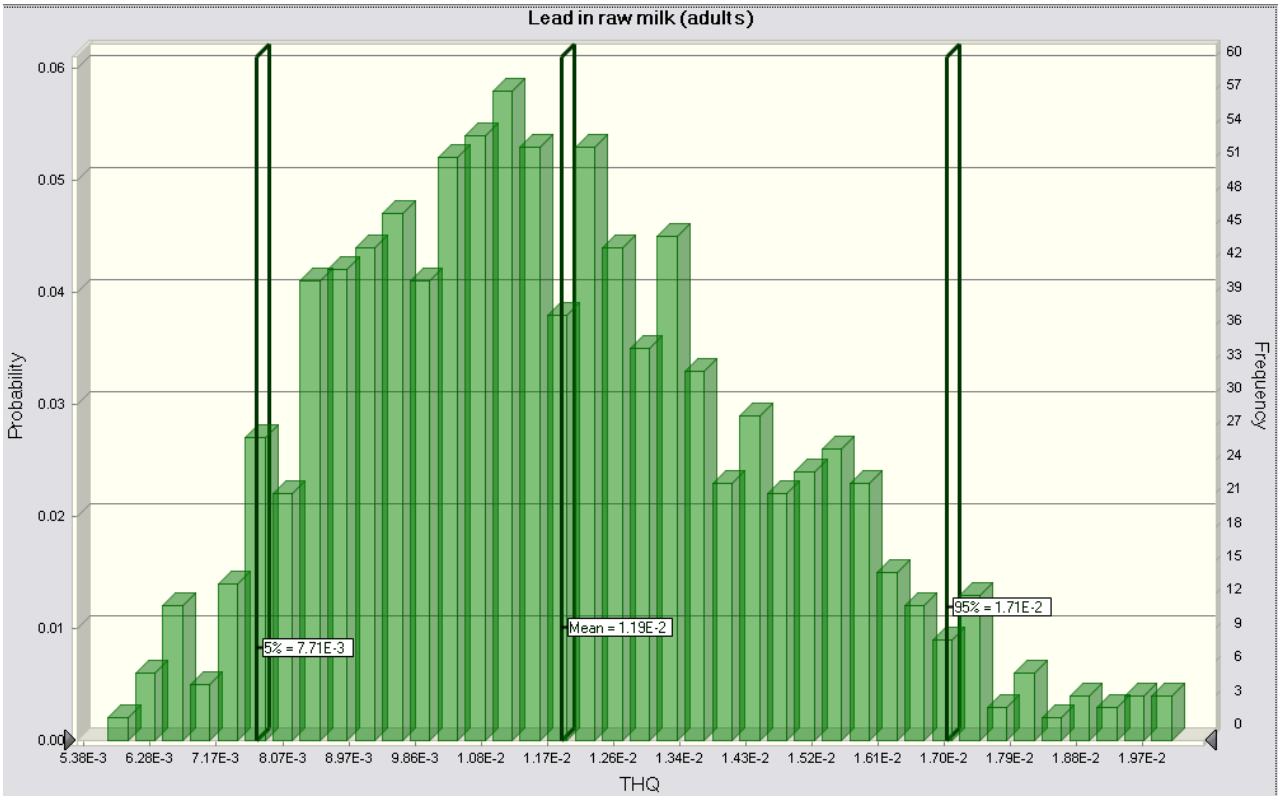
µg/kg BW/day) (38). Analysis of samples showed that concentrations of lead and cadmium in all milks from east of Iran were lower than tolerable weekly intake (TWI) levels of these heavy metals. Results of another study in Croatia showed that the TWI of lead in cow and goat milks included approximately 1.37 and 1.84%, respectively (14, 39). These results showed that lead concentration in the milk samples included no risks to consumer health including adults, children and pregnant women. A similar report showed the presence of less than permitted levels of lead and cadmium in dairy products in Korea (37). The PTWI of cadmium for adults include 7 µg/kg BW. This equals 47.52 µg for lead and 6.70 µg for cadmium for individuals with an average weight of 70 kg. Therefore, cadmium in the milk samples included no health risks to consumers. in the present study, the THQ distribution was calculated using the Monte Carlo approach (Fig. 1). Nearly 95% of iterations for lead and cadmium were below hazard quotient thresholds (1). The estimated mean and median values of THQ for the lead and cadmium were below 1. Therefore, levels of human exposure to these elements could not cause any deleterious effects and it could be suggested that consumption of milk samples from east of Iran is relatively safe. Sobhanardakan *et al.* demonstrated that the average health risk index (HRI) through consumption of raw cow milk was estimated as 2.15E-03 and 6.81E-04 for children and adults, respectively. The average HRI through consumption of pasteurized cow milk was estimated as 2.65E-03 and 5.68E-04 for children and adults, respectively. They reported mean HRI values of lower than 1 for the analyzed Cd, Cu, Pb and Zn in children and adults through consumption of raw and pasteurized cow milks. (40). Meshref *et al.* found that the THQ values of metals (Pb, Cd, Fe, Cu and Zn) in Egyptian population through consumption of milks included 2E−01, 1.7E−01, 7E−02, 8E−03 and 1E−01 mg/kg BW/day, respectively. Results showed consumption of milk in this area was nearly free of risks (41).



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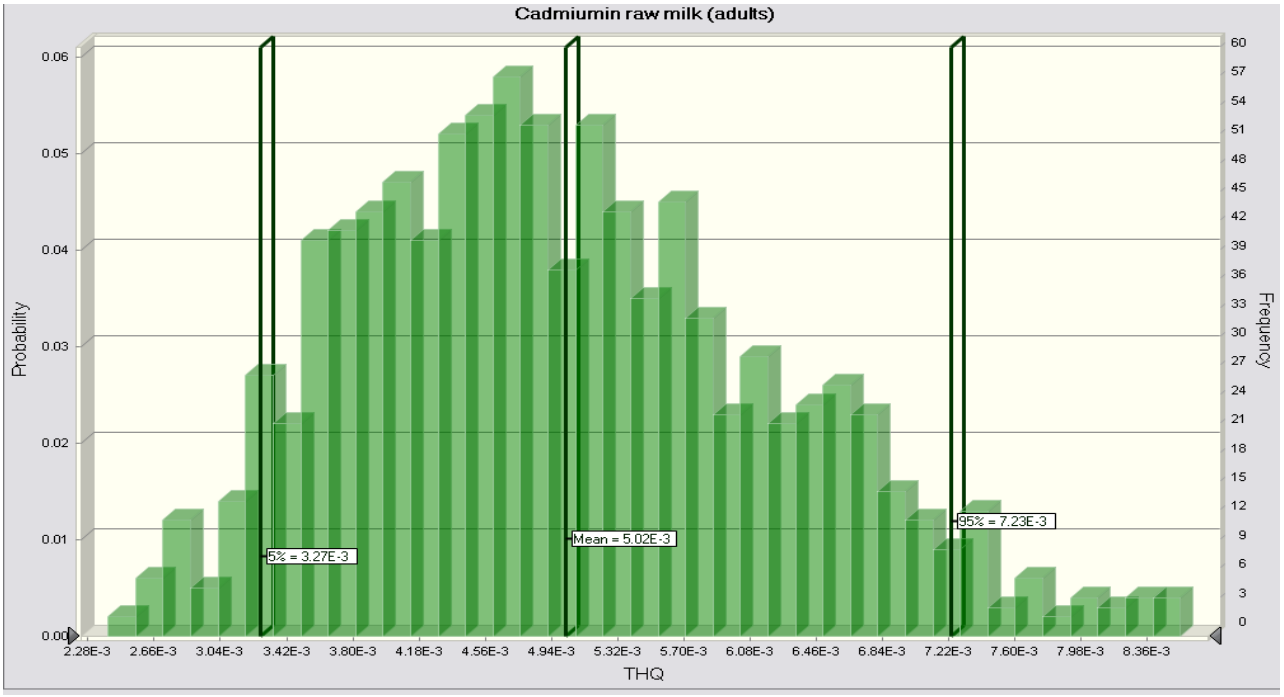

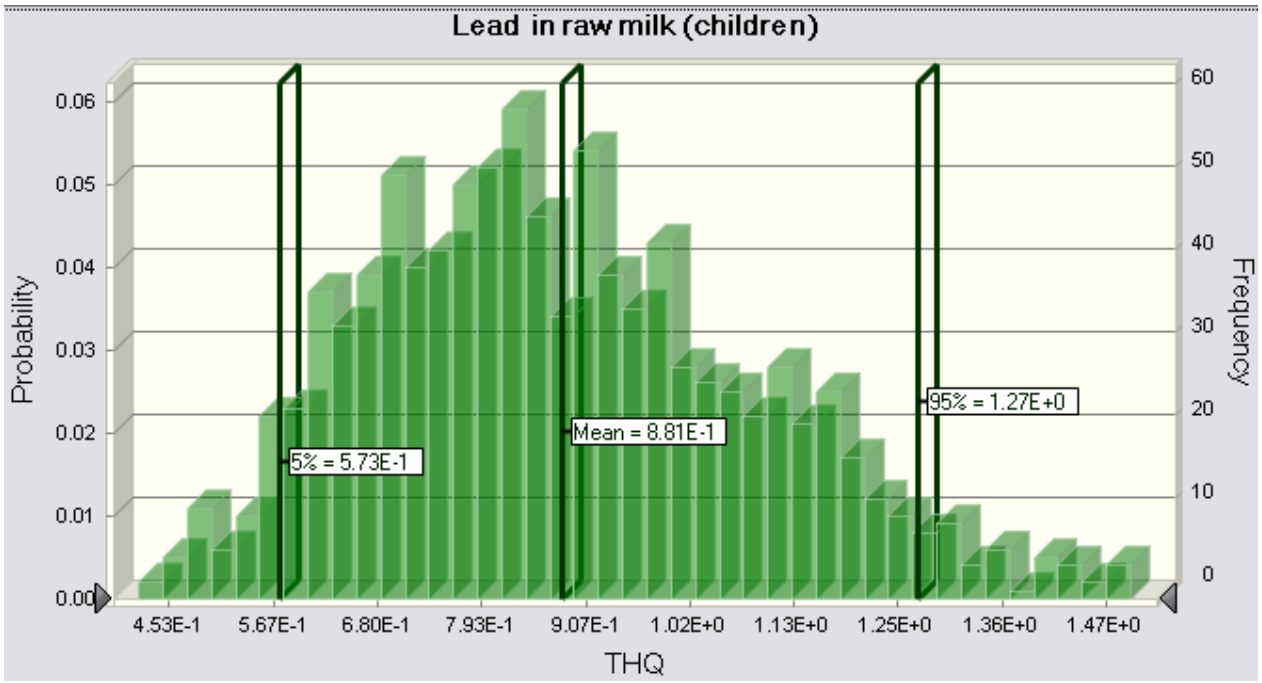

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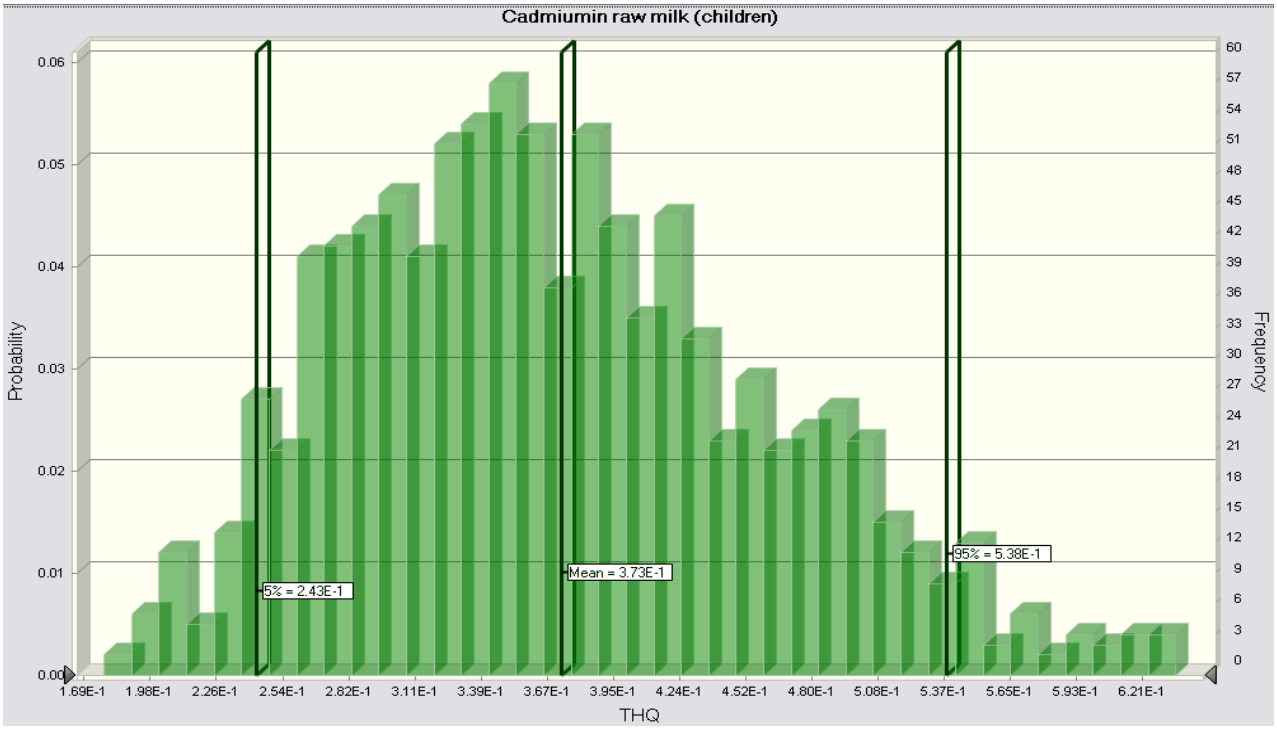

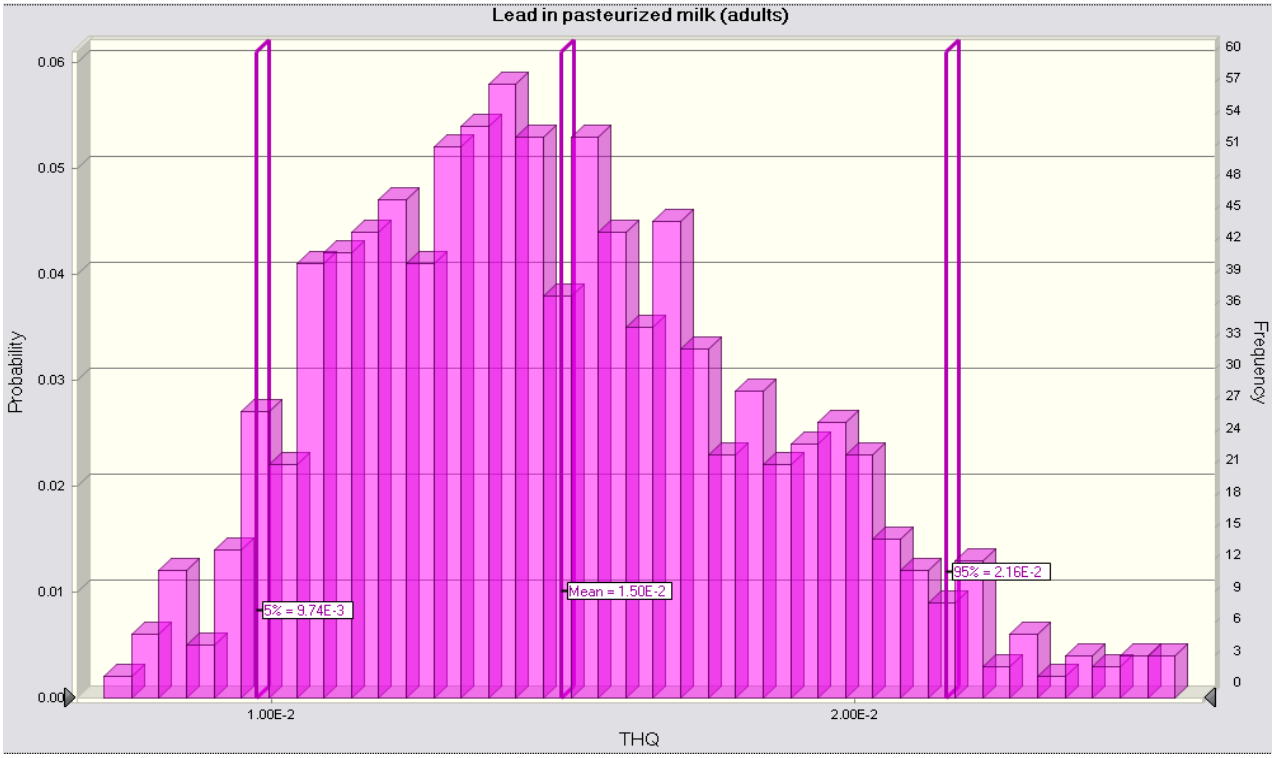

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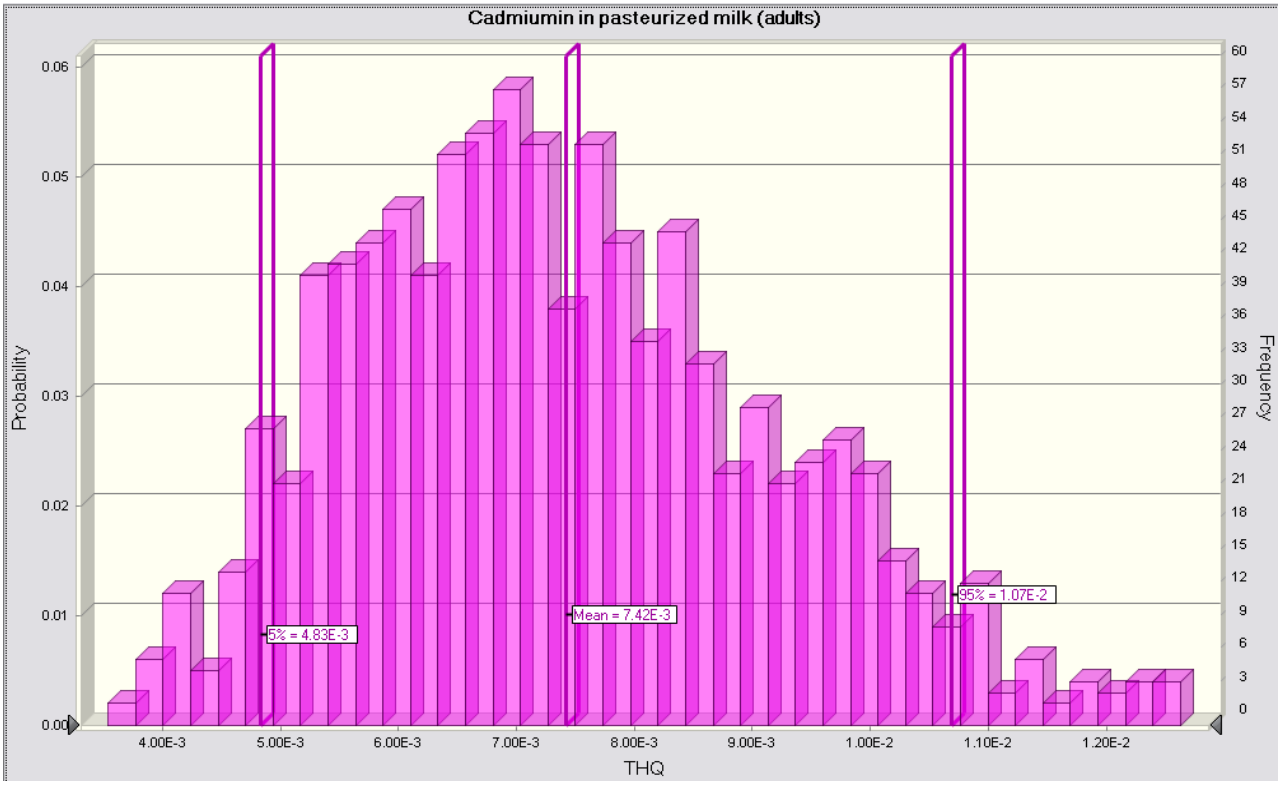

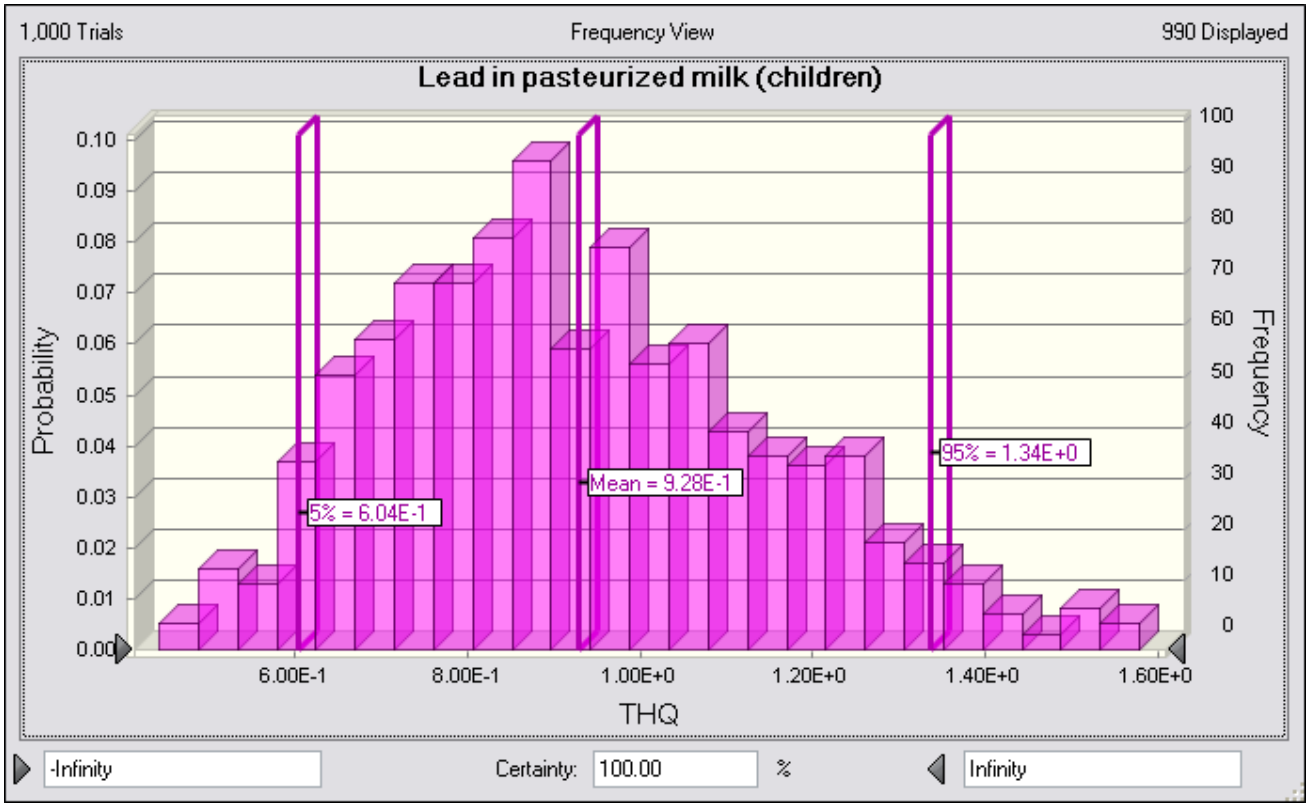

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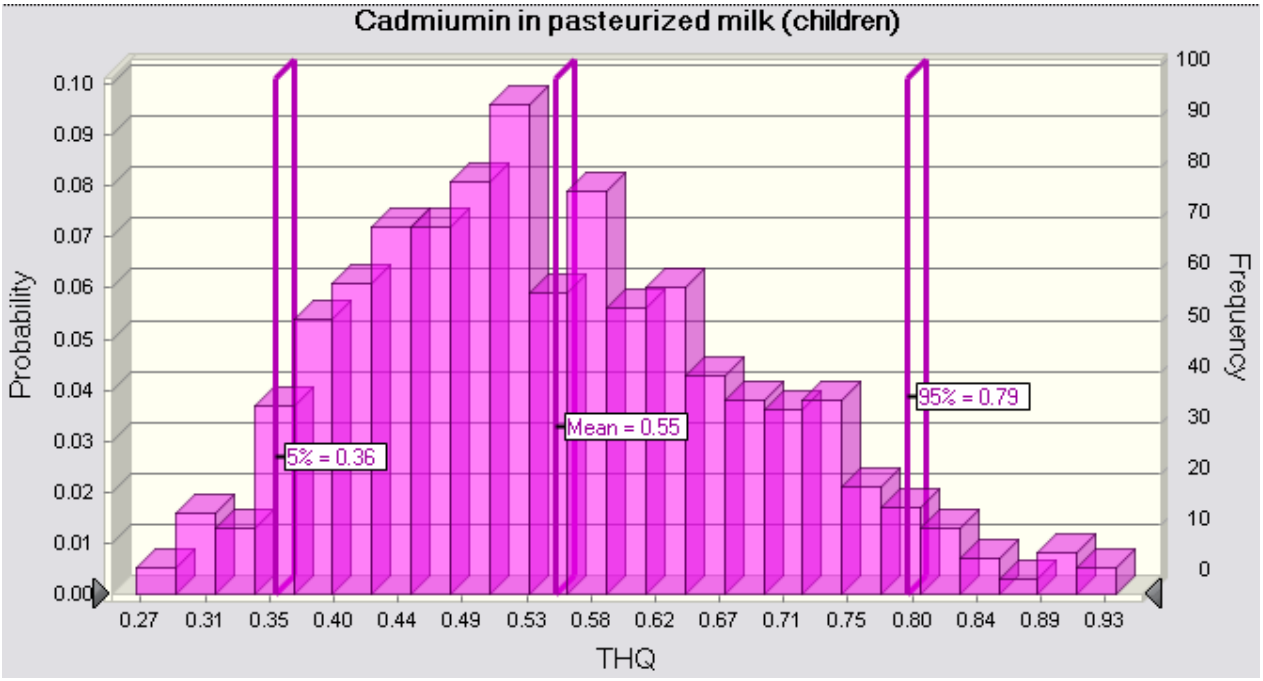

Figure 1. Simulation of THQ distribution for cadmium and lead through 10,000 iterations

Conclusion

Contaminant monitoring helps to improve safety and quality of foods and to assess potential risks by providing information on food contamination in societies. In this study, heavy metals including lead and cadmium were assessed in milks produced in various regions of Eastern Iran. Regarding milk quality data acquired from the exposure assessment, the THQ value was simulated for lead and cadmium. Findings showed that the THQ value was below 1 for all the samples. Therefore, all the male and female age groups were not at health risks due to milk consumption. However, products with greater consumption volumes should receive further attention. Continuous monitoring plays an important role in protecting consumer health against food contaminations.

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