

Exposure to Aflatoxin M1 through Milk Consumption in Tehran Population, Iran

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Abstract

Milk would be contaminated with Aflatoxin M1 (AFM₁), if it was obtained from lactating animal which fed with feedstuffs containing Aflatoxin B1 (AFB₁). AFM₁ is classified as group 2B, possibly carcinogenic to humans and its exposure to AFM₁ through milk consumption is a public concern. The purpose of this study was to determine the AFM₁ exposure through liquid milk consumption for adult consumers in Tehran. Forty-five samples including raw, pasteurized, and UHT milk samples were collected from markets in different cities of Tehran province in January and February 2017. The AFM₁ was determined by HPLC method after immunoaffinity column clean up. Also, the milk intake was calculated using household budget survey. Finally, the daily intake of AFM₁ through milk consumption was estimated using a deterministic approach. From total 45 samples, AFM₁ was detected in 36 (80%) samples, although none of the analyzed samples were exceeded Iran legal limit of 0.1 µg/kg. On the basis of the average milk intake, the mean daily exposure to AFM₁ was estimated between 0.03 ng/ Kg BW per day (lower bound estimate) and 0.06 ng/ Kg BW per day (upper bound estimate) and the 95th percentile daily exposure was calculated at 0.14 ng/ Kg BW per day. According to these values, it should be expected that the adults of Tehran population are not exposed to a significant risk of Hepatocarcinoma associated with AFM₁ intake through milk consumption.

Keywords: Aflatoxin M₁; Milk; Exposure assessment; Tehran; Iran.

Introduction

Aflatoxin M₁ (AFM₁) is a monohydroxylated metabolite of aflatoxin B₁ (AFB₁) (1, 2). Feed may be contaminated with AFB₁ where the environmental conditions are favorable for mold growth, and when AFB₁ contaminated

feed consume by dairy ruminant animals, AFB₁ is transformed to AFM₁ by means of microsomal cytochrome P450-associated enzymes in liver and excreted in milk at a rate of 0.3-6.2 percent of ingested AFB₁, depending on the AFB₁ amount of feed (2-4).

AFM₁ contamination of milk and dairy products has been reported from many countries (5-8), most of which are located in the Mediterranean and the Middle East region,

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where environmental conditions is suitable for mold growth in agricultural products used as animal feed (6). It is noticeable that the presence of AFM₁ in milk and dairy products were reported from Iran in many published articles during the last decade (7–9). Furthermore, some studies from Iran have demonstrated high frequency rate of AFM₁ contamination in milk and dairy products (7).

In terms of food safety, the importance of AFM₁ is due to its carcinogenic potency (1, 5). The International Agency for Research on Cancer (IARC) categorized AFM₁ as a Group 2B human carcinogen in 1993 (1, 10). Therefore, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has not established a maximum tolerable daily intake (TDI) for AFM₁, because AFM₁ intake levels even less than 1 ng/kg body weight (BW) per day increase the risk of liver cancer, so recommended AFM₁ level of milk and dairy products should be as low as reasonably achievable (ALARA) (7, 11). The greatest potential of milk for introducing AFM₁ into the human diet has been demonstrated, so milk and dairy products consumption contribute significantly for the human exposure to AFM₁ which is a serious public concern (Ruangwises & Ruangwises, 2010). The common AFM₁ exposure assessment plan is based on the combination of the AFM₁ occurrence data with milk intake data using a deterministic approach (12). However, the AFM₁ levels in milk is usually low, and only long-term intake of such low levels is associated with the occurrence of diseases such as Hepatocarcinoma in humans (1). So, the main health concern is relevant to areas where have high milk consumption per capita, as well as children and adolescents who have the higher proportion of milk intake per kg of body weight. As a result, considering food consumption pattern and economic considerations (8), the legal maximum limits for AFM₁ are different in various countries (2, 8, 13, 14).

Several methods have been developed for measuring AFM₁ in milk such as ELISA as a screening method, and high performance liquid chromatographic techniques

(HPLC) after immunoaffinity clean-up as a confirmatory method (5).

Several studies have reported the exposure to AFM₁ through milk and dairy products consumption from different countries in worldwide (11, 12, 15–18). At the international level, AFM₁ daily intake through milk consumption by European Union, Latin America, Far Eastern, Middle Eastern and African population was respectively estimated as 0.11, 0.058, 0.20, 0.10, and 0.002 ng/kg BW per day, within the framework of GEMS/Food regional diets (5, 19)

In Iran, AFM₁ contamination of varied portion of analyzed milk samples was reported in almost all published studies so far (20) and based on the obtained results, occurrence and levels of AFM₁ contamination seem to be a public health concern in winter season and humid climate regions of Iran, particularly for children (2, 21). However, AFM₁ exposure through milk and traditional dairy product consumption in an adult population of four west (Kermanshah, Ilam, Hamadan, and Kurdistan) provinces is only one published report regarding AFM₁ intake from Iran (21).

The purpose of this study was to determine the exposure to AFM₁ through raw, pasteurized and UHT milk consumption in an adult urban population of Tehran province in Iran.

Experimental

Sampling

A total of 45 milk samples, including 25 raw and 20 Heat-treated milk (including 16 Pasteurized and 4 UHT milk) the samples were obtained from markets in different cities of Tehran province, during January and February 2017.

Raw milk samples were collected with sampler jars directly from milk-holding tanks in the traditional dairy product markets. After stirring the milk-holding tank, the equal amount of milk was collected from each tank in a market, and then pooled together, and finally, 500 mL milk sample was transferred to a disposable pet container.

Pasteurized and UHT milk samples were obtained from different supermarkets or

hypermarkets in original packaging. Only one packaging was selected from each available brand. Then, 500 mL milk sample from each pack was transferred as a sample to a disposable pet container.

Soon after collection, the samples were transported to the laboratory in an icebox with ice packets, and then stored at $-20\text{ }^{\circ}\text{C}$ and protected against light until further analysis for AFM₁.

Apparatus, chemicals and reagents

Agilent Technologies 1200 Series HPLC system (USA) consisted of binary pumps and a fluorescence detector was used to determine AFM₁ and equipped with a custom built oven column.

Separation was achieved using an Agilent Eclipse XDB- C18 column ($4.6 \times 150\text{ mm}$, $5\text{ }\mu\text{m}$).

Immunoaffinity column obtained from Libios (PuriFast Afla, Libios, France).

Chemicals and reagents were HPLC grade including: Acetonitrile (Merck, Germany), Methanol (Merck, Germany), Deionized Water (Heal Force, China), Sodium Chloride (Merck, Germany), Potassium Chloride (Merck, Germany), Potassium Dihydrogen Phosphate (Aldrich, Germany), Disodium Hydrogen Phosphate (Carlo Erba, Italy), Nitric Acid 65% (Merck, Germany), and Potassium Bromide (Merck, Germany).

AFM₁ stock standard solution was prepared from Sigma Chemical Co. (Sigma, USA) and kept frozen at $-20\text{ }^{\circ}\text{C}$ prior to the experiment. Working standard solutions AFM₁ at concentrations of 0.25, 0.50, 0.75, 1.00, 1.25, and 1.50 $\mu\text{g/L}$ in mobile phase were used to obtain the calibration curve.

Extraction and clean up procedure

According to the official national standard of ISIRI, No. 7133 based on ISO 14501/IDF 17122), the frozen milk samples were thawed using a water bath at $35\text{ }^{\circ}\text{C}$ to $37\text{ }^{\circ}\text{C}$, and then liquid milk was centrifuged at $4500 \times g$ for 15 minutes and upper fat layer discarded completely.

The skimmed milk was filtered through a paper filter (GVS Filter Technologies; Italy)

and then 50 mL of it was passed through immunoaffinity column at flow rate of 1 mL/min.

Immunoaffinity column was previously brought to the room temperature by passing 10 ml of Phosphate buffered saline (PBS)]. Next, 15 mL of PBS was used for washing sample container and then passed through immunoaffinity column. The column was washed with a mixture of acetonitrile and methanol (3:2 v/v), twice (each time with 500 μL).

The eluate was collected in a conical tube and evaporated to dryness using a gentle stream of nitrogen. The residue was dissolved in 1 mL of mobile phase and then a 200 μL aliquot was injected into LC system and filtered through a syringe filter ($0.2\text{ }\mu\text{m}$ PTFE; USA).

Quantitative analysis by HPLC

The HPLC conditions for quantitative analysis of AFM₁ were as follows: column temperature $40\text{ }^{\circ}\text{C}$ and mobile phase consisted of water: methanol: acetonitrile (60:30:10 v/v) + 350 μL HNO₃ 4M + 120 mg/L KBr pumped at a flow rate of 1 mL/min. Excitation and emission wavelengths of fluorescence detector were 362 and 435 nm, respectively. The retention time for AFM₁ was 5.8 min.

For identification of AFM₁ peak in the sample chromatogram, its retention time was compared with that of the analyzed AFM₁ standard under the same conditions. Using the equation of calibration curve, the area under the curve of sample chromatogram was calculated for quantitation of AFM₁. The limits of detection (LOD) and quantitation (LOQ) of the current method were 0.01 and 0.03 $\mu\text{g/L}$, respectively.

Statistical Analysis

Mean, standard deviation (SD), and 95 percentile of AFM₁ concentration in milk samples were statistically analyzed by the Data Analysis tools of Microsoft Excel 2010 for data analysis.

Calculation of exposure

In this study, daily intake of AFM₁ was

calculated using the deterministic approach explained by International Program on Chemical Safety (IPCS) (23).

Based on Global Environment Monitoring System (GEMS)/Food guidelines, as the proportion of censored data (results reported below LOD and/or LOQ) exceeded 60%, two scenarios were adopted and used for calculation purposes:

(1) the upper bound of mean (UB) computes after replacing the LOQ instead of the results that were lower than LOQ and LOD instead of the results that were lower than LOD; (2) the lower bound of mean (LB) computes by replacing the LOD instead of the results were lower than LOQ and zero instead of the results, lower than LOD (23–25).

The milk consumption per capita in Iran was calculated using the average milk consumption by a household from March 2016 to February 2017 as provided by the Household Budget Survey in Urban Areas of Iran in 2017 divided by the average size of urban households in this year (26). Then, milk consumption per capita by urban population of Tehran province was estimated by comparison whole country with Tehran household expenditure for purchasing milk types (27).

Taking into account the variability that exists in food consumption patterns within our studied population, the milk consumption per capita was calculated using coefficients obtained from the study performed by Nasimi *et al.* They demonstrated per capita milk consumption of two top income decile is almost three times more than two lowest income decile (28). Moreover, the pattern of household milk consumption is assumed to be raw milk or heat-treated milk or both.

Eventually, the mean and 95 percentile exposure levels (p95) to AFM₁ were calculated by combining the mean and percentile 95 of the AFM₁ concentrations with the milk intake using the following formula:

Daily intake [ng/kg BW /day] =

$$\frac{[\text{AFM1 concentration (ng/kg)}][\text{Daily milk consumption (Kg/day)}]}{[\text{body weight (BW)kg}]}$$

Results

Method performance

Each day a set of working standard solutions were injected to construct the calibration curve. The accepted linearity of the calibration of minimum $R^2 > 0.98$ was obtained at the working range. For quality control, recovery test was performed by spiking of the blank milk samples with known amounts of AFM₁ (0.1 µg/L). Mean recovery rates and relative standard deviations were $90.6 \pm 5.7\%$.

Occurrence of AFM1 in milk

AFM₁ was detected in 36 (80%) from 45 analyzed milk samples. However, the AFM₁ level was 0.03 µg/kg (LOQ) or higher in 9 (20%) samples.

The concentration of AFM₁ in 12 from 25 raw milk (48%) and 15 from 20 (75%) heat-treated milk positive samples were lower than LOQ. Distribution of AFM₁ contamination was presented in Figure 1. As shown in this Figure, AFM₁ concentration in 3 (6.66%) of the milk samples exceeded the EU maximum tolerance limit for AFM₁ (0.05 µg/kg), although none of the analyzed samples were exceeded Iranian legal limit (0.1 µg/kg) (14) and the Codex Alimentarius criterion of 0.5 µg/kg (8, 13).

The upper and lower limit of mean AFM₁ concentrations was 0.016 and 0.030 µg/kg and its 95th percentile was 0.667 µg/kg, whereas mean (\pm SD) of AFM₁ levels for the positive samples was 0.048 ± 0.019 µg/kg. The descriptive data of AFM₁ contamination occurrence by type of sample was presented in Table 1.

AFM₁ intake estimate

The milk consumption per capita by urban population of Tehran province was calculated 27, 54, and 81 kg/year (74, 148 and 222 gr/day) for population groups with high, moderate, and low milk consumption,

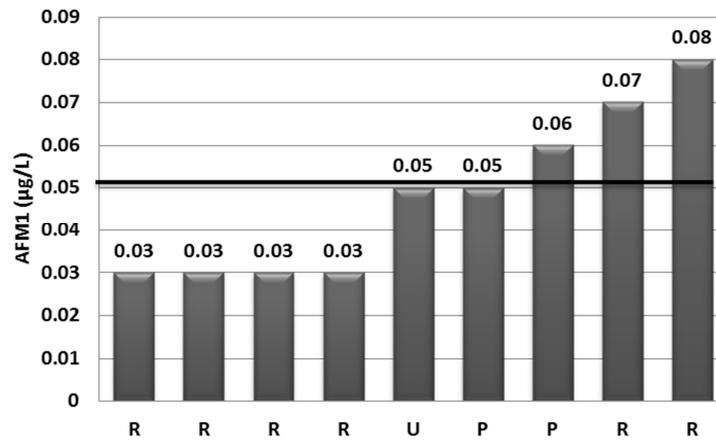


Figure 1. Distribution of AFM₁ concentration of the analyzed milk samples above than the limit of quantification value (0.03 µg/kg). The mean of AFM₁ concentration for these samples (0.047 µg/L) is represented with a horizontal line (R: raw milk, P: pasteurized milk, U: UHT milk).

Table 1. AFM₁ contamination in different types of milk samples collected from markets of Tehran province during January and February, 2017.

Descriptive data		Raw milk	Heat-treated milk ^a
Frequency rate (%)		18/25 (72)	18/20 (90)
Mean (± SD) µg /kg	Positive samples	0.045 (0.234)	0.0533 (0.0057)
	Total samples ^b	0.0155- 0.0315	0.0156-0.0280
Percentile 95	Positive samples	0.075	0.059
	Total samples	0.062	0.050

^a: Including Pasteurized milk and UHT milk.

^b: Upper limit was Calculated by replacing the results below LOQ by LOQ and results below LOD by LOD, Lower limit was calculated by replacing the results below LOQ by LOD and results below LOD by zero.

respectively.

The average body weight for adults was assumed 70 kg. Accordingly, the mean daily exposure to AFM₁ was calculated with the range between 0.03 (lower bound estimate) and 0.06 (upper bound estimate) ng/Kg

BW per day for each member of the urban households in Tehran province.

The Mean and 95 percentile (p95) exposure to AFM₁ through raw and heat-treated milk consumption were presented in Table 2.

Table 2. AFM₁ intake through milk consumption by each urban household's member of Tehran province.

Milk type	Milk consumption pattern			AFM ₁ intake (ng/ person per day)			AFM ₁ intake ^g (ng/ Kg BW per day)		
	Consumer group	Milk intake (g/day) ^c	Percentile (P95) ^f	Mean		Percentile (P95) ^f	Mean		Percentile (P95)
				LB ^d	UB ^e		LB	UB	
Raw milk	<i>High consumer</i>	222	13.21	3.44	6.99	13.21	0.05	0.10	0.19
	<i>Moderate consumer</i>	148	8.86	2.29	4.66	8.86	0.03	0.07	0.13
	<i>Low consumer</i>	74	4.40	1.15	2.33	4.40	0.02	0.03	0.06
Heat-treated milk ^a	<i>High consumer</i>	222	17.09	3.46	6.22	17.09	0.05	0.09	0.24
	<i>Moderate consumer</i>	148	11.40	2.31	4.14	11.40	0.03	0.06	0.16
	<i>Low consumer</i>	74	5.70	1.15	2.07	5.70	0.02	0.03	0.08
Liquid milk ^b	<i>High consumer</i>	222	14.87	3.46	6.56	14.87	0.05	0.09	0.21
	<i>Moderate consumer</i>	148	9.92	2.30	4.37	9.92	0.03	0.06	0.14
	<i>Low consumer</i>	74	4.96	1.15	2.19	4.96	0.02	0.03	0.07

a: Including Pasteurized and UHT milk; b: Including Raw, Pasteurized and UHT milk; c: The pattern of household milk consumption is assumed to be only raw milk or only heat-treated milk or both; d: LB: Lower bound estimate; e: UB: Upper bound estimate; f: P95: 95 Percentile; g: The average body weight for adults is assumed 70 kg.

Discussion

Milk and dairy products are an important part of the human diet, notably for infants and children, due to its richness in certain nutrients such as protein, calcium, riboflavin, phosphorus, potassium, vitamins A and D, and its usability at all ages (29). However, they could contain some contaminants such as AFM₁ (9) and AFM₁ intake through milk consumption is an important health concern because of AFM₁ carcinogenic properties, especially as there are no preventing procedures for the complete elimination of AFB₁ in feeds, as well as suitable weather conditions for the growth of fungi and production of mycotoxins in feed. On the other hand, the resistance of AFM₁ to the heat treatment and mild acidic conditions used in dairy processing were demonstrated (30, 31). So, the dairy products are contaminated with AFM₁ if raw milk used for processing is contaminated with AFM₁ (32, 33). The mixing of bulk milk consignments of different contamination levels is the only process currently applied (34).

Our results were lower than AFM₁ exposure level reported from four west provinces of Iran (0.242 ng/kg BW per day) (21), Sao Paulo, Brazil (0.18 and 0.14 ng/kg BW per day) (15, 16), Catalonia region, Spain (0.036 and 0.043 ng/ person per day for male and female, respectively) (12), and Serbia (1.420, 0.769 and 0.503 ng/kg BW per day during February, April and May 2013) (11) and higher than AFM₁ exposure level reported from France (0.01 ng/kg BW per day) (17), while being agreement with a report from Rabat, Morocco (3.26 ng/person per day) (18). Level of AFM₁ contamination and milk intake per capita are the most important factors affecting the exposure reported in these studies.

The estimated mean of AFM₁ concentration in the present study was higher than the previous values reported from Iran by Sheikhloie and Safarpour (35) and Nowrozi and Kazemi (36), while being lower than the AFM₁ average reported from Iran by kamkar *et al.* (37), Rezaei *et al.* (38) and mashak *et al.* (39). However, the reported results by

Movassaghazani and Ghorbani (40) and Sohrabi and Gharahkoli (41) from Iran were similar to our results. Season of sampling and climate condition of the study area; number and type of analyzed samples, used method for AFM₁ analysis were the factors influencing the AFM₁ concentration reported by different researchers. It is remarkable, all samples in our study were collected in the winter season and, as reported by some researchers, the AFM₁ contamination of raw milk in this season is significantly at higher level compared with other seasons. This is because of the lactating animals are fed with greater amounts of silage and concentrate feeds in cold seasons which may be contaminated with higher levels of AFB₁ (21, 42, 43). However, the occurrence of AFM₁ in samples collected from a modern dairy farm in winter season is higher than summer season versus traditional farm (43).

The lower consumption of milk in Iran than the recommended daily intake by optimal food basket (44) was another reason for being low AFM₁ exposure that was obtained in our study. However, AFM₁ intake in high consumers was up to three times more than low consumers, because of the milk and dairy products expending are strongly dependent on household income (28). Meanwhile, AFM₁ exposure in children and adolescents who have more proportion of milk intake per kg of body weight are higher than adults. Moreover, AFM₁ exposure in Tehran population will increase in the long term, because of an expected augment in milk consumption to the recommended daily intake by enhancing household livelihood and public knowledge.

Conclusion

This study represents one of the first insights into the AFM₁ exposure through milk consumption in Iran population. Although the levels of AFM₁ contamination in our collected milk samples and per capita milk consumption of our study population and so estimated AFM₁ intake in an adult of Tehran population were low, the contribution of such low levels of AFM₁ intake in increasing the risk of hepatocarcinoma could not be ignored,

notably regarding an expected increase in milk consumption to the recommended daily intake in the long term. Therefore, systematic AFM₁ monitoring program in raw milk should be performed in along the time. Moreover, as an important strategy to protect consumers against AFM₁ intake, the conditions of harvest, postharvest, storage, and dairy feedstuffs production should be improved and regularly controlled in feedstuffs along the supply chain require prompt attention regarding AFB₁ issues by veterinary competent authority.

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References

- (1) IARC. IARC monographs on the evaluation of carcinogenic risks to humans, some traditional herbal medicines, some Mycotoxins, naphthalene and styrene. Vol. 82. Lyon, France (2002) [cited 2017 Oct 19]. Available from: URL: <http://monographs.iarc.fr/ENG/Monographs/vol82/mono82.pdf>
- (2) Kamkar A, Fallah AA and Mozaffari Nejad AS. The review of aflatoxin M₁ contamination in milk and dairy products produced in Iran. *Toxin Rev.* 33: 160–8.
- (3) Ayar A, Sert D and Çon AH. A study on the occurrence of Aflatoxin in raw milk due to feeds. *J. Food Saf.* (2007) 27: 199–207.
- (4) Tavakoli HR, Kamkar A, Riazipour M and Mozaffari Nejad AS. Assessment of aflatoxin M1 levels by enzyme-linked immunosorbent assay in yoghurt consumed in Tehran, Iran. *Asian J. Chem.* (2013) 25: 2836–8.
- (5) JECFA. Fifty-sixth meeting of the joint FAO/WHO expert committee on additives and contaminants. In: Safety evaluation of certain mycotoxins in food: Aflatoxin M1. WHO Food Additives Series. World Health Organization, IPCS—International Programme on Chemical Safety, Geneva (2001) 1–102.
- (6) EFSA. Report for 2010 on the results from the monitoring of veterinary medicinal product residues and other substances in live animals and animal products. Technical report of European Food Safety Authority (EFSA). Parma, Italy (2012).
- (7) Duarte SC, Almeida AM, Teixeira AS, Pereira AL, Falcão AC, Pena A and lino CM. Aflatoxin M1 in marketed milk in Portugal: Assessment of human and animal exposure. *Food Control* (2013) 30: 411–7.
- (8) Ismail A, Akhtar S, Levin RE, Ismail T, Riaz M and Amir M. Aflatoxin M1: Prevalence and decontamination strategies in milk and milk products. *Crit. Rev. Microbiol.* (2016) 42: 418–27.
- (9) Heshmati A and Milani JM. Contamination of UHT milk by aflatoxin M1 in Iran. *Food Control* (2010) 21: 19–22.
- (10) IARC. IARC monographs on the evaluation of carcinogenic risks to humans. Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins. Vol. 56. (1993).
- (11) Škrbić B, Živančev J, Antić I and Godula M. Levels of aflatoxin M1 in different types of milk collected in Serbia: Assessment of human and animal exposure. *Food Control* (2014) 40: 113–9.
- (12) Cano-Sancho G, Marin S, Ramos AJ, Peris-Vicente J and Sanchis V. Occurrence of aflatoxin M1 and exposure assessment in Catalonia (Spain). *Rev. Iberoam. Micol.* (2010) 27: 130–5.
- (13) FAO. Mycotoxin regulations in 2003 and current developments. In: *Worldwide Regulations for Mycotoxins in Food and Feed in 2003* (2004) 9–28.
- (14) ISIRI. Food & Feed - Mycotoxins- Maximum Tolerated level (Amendment No.1). National Standard No. 5925 Institute of Standards and Industrial Research of Iran, Iran, Karaj: Institute of Standard and Industrial Research of Iran (2006).
- (15) Shundo L, Navas SA, Lamardo LCA, Ruvieri V and Sabino M. Estimate of aflatoxin M1 exposure in milk and occurrence in Brazil. *Food Control* (2009) 20: 655–7.
- (16) Jager AV, Tedesco MP, Souto PCMC and Oliveira CAF. Assessment of aflatoxin intake in São Paulo, Brazil. *Food Control* (2013) 33: 87–92.
- (17) Leblanc J-C, Tard A, Volatier J-L and Verger P. Estimated dietary exposure to principal food mycotoxins from The First French Total Diet Study. *Food Addit. Contam.* (2005) 22: 652–72.
- (18) Zinnedine A, González-Osnaya L, Soriano JM, Moltó JC, Idrissi L and Mañes J. Presence of aflatoxin M1 in pasteurized milk from Morocco. *Int. J. Food Microbiol.* (2007) 114: 25–9.
- (19) IPCS. International Programme on Chemical Safety of WHO. Safety Evaluation of Certain Food Additives and Contaminants: Aflatoxins. WHO food additives series 40. World Health Organization, Genève, Switzerland (1998).
- (20) Hedayati MT, Omran SM, Soleymani A and Armaki MT. Aflatoxins in Food Products in Iran: a Review of the Literature. *Jundishapur J. Microbiol.* (2016) 9: 1–8.
- (21) Bahrami R, Shahbazi Y and Nikousefat Z. Aflatoxin M1 in milk and traditional dairy products from west part of Iran: Occurrence and seasonal variation with an emphasis on risk assessment of human exposure. *Food Control* (2016) 62: 250–6.
- (22) ISIRI. Milk and milk products. Determination of Aflatoxin M1 by HPLC Method and Immunoaffinity Column Clean up-Test Method. National Standard No. 7133 (First revision), 7133 (First revision) Institute of

- Standards and Industrial Research of Iran, Iran, Karaj (2011).
- (23) IPCS. International Programme on Chemical Safety of WHO. Principles and methods for the risk assessment of chemicals in food, Chapter 6: Dietary exposure assessment of chemicals in food. Environmental Health Criteria; 240. World Health Organization, Genève, Switzerland (2009).
- (24) GEMS/Food-EURO. GEMS / Food-EURO Second Workshop on Reliable Evaluation of Low-Level Contamination of Food: Report on a Workshop in the Frame of GEMS/Food-EURO. (1995).
- (25) EFSA. Management of left-censored data in dietary exposure assessment of chemical substances. *Eur. Food Saf. Auth. J.* (2010) 8: 1557.
- (26) CBIRI. Results of Household Budget Survey in Urban Areas of Iran in 2016. Tehran: Iran (2017).
- (27) Statistical Center of Iran. The results of the Iranian Urban and Rural Household Income and Expenditure Survey - The Year 2016 (2017).
- (28) Nasimi A. Comparison Of Achievements in Products Situation, Annual Consumption Commerce and Price Building of Milk and Dairies Products, Between World Level and Iran (Using SWOT Analyzing Method). Tehran, Iran (2005).
- (29) Iqbal SZ, Jinap S, Pirouz AA and Ahmad Faizal AR. Aflatoxin M1 in milk and dairy products, occurrence and recent challenges: A review. *Trends Food Sci. Technol.* (2015) 46: 110–9.
- (30) Kos J, Lević J, Duragić O, Kokić B and Miladinović I. Occurrence and estimation of aflatoxin M1 exposure in milk in Serbia. *Food Control* (2014) 38: 41–6.
- (31) Tajkarimi M, Aliabadi-Sh F, Salah Nejad A, Poursoltani H, Motallebi AA and Mahdavi H. Aflatoxin M1 contamination in winter and summer milk in 14 states in Iran. *Food Control* (2008) 19: 1033–6.
- (32) Oruc HH, Cibik R, Yilmaz E and Kalkanli O. Distribution and stability of Aflatoxin M₁ during processing and ripening of traditional white pickled cheese. *Food Addit. Contam.* (2006) 23: 190–5.
- (33) Taherabadi MS, Gharavi MJ, Javadi I, Alimohammadi M, Moghadamnia H, Mosleh N, Farajollahi MM and Sharif M. The Level of Aflatoxin M1 in Raw and Pasteurized Milk Produced in Alborz Province , Iran. *Jundishapur J. Nat. Pharm. Prod.* (2016) 11: 1–4.
- (34) Colak H. Determination of aflatoxin M1 levels in Turkish White and Kashar cheeses made of experimentally contaminated raw milk. *J. Food Drug Anal.* (2007) 15: 163–8.
- (35) Sheikhoie H and Safarpour B. Comparison of Aflatoxin M1 in Tehran Traditional and Industrial Cattle-Farm Milk in Spring. *Int. J. Rev. Life Sci.* (2015) 5: 1167–72.
- (36) Nowrozi H and Kazemi A. Determination of Aflatoxin M1 in Fresh (Raw and Pasteurized) Cow Milk in Khuzestan Province. *Jundishapur Sci. Med. J.* (2014) 13: 327–333 (In persian).
- (37) Kamkar A, Yazdankhah S, Mohammadi Nafchi A and Mozaffari Nejad AS. Aflatoxin M1 in raw cow and buffalo milk in Shush city of Iran. *Food Addit. Contam. Part B Surveill.* (2014) 7: 21–4.
- (38) Rezaei M, Eshagi gorji M, Shariatifar N, Hosseini MA and Habibi S. Occurrence of Aflatoxin M1 in milk in Qom , Iran. *Ital. J. Food Sci.* (2014) 26: 325–8.
- (39) Mashak Z, Sohi HJ, Heshmati A and Nejad ASM. Assessment of Aflatoxin M1 contamination in UHT flavored milk samples in Karaj, Iran. *Iran. J. Pharm. Res.* (2016) 15: 407–11.
- (40) Movassaghazani MH and Ghorbani M. Incidence of Aflatoxin M 1 in Human and Cow Milk in Kashan, Iran. *J. Food Qual. Hazards Control* (2017) 4: 99–102.
- (41) Sohrabi N and Gharahkoli H. A seasonal study for determination of aflatoxin M1 level in dairy products in Iranshahr, Iran. *Curr Med. Mycol.* (2016) 2: 29–33.
- (42) Tajik H, Moradi M, Razavi Rohani SM and Hadian M. Determination of Aflatoxin M1 in Pasteurized and UHT Milk in West-Azerbaijan Province of Determination of Aflatoxin M 1 in Pasteurized and UHT Milk in West-Azerbaijan Province of Iran. *J. Food Qual. Hazards Control* (2016) 3: 37–40.
- (43) Tajkarimi. Seasonal survey in content M1 aflatoxin in raw milk taken from 15 dairy factory. *Pajouhesh & Sazandegi* (2007) 2–9.
- (44) Secretariat of supreme council for health and food security. National Nutrition and Food Security Document (2012-2020). Ministry of Health, Treatment and Medical Training. (2004).