Journal of Environmental Health Science and Engineering (2021) 19:165–171
https://doi.org/10.1007/s40201-020-00590-w

RESEARCH ARTICLE

Determination of melamine contamination in chocolates containing powdered milk by high-performance liquid chromatography (HPLC)

Roghayeh Abedini[1] · Gholamreza Jahed Khaniki[1] · Ebrahim Molaee Aghaee[1] · Parisa Sadighara[1] · Shahrokh Nazmara[1] · Behrouz Akbari-Adergani[2] · Maziar Naderi[1]

Received: 11 June 2020 / Accepted: 7 December 2020 / Published online: 7 January 2021

Abstract

Melamine is widely being reported as a food adulterant. Although its toxicity is currently recognized, melamine adulterations of dairy products are ongoing to apparently increase the amount of protein. The study was conducted to investigate the determination of melamine amounts in chocolates containing powdered milk. In this study, 60 samples of chocolates containing powdered milk, both imported and domestic brands, were collected. The samples were prepared by solid phase extraction (SPE) and analyzed using high-performance liquid chromatography (HPLC). According to the results, melamine was found in about 94% of imported samples and about 77% of Iranian samples. Melamine concentration in imported samples ranged from 0.032 to 2.692 mg/kg, while in Iranian ones it ranged from 0.013 to 2.600 mg/kg. The mean melamine concentrations of foreign and Iranian samples were 0.685 ± 0.68 and 0.456 ± 0.73 mg/kg, respectively. Moreover, the limit of detection (LOD) and limit of quantification (LOQ) values of melamine were 0.017 and 0.052 μg/ml, respectively. The recovery rate (R%) at fortified levels of 1–2 mg/kg was found to be 89.20–95.69% with an RSD (Relative Standard Deviation) of 1.8–2.7%. Based on the study results, melamine was present in 85% of all samples and the melamine level in one Iranian brand and one imported brand was higher than the Codex Organization standard. However, the consumption of chocolates containing these low levels of melamine does not constitute a health risk for consumers.

Keywords Melamine · Chocolate · Powdered milk · HPLC

Introduction

Recently, food and dairy products contaminated with melamine have created a widespread food safety concern [1]. Melamine $C_3H_6N_6$ (2, 4, 6-triamino-1, 3, 5-triazine) is a triazine heterocyclic organic compound (Fig. 1) that is illegally added to milk during processing to increase the apparent protein content [2]. Ingestion of melamine may lead to reproductive damage, or bladder or kidney stones, and bladder cancer [3]. An incident of the contamination of milk with melamine has resulted in numerous cases of renal complications in children, and six deaths have been attributed to the consumption of contaminated products [4, 5]. The toxic effects of melamine consumption are usually associated with high doses and the concentrations of melamine in contaminated samples ranged from 90 to 620 g mL^{-1} [6]. Tolerable daily intake (TDI) is 0. 63 mg kg^{-1} (0.63 g mL^{-1}) body weight per day as recommended by the Food and Drug Administration (FDA) for food and food ingredients other than infant formula [7]. Melamine contamination in foods with animal origin such as milk and dairy products, meat and meat products, eggs, fish and non-animal sources such as wheat, rice and beverages have been reported [8–14]. One of the major sources of melamine migration is triazine pesticides such as cyromazine [13, 15, 16]. Cyromazine is also added to animal feed to remove insects and, so melamine can be found in the body of exposured animals [12, 14, 17]. Contamination can also be due to legal use in food packaging, environment, nitrogen supplements used in

✉ Gholamreza Jahed Khaniki
ghjahedkh@yahoo.com

✉ Behrouz Akbari-Adergani
analystchemist@yahoo.com

[1] Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

[2] Food & Drug Laboratory Research Center, Food & Drug Organization, Ministry of Health and Medical Education, Tehran, Iran

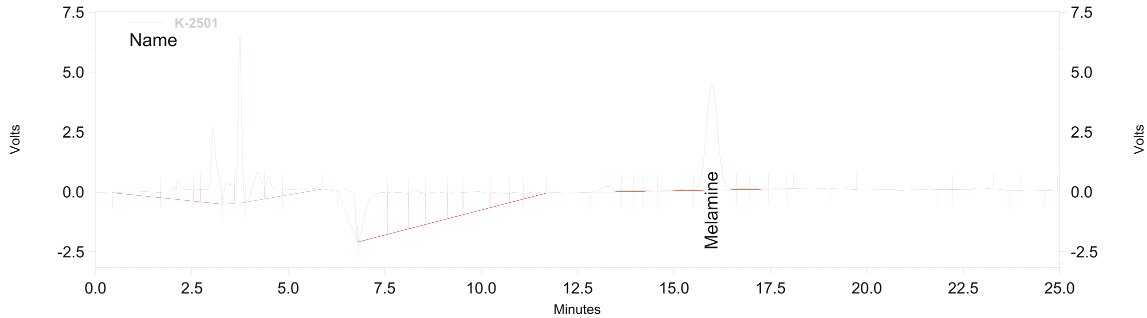

Fig. 1) A chromatogram of a chocolate sample

animal feed, degradation of veterinary drugs and fertilizers [17–19]. The World Health Organization (WHO) sets in foods of animal origin in the list of foods which need to be monitored for melamine levels to ensure foods have not been affected by melamine fraud [13]. In 2007, cases of melamine adulteration had been reported in pet food [20]. In 2008, high concentrations of melamine were reported in contaminated infant formula China [17]. The adulterated products resulted in illnesses of 294,000 individuals, hospitalization of 50,000, and death of six children [21]. The concentration of melamine in milk was as high as 2.563 mg/kg in some samples [22]. Melamine contamination has been detected in milk-based products such as powderd milk. In a study conducted in Africa, from 49 powderd milk brands, 6% of the samples were contaminated with melamine, and the concentration range of the compound was between 0.5 and 5.5 mg/kg [23]. To protect public health and food safety, many countries have established maximum residue limits (MRLs) for melamine in various products [17]. The FDA has determined that 1 mg/kg is the maximum limit allowed in infant formula and 2.5 mg/kg for other foods [21]. In a study, Londono et al. carried out a survey on melamine contamination in milk powder in Uruguay [24]. Besides, Wen et al. conducted a study on melamine-contaminated milk formula and its impact on children [22]. The methods used for protein estimation in food samples such as the Kjeldahl and Dumas methods measure the nitrogen content and cannot distinguish the nitrogen of protein and non-protein sources (melamine) [14, 18]. Therefore, melamine adulterated food products easily passed the quality checks [21]. Based on the previous studies, HPLC has been approved as a powerful technique for the analysis of melamine in biological, environmental, and food materials due to its high sensitivity, high specificity, shortest detection time, and low cost compared to the other methods [18]. Powdered milk-based chocolates are very popular products among all age groups in the world. Few studies have been performed to measure melamine in such products. The objective of this study was to evaluate the presence and determination of melamine in chocolates containing powdered milk in the available imported and Iranian brands in Iran using SPE column and HPLC analysis. Investigating the presence of melamine in powdered milk-based chocolates and comparing various brands together is a new approach that has not been carried out in Iran and many countries. In addition, the study of Iranian brands and even some imported brands in terms of melamine content was survied for the first time. Hence, the study has significant innovations in the field of milk-based food products.

Materials and methods

Sample collection

A total of 60 overused samples of chocolate (15 Iranian brands and 15 imported brands) were prepared. From each brand, 2 samples were randomly collected from large stores in Tehran (during 2018–2019) and were then transported to the food laboratory.

Reagents and chemicals

Melamine analytical standard (HPLC grade with 99% purity) was purchased from Sigma-Aldrich Co., St. Louis, MO. Ammonia solution (25% *v*/v), acetonitrile (ACN, HPLC grade), methanol (HPLC grade), trichloroacetic acid (93%, HPLC grade), sodium hexasulfonic acid and citric acid were purchased from Merck Co. (Darmstadt, Germany). All chemicals used in this study were reagents of the highest grade and were used without further treatment.

Preparation of standard solutions

To prepare standard solution (1000.0 μg/ml), 100.0 mg of melamine was weighed into a 100-ml volumetric flask, dissolved with methanol: water solution (50:50 v/v) and sonicated. Suseqently, the solution was diluted with the mobile phase into a series of standard working solutions (from 0.2 to 1.2 μg/ml) and injected into HPLC and then, calibration curve with $r^2 = 0.99$ was created.

Sample preparation

Fiftheen ml of 1% trichloroacetic acid and 5 ml of acetonitrile were added to 2 g of chocolate in a 50-ml centrifuge tube, and the mixture was sonicated for 30 min. The sample was shaken at 70 rpm over 30 min; then, it was centrifuged at 4000 rpm for 15 min and filtered using filter paper. The filtered solution was volumed up to 25 ml with 1% trichloroacetic acid, and centrifuged for 5 min. The supernatant was diluted with 5 ml of distilled water. After that, sample clean-up procedure was performed using solid phase extraction (SPE). The cleaned up sample was dried under a stream of nitrogen gas and dissolved in 1 ml of mobile phase. The final solution was filtered through a 0.45 μm syringe filter. Finally, 100 μl of the obtained solution was injected into the HPLC.

HPLC parameters

KNAUER 0000 High Pressure Liquid Chromatography (HPLC) Machine (Germany) equipped with 4.6 * 250 mm C18 Column (ODS-3) and UV detector at 242 nm wavelength was used. The mobile phase was applied at a determined ratio (450 ml of buffer solution and 50 ml of methanol suitable for liquid chromatography) at a flow rate of 1 ml/min.

Method validation

Validity, limit of detection (LOD), limit of quantification (LOQ), as wel as precision and accuracy were used to validate the values measured by HPLC based on statistical analysis.

Linearity

To investigate the linearity of the relationship between the measured concentration and the response area (UV absorbed), 6 concentrations (0.2 to 1.2 μg/ml) from the stock melamine solution were considered and then any concentrations were prepared 3 times and eventually injected into HPLC. In the following, the means of the concentrations were calculated, and they were plotted against the amount of response areas. For interpretation of the linearity of the calibration curve, their correlation coefficient (R^2) was calculated.

Data analysis

To non-normalize the data in this study, nonparametric tests of Kruskal Wallis and Mann Whitney were used for data analysis. The data analysis was performed using SPSS v. 21.

Results and discussion

Method validation

Calculation of LOD, LOQ

The LOD and the LOQ were calculated by eqs. 1 and 2:

$$\mathrm{LOD} = 3\mathrm{S_a}/\mathrm{b} \quad (1)$$

$$\mathrm{LOQ} = 10\mathrm{S_a}/\mathrm{b} \quad (2)$$

where S_a is the standard deviation of the response areas, b is the slope of the calibration curve, and also 3 and 10 are signal-to-noise ratio (SNR).

Determination of accuracy and precision

Accuracy and precision indicate the degree of dispersion within a series on the determination of the same sample. Two spiked samples with 1 and 2 mg/l melamine concentration were prepared and analyzed on the same day (accuracy) and two for consecutive days (precision), and then the relative standard deviation (RSD%) was calculated. Each sample was injected into HPLC three times.

The mean and standard deviation were calculated for each concentration and also the relative standard deviation (RSD%) and recovery percentage (R%) of each concentration were calculated (Eq. 3 and Eq. 4).

$$\%RSD = \left(s/|\overline{x}|\right)*100 \quad (3)$$

$$\%\mathrm{R} = \frac{\mathrm{Mean\ repetitions\ of\ each\ concentration\ measured}}{\mathit{Injected\ concentration}}*100 \quad (4)$$

where, s is the sample standard deviation and $\bar{x}$ represents the sample mean.

Furthermore, the LOD and LOQ of the method were 0.017 and 0.052 μg / ml, respectively. The R% of spiked samples with a concentration of 1–2 mg/kg was found to be 89.20–95.69% with an RSD of 1.8–2.7% (Table 1). The RSD less than 6% and mean recovery rate of 92.44% indicate acceptable accuracy.

Table 1) Recovery percentage of spiked samples

Sample	*Spike level*	*Recovery (%)*	*RSD[1] (%) No.: 3*
1	2	95.69	1.8
2	1	89.20	2.7
		92.44	2.3

[1] RSD: Relative Standard Deviation

Melamine amounts in the chocolate brands

In Table 2, the melamine concentrations of the defferent chocolate brands with two repetitions has been shown. The mean concentration of melamine in Iranian and non-Iranian chocolate brands were 0.456 and 0.685 mg/kg, respectively. Also, the concentration range in Iranian chocolate samples was 0.013 to 2.600 mg/kg and in imported brands 0.032 to 2.692 mg/kg (Table 3). The results showed that out of 60 samples, 51 were melamine-contaminated samples. Melamine was found in 76.66% of Iranian samples and 93.33% of imported ones. The mean concentration of imported samples was higher than that of Iranian ones, and the percentage of positive samples was higher in this group. The highest amount of melamine was observed in an imported and the least in an Iranian brand. In Fig. 1, a sample chromatogram of a sample with 16-min retention time of melemine is demonstrated.

The Iranian National Standards Organization (INSO) has not yet accepted legislation to control melamine concentration in food products of animal origin, and our results were therefore compared with standard levels [25]. The FDA and many countries set 2.5 mg/kg as the standard level [18]. The results indicated that the concentration mean of melamine in imported samples was higher than that of Iranian samples and the difference between the melamine concentrations of the Iranian and imported samples was statistically significant (P value = 0.02). However, the difference between the mean concentrations of melamine in two groups of the samples was statistically insignificant (P value >0.05). Moreover, the difference between the melamine concentrations of the taffy and non-taffy chocolates was statistically insignificant (P value

Table 2) Melamine concentration of the different brands of chocolate

Chocolate brand	*Melamine Con. (mg/kg) (With two repetitions)*	*Chocolate brand*	*Melamine Con. (mg/kg) (With two repetitions)*
1	1.062	**16**	0.265
	0.517		0.422
2	1.355	**17**	0.015
	0.877		0.000
3	1.430	**18**	0.013
	1.005		0.452
4	1.002	**19**	2.367
	0.187		0.560
5	0.092	**20**	0.477
	0.080		2.145
6	1.227	**21**	0.412
	0.000		0.562
7	0.032	**22**	0.387
	0.875		0.000
8	0.405	**23**	0.610
	1.477		0.027
9	0.517	**24**	1.587
	0.397		0.070
10	1.840	**25**	2.600
	0.125		0.030
11	0.385	**26**	0.032
	1.842		0.000
12	2.692	**27**	0.000
	0.160		0.000
13	0.150	**28**	0.327
	0.322		0.240
14	0.250	**29**	0.000
	0.120		0.050
15	0.135	**30**	0.000
	0.000		0.020

Table 3) The mean and range of melamine concentrations in Iranian and non-Iranian chocolate samples

Sample	*Brand*	*Sample volume*	*Concentration mean*	*Standard deviation*	*Concentration range (mg/kg)*	*Positive cases (%)*
Chocolate	Iranian	30	0.456	0.73	0.013–2.600	76.66
Chocolate	Imported	30	0.685	0.68	0.032–2.692	93.33
Sum		60				85

>0.05). Melamine was found in about 94% of imported samples and about 77% of Iranian ones. Melamine concentration in imported samples ranged from 0.032 to 2.692 mg/kg, while in Iranian ones it ranged from 0.013 to 2.600 mg/kg. The mean melamine concentrations of foreign and Iranian samples were 0.685 ± 0.68 and 0.456 ± 0.73 mg/kg, respectively (Table 3). The melamine content of one Iranian brand and one imported brand was higher than the Codex Organization standard. The melamine levels in all samples except two samples (one Iranian brand with 2.6 mg/kg and one imported brand with 2.692 mg/kg melamine) were less than the standard limit of melamine in foods (2.5 mg/kg). Maleki et al. analyzed 69 samples of milk powder by HPLC, in which 65% were melamine-contaminated samples and also 10 samples were above the permissible limit, which was in accordance with the results of the present study [26]. Londono et al. carried out a study on the melamine contamination in milk powder in Uruguay. The values for positive samples ranged from 0.017 to 0.082 mg kg^{-1}. Nine samples were positive and also the mean of melamine concentration was reported to be 0.028 mg kg^{-1} [24]. In India, ten brands of milk powder were analyzed by HPLC and the results showed that melamine was lower than the standard limit in all ten brands [27]. Moreover, in Canada, about 250 samples of dairy products were tested and the amount of melamine in their samples was lower than the permissible limit, concluding that this amount of melamine was at the basic level and probably not manipulated [28]. In another study, samples of milk powder in Canada were analyzed, where melamine concentrations were reported to be lower than the established limit, and the origin of the amount of melamine was not detected by fraud [29]. In the present study, the melamine concentration in imported samples was higher than that of Iranian samples, which may be due to the more contaminated powdered milk used in these countries because of the consumption of melamine contaminated feeds by livestock [30]. Another possibility could be the difference in the ingredients used and formulated in Iranian and imported chocolates [31]. Furthermore, differences in the type of materials used for packaging and manufacturing equipments and compounds used to disinfect these facilities in different countries can be significant for other reasons [32].

Migration of melamine from food packaging materials and plastic containers and utensils was a major concern during the 2008 melamine contaminated infant formula crisis [33]. Chik et al. analyzed melamine migration from melamine food contact packages [34]. In their study, the migration of melamine was determined for 41 types of retail melamine-ware products. For the packages tested with distilled water, melamine migration was [median (interquartile range)] 22.2 (32.6), 49.3 (50.9), 84.9 (89.9) ng/ml at room temperature (25 °C), 70 °C and 100 °C, respectively [34]. In 3% acetic acid, melamine migration was 31.5 (35.7), 81.5 (76.2), 122.0 (126.7) ng/ml at room temperature (25 °C), 70 °C and 100 °C, respectively. Their study reported that excessive heat and acidity may directly affect melamine migration from melamine-ware products [34]. However, their results showed that melamine migration in the tested items was well below the specific migration limit (SML) of 30 mg/kg (30,000 ng/ml) set out in European Commission Directive 2002/72/EC [34]. Another reason for the differences in the amount of melamine in the produced chocolates may be the amount and type of pesticides, fertilizers, nitrogen supplements in animal feed and veterinary medicines in different countries [35]. A specialized committee formed by the WHO stated that low melamine concentrations (e.g., less than 1 mg/kg) in foods may be considered as the permissible level that shows no health concern [36]. Accordingly, with the exception of two cases in which the levels were higher than the standard limit, the presence of low levels of melamine in chocolates probably had a reason other than manipulation in consumed powder milk. In fact, due to the widespread legal use of melamine as a raw organic matter in the industry such as preparation of food containers, use of disinfectants, fertilizers and animal feed, the compound can be present at the basic levels in food .

Conclusion

Melamine contamination of human and animal food has become an ongoing public health concern. According to the results, melamine was present in 85% of all the samples, and also the melamine level in one Iranian brand and one imported brand was higher than the Codex Organization standard. The above-standard values in these two brands can not be a sign of food fraud and is probably due to the presence of melamine in chocolate packaging and melamine in animal feed, which is indirectly detected through milk powder in chocolate contents.

Moreover, the LOD and LOQ of the method were sufficient to detect melamine in milk and dairy products under the safety limits recommended by the Codex Alimentarius Commission. The proposed method can be used for the routine determination of melamine residues in milk and dairy products. Consumption of chocolates containing these low levels of melamine does not constitute a health risk for consumers. However, monitoring of melamine residues is necessary in order to prevent the excessive consumption of contaminated food products. Furthermore, it is necessary that maximum residue levels and tolerable daily intake values for melamine be defined and monitored daily in Iranian food products. As a result, if melamine is identified in samples of milk-based products, it must be inhibited from the marketplace.

Acknowledgments This work is a part of MSc thesis in food safety and hygiene course in School of Public Health at Tehran University of Medical Sciences and This study has been funded and supported by Tehran University of Medical Sciences (TUMS); Grant no. 97-02-27-38033. The authors are grateful for the financial support provided by TUMS. Also, we appreciate the collaboration of the Department of Environmental Health Engineering Laboratories of the Tehran University of Medical Sciences.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

1. Venkatasami G, Sowa JR Jr. A rapid, acetonitrile-free. HPLC method for determination of melamine in infant formula, Analytica Chimica Acta. 2010;665:227–30.
2. Ramalingam K, Devasena T, Senthil B, Kalpana R, Jayavel R. Silver nanoparticles for melamine detection in milk based on transmitted light intensity. IET Sci Meas Technol. 2016;11:171–8.
3. Hau AK-C, Kwan TH, Li PK-T. Melamine toxicity and the kidney. Journal of the American Society of Nephrology. 2009;20:245–50.
4. Chan E, Griffiths S, Chan C. Public-health risks of melamine in milk products. Lancet. 2008;372:1444–5.
5. Filigenzi MS, Puschner B, Aston LS, Poppenga RH. Diagnostic determination of melamine and related compounds in kidney tissue by liquid chromatography/tandem mass spectrometry. J Agric Food Chem. 2008;56:7593–9.
6. Chan ZC, Lai W-F. Revisiting the melamine contamination event in China: implications for ethics in food technology. Trends Food Sci Technol. 2009;20:366–73.
7. Dobson RL, Motlagh S, Quijano M, Cambron RT, Baker TR, Pullen AM, et al. Identification and characterization of toxicity of contaminants in pet food leading to an outbreak of renal toxicity in cats and dogs. Toxicol Sci. 2008;106:251–62.
8. Zhu X, Wang S, Liu Q, Xu Q, Xu S, Chen H. Determination of residues of cyromazine and its metabolite, melamine, in animal-derived food by gas chromatography− mass spectrometry with derivatization. J Agric Food Chem. 2009;57:11075–80.
9. Andersen WC, Turnipseed SB, Karbiwnyk CM, Clark SB, Madson MR, Gieseker CM, et al. Determination and confirmation of melamine residues in catfish, trout, tilapia, salmon, and shrimp by liquid chromatography with tandem mass spectrometry. J Agric Food Chem. 2008;56:4340–7.
10. Deabes M, El-Habib R. Determination of melamine in infant milk formula, milk powder and basaa fish samples by HPLC/DAD. J Environ Anal Toxicol. 2012;2:2161–0525.1000137.
11. Valat C, Marchand P, Veyrand B, Amelot M, Burel C, Eterradossi N, et al. Transfer of melamine in some poultry products. Poult Sci. 2011;90:1358–63.
12. Wang P-C, Lee R-J, Chen C-Y, Chou C-C, Lee M-R. Determination of cyromazine and melamine in chicken eggs using quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction coupled with liquid chromatography–tandem mass spectrometry. Anal Chim Acta. 2012;752:78–86.
13. W.H. Organization, Toxicological and health aspects of melamine and cyanuric acid: report of a WHO expert meeting in collaboration with FAO, supported by Health Canada, Ottawa, Canada, 1–4 December 2008, (2009).
14. Haughey SA, Graham SF, Cancouët E, Elliott CT. The application of near-infrared reflectance spectroscopy (NIRS) to detect melamine adulteration of soya bean meal. Food Chem. 2013;136:1557–61.
15. Sun F, Ma W, Xu L, Zhu Y, Liu L, Peng C, et al. Analytical methods and recent developments in the detection of melamine. TrAC Trends Anal Chem. 2010;29:1239–49.
16. P. Basuri, A. Baidya, T. Pradeep (2019), Sub-ppt level detection of analytes by superhydrophobic pre-concentration paper spray ionization mass spectrometry (SHPPSI MS), Anal Chem.
17. Filazi A, Sireli U, Ekici H, Can H, Karagoz A. Determination of melamine in milk and dairy products by high performance liquid chromatography. J Dairy Sci. 2012;95:602–8.
18. Shakerian A, Khamesipour F, Rahimi E, Kiani P, Shahraki MM, Pooyan M, et al. Melamine levels in food products of animal origin in Iran. Revue de Medecine Veterinaire. 2018;169:7–9.
19. Dorne JL, Doerge DR, Vandenbroeck M, Fink-Gremmels J, Mennes W, Knutsen HK, et al. Recent advances in the risk assessment of melamine and cyanuric acid in animal feed. Toxicol Appl Pharmacol. 2013;270:218–29.
20. Kumar N, Kumar H, Mann B, Seth R. Colorimetric determination of melamine in milk using unmodified silver nanoparticles. Spectrochim Acta A Mol Biomol Spectrosc. 2016;156:89–97.
21. Domingo E, Tirelli AA, Nunes CA, Guerreiro MC, Pinto SM. Melamine detection in milk using vibrational spectroscopy and chemometrics analysis: a review. Food Res Int. 2014;60:131–9.
22. Wen JG, Liu XJ, Wang ZM, Li TF, Wahlqvist ML. Melamine-contaminated milk formula and its impact on children. Asia Pac J Clin Nutr. 2016;25:697.
23. D. Schoder, C.R. McCulloch, Food fraud with melamine and global implications, in: Chemical hazards in foods of animal origin, Wageningen Academic Publishers, 2019, pp. 1–9.
24. García Londoño VA, Puñales M, Reynoso M, Resnik S. Melamine contamination in milk powder in Uruguay. Food Addit Contam Part B. 2018;11:15–9.
25. Shakerian A. E.G. SAVABI. Detection and determination of Melamine in infant formula by ELISA method. 2012.
26. Maleki J, Nazari F, Yousefi J, Khosrokhavar R, Hosseini M-J. Determinations of melamine residue in infant formula brands available in Iran market using by HPLC method. Iranian journal of pharmaceutical research: IJPR. 2018;17:563.
27. T. Ananthakumar, A.J. Suresh, V. Niraimathi, Detection of Melamine Residue in Raw Milk and Milk Related Products by UV spectrophotometry.
28. Tittlemier SA, Lau BP-Y, Menard C, Corrigan C, Sparling M, Gaertner D, et al. Baseline levels of melamine in food items sold in Canada. I. Dairy products and soy-based dairy replacement products. Food Addit Contam. 2010;3:135–9.

29. Braekevelt E, Lau B-Y, Feng S, Ménard C, Tittlemier S. Determination of melamine, ammeline, ammelide and cyanuric acid in infant formula purchased in Canada by liquid chromatography-tandem mass spectrometry. Food Addit Contam. 2011;28:698–704.
30. Zhu H, Kannan K. Continuing occurrence of melamine and its derivatives in infant formula and dairy products from the United States: implications for environmental sources. Environ. Sci. Technol. Lett. 2018;5:641–8.
31. Yong-Ning W, Yun-Feng Z, Jin-Guang L, M.A. Group. A survey on occurrence of melamine and its analogues in tainted infant formula in China. Biomed Environ Sci. 2009;22:95–9.
32. Bradley E, Castle L, Day J, Ebner I, Ehlert K, Helling R, et al. Comparison of the migration of melamine from melamine–formaldehyde plastics ('melaware') into various food simulants and foods themselves. Food Addit Contam Part A. 2010;27:1755–64.
33. Jie L, Jing X, Da-Jin Y, Zhu-Tian W, Jiang D-G, Cong-Rong F, et al. Study on migration of melamine from food packaging materials on markets. Biomed Environ Sci. 2009;22:104–8.
34. Chik Z, Haron DM, Ahmad E, Taha H, Mustafa A. Analysis of melamine migration from melamine food contact articles. Food Addit Contam Part A. 2011;28:967–73.
35. Shi X, Dong R, Chen J, Yuan Y, Long Q, Guo J, et al. An assessment of melamine exposure in Shanghai adults and its association with food consumption. Environ Int. 2020;135:105363.
36. A. Bermudez, K. Zavarize, D. Ledoux, Pathology and mortality associated with graded levels of melamine fed to young broiler chickens, Proceedings of the American Association of Veterinary Laboratory Diagnosticians. Greensboro, NC. Davis, CA, American Association of Veterinary Laboratory Diagnosticians, 112 (2008).