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Food Additives & Contaminants: Part B
Surveillance

ISSN: 1939-3210 (Print) 1939-3229 (Online) Journal homepage: http://www.tandfonline.com/loi/tfab20

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To cite this article: Víctor Alonso García Londoño, Martín Puñales, Marcela Reynoso & Silvia Resnik (2017): Melamine contamination in milk powder in Uruguay, Food Additives & Contaminants: Part B, DOI: 10.1080/19393210.2017.1389993

To link to this article: https://doi.org/10.1080/19393210.2017.1389993

Accepted author version posted online: 06 Nov 2017.
Published online: 23 Nov 2017.

FOOD ADDITIVES & CONTAMINANTS: PART B, 2017
https://doi.org/10.1080/19393210.2017.1389993

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ARTICLE

Melamine contamination in milk powder in Uruguay

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ABSTRACT

Forty samples of milk powder purchased in Uruguay were analysed to assess melamine (MEL) levels. Trichloroacetic acid and acetonitrile were used to extract and precipitate milk proteins previously to clean up of the samples by solid-phase extraction and then were determined by liquid chromatography coupled to ultraviolet detection. The limit of detection (LOD) and limit of quantification (LOQ)of MEL were 0.006 and 0.019 mg kg^{-1}, respectively. Milk was fortified with MEL at three levels, producing average recoveries higher than 83.8%. The values for positive samples ranged from 0.017 to 0.082 mg kg^{-1}. Nine samples were positive. Three of them had concentrations between LOD and LOQ. The mean MEL contamination was 0.028 mg kg^{-1}. Consumption of milk powder containing these levels of MEL does not constitute a health risk for consumers.

ARTICLE HISTORY
Received 3 February 2017
Accepted 30 September 2017

KEYWORDS
Melamine; milk powder; HPLC

Introduction

Melamine (MEL) (1,3,5-triazine-2,4,6-triamine, MW 126) is a polar organic compound with a nitrogen-rich heterocyclic triazine. It is an industrial chemical widely used in the manufacture of laminates, plastic, fire retardant products, coatings, commercial filters, glues or adhesives and fertilizer urea mixtures (Chik et al. 2011; Zhang et al. 2011). MEL is often combined with formaldehyde in the manufacturing of plasticware because it is heat-resistant and durable (Venkatasami and Sowa 2010). It can also be present as a metabolite resulting from degradation of cyromazine, an insecticide and a veterinary drug (EFSA 2010). Since 1958–1978, it was added to cattle feed as a non-protein nitrogen supplement; however, this use was discontinued because ruminants could not hydrolyse it completely (Sun et al. 2010a).

MEL can be added on purpose to increase the apparent protein content, due to its high nitrogen content (67%), because it can be measured by traditional protein methods, such as Kjeldahl or Dumas (Venkatasami and Sowa 2010; Sun et al. 2010b). In 2007, it was discovered that MEL was added into animal feed which caused the death of hundreds of pets, mainly in the United States of America and Canada (Tittlemier 2010). The contamination of milk with MEL in China, in the fall of 2008, likely caused 300,000 cases of renal complications in children, directly resulting from consumption of tainted product (Xiang et al. 2011). Since then, MEL presence in food has drawn the attention of scientists (Sun et al. 2010a).

A variety of toxic effects from MEL, including nephrolithiasis, chronic kidney inflammation and bladder carcinoma have all been studied in animals (Hau et al. 2009). It is rapidly absorbed from the gastrointestinal tract and excreted from the body. Humans may also be more susceptible to precipitation of MEL with uric acid because humans excrete more uric acid in the urine than most mammals. In newborns until 1 year, excretion of uric acid in the urine is higher than in adults and for this reason it can be argued that babies will be even more sensitive to MEL nephrotoxicity than adults (EFSA 2010). To safeguard public health and to rebuild consumer confidence, various national and international health organisations have responded by establishing regulations on MEL content over a wide variety of foods. The Codex Alimentarius Committee has established maximum limits of 1 mg kg^{-1} in powdered infant formula, 2.5 mg kg^{-1} in food other than infant formula, in feed in 2010 and 0.15 mg kg^{-1} for MEL in liquid infant formula in 2012. The tolerable daily intake of MEL was set at 0.063 mg kg^{-1} body weight and was officially adopted as a safety guidance by the United States Food and Drug Administration (US FDA),

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European Food Safety Authority and the World Health Organization (Pei et al. 2011).

Milk is an important source of proteins and it is a widely consumed food, not only by children but also by adults. Therefore, it is important to monitor MEL in raw milk, milk powder and milk products. The sources of MEL have been divided into background levels, which refer to levels in food that do not result from adulteration or misuse, and "adulteration" levels, including misuse, which refer to intentional addition of MEL to food or unapproved use of MEL or substances that can degrade to form MEL (EFSA 2010). Background includes expected levels from the environment, food processing, packaging materials, residues from the legitimate use of triazine pesticides or veterinary drugs, and legitimate use of MEL in fertilizers or cyanuric acid in feed additives. Until 2007, MEL was not routinely monitored in food, but after Chinese reports of babies' death by MEL ingestion, determination of MEL in milk powder and liquid milk was considered in laboratories all over the world (Hassani et al. 2013). The average contamination levels are summarised in Table 1. Schoder (2010) published MEL concentrations between 0.5 and 5.5 mg kg^{-1} in milk powder in infant formula exported to the African market from European, African and New Zealand origin. In 2012, Filazi et al. analysed 300 samples of milk and dairy products purchased from major retailers in Turkey and found a mean level of MEL contamination in milk powder of 694 ± 146 µg kg^{-1}. Hassani et al. (2013) analysed the occurrence of MEL in milk powder on the Iranian market. They found that from nine randomly selected milk powder samples of different brands, eight were contaminated with MEL, ranged from 1.50 to 30.2 µg g^{-1}. The aim of this work has been to determine background concentrations of MEL in whole milk and skimmed milk powder samples produced and consumed in Uruguay.

Materials and methods

Chemicals

MEL standard (99% purity) was purchased from Fluka (Sigma-Aldrich, St. Louis, MO, USA). Solvents were high-performance liquid chromatography (HPLC)-grade, acetonitrile (ACN) from TEDIA (Fairfield, OH, USA) and methanol from TEDIA (Fairfield, OH, USA). Water HPLC grade was obtained from water purification system NANO pure Diamond (Barnstead International, Dubuque, IA, USA). 1-heptanesulphonic acid sodium salt (chromatographic grade) from Carlo Erba (Rodano, MI, Italy) was used as an ion pair reagent. Citric acid and ammonium hydroxide analytical grade were from Merck (Steinheim, Germany) and trichloroacetic acid (TCA) from Baker (Phillipsburg, NJ, USA). MEL stock solution (1000 µg ml^{-1}) was prepared by dissolving 100 mg of solid MEL in ACN: water (74:26, v/v). The stock solution was protected from light and stored at 4°C. Working standard solutions were obtained by appropriately diluting the stock solution with deionised water.

Samples

Forty samples milk powder (whole n = 35; skimmed n = 5) produced in Uruguay were purchased in supermarkets of "Montevideo", "Maldonado" and "Treinta y Tres" during 2013 and 2014. Sampling was performed in accordance with Regulation No. 836/2011 (European Commission 2011).The samples were picked randomly from the shelves in the supermarket, where the number of products found of each trademark was always less than 10. The package size ranged between 0.5 and 1 kg. When the package sizes were smaller than 1 kg, more packages were bought to have a representative lot sample. Samples were kept in their original packages,

Table 1. Melamine levels in milk powder samples.

Reference	Country	Matrix	N	Positives samples (%)	Mean (mg kg^{-1})	Min (mg kg^{-1})	Max (mg kg^{-1})	Method
Hassani et al. (2013)	Iran	Milk powder	9	100	9.64	1.50	30.32	LC-MS/MS
		Liquid milk	5	100	1.05	0.11	1.48	
Reynoso et al. (2015)	Argentina	Milk powder	20	5	–	–	0.091	HPLC/DAD
	Brazil	Milk powder	11	0	–	–	–	
Mohamed and Roquaia (2012)	Saudi Arabia	Infant milk formula	8	100	146.9	9.49	254.0	HPLC/DAD
	Saudi Arabia	Growing up milk formula	8	100	96.1	5.97	251.23	
	China	Sweetened full cream milk powder	6	100	34.2	29.1	39.7	
Filazi et al. (2012)	Turkey	Milk powder	50	8	0.694	0.505	0.86	HPLC/DAD
		Powdered infant formula	50	0	–	–	–	
Feng et al. (2012)	China	Milk powder	3	100	0.851	0.793	0.902	HPLC/DAD
Tittlemier et al. (2010)	Canada	Milk powder	2	100	0.00802	0.00528	0.0122	LC-MS/MS
This study	Uruguay	Milk powder	40	23	0.0063	<LOD	0.082	HPLC/DAD

properly identified, and stored under refrigeration (4 ± 1°C) until analysis.

Method of analysis

Sample extraction and clean up

The method employed for extraction and "clean up" of the samples corresponds to a modification of the note of application DIONEX 224 (DIONEX 2009). Briefly, 2 g of milk powder were weighed in a polypropylene tube and 15 ml 1% TCA and 5 ml ACN, were added to precipitate proteins. The mixture is vortexed for a minute, sonicated for 30 min (Branson Ultrasonics, model 2510E-MT, Danbury, LT, USA) and stirred for 10 min in a mechanical Shaker at 420 rpm (Vicking Shaker Pro, Argentina). The extracts were centrifuged at 10,000 rpm for 10 min. The supernatant was transferred to a 25 ml flask and filled to volume with 1% TCA solution. Approximately, 10 ml sample were centrifuged again at 3600 rpm for 10 min and 5 ml of the supernatant fluid was added to 5 ml of water. Purification was carried out with solid-phase extraction column Strata^{TM}-X-C-33 µm, "Cation Mixed-Mode Polimeric Sorbent," 60 mg/3 ml (Phenomenex, Torrance, CA, USA). The column was conditioned with 3 ml MeOH, followed by 3 ml water and loaded with 10 ml of extract added with water, washed with 3 ml MeOH and 3 ml water. The column was dried for 2 min at 10 mm of Hg. MEL was eluted with 7 ml 5% ammonia in MeOH. The eluate is dried at ambient temperature with a nitrogen flow and reconstituted with 1 ml of mobile phase, filtered by a nylon membrane of 0.45 mm and placed in vials.

High-performance liquid chromatography with diode-array detection

The HPLC system consisted of a Waters Alliance module 2695 coupled to a diode-array detector (DAD) Waters 2698 (Milford, MA, USA). The separation was performed with a BDS Hypersil C8 column (5 µm, 250 × 4.6 mm; Thermo Scientific, Waltham, MA, USA) at 30°C. The mobile phase was a solution of 10 mM sodium 1-heptanesulfonate and 10 mM citric acid (pH 2.7) with ACN (92:8, v/v). The flow was 1.0 ml min^{-1} and the injection volume was 90 µl. The DAD was set at a resolution of 1.2 mm and a wavelength scanning range from 210 to 400 nm. The UV spectrum of the standard was used to confirm positive samples and quantification of MEL was performed at 236.3 nm.

Results and discussion

Analytical quality assurance

The precision and accuracy of the method were determined by spiking MEL standard solution on a non-contaminated whole milk powder sample at three different concentration levels (0.2, 2 and 10 mg kg^{-1}) and analysed in triplicate. The recovery obtained for the three levels evaluated was equal to or greater than 83.8% and the average was 85.3%. Triplicate determinations were made on all samples. The relative standard deviation (RSDr)% ranged between 0.5% and 9.9%. The limit of detection (LOD) was defined as the concentration at which the signal-to-noise ratio was 3:1. The limit of quantification (LOQ) was defined as the lowest concentration of analyte that could be determined with acceptable precision and accuracy and defined as the concentration at which the signal-to-noise ratio was 10:1. LOD and LOQ were 0.006 and 0.019 mg kg^{-1}, respectively (Table 2). Quantification was carried out by constructing a calibration curve with successive dilutions of the standard stock solution. The linearity of the calibration curve was presented by three orders of magnitude. The linear correlation coefficient (R^2) was greater than 0.999.

MEL content

MEL was detected in nine milk powders (23%), seven of which were whole and two skimmed milk, with levels ranging from 0.017 to 0.082 mg kg^{-1}. Three of these nine had concentrations between LOD and LOQ (Table 1). Results of these milk powders from Uruguay were similar to those found by Feng et al. (2012), of analysis conducted after the incident in Chine in 2008. Another study performed with milk powder samples of China (Mohamed and Roquaia 2012) showed concentrations of MEL with a maximum of 39.7 mg kg^{-1}, while samples from Turkey, collected at the same period (Filazi et al. 2012) revealed 8% of MEL contamination with a maximum of 0.86 mg kg^{-1}. In 2013, Reynoso et al. (2015) analysed 31 samples of milk powder purchased in the Argentinean market and the South of Brazil (the same geographical region to the present study) and only 1 of the analysed samples was positive, with a MEL concentration of 0.091 mg kg^{-1}. Due to the China incident both the European Commission and the US FDA applied a maximum limit of 2.5 mg kg^{-1} for MEL in imported foods, particularly foods containing powdered milk from China, and 1 mg kg^{-1} in infant formula. In 2010, the Codex Alimentarius Commission

Table 2. Performance characteristics of the analytical method.

Analyte	Matrix	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	Average recovery (%)	RSDr range (%)	Accreditation Yes/No
MEL	Milk powder	0.006	0.019	85.3	0.5–9.9	
Sample_id	Analyte	Value	Is_less_than	Units	Uncertainty	No
1	Melamine	<0.006	<	mg kg^{-1}		
2	Melamine	<0.006	<	mg kg^{-1}		
3	Melamine	<0.006	<	mg kg^{-1}		
4	Melamine	<0.006	<	mg kg^{-1}		
5	Melamine	<0.006	<	mg kg^{-1}		
6	Melamine	<0.006	<	mg kg^{-1}		
7	Melamine	0.022		mg kg^{-1}	±6.2%	
8	Melamine	0.019		mg kg^{-1}	±4.7%	
9	Melamine	0.017		mg kg^{-1}	±9.1%	
10	Melamine	<0.006	<	mg kg^{-1}		
11	Melamine	<0.006	<	mg kg^{-1}		
12	Melamine	<0.006	<	mg kg^{-1}		
13	Melamine	<0.006	<	mg /kg^{-1}		
14	Melamine	<0.006	<	mg kg^{-1}		
15	Melamine	<0.006	<	mg kg^{-1}		
16	Melamine	<0.006	<	mg kg^{-1}		
17	Melamine	<0.006	<	mg kg^{-1}		
18	Melamine	<0.006	<	mg kg^{-1}		
19	Melamine	0.082		mg kg^{-1}	±3.2%	
20	Melamine	<0.006	<	mg kg^{-1}		
21	Melamine	0.034		mg kg^{-1}	±5.3%	
22	Melamine	<0.006	<	mg kg^{-1}		
23	Melamine	0.017		mg kg^{-1}	±6.4%	
24	Melamine	0.019		mg kg^{-1}	±4.2%	
25	Melamine	<0.006	<	mg kg^{-1}		
26	Melamine	<0.006	<	mg kg^{-1}		
27	Melamine	<0.006	<	mg kg^{-1}		
28	Melamine	<0.006	<	mg kg^{-1}		
29	Melamine	<0.006	<	mg kg^{-1}		
30	Melamine	<0.006	<	mg kg^{-1}		
31	Melamine	<0.006	<	mg kg^{-1}		
32	Melamine	<0.006	<	mg kg^{-1}		
33	Melamine	<0.006	<	mg kg^{-1}		
34	Melamine	0.023		mg kg^{-1}	±5.5%	
35	Melamine	0.017		mg kg^{-1}	±3.6%	
36	Melamine	<0.006	<	mg kg^{-1}		
37	Melamine	<0.006	<	mg kg^{-1}		
38	Melamine	<0.006	<	mg kg^{-1}		
39	Melamine	<0.006	<	mg kg^{-1}		
40	Melamine	<0.006	<	mg kg^{-1}		

adopted the same recommended limits (WHO 2008; EFSA 2010). The results of the present study were below the proposed maximum levels.

Conclusions

Taking into account that the average concentration of positive samples was 0.028 mg kg^{-1}, with 23% of all tested samples contaminated, the background level of MEL consumed in Uruguay does not pose a risk upon consumers and is not a priority for control routine in this country.

Acknowledgements

This work was supported by the UBA (Universidad de Buenos Aires), CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), CIC (Comisión de Investigaciones Científicas de la Provincia de Buenos Aires) and FICTB (Fundación de Investigaciones Científicas Teresa Benedicta de la Cruz).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the UBA (Universidad de Buenos Aires) [20020120100157]; CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas) [Resolución D No 4883]; CIC (Comisión de Investigaciones Científicas de la Provincia de Buenos Aires) [Resolución No 048/2016]; and FICTB (Fundación de Investigaciones Científicas Teresa Benedicta de la Cruz) [FICTBC 029/15].

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