Mycotoxin Research (2021) 37:315–325
https://doi.org/10.1007/s12550-021-00444-w

ORIGINAL ARTICLE

Quantitative risk assessment for aflatoxin M_1 associated with the consumption of milk and traditional dairy products in Argentina

D. Costamagna[1] · **M. Gaggiotti**[1] · **M. L. Signorini**[1]

Received: 9 April 2021 / Revised: 29 September 2021 / Accepted: 30 September 2021 / Published online: 9 October 2021

Abstract

A quantitative risk assessment for exposure to aflatoxin M1 (AFM_1) related to the consumption of milk and traditional dairy products of Argentina was developed. The frequency and concentration of AFM_1 was modelled at various stages through the milk processes, considering Argentinean practices. Concentration of AFM_1 (0.046 μg/l, 95%CI = 0.002–0.264 μg/l) in raw milk was estimated. The AFM_1 concentration in milk was sensitive to the carry-over rate ($r = 0.80$), and milk yield in the first third of lactation during the spring–summer season ($r = 0.11$). AFB_1 levels in silage ($r = 0.22$), pasture during the spring–summer season ($r = 0.11$), concentrate ($r = 0.08$), and cotton seed ($r = 0.05$) were the factors most correlated with AFM_1 concentrations. Although the results showed that MoE values for the mean and median exposure to AFM_1 were < 10,000 in infants, toddlers, and other children, the additional cancer risk due to exposure to AFM_1 in infants, toddlers, and other children was 0.007, 0.005, and 0.0009 additional cases per year per 100,000 individuals, respectively, which indicates no health concern. In addition, the percentages of the population exceeding HI values (HI > 1) for exposure to AFM_1 for infants, toddlers, and other children were 45%, 49.1%, and 40.6%, respectively. Under this scenario, the most susceptible population at risk was children < 10 years old; therefore, it is necessary to establish measures to prevent contamination of AFM_1 in milk and milk products.

Keywords Aflatoxin M1 · Exposure assessment · Risk characterization; Margin of exposure (MoE) · Milk and dairy products

Introduction

Aflatoxins are common contaminants which can be found in many types of food and feed. Aflatoxin B_1 (AFB_1) is the most toxic metabolite, which has been shown to have teratogenic, mutagenic, and carcinogenic effects (IARC 1993). AFM_1 is the major hydroxylated derivative of AFB_1, which is formed in the liver by P450 cytochrome enzymes and is excreted into milk via the mammary gland of dairy cows fed AFB_1-contaminated feed (Fallah 2010). AFM_1 is highly resistant to thermal treatments such as sterilization and pasteurization (Galvano et al. 1996). It can damage DNA when entering the body, ultimately leading to mutagenic and carcinogenic effects, with the liver being the main target organ (Fung and Clark 2004). AFM_1 has been classified as a Group 2B carcinogen (possibly carcinogenic to humans) by the International Agency for Research on Cancer (IARC 2002). AFM_1 is considered to be one of the most important xenobiotic compounds in milk because it is relatively stable and resists heat treatment during the production of milk and milk products. Therefore, it is likely that AFM_1 is present in dairy products when milk is contaminated (Colak 2007; Oruc et al. 2006).

Milk is considered to be a complete and natural food for consumers of all ages due to its high nutritional value. It has been shown to have the greatest potential for dietary intake of aflatoxins as it is one of the most important foods in the human diet (Kos et al. 2014). High milk consumption in all age groups, especially in children, appears to be one of the most important dietary exposure factors for AFM_1 (Rahimi et al. 2010; Prandini et al. 2009).

✉ M. L. Signorini
signorini.marcelo@inta.gob.ar

[1] Instituto de Investigación de la Cadena Láctea – IdICaL (INTA – CONICET), Ruta 34, Km 227, Rafaela (C.P. 2300), Santa Fe, Argentina

In order to protect public health, it is imperative to implement risk management measures with the aim of reducing exposure to AFM_1 due to the consumption of milk and dairy products. One of the most widely accepted tools to scientifically support control management measures is risk assessment. This methodology allows to estimate the probability of an adverse event, known or potential, resulting from the exposure of humans to foodborne hazards; in addition, it is the most important scientific basis for the laws that regulate the international food trade (FAO-WHO 2006). Quantitative risk assessment for AFM_1-contaminated milk and dairy products is one of the most useful methods to evaluate the severity and probability of liver cancer risk (Tsakiris et al. 2013). The objective of this study was to assess the risk to human health from the consumption of milk and milk products contaminated with AFM_1 for different age categories by calculation of cancer risk, considering the prevalence of the hepatitis B virus (HBV), margin of exposure (MoE), and hazard index (HI).

Material and methods

A quantitative risk assessment of AFM_1 throughout the dairy chain in Argentina, from the occurrence of AFB_1 in dairy cows feed and the carry-over of AFB_1 into AFM_1 in milk until the transference of AFM_1 from milk to dairy products, was performed. This quantitative risk assessment may be used as a scientific basis to establish risk management measures to reduce human health hazards due to this aflatoxin.

Model development

The frequency and concentration of aflatoxins were modelled at various stages along the dairy chain to estimate exposure to AFM_1 by consumption of fluid milk, powdered milk, and soft and hard cheeses. The conceptual model upon which the mathematical model was based on is depicted in Supplementary Figure S1. The model was created in Microsoft Excel 2007 with the add-on package @Risk (version 7.5, Palisade Corporation, New York, USA).

The model was developed using inputs from our own data, previously collected from Argentinean dairy systems. The Monte Carlo Model Simulation technique (applying 5000 iterations) was used to create the output distributions, which reflect the inherent uncertainty and variability in each input variable. The number of iterations provided an adequate convergence of the simulation statistics ($< 1\%$).

Data collection and data characterization

The data used to model the ingredients that make up diets in dairy feed, the frequency and concentration of AFB_1 in these ingredients, the frequency and concentration of AFM_1 in raw milk, and the carry-over rate of AFB_1 to AFM_1 in raw milk were obtained from a previous study conducted in 34 dairy farms located in the most important milk production region in Argentina during the autumn–winter of 2016 and spring–summer of 2017 (Costamagna et al. 2018; 2019). Information about the frequency and concentration of AFB_1 in the different ingredients was complemented from a database generated by the National Institute of Agricultural Technology (Rafaela, Argentina) collected during the 2000–2015 period in Argentina's central dairy region (Michlig et al. 2016).

The concentration factor of AFM_1 from raw milk to powdered milk and soft and hard cheeses was estimated from a study previously conducted in a semi-intensive voluntary milking dairy system (VMS, DeLaval Group, Tumba, Sweden) located in the National Institute of Agricultural Technology (Rafaela, Argentina). The details of this study were published by Costamagna et al. (2019).

Model inputs

All the probability distributions used in the quantitative risk assessment model are presented in Supplementary Table S1.

Animals and diets

The amount of each ingredient in the diet for dairy cows in Argentina depends on two factors: (a) the season (*S*) (autumn–winter and spring–summer) and (b) the milk production level (low and high milk production) (*MP*). Information about the quantity of each ingredient offered in the cows' diets is depicted in Table 1. These diets (ingredients and quantities) were a reflection of the diets used in Argentina's central dairy region and were obtained from sampling previously conducted in 34 dairy farms located in the most important milk production region in Argentina

Table 1 Mean diet composition of low and high milk production cows in autumn–winter and spring–summer seasons for the central dairy region of Argentina

Ingredient	Quantity in the diet (kg/DM)			
	Autumn–winter		Spring–summer	
	LP cows	HP cows	LP cows	HP cows
Silage	7	8		5
Concentrate	4.5	5.5	4	5
Alfalfa hay	1.5	2	2	2
By-products	2	4	1	2
Cotton seed	1	1	1	1
Pasture	2	3	10	8

LP low production, *HP* high production, *DM* dry matter during the autumn–winter of 2016 and spring–summer of 2017 (Costamagna et al. 2019). The same probability was considered for each season by using a Bernoulli distribution with probability equal to 0.5.

The cows in the first 3 months of lactation were considered as high milk production (ML_{early}), and those from the fourth month to the end of lactation were considered as low milk production (ML_{late}). In order to develop the model, lactation cows from any of the 10 months of lactation (ML) were considered as having the same probability by using a discrete uniform distribution. From this information, it was possible to calculate the ingredients used in the diets offered to cows according to their productive level and climatic season. The model did not consider variations due to dairy cattle breeds, since the only breed used is the Holstein breed in the most important milk production region in Argentina.

Estimation of AFB_1 intake by dairy cows

This information was obtained from a database generated by the National Institute of Agricultural Technology (Rafaela, Argentina), collected during the 2000–2015 period in Argentina's central dairy region (Michlig et al. 2016), and from sampling previously conducted in 34 dairy farms located in the most important milk production region in Argentina during autumn–winter of 2016 and spring–summer of 2017. The average size of the herd in the dairy farms analysed was 175 dairy cows (range = 52–600 cows) and a daily milk production of 4008 l (range = 1000–17,000 l). The daily milk production per cow was, on average, 22 l (range = 15–31 l) (Costamagna et al. 2019).

The determination of AFB_1 in feedstuff samples was analysed using the RIDASCREEN test kits (Product No. 5202, R-Biopharm, Germany). This test kit is sufficient for 96 determinations (including the calibration curve). The limit of detection (LOD) was < 1.7 μg/kg, and 93% mean recovery rate for naturally contaminated reference materials. The test was used according to the manufacturer's instructions.

Out of 1762 samples, 1088 (62%) were positive for AFB_1 with a mean value of 6.4 ± 29.2 μg/kg dry matter (DM). The highest frequency was detected in silages ($n = 870$), where 564 samples (64.8%) were positive for AFB_1 with a mean value of 11.66 ± 48.66 μg/kg DM, followed by cotton seed ($n = 96$), where 65 samples (67.7%) were positive for AFB_1 with a mean value of 16.08 ± 24.15 μg/kg DM, pastures ($n = 142$), with 89 samples (62.7%) positive with a mean value of 6.06 ± 3.65 μg/kg DM, concentrates ($n = 393$), where 239 samples (60.8%) were positive for AFB_1 with a mean value of 7.87 ± 13.56 μg/kg DM, hays ($n = 95$), where 42 samples (44.2%) were positive for AFB_1 with a mean value of 6.00 ± 6.66 μg/kg DM, grains ($n = 120$) with 47 samples (39.2%) positive for AFB_1 with a mean value of 15.89 ± 24.29 μg/kg DM, and finally agroindustry by-products ($n = 46$), where 15 samples (32.6%) were positive for AFB_1 with a mean value of 9.74 ± 13.86 μg/kg DM.

The presence of AFB_1 in feeds was modelled by a Bernoulli distribution, and variability in the frequency was modelled using a Beta distribution based on the information obtained from the database. On the other hand, the AFB_1 concentration in each ingredient was modelled using lognormal distributions by considering the frequency distribution observed for each of them (Supplementary Table S1). In those samples where the AFB_1 concentration was below the limit of detection, a uniform distribution was used with a minimum value = 0 and a maximum value = ELISA limit of detection. Finally, the total amount of AFB_1 ingested by the cows was calculated as the sum of the AFB_1 level of each ingredient of the diet per kilograms of dry matter (CDM) of each feed offered (Supplementary Table S1).

Carry-over rate calculation

The information provided by Costamagna et al. (2019) was used to calculate the frequency and concentration of AFM_1 in milk and to estimate the carry-over rate of AFB_1 to AFM_1 in raw milk (CC_{AFB1}). The determination of AFM_1 has been based on an enzyme-linked immunoassay (ELISA) using the RIDASCREEN test kit (Rbiopharm, Germany, Product N°: R1101). This test kit is sufficient for 96 determinations (including the calibration curve). The basis of the test is the antigen–antibody reaction. The mean lower detection limit (LOD) is 5 ng/l for milk and 50 ng/l for cheese and 96% mean recovery rate for contaminated reference materials at levels of 10–80 ng/l. The test was used according to the manufacturer's instructions.

In this study, the AFM_1 frequency in raw milk was 78% ($n = 53$), with a mean value of 0.014 ± 0.017 μg/l. The AFM_1 concentration was not influenced by the climatic season ($P = 0.541$). The carry-over rate from feed to raw milk was calculated as the percentage of AFB_1 consumed that was excreted as AFM_1 in raw milk. The total AFB_1 consumed by the cows was calculated as the sum of the AFB_1 concentration in each ingredient (in μg/kg) multiplied by the total amount of each ingredient consumed (in kg). On the other hand, the total amount of AFM_1 excreted in milk was calculated by considering the AFM_1 concentration in milk (in μg/l) multiplied by the total amount of milk produced (in l). The carry-over rate was calculated as (Eq. 1)

$$CC = \frac{\text{AFM1 excreted in raw milk}\left(\frac{\mu g}{cow}\right)}{\text{AFB1 consumed}\left(\frac{\mu g}{cow}\right)} \tag{1}$$

The average AFB_1 carry-over rate considered for this study was 0.70%, with a variation between 0.02 and 7.3%.

This information was included in the model (using a PERT distribution) to consider the variability in the estimation of the carry-over rate from AFB_1 to AFM_1.

Concentration factor

The AFM_1 concentration in dairy products was calculated from the concentration factor for powdered milk and hard and soft cheeses using the information reported by Costamagna et al. (2019) and Costamagna (2019). Briefly, a total of 36 cows were sampled according to the lactation stage: (a) < 90 days of lactation (high milk production), (b) between 90 and 150 days (medium milk production), and (c) > 150 days of lactation (low milk production). From the total raw milk collected from each of the 36 cows, one portion (1 l) was used immediately for soft cheeses manufacturing, another portion (0.500 l) was used for powdered milk, and the last portion (0.250 l) was used for the AFM_1 analysis. Finally, pool milk (180 l) was used for hard cheeses manufacturing. The manufacturing of dairy products was performed in a pilot scale in the process area of the laboratory of milk quality and agroindustry of the national agricultural technology institute. The concentration factor (*CF*) was calculated as (Eq. 2)

$$\mathrm{CF} = \frac{\text{Concentration of AFM1 in dairy product}\left(\frac{\mu g}{kg}\right)}{\text{Concentration of AFM1 in fluid milk}\left(\frac{\mu g}{kg}\right)} \tag{2}$$

The concentration factors were 5.5, 11.8, and 7.9 for soft cheese (CF_{sc}), hard cheese (CF_{hc}), and powdered milk (CF_{mp}), respectively. The concentration factors were calculated by modelling the frequency distribution of the carry-over rates calculated for each dairy product processed (using a Lognormal and Pert distributions).

Dietary exposure assessment

The estimate of the AFM_1 mean ingestion in ng/kg per body weight (*bw*) per day (T_{Exp}) through milk and dairy product consumption was calculated by combining the average AFM_1 concentration found in the fluid milk and dairy products evaluated and the mean intake of milk, powdered milk, and soft and hard cheese ingested by Argentinean consumers (T_{Cons}), in addition to the body weight range for different age categories according to the following equation (Eq. 3):

Considering that the different population strata have particular consumption patterns and different susceptibilities, six population groups were considered in the risk assessment: infants (< 12 months old), toddlers (≥ 12 months to < 36 months old), other children (≥ 36 months to < 10 years old), adolescents (≥ 10 years to < 18 years old), adults (≥ 18 years to < 65 years old), and the elderly (≥ 65 years to < 75 years old) (EFSA 2005). The mean (M), standard deviation (SD), and consumption frequency (*F*) of pasteurized fluid milk, powdered milk, soft cheese and hard cheese, and the *bw* range for different age categories are depicted in Table 2.

The consumption and consumption frequency data of each product were obtained from the Argentinean National Nutrition and Health Survey (Argentinean Ministry of Health 2012). The frequency of consumption data were obtained from a national survey that interviewed consumers and asked what their family group had consumed during the last 24 h. Therefore, frequency of consumption refers to the probability that a person has consumed milk or milk products on a particular day. If the person claimed to have consumed the food, they were asked what quantity or volume of said food they had ingested throughout the day.

In order to model the consumption variability of each product for each population stratum, lognormal distributions were used from the mean and standard deviation data for each product (Supplementary Table S1). The consumption frequency was modelled as a probability using a Bernoulli distribution for each dairy product (Supplementary Table S1). The *bw* of the six population groups considered was obtained from an evaluation conducted in Argentina (Ortiz 2012) and modelled by Uniform distributions.

Risk characterization

The risk of AFM_1 was characterized using cancer risk (JECFA 2017), margin of exposure (MoE) (EFSA 2005), and hazard index (HI) (Kuiper-Goodman 1990) approaches.

Cancer risk

JECFA assessed the cancer potency for exposure to 1 ng AFB_1/kg bw/day in 100,000 populations. The resulting upper boundaries are 0.049 additional cancer cases per 100,000 for $HBsAg^-$ populations and 0.562 additional cancer cases per 100,000 for $HBsAg^+$ populations (JECFA 2017). AFM_1 was about one-tenth as potent as

$$\text{Dietary Exposure}\left(\frac{\text{ng}}{\text{kg}}/\text{bw}/\text{day}\right) = \frac{\sum(\text{AFM1 concentration in milk product} \times \text{consumpion of milk product})}{\text{body weight (kg)}} \tag{3}$$

Table 2 Description of the mean (M), standard deviation (SD), and consumption frequency (F) of milk and traditional dairy products of Argentina, and body weight range for different age categories

Population group	Fluid milk (ml)			Powder milk (g)			Soft cheese (g)			Hard cheese (g)			Body weight range (kg)
	M[a]	SD[b]	*F*[c] (%)	M[a]	SD[b]	*F*[c] (%)	M[a]	SD[b]	*F*[c] (%)	M[a]	[b]SD[b]	*F*[c] (%)	
Infants (≤12 months)	482.40	289.00	51.30	60.50	38.00	32.30	32.80	20.00	5.80	6.00	5.10	6.40	7.60–10.50
Toddlers (12 to < 36 months)	400.40	265.20	63.80	51.20	35.80	28.10	39.60	26.50	5.80	7.10	7.30	6.50	9.70–14.70
Other children (36 months to < 10 years)	400.40	265.20	63.80	51.20	35.80	28.10	39.68	26.50	5.80	7.10	7.30	6.50	14.40–27.40
Adolescents (10–< 18 years)	191.60	265.20	38.00	29.30	15.70	7.10	58.80	48.20	8.90	8.90	7.80	5.90	38.60–72.10
Adults (18 to < 65 years)	191.60	265.20	38.00	29.30	15.70	7.10	58.80	48.20	8.90	8.90	7.80	5.90	58.00–82.00
Elderly (65 to < 75 years)	191.20	265.20	38.00	29.30	15.70	7.10	58.80	48.20	8.90	8.90	7.80	5.90	57.80–79.20

[a] Mean

[b] Standard deviation

[c] Consumption frequency

AFB_1 in carcinogenicity studies, even in sensitive species such as the rainbow trout and the Fischer rat (JECFA 2017). Therefore, the carcinogenic potency of AFM_1 was calculated to be 0.0562 additional cancer cases per 100,000 for $HBsAg^+$ populations and 0.0049 additional cancer cases per 100,000 for $HBsAg^-$ populations.

The cancer risk of AFM_1-induced hepatocellular carcinoma (HCC) was calculated by multiplying the probability of cancer with the estimates of mean and median AFM_1 exposure according to Eq. 4:

$$\text{Cancer risk} = \text{Pcancer} * \text{Exposure}\,\left(\frac{\frac{ng}{kg}\text{pc}}{\text{day}}\right) \tag{4}$$

According to the Argentinean Ministry of Health (2020), the prevalence of $HBsAg^+$ in Argentina is 1.2 cases per 100,000 persons. However, the incidence rate varies in the different subpopulations (Supplementary Table S1). Here, cancer potency (P_{cancer}) deals with the percentage of population (*Pop*) for both carriers (%Pop.$HBsAg^+$) and non-carriers (%Pop.$HBsAg^-$) of the hepatitis B virus (HBV) infection in the population of Argentina, as well as with the carcinogenic potency of AFB_1 (*P*), including 0.0049 additional cancer cases per 100,000 for chronic hepatitis B virus surface antigen negative ($HBsAg^-$) populations and 0.0562 additional cancer cases per 100,000 for $HBsAg^+$ populations (Eq. 5) (JECFA 2017):

$$\begin{aligned} P\text{cancer} =& (\text{PHBsAg} + \times \%\text{PopHBsAg+}) \\ &+ (\text{PHBsAg} - \times \%\text{PopHBsAg}-) \end{aligned} \tag{5}$$

Margin of exposure

To obtain MoE, it is suggested to use the benchmark dose (BMD), which is the dose that causes a low but measurable response, or the benchmark dose lower confidence limit 10% (BMDL10), which is an estimation of the lowest dose that is 95% certain to cause no more than 10% cancer incidence (EFSA 2005). MoEs were calculated at mean and median exposures to AFM_1 concentration by dividing the reference value of 570 ng/kg bw/day (AFM_1 potency for hepatocellular carcinoma based on a 2-year study in male Fischer rats) (Udovicki et al. 2019) by the estimated daily intakes (EDI) in humans (Eq. 6) (EFSA 2005):

$$\text{MoE} = \frac{\text{mean and median exposure AFM1}}{570} \tag{6}$$

A MoE value equal to or higher than 10,000 would be of little concern from a public health point of view.

Hazard index

The hazard index (HI, expressed as ng/kg bw) was considered to evaluate the non-carcinogenic and carcinogenic effects of AFM_1 due to consumption of milk and dairy products. This index was computed based on TD 50 (threshold dose per body weight which is divided by 5000) of AFM_1 (10.38 mg/kg bw/day, the dose that induces tumours in half of the tested animals) by an uncertainty factor of 50,000, which is a value equal to a risk level of 1:100,000 (Kuiper-Goodman 1990). The EDI is then divided by the derived value (0.2 ng/kg bw/day) to obtain the respective HI (Eq. 7):

$$\mathrm{HI} = \frac{\mathrm{EDI}}{0.2} \tag{7}$$

A HI index of AFM_1 higher than 1 indicates liver cancer risk to consumers.

Sensitivity analysis

In order to determine the impact of each input variable on the outputs variable (AFM_1 in milk and dairy products, EDI), a sensitivity analysis was conducted by using Pearson's correlation coefficient to determine the degree of association. The sensitivity analysis was performed using the @ Risk® version 7.5 software (Palisade, New York).

Model assumptions

The following assumptions were applied to the model. Considering that assumptions can have an impact on the obtained results, they should be taken into account when considering risk assessment outputs: the model considered that the proportion of milking cows was the same in each season. In order to develop the model, the length of lactation was considered to be the same for all cows, and each cow could be in any of the ten months of lactation with the same probability; even considering that the diets (ingredients and quantities) were a reflection of the diets used in Argentina's central dairy region, they could change regularly depending on ingredient availability and pricing, and the dilution effect of whole milk with different AFM_1 concentrations at the level of the processing factories was not considered. The frequency and concentration of AFB_1 in feed ingredients and AFM_1 in raw milk and milk products were estimated based on an ELISA test without any other confirmatory analysis. Finally, this risk assessment considered the exposure to AFM_1 by consumption of pasteurized fluid milk, powdered milk, soft cheese, and hard cheese. Other milk products such as sour milk, cream milk, butter, and whey were not considered.

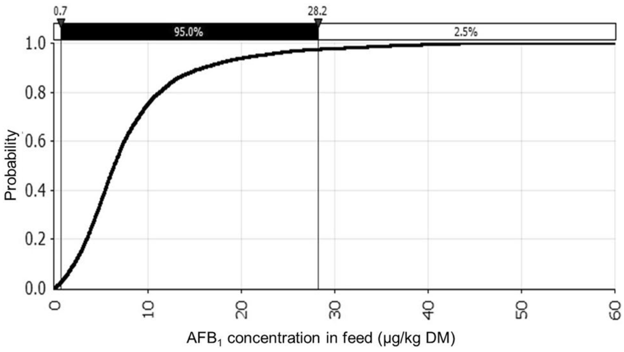

Fig. 1 Cumulative probability distribution for aflatoxin B_1 concentration in feedstuff of dairy cattle

Results and discussion

AFB_1 occurrence in feeds

The AFB_1 concentration in dairy cattle diets was estimated to be 8.20 µg/kg DM (Fig. 1). The uncertainty about the true mean value (95% confidence interval) was calculated for AFB_1 (0.7–28.2 µg/kg DM). The European Union (EU) (European Commission 2002) and the Southern Common Market (MERCOSUR) (MERCOSUR 2002) regulations establish a maximum level of 5 and 20 µg/kg AFB_1 in dairy cattle feed, respectively. According to the estimates generated by this risk model, approximately 64% and 6% of the diets offered to dairy cattle in Argentina's central dairy region would present higher levels than those established by international regulations.

AFM_1 occurrence in milk

The average AFM_1 concentration in bovine milk was estimated to be 0.046 µg/l (95%CI = 0.002–0.264 µg/l) (Fig. 2). Considering the risk of carcinogenicity associated with the

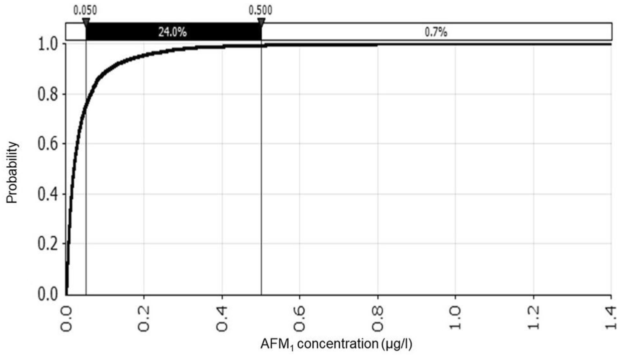

Fig. 2 Cumulative probability distribution for aflatoxin M_1 concentration in milk

Table 3 Estimated daily intake (EDI) of AFM$_1$ through milk and dairy product consumption in different age categories

Age categories	AFM$_1$ estimated daily intake (ng/kg bw/day)		
	Mean	Median	CI (95%)
Infants	1.42	0.10	0.00–12.00
Toddlers	1.05	0.19	0.00–7.80
Other children	0.59	0.10	0.00–4.40
Adolescents	0.09	1.00×10^{-4}	$0.00–7\times10^{-4}$
Adults	8.69×10^{-5}	1.32×10^{-8}	$0.00–7.00\times10^{-4}$
Elderly	8.59×10^{-5}	5.52×10^{-8}	$0.00–7.00\times10^{-4}$

exposure to AFM$_1$, and the fact that milk and milk products are consumed daily, most countries have established maximum residue levels (MRL). MRL for AFM$_1$ in milk varies from 0.05 µg/l in the EU (European Commission 2006) to 0.5 µg/l, as established by the US Food and Drug Administration (FDA) (FDA 2011) and MERCOSUR (MERCOSUR 2002). Taking into account these regulations, 0.7% of the milk produced in Argentina's central dairy region exceeds the MRL accepted by the FDA and MERCOSUR. However, 24% and 43% of the milk produced in Argentina's central dairy region exceeds the maximum level established by European regulations for fluid milk and baby food, respectively.

Dietary exposure assessment to AFM$_1$ due to milk and dairy product consumption

Daily intake of AFM$_1$ (ng/kg bw/day) through milk and dairy product consumption in different age categories is shown in Table 3. The risk of AFM$_1$ exposure was lowest in milk and dairy products for consumers from the adolescent, adult, and the elderly categories. Milk and dairy product consumers from the infant, toddler, and other children categories were found to have the highest risk of AFM$_1$ exposure.

The EDI for each population group analysed was correlated with the carry-over rate of AFM$_1$ to milk and milk products, and the consumption frequency of these products (Fig. 3). High milk consumption by all age categories and especially by infants appears to be one of the most important exposure factors through diet for AFM$_1$, as they consume more milk relative to their body weight than other age categories evaluated. Similar results were recorded by other researchers (Rahimi et al. 2010; Prandini et al. 2009; Udovicki et al. 2019).

The intake of AFM$_1$ established by JECFA (2001) for a general adult population was 0.058 for the Latin American diet, assuming a constant body weight of 60 kg, which was higher than that obtained in this risk assessment model. In Argentina, the first quantitative risk assessment for mycotoxins in milk has reported a daily intake for adults of 0.122 ng/

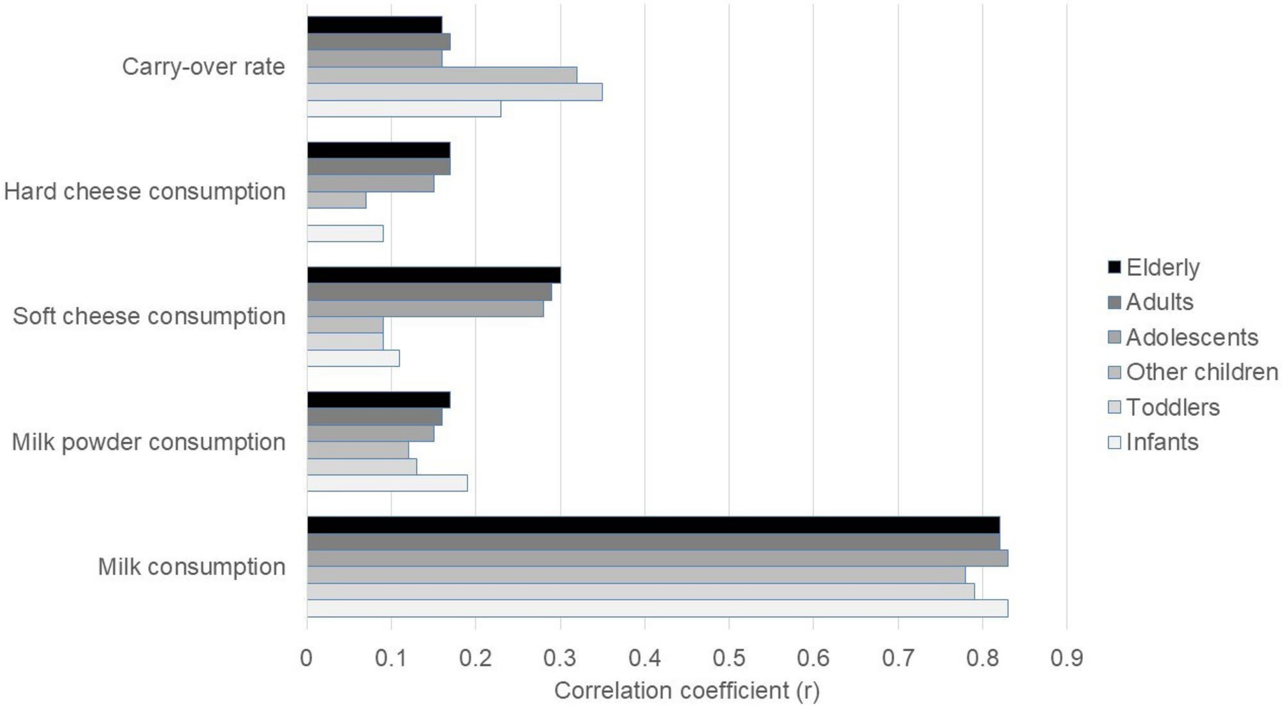

Fig. 3 Sensitivity analysis for consumption of AFM$_1$ for body weight for population group

Table 4 Estimation of liver cancer risk, MoE, and HI of AFM$_1$ through milk and dairy products consumption in different age categories

Age categories	Liver Cancer risk		MoE			HI		
	Mean[a]	Median[b]	Mean[a]	Median[b]	%Pop>10,000	Mean[a]	Median[b]	% Pop >1
Infants	0.00700	0.0005	1578341	5628	45.7	7.1	0.505	45
Toddlers	0.00519	0.0009	1306637	3049	38.2	5.27	0.933	49.1
Other children	0.00491	0.0049	3945155	5623	44	2.98	0.506	40.6
Adolescents	0.00489	0.0049	2636727	4488188	76.5	0.436	0.0006	9.9
Adults	0.00488	0.0049	$1.34\text{x}10^{9}$	4488188	100	$6.17\text{x}10^{-4}$	$6.35\text{x}10^{-4}$	0.0
Elderly	0.00488	0.0049	$2.66\text{x}10^{9}$	4488188	100	$6.09\text{x}10^{-4}$	$6.35\text{x}10^{-4}$	0.0

[a] Calculated with mean daily intake of AFM$_1$ and mean milk and dairy products consumption rate

[b] Calculated with median daily intake of AFM$_1$ and mean milk and dairy products consumption rate

[c] Percentage of population with a MoE value equal or higher than 10,000

[d] Percentage of population with HI value higher than 1

kg bw/day (Signorini et al. 2012). The lower intake in the adult population calculated in the present study may be due to, on the one hand, a better fit of the model as a consequence of the incorporation of real data from the Argentinean production system and, on the other hand, to this risk assessment having considered exposure due to the consumption not only of fluid milk but also of three traditional dairy products.

This is the first study to assess the risk of exposure to milk AFM$_1$ in different age groups in Argentina. This study found that of the 6 age groups assessed, infants, toddlers, and other children had the highest risk of exposure to milk AFM$_1$. The EDI obtained in this study for toddlers and other children was higher than the EDI reported in similar age categories in a recent study (Xiong et al. 2021) in central China. According to a study reported by Shundo et al. (2009) for children (2–4 years old), the EDI was 1.04 ng/kg bw/day, similar to that obtained in our study for the toddler age category.

Risk characterization

The risk of exposure to AFM$_1$ through milk and dairy product consumption was characterized using the liver cancer risk approach, MoE, and HI (Table 4).

Infants are commonly recognized as populations vulnerable to the effects of AFM$_1$, as they consume more milk relative to their body weight than adults. The additional cancer risk due to mean exposure to AFM$_1$ associated with milk and dairy product consumption in infants and adults was 0.007 and 0.00488 cases per 100,000 individuals per year, respectively, which indicates no health concern. Our results are similar to those reported in an assessment by EFSA (2020); the estimated cancer risk (mean and upper bound) ranged between 0.002–0.035, 0.008–0.032, 0.003–0.018, 0.001–0.006, 0.001–0.004, and 0.001–0.003 aflatoxin-induced cancers per 100,000 person-years for infants, toddlers, other children, adolescents, adults, and the elderly, respectively, and it was based on the mean potency estimates and hepatitis B virus/hepatitis C virus prevalence of 0.2%. A similar low cancer risk level of 0.0036 cases per 100,000 has been reported in Serbia for people younger than 22 years via milk and yoghurt consumption (Udovicki et al. 2019).

When the MoE value is 10,000 or higher, it is considered that there is a low risk of a negative impact on public health, according to the EFSA scientific committee guidance (EFSA 2005). Our results showed that MoE values for mean and median exposure to AFM1 were < 10,000 in infants, toddlers, and other children, indicating a health concern due to exposure to AFM1 through consumption of milk and dairy products. However, the percentage of the population that exceeded this value was 45.7%, 38.2%, and 44%, respectively (Table 4).

The HI values for mean and median exposure to AFM$_1$ for infants, toddlers, and other children were greater than one, which indicates a health concern for these categories even though less than half of the population exceeded this level within each population group (45%; 49.1%; 40.6%, respectively) (Table 4).

Our findings were in accordance with the report by the CONTAM Panel regarding the younger age groups, which raises a health concern for these age categories (EFSA 2020). Other researchers have also assessed people's risk of exposure to AFM$_1$ in milk, and found that younger children (2–4 years) had the highest risk of exposure to milk AFM$_1$, with an EDI, MoE, and HI similar to the report for our study (Tsakiris et al. 2013; Bahramia et al. 2016; Udovicki et al. 2019; Guo et al. 2020; Xiong et al. 2021). As children are susceptible to the negative effects of AFM$_1$, children's exposure risk to AFM$_1$ in milk and dairy products should also be a focus of attention.

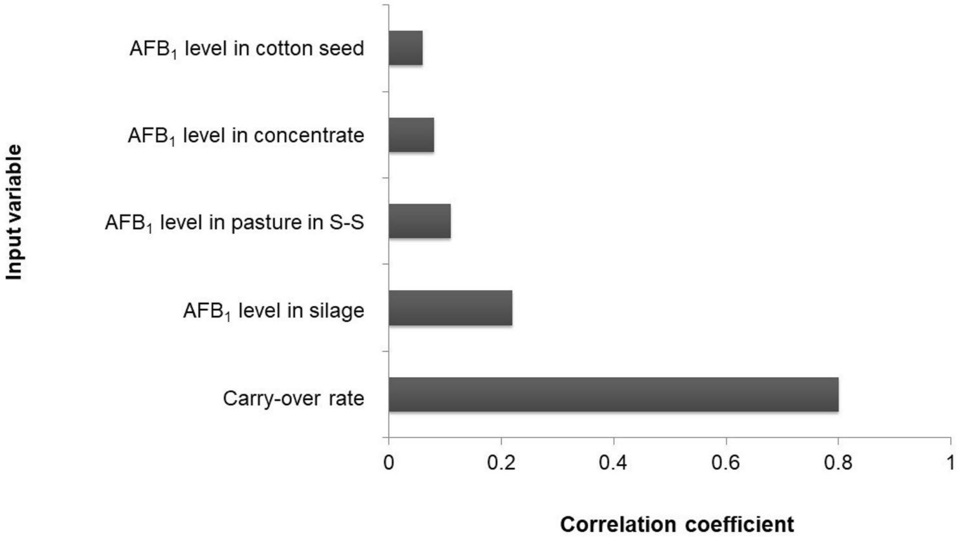

Fig. 4 Sensitivity analysis for aflatoxins concentration in bovine milk

Sensitivity analysis

The AFM_1 concentration in bovine milk was sensitive to the carry-over rate ($r = 0.80$), and to the AFB_1 level on silage ($r = 0.22$), pasture during the spring–summer (S–S) season ($r = 0.11$), commercial concentrate ($r = 0.08$), and cotton seed ($r = 0.05$) (Fig. 4).

The AFB_1 carry-over rate was identified in the model as the variable with the greatest impact, since it is a variable that is highly correlated to the AFM_1 concentration in milk. Studies have shown considerable variability regarding the percentage of aflatoxins transformed into AFM_1 and the amount of this mycotoxin present in milk (Prado et al. 1999).

Bakirci (2001) has stated that there is a linear relationship between the amount of AFM_1 in milk and the AFB_1-contaminated feed consumed by cows. Silage, pasture, commercial concentrate, and cotton seed feeds were ingredients associated with the AFM_1 level in raw milk. The proportion of AFB_1 contributed by silage and pasture was, on average, 52.2% of the total diet. These two ingredients are part of the diet throughout the year, their proportion being changed in the different seasons. In autumn–winter, silage represents 31–38% of the dry matter offered to cows, whereas during spring–summer, it contributes 19–25%. On the other hand, pasture represents 20–25% of the dry matter offered to cows in autumn–winter, whereas during spring–summer, pasture is abundant in Argentina's central dairy region, contributing 40–70% of the diet. Generally, pasture is not associated with the presence of AFB_1 when compared to other feeds that are part of the diet. However, recent results have reported an AFB_1 frequency in pastures of 91% (Signorini et al. 2012). These results could be due to the presence of *Aspergillus* in the plants and, under conditions of water stress and high variability in the rest of the climatic conditions, the toxin could be generated.

Silage is one of the most important cattle feeds and one of the main sources of fungi (Richard et al. 2007). In this sense, there are good practices that should be applied to reduce AFB_1 levels in silages. Some examples of these practices are taking into account the optimal harvesting times of the plant (moisture content) and ensuring the maintenance of an anaerobic atmosphere, which allows for correct fermentation and storage without risk of contamination since most of the fungi-producing toxins are aerobic (Fink-Gremmels 2005).

Concentrated feeds and cotton seed were the other highly correlated ingredients with the aflatoxin level in dairy milk, coinciding with results obtained in previous studies (Michlig et al. 2016). Commercial feed is composed of several grains, harvested, and stored in very diverse conditions and, in some cases, of contaminated grains that could not be used in formulating diets of a monogastric. Compared with monogastric animals, ruminants are generally considered to be less susceptible to aflatoxicosis, based on the assumption that the rumen flora degrades and deactivates the mycotoxins present in the feed (Diaz et al. 2004). However, there are factors that could affect AFB_1 detoxification in the rumen with the consequent appearance of higher AFM_1 concentrations in milk. Costamagna et al. (2019) have observed that when the particle size of the ration was not ideal, cows made a greater feed selection, preferably of short fibre, causing a shorter stay time of the feed in the rumen and thus obtaining a higher carry-over rate. The AFB_1 prevention in grains can be achieved by selecting varieties resistant to toxigenic fungi, by performing an adequate rotation of the crop and, during the harvest period, it is important to avoid excessive damage to the grains, which may predispose them to become infected during storage (Campagnollo et al. 2016). Poor storage conditions of products and by-products in dairy farms were associated with the frequency of aflatoxin in milk (Costamagna et al. 2018). The control of conditions during the grain post-harvest and by-products storage should be considered, since they could favour fungi growth and the subsequent production of toxins (Kumera and Ali Mohammed 2017).

Knowledge of these risk factors is of great importance to define Good Management Practices. Examples of these may be mixing the contaminated material with feeds having lower concentrations or being destined to other animal categories of lower susceptibility than lactating cows, or the addition of mycotoxin sequestrants in the diet (Gallo et al. 2010; Masoero et al. 2009).

Scenario analysis

Considering a scenario where all the milk consumed in Argentina had an AFM_1 contamination of 0.05 µg/kg (fluid milk) and 0.025 µg/kg (infant formula) established by the EU, the AFM_1 intake in the diet of infants, who are considered to be the population with the highest risk, would be 0.0556 ng/person/day and 0.0278 ng/person/day, where 55% and 53% would exceed the TDI, respectively. On the other hand, considering an additional scenario where all the milk produced in Argentina was within the limits established by the FDA and MERCOSUR (0.5 µg/l), the total exposure to AFM_1 was estimated at 0.556 ng/person/day, where 65% would exceed the TDI. Under this scenario, the most susceptible population could be at risk, and measures to prevent AFM_1 contamination in milk and milk products should be established.

In conclusion, this study has shown that the dairy cattle diet presents considerable levels of aflatoxins, being silages, pastures, commercial feed, and cotton seed the ingredients most correlated with the aflatoxin level in milk. These results highlight the need to include these feeds under monitoring and control programs, as well as the implementation of good management practices to prevent and/or inhibit fungi growth. Although the levels of aflatoxins found in animal diets are important, significantly high levels of aflatoxins were not perceived in milk, thus remaining within the limits established by international legislation. However, it must be considered that the global trend is to establish stricter quality requirements, and therefore, in the face of a reduction in the maximum residue limits, a serious impact on national production would be expected.

According to the potential risks for human safety derived from the consumption of AFM_1 in milk and milk products, the daily intake estimated has shown that the amount of AFM_1 is higher than the recommended allowable intake in the infant population categories evaluated, while it is the lowest for the adult population. Even though these exposures are not alarming, they should be evaluated over time. Our results show that aflatoxin exposure from milk and dairy products contributes relatively little to the incidence of liver cancer. Nonetheless, risk managers should take action based on cumulative exposure from all sources of aflatoxins.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s12550-021-00444-w.

References

Argentinean Ministry of Health (2012) Alimentos Consumidos en Argentina Resultados de la Encuesta Nacional de Nutrición y Salud ENNyS 2004/5. Buenos Aires: Ministerio de Salud. http://www.msal.gob.ar. Accessed 19 May 2021

Argentinean Ministry of Health (2020) Las hepatitis virales en la Argentina. Ministerio de Salud. https://bancos.salud.gob.ar/sites/default/files/2021-01/boletin-n2-hepatitis-virales-en-la-argentina-2020.pdf. Accessed 7 June 2021

Bahramia R, Shahbazia Y, Nikousefat Z (2016) Aflatoxin M_1 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 62:250–256. https://doi.org/10.1016/j.foodcont.2015.10.039

Bakirci I (2001) A study on the occurrence of aflatoxin M_1 in milk and milk products produced in Van province of Turkey. Food Control 12:47–51. https://doi.org/10.1016/S0956-7135(00)00020-7

Campagnollo FB, Ganev KC, Khaneghah AM, Portela JB, Cruz AG, Granato D, Corassin CH, Oliveira CAF, Sant'Ana AS (2016) The occurrence and effect of unit operations for dairy products processing on the fate of aflatoxin M_1: a review. Food Control 68:310–329. https://doi.org/10.1016/j.foodcont.2016.04.007

Colak H (2007) Determination of aflatoxin M_1 levels in Turkish white and Kashar cheeses made of experimentally contaminated raw milk. J Food Drug Anal 15:163–168. https://doi.org/10.38212/2224-6614.2428

Costamagna D (2019) Contaminantes en leche, productos y subproductos lácteos: evaluación de factores de riesgo y estrategias de intervención. https://inta.gob.ar/documentos/contaminantes-en-leche-productos-y-subproductos-lacteos-evaluacion-de-factores-de-riesgo-y-estrategias-de-intervencion

Costamagna D, Gaggiotti M, Chiericatti CA, Costabel L, Audero GML, Taverna M, Signorini ML (2019) Quantification of aflatoxin M_1 carry-over rate from feed to soft cheese. Toxicol Rep 6:782–787. https://doi.org/10.1016/j.toxrep.2019.07.004

Costamagna D, Gaggiotti MC, Signorini M (2018) Ocurrencia natural de aflatoxina M_1 en leche de tanque proveniente de tambos de la cuenca lechera central de Argentina y factores de riesgo asociados. Información Técnica de Producción Animal Publicación Miscelánea 4: 2314–3126. https://repositorio.inta.gob.ar/xmlui/bitstream/handle/20.500.12123/9573/INTA_CRSantaFe_EEARafaela_Informacion_tecnica_Produccion_Animal_2018.pdf?sequence=1&isAllowed=y

Diaz DE, Hagler WM, Blackwelder JT, Eve JA, Hopkins BA, Anderson KL, Jones FT, Whitlow LW (2004) Aflatoxin binders II: reduction of aflatoxin M_1 in milk by sequestering agents of cows consuming aflatoxin in feed. Mycopathol 157:233–241. https://doi.org/10.1023/B:MYCO.0000020587.93872.59

EC- European Commission (2002) Directive (2002/32/EC) of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. Off J Eur Union C 221:232. Last consolidated version available from: https://www.eumonitor.eu/9353000/1/j9vvik7m1c3gyxp/vhckn6qup9us

EC- European Commission (2006) Commission regulation (Nº 1881/2006) of 19 December 2006, setting maximum levels for certain contaminants in foodstuffs. Off J Eur C128: 132. Last consolidated version available from: https://eur-lex.europa.eu/legal-content/DE/AUTO/?uri=CELEX:02006R1881-20180319

EFSA - European Food Safety Authority (2005) Opinion of the scientific committee on a request from EFSA related to a harmonized approach for risk assessment of substances which are both Genotoxic and carcinogenic. EFSA J 3:282. https://doi.org/10.2903/j.efsa.2005.282

EFSA - European Food Safety Authority (2020) Panel on contaminants in the food chain (CONTAM) risk assessment of aflatoxins in food. EFSA J 18(3):e06040. https://doi.org/10.2903/sp.efsa.2020.EN-1798

Fallah A (2010) Assessment of aflatoxin M_1 contamination in pasteurized and UHT milk marketed in central part of Iran. Food Chem Toxicol 48:988–991. https://doi.org/10.1016/j.fct.2010.01.014

FAO WHO-Food and Agriculture Organization of the United Nations World Health Organization (2006) Food safety risk analysis: a guide for national food safety authorities. FAO Food Nutr Paper 87. https://apps.who.int/iris/handle/10665/43718

FDA- Food and Drug Administration (2011) Guidance for industry: action levels for poisonous or deleterious substances in human food and animal feed. Last consolidated version available from: http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ChemicalContaminantsandPesticides/ucm077969.htm

Fink-Gremmels J (2005) The Mycotoxin Blue Book. En: Diaz, D. Mycotoxin in forages. Ed. Nottingham University Press, United Kindon. ISBN 1–904761–19–4. Pag 249–268

Fung F, Clark RF (2004) Health Effects of mycotoxins: a toxicological overview. J Toxicol: Clin Toxicol 42:217–234. https://doi.org/10.1081/CLT-120030947

Gallo A, Masoero F, Bertuzzi T, Piva G, Pietri A (2010) Effect of the inclusion of adsorbents on aflatoxin B_1 quantification in animal feedstuffs. Food Addit Contam 27:54–63. https://doi.org/10.1080/02652030903207219

Galvano F, Galorafo V, Galvano G (1996) Occurrence and stability of aflatoxin M_1 in milk and milk products: a worldwide review. J Food Prot 59:1079–1090. https://doi.org/10.4315/0362-028X-59.10.1079

Guo Y, Han X, Peng S, Yue T, Wang Z (2020) Occurrence and risk assessment of aflatoxin M_1 in commercial milk in Shaanxi. Chinese J Northwest A&f University 48(5):131–137

IARC- International Agency for Research on Cancer (1993) Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. In IARC monograph on the evaluation of carcinogenic risks to humans 56:19–23 Lyon France World Health Organization IARC

IARC- International Agency for Research on Cancer (2002) Monograph on the evaluation of carcinogenic risk to humans 82: 171 Lyon France World Health Organization IARC

JECFA - Joint FAO/WHO Expert Committee on Food Additives (2001) Safety evaluation of certain mycotoxins in food. In Prepared by the fifty- sixth meeting of the JECFA (Join FAO/WHO Expert Committee on Food Additives. WHO Foods Additives Series 47. https://apps.who.int/iris/handle/10665/42467

JECFA - Joint FAO/WHO Expert Committee on Food Additives (2017) Evaluation of certain contaminants in food: eighty-third report of the joint FAO/WHO expert committee on food additives. WHO Technical Report Series 1002: 1–166. https://apps.who.int/iris/handle/10665/254893

Kos J, Lević J, Duragić O, Kokić B, Mastilović J (2014) Occurrence and estimation of aflatoxin M_1 exposure in milk in Serbia. Food Control 38:41–46. https://doi.org/10.1016/j.foodcont.2013.09.060

Kuiper-Goodman T (1990) Uncertainties in the risk assessment of three mycotoxins: aflatoxin, ochratoxin and zearalenone. Can J Physiol Pharmacol 68:1017–1024. https://doi.org/10.1139/y90-155

Kumera N, Ali Mohammed I (2017) Mycotoxin occurrence in grains and the role of postharvest management as a mitigation strategies. A Review Food Control 78:412–425. https://doi.org/10.1016/j.foodcont.2017.03.012

Masoero F, Gallo A, Diaz D, Piva G, Moschini M (2009) Effects of the procedure of inclusion of a sequestering agent in the total mixed ration on proportional aflatoxin M_1 excretion into milk of lactating dairy cows. Ani Feed Sci Technol 150:34–45. https://doi.org/10.1016/j.anifeedsci.2008.07.009

MERCOSUR (2002) Reglamento técnico MERCOSUR sobre límites máximos de aflatoxinas admisibles en leche, maní y maíz. GMC/RES. Nº 25/02 [MERCOSUR technical regulation on maximum limits of admissible aflatoxins in milk, peanuts and corn]. http://www.puntofocal.gov.ar/doc/r_gmc_25-02.pdf. Accessed 12 April 2021

Michlig N, Signorini M, Gaggiotti M, Chiericatti C, Basílico JC, Repetti MR, Beldomenico HR (2016) Risk factors associated with the presence of aflatoxin M_1 in raw bulk milk from Argentina. Food Control 64:151–156. https://doi.org/10.1016/j.foodcont.2015.12.025

Ortiz S (2012) Evaluación del crecimiento de niños y niñas. Fondo de las Naciones Unidas para la Infancia (UNICEF) 86 ISBN: 978–92–806–4642–9

Oruc HH, Cibik R, Yilmaz E, Kalkanli O (2006) Distribution and stability of aflatoxin M_1 during processing and ripening of traditional white pickled cheese. Food Addit Contam 23(2):190–195. https://doi.org/10.1080/02652030500389048

Prado G, Oliveira MS, Abrantes FM, Santos LG, Soares CR, Veloso T (1999) Ocorrencia de aflatoxina M_1 em leite consumido na cidade de Belo Horizonte - Minas Gerais/Brasil - agosto/98 - a abril/99. Ciencia e Tecnologia De Alimentos 19:420–423. https://doi.org/10.1590/S0101-20611999000300022

Prandini A, Tansini G, Sigolo S, Filippi l, Laporta M, Piva G (2009) On the occurrence of aflatoxin M_1 in milk and dairy products. Food ChemToxicol 47:984–999. https://doi.org/10.1016/j.fct.2007.10.005

Rahimi E, Bonyadian M, Rafei M, Kazemeini HR (2010) Occurrence of aflatoxin M_1 in raw milk of five dairy species in Ahvaz Iran. Food Chem Toxicol 48:129–131. https://doi.org/10.1016/j.fct.2009.09.028

Richard E, Heutte N, Sage L, Pottier D, Bouchart V, Lebailly P, Garon D (2007) Toxigenic fungi and mycotoxins in mature corn silage. Food Chem Toxicol 45:2420–2425. https://doi.org/10.1016/j.fct.2007.06.018

Shundo S, Navas SS, Conceicao L, Lamardo A, Ruvieri V, Sabino M (2009) Estimate of aflatoxin M1 exposure in milk and occurrence in Brazil. Food Control 20: 655-657. https://doi.org/10.1016/j.foodcont.2008.09.019

Signorini ML, Gaggiotti M, Molineri A, Chiericatti CA, Zapata de Basílico ML, Basílico JC (2012) Exposure assessment of mycotoxins in cow's milk in Argentina. Food Cheml Toxicol 50:250–257. https://doi.org/10.1016/j.fct.2011.09.036

Tsakiris IN, Tzatzarakis MN, Alegakis AK, Vlachou MI, Renieri EA, Tsatsakis AM (2013) Risk assessment scenarios of children's exposure to aflatoxin M_1 residues in different milk types from the Greek market. Int J Food Microbiol 56:261–265. https://doi.org/10.1016/j.fct.2013.02.024

Udovicki B, Djekic I, Kalogianni EP, Rajkovic A (2019) Exposure assessment and risk characterization of Aflatoxin M1 intake through consumption of milk and yoghurt by student population in Serbia and Greece. Toxins 11:205. https://doi.org/10.3390/toxins11040205

Xiong J, Zhang L, Zhou H, Lei M, Liu J, Ye C, Wu W, Wang C, Wu L, Qiu Y (2021) Aflatoxin M_1 in pasteurized, ESL and UHT milk products from central China during summer and winter seasons: prevalence and risk assessment of exposure in different age groups. Food Control 125:107908. https://doi.org/10.1016/j.foodcont.2021.107908