Aflatoxin M1 in Albanian pasteurized milk: occurrence, regulatory compliance, and human health risk assessment

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Abstract. Milk and dairy products represent indispensable nutritional components in the human diet, particularly in Albania, where daily milk intake is widespread. Among natural food contaminants, mycotoxins are of special public health relevance owing to their chronic dietary exposure potential. This study investigated the occurrence and exposure risk of aflatoxin M1 (AFM1), a hydroxylated metabolite of aflatoxin B1 (AFB1), in commercially available cow milk in Tirana, Albania, during the autumn and winter seasons of 2025–2026. A total of 76 milk samples were analyzed by High-Performance Liquid Chromatography coupled with Fluorescence Detection (HPLC-FLD). Detected AFM1 concentrations ranged from the limit of detection (LOD) to 248 ng/L, with a mean of 145 ng/L. AFM1 was found in 59.5% of samples, and 17.1% surpassed the EU maximum residue limit (MRL) of 50 ng/L. Dietary exposure estimates, including the Estimated Daily Intake, Hazard Index, Margin of Exposure, and cancer risk indicators, point to an elevated risk for the adult Albanian population. These results emphasize the critical importance of continuous AFM1 surveillance and upstream control of AFB1 contamination in animal feed.

1 Introduction

Milk holds a central role in human nutrition and has been integral to dietary patterns across generations and continents. As a foundational dietary component from infancy through adulthood, it serves as a major route of human exposure to food contaminants, among which mycotoxins constitute a recognized global health hazard [1]. These secondary fungal metabolites are widespread in contaminated food commodities and remain a priority concern for food safety regulatory bodies worldwide [2].

Among mycotoxins, aflatoxins — and particularly aflatoxin B1 (AFB1) — rank as the most toxicologically significant natural contaminants in food and feed. AFB1 is biosynthesized primarily by two aflatoxigenic mold species, Aspergillus flavus and A. parasiticus [3], and is frequently detected in a range of agricultural products, including maize, groundnuts, dried cereals, rice, and cottonseed, stored under humid or warm conditions conducive to fungal proliferation. Aflatoxins commonly co-occur with other mycotoxins

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across different production regions [4], and their presence in animal feed is directly linked to substantial economic losses across the livestock and food industries.

Dairy cattle are particularly susceptible to AFB1 carry-over because the toxin undergoes hepatic biotransformation and is subsequently secreted into milk as aflatoxin M1 (AFM1), its hydroxylated derivative. AFM1 is thus an important contaminant in milk and dairy products. Chronic human exposure to AFM1 is a significant public health concern, as it exerts both acute and chronic toxic effects. At the molecular level, AFM1 — like AFB1 is bioactivated by hepatic cytochrome P450 monooxygenases (CYP) into a reactive 8,9-epoxide intermediate that forms covalent DNA and protein adducts, thereby disrupting cellular metabolic processes and underpinning the mutagenic and carcinogenic properties associated with aflatoxin exposure. High-level acute exposure may result in aflatoxicosis, potentially leading to hepatic failure [3,4].

At the cellular level, reactive oxygen species generated during aflatoxin metabolism may induce oxidative DNA damage, contributing to cytotoxicity and hepatocellular carcinoma development [2]. The toxicological profile of AFM1 encompasses carcinogenicity, mutagenicity, teratogenicity, hepatotoxicity, neurotoxicity, genotoxicity, and immunosuppression. Although initially classified as a Group 2B possible human carcinogen, updated evaluations have elevated its classification to Group 1 (definite human carcinogen) [2].

The presence of AFM1 in milk represents a particular concern because milk is consumed by all demographic groups, including those most vulnerable to mycotoxin exposure, such as infants and young children. Regulatory authorities at both national and international levels have established maximum residue limits for AFM1 in milk and dairy products to safeguard public health. High prevalence of AFM1 has been documented in raw milk across various regions, representing a notable public health challenge given the high daily consumption rates, although considerable geographic variability exists. Furthermore, standard thermal treatments, including pasteurization and ultra-high-temperature (UHT) processing, are insufficient to fully eliminate AFM1 due to its considerable heat stability [5].

Globally, the incidence of AFM1 contamination in milk is inversely correlated with the stringency of regulatory oversight and the economic development status of producing countries. The highest contamination levels have been reported in African nations — for instance, concentrations reaching 4563 ng/L in Kenya and elevated levels in Nigeria [6] — while substantial regional heterogeneity is also observed across Asia, with data from China [5], Iran [7], Pakistan [8], and Turkey [9] reflecting variability attributable to husbandry practices, feed quality, seasonal influences, and differences between lactating species. In Europe, AFM1 contamination is comparatively lower but remains prevalent, particularly in southern European nations such as Italy [10].

Climate change has been identified as a significant driver of increasing aflatoxin levels in European food supplies [3]. In Albania specifically, AFB1 contamination has historically been documented primarily in maize and wheat. The carry-over of this contamination into milk and dairy products through AFM1 has been increasingly discussed in the context of shifting climatic conditions. Evidence from EU member states such as Italy [10] and from accession candidate countries including Serbia [11], Albania [12], and Kosovo [13] collectively supports the conclusion that AFM1 contamination in milk represents an escalating global food safety risk.

2 Materials and methods

2.1 Chemicals and standards

The certified reference standard of AFM1 was procured from RomerLab (Vienna, Austria). All solvents employed in the preparation of the mobile phase were of HPLC grade and sourced from Fluka (Brussels, Belgium). Sample extracts and homogenates were filtered through Whatman No. 4 filter paper and 0.45 µm membrane filters (Whatman plc, Maidstone, UK). Ultrapure water was generated using a Millipore Elix Essential purification system (Bedford, MA, USA). AFM1-specific immunoaffinity columns (AflaCean AFM1, LGC, Germany), stored at 4°C until use, were applied for matrix cleanup.

2.2 Preparation of AFM1 standard solutions

A primary stock solution at a concentration of 0.1 µg/mL was prepared by diluting a certified AFM1 reference standard (0.993 µg/mL in acetonitrile) and stored in a freezer at −20°C. A secondary working stock at 0.01 µg/mL was obtained by stepwise dilution in a mixed solvent system (MeOH/AcCN/H2O, 23/17/60, v/v/v) and kept in sealed vials at below 4°C until use for calibration curve preparation. Working calibration solutions at concentrations of 2, 76, 126, 251, 501, and 1260 ng/L were prepared. Samples with AFM1 concentrations exceeding the upper calibration limit were diluted accordingly, with appropriate dilution factors applied during quantification, in accordance with EN ISO 14501:2007.

2.3 Materials

A total of 76 (n = 76) pasteurized and UHT cow milk samples were acquired from retail outlets and food markets located in Tirana, the capital of Albania. Sample collection was carried out between October 2025 and February 2026, and all samples were transported immediately to the laboratory under refrigerated conditions.

2.4 Sample extraction

Milk samples were pre-warmed to approximately 37°C in a water bath and subsequently centrifuged at 3000 g/min to remove the fat layer. The defatted milk was passed through Whatman No. 4 filter paper, and a 50 mL aliquot was transferred into a syringe barrel fitted with an AFM1 immunoaffinity column (IAC). The sample was applied to the column at a controlled flow rate of 1–2 mL/min. The column was subsequently washed with 20 mL of ultrapure water, and residual water was removed by passing air through the column bed.

Bound AFM1 was eluted using AcCN/MeOH (60:40, v/v) with a contact time of at least 60 seconds. The IAC column was then rinsed with 1 mL of mobile phase, followed by 1 mL of H2O. The final eluate was filtered through a membrane filter prior to HPLC injection.

2.5 Instrumentation

Chromatographic quantification of AFM1 was performed using an Agilent HPLC 1260 Infinity system equipped with a quaternary pump and a fluorescence detector. Data acquisition and processing were conducted via ChemStation software (OpenLab edition). The fluorescence detector was configured with an excitation wavelength of 360 nm and an emission wavelength of 440 nm. Chromatographic separation was achieved on a Zorbax-C18 column (5 µm particle size, 4.6 × 250 mm) maintained at 30°C, with isocratic elution using

a mobile phase of methanol/acetonitrile/water (23:17:60, v/v/v) at a flow rate of 1 mL/min and an injection volume of 100 µL.

2.6 Validation

Method performance was evaluated for linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, and selectivity (Table 1). Linearity was determined by constructing five-point solvent-matched calibration curves in triplicate across a concentration range of 0.002–1.26 µg/L, using peak area as the response variable plotted against AFM1 concentration.

Table 1. Validation protocol to the AFM1 analysis in milk by HPLC-FLD.

			Reproducibility (n = 3)			
LOD (ng/L)	**LOQ (ng/L)**	**Spiked Level (ng/L)**	**Day 1**		**Day 2**	
			Mean Recovery (%)	**RSD (%) n=3**	**Mean Recovery (%)**	**RSD (%) n=3**
2	6	10	101.5	5.93	95.2	4.78
		50	92.4	4.8	93.1	4.27
		100	84.1	3.92	86.2	4.52

2.7 Risk Assessment

2.7.1 Estimated Daily Intake

Dietary exposure to AFM1 via milk consumption was quantified using the Estimated Daily Intake (EDI) framework.

$$EDI\ (ng/L \cdot bw/day) = [CAFM1\ (ng/L) \times DC\ (L/day)] / Bw\ (kg) \quad (1)$$

Where CAFM1 is the measured AFM1 concentration in the milk sample (ng/L), DC is the mean daily milk consumption volume derived from the WHO/GEMS database (Cluster G02), estimated at 0.441 L/day for the Albanian population [14], and Bw represents adult body weight as specified by EFSA.

2.7.2 The Hazard Index (HI)

The Hazard Index (HI) was applied to characterize the non-carcinogenic health risk associated with chronic AFM1 exposure through milk, expressed as the ratio of the EDI to the Reference Dose (RfD).

$$HI = EDI\ (ng/kg \cdot bw/day) / RfD\ (ng/kg \cdot bw/day) \quad (2)$$

A tolerable daily reference dose of 0.2 ng/kg body weight/day was adopted as the health-based guidance value for AFM1 exposure through contaminated milk consumption [3].

2.8 Risk Characterization

2.8.1 Margin of Exposure

Risk characterization across Albanian age groups was conducted using the Margin of Exposure (MoE) approach as formulated by EFSA, which provides a risk index for compounds exhibiting genotoxic carcinogenicity following oral exposure [3]. The MoE for each population subgroup was derived using:

$$\text{MoE} = \text{BMDL10} / \text{EDI (ng/L} \cdot \text{bw/day)} \quad (3)$$

The BMDL10 corresponds to the lower confidence bound of the benchmark dose associated with a 10% increase in liver cancer incidence attributable to aflatoxin exposure. MoE values below 10,000 indicate a potential public health concern. EFSA has endorsed a potency conversion factor of 0.1 between AFB1 and AFM1, yielding an effective BMDL10 of 4 μg/kg bw/day applicable to AFM1 risk assessment in the present study.

2.8.2 Aflatoxin B1 Cancer Potency Estimates

The incremental hepatocellular carcinoma (HCC) risk attributable to dietary AFB1 exposure was expressed as additional cases per 100,000 persons per year per ng/kg bw/day. As established by JECFA, the cancer potency coefficients for AFB1 are 0.01 for hepatitis B surface antigen-negative individuals (HBsAg−) and 0.3 for hepatitis B surface antigen-positive individuals (HBsAg+) [3].

$$P_{cancer} = (0.01 \times HBsAg^{-}) + (0.3 \times HBsAg^{+}) \quad (4)$$

Applying the above potency coefficients, the estimated cancer risk is:

$$P_{cancer} = (0.01 \times 0.98) + (0.3 \times 0.02) \quad (5)$$

3 Results and Discussion

3.1 AFM1 occurrence in Pasteurized milk

The analytical findings from AFM1 determination in the 76 pasteurized milk samples are presented in Table 2. Detection was confirmed in 59.5% of all samples, indicating a high prevalence of contamination in the surveyed milk supply. This contamination rate is comparatively elevated compared with several previously published studies from other regions, while aligning with data from countries with warm climates that support the proliferation of *Aspergillus flavu*s — the principal AFB1-producing mold and an indirect source of AFM1 in dairy milk.

Table 2. AFM1 contamination in Pasteurized Milk — Incidence and Concentration Data.

Parameter	n	Incidence (%)	Mean (ng/L)	Min (ng/L)	Max (ng/L)	Above EU MRL (50 ng/L) (%)
Pasteurized milk	76	59.5	145	2	248	17.1

Note 1: n = number of samples analyzed; MRL = Maximum Residue Level; EU MRL for AFM1 in milk = 50 ng/L (EC No. 1881/2006). Values are reported as detected concentrations in positive samples.

The arithmetic mean AFM1 concentration across positive samples was 145 ng/L, with individual values ranging from 2 to 248 ng/L. This pronounced variability likely reflects differences in feed composition, animal husbandry standards, the geographic origin of sampled milk, and seasonal fluctuations in mycotoxin contamination at the feed level. Critically, the recorded mean concentration of 145 ng/L is nearly threefold the EU MRL of 50 ng/L set under Commission Regulation (EC) No. 1881/2006, constituting a significant food safety signal.

With respect to regulatory compliance, 17.1% of analyzed samples exceeded the 50 ng/L EU threshold. While this fraction represents a numerical minority of the total sample set, its public health implications are disproportionate, as AFM1 has been reclassified as a Group 1 definite human carcinogen by the International Agency for Research on Cancer (IARC). Sub-threshold chronic dietary exposure has been linked to hepatotoxic and immunosuppressive outcomes, with heightened vulnerability among infants, young children, and immunocompromised individuals with high dairy consumption.

The thermostability of AFM1 renders it highly resistant to conventional dairy processing. Standard pasteurization at 72°C for 15 seconds reduces AFM1 concentrations by no more than 20%, leaving the bulk of contamination intact in the processed product. This underscores the necessity of implementing preventive controls at the pre-harvest stage — including optimizing feed storage conditions, incorporating mycotoxin sequestrants, and systematically monitoring feedstuffs — rather than relying solely on post-harvest thermal treatments.

Collectively, these findings indicate an urgent need to strengthen monitoring programs and regulatory enforcement throughout the surveyed milk supply chain. Future research should expand the sampling frame, extend seasonal coverage, and explore comparisons with stricter Codex Alimentarius standards or more restrictive national regulations to provide a broader regulatory context for the data.

A prior two-year study conducted in Albania reported AFM1 detection in 52.10% of milk samples collected during 2019–2020 [12], with a mean concentration of 22 ng/L and a maximum of 217 ng/L. The proportion of samples exceeding the EU MRL was 5.88%, and notable inter-annual variation was observed — contamination rates were markedly higher in 2019 (81.36%) compared to 2020 (23.33%).

Published data from Iran, based on a systematic review, indicate AFM1 prevalence of 64% (95% CI: 53–75%) in raw milk with a mean of 39.7 ng/L (32–47 ng/L), 95% (89–98%) in pasteurized milk with a mean of 62 ng/L (41–84 ng/L), and 71% (56–84%) in sterilized milk averaging 60 ng/L (31–89 ng/L), with 25% (18–32%) of milk samples exceeding regulatory limits.

AFM1 is formed through hepatic hydroxylation of AFB1 ingested via contaminated feed and is subsequently excreted into milk by lactating animals. The toxin carries over into diverse dairy products and has also been detected in human breast milk [3]. In Pakistan, a large-scale market survey by Iqbal and colleagues found AFM1 in 71% of the milk and dairy products analyzed, with 58% exceeding EU regulatory limits [8]. The occurrence of AFM1 in milk is broadly influenced by geographic location, environmental conditions, climate, and the socioeconomic context of dairy production systems. Seasonal patterns are also notable:

contamination tends to peak during colder months when animals are more reliant on stored compound feeds with potentially elevated AFB1 loads [8]. Data from China confirm this seasonal trend, with AFM1 concentrations in raw milk being substantially higher in winter (123 ng/L) than in spring (29 ng/L), summer (31.9 ng/L), or autumn (31.6 ng/L) [5].

3.2 Risk assessment to AFM1 exposure

Based on WHO/GEMS Cluster G02 dietary data, the Albanian adult population has an estimated daily milk consumption of 0.441 L, with an assumed average body weight of 70 kg per WHO reference values. These parameters were used as inputs for the exposure assessment, risk quantification, and risk characterization calculations presented in Tables 3 and 4.

The EDI derived from mean AFM1 concentrations was 0.914 ng/kg bw/day, while the maximum scenario yielded 1.563 ng/kg bw/day.

HI values exceeding 1.0 indicate a non-negligible health risk from chronic AFM1 exposure in milk-consuming populations. Both the mean- and maximum-concentration scenarios produced HI values above this threshold, indicating an elevated systemic risk for the adult Albanian population.

The MoE for the mean concentration scenario was 4378.3, while that for the maximum exposure scenario was 2559.9 — both substantially below the EFSA threshold of 10,000, which defines the boundary between negligible and significant public health concern.

Table 3. EDI, HI, and MoE among adult Albanians, from AFM1 exposure due to milk consumption.

Value	AFM1 (ng/L)	EDI (ng/kg-bw/day)	HI	MoE
Mean	145	0.914	4.568	4378.3
Max	248	1.563	7.813	2559.9

Cancer risk estimation was carried out following the approach of Serraino et al. (2019) [9], with an assumed HBV carrier prevalence of 2% among Albanians, based on Italy-Albania geographic and epidemiological proximity. The estimated population-at-risk from AFM1 exposure through milk was 0.0144 per 100,000 persons under the mean concentration scenario, rising to 0.0247 under the maximum concentration scenario.

$$\text{Population at risk} = (\text{risk potency} \times \text{BMDL}_{10}) / \text{MoE} \quad (6)$$

Table 4. Cancer risk estimation among adult Albanians exposed to AFM1.

Scenario	$BMDL_{10}$ (μg/kg bw/day)	Risk potency	MoE	Population at risk
Mean	4000	0.0158	4378.3	0.0144
Max	4000	0.0158	2559.9	0.0247

The cancer potency coefficient (0.0158 additional HCC cases per 100,000 persons per year per ng/kg bw/day) is derived from epidemiological modeling associating dietary aflatoxin exposure with hepatocellular carcinoma risk, as used by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The population-at-risk estimate was obtained by multiplying the cancer potency factor by the BMDL10 and dividing by the MoE at the

mean dietary exposure level [15]. EFSA considers MoE values ≥10,000 as indicative of low public health concern for genotoxic carcinogens. Both calculated MoE values fall well below this threshold, signaling a potential health concern — particularly under high-consumption scenarios (MoE ≈ 2559). While the absolute cancer incidence estimates attributable to AFM1 exposure are low in magnitude, they cannot be disregarded given the genotoxic nature of aflatoxins and the absence of a demonstrable safe exposure threshold. Accordingly, even modest estimated risk levels warrant consideration in food safety risk management.

4 Conclusion

AFM1 contamination in pasteurized milk consumed by Tirana residents was detected at a notably high prevalence of 59.5% during the study period. The mean contamination level of 145 ng/L — approximately three times the EU regulatory limit — represents a significant public health concern warranting prompt intervention in the dairy production sector. The primary contributing factor identified is the quality of animal feed, and the adoption of best agricultural practices is recommended as the most impactful corrective measure. Routine aflatoxin monitoring at both the farm and dairy-processing levels is essential for producers and regulatory authorities alike. Health risk indicators, including the MoE and cancer risk estimates, demonstrate an elevated risk profile for consumers with high milk and dairy intake. While the estimated cancer burden attributable to AFM1 remains relatively low in absolute terms, the genotoxic and threshold-free nature of aflatoxin carcinogenicity means that even marginal risks are of regulatory significance. These findings collectively support the case for more stringent regulatory frameworks to reduce population-level AFM1 exposure.

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