





A 20-Year Data Review on the Occurrence of Aflatoxin M1 in Milk and Dairy Products in Mediterranean Countries—Current Situation and Exposure Risks

Eleni Malissiova ^{1,2,*}, Garyfallenia Tsinopoulou ³, Efrosini S. Gerovasileiou ², Ermioni Meleti ¹, Georgia Soultani ⁴, Michalis Koureas ⁵, Ioannis Maisoglou ⁶ and Athanasios Manouras ^{2,6}

- ¹ Food of Animal Origin Laboratory, Animal Science Department, University of Thessaly, 41500 Larisa, Greece; ermeleti@uth.gr
- ² Food Technology PS, University of Thessaly, 43100 Karditsa, Greece; gerovasileiou@yahoo.com (E.S.G.); amanouras@uth.gr (A.M.)
- ³ Department of Veterinary Medicine, University of Thessaly, 43100 Karditsa, Greece; gatsinop@gmail.com
- ⁴ Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, 15771 Athens, Greece; georgiasoultani@gmail.com
- ⁵ Department of Hygiene and Epidemiology, Faculty of Medicine, University of Thessaly, 41500 Larisa, Greece; mkoureas@uth.gr
- ⁶ Food Chemistry, Biochemistry and Technology Laboratory, Nutrition and Dietetics Department, University of Thessaly, 42132 Trikala, Greece; ioanmais@uth.gr
- * Correspondence: malissiova@uth.gr; Tel.: +30-2410684388



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Abstract: Aflatoxin M1 (AFM1) is a major carcinogenic compound found in milk and dairy products, posing a constant risk to consumers in the Mediterranean region. This study systematically reviewed AFM1 presence in these products in Mediterranean countries over a period of 20 years and estimated the relevant nutritional exposure for consumers. Using data from three databases, 596 articles were retrieved with 123 meeting the inclusion criteria. The frequency of AFM1 occurrence was 40% regardless of milk type, with non-cow milk showing an increasing trend. Moreover, the Estimated Daily Intake of AFM1 from milk and dairy product consumption and the associated Hazard Index were determined. Notably, Southeastern Mediterranean countries presented higher contamination levels, leading to elevated Estimated Daily Intake and Hazard Index values. Thermally processed milk showed high contamination levels, and among dairy products, cheeses were the most contaminated. This review highlights AFM1 as a persistent hazard in the dairy sector, underscoring the need for effective interventions throughout the milk production stages.

Keywords: aflatoxin M1; Mediterranean countries; milk; dairy products; exposure risk

1. Introduction

Aflatoxins (AF) are mycotoxins produced by strains of *Aspergillus flavus*, *Aspergillus parasiticus*, and less of *Aspergillus nomius* [1]. Known for their high toxicity and mutagenic, teratogenic, and carcinogenic effects, aflatoxins are found in various products such as cereals, peanuts, dried fruits, milk, dairy products, and feed [2–4]. Specifically, aflatoxin B1 (AFB1), commonly found in the feed of lactating animals, is metabolized in the liver into a 4-hydroxylated derivative and then excreted in milk as aflatoxin M1 (AFM1) [2,5]. It is estimated that about 1–6% of AFB1 in feed is converted to AFM1 in milk, influenced by factors such as individual animals, milking season, and yield [2]. Based on multiple research studies on the cancer-causing effects in humans, the International Agency for Research on Cancer (IARC) has classified both AFB1 and AFM1 as a class 1 compound [6].

The European Union (EU) has established an acceptable limit for AFM1 concentration in milk of 50 ng/kg and 25 ng/kg for infant milk products [7]. The maximum level established by the FDA is 500 ng/kg in milk [8], and the FAO/WHO Joint Expert Committee

on Food Additives has accepted this limit [9]. Nevertheless, there is no established limit in the EU for dairy products, such as cheese. Across the globe, various limits apply to different products. Indicatively, Switzerland sets a limit of 250 ng/kg for cheese, Serbia sets a limit of 500 ng/kg for milk, and Iran sets a limit of 200 ng/kg for cheese [10].

The food industry, especially in Mediterranean countries, has been pressured into finding out how it may inhibit the growth of toxigenic moulds and the synthesis of mycotoxins in raw materials and end products, while the general public requires high-quality, preservative-free, safe, and mildly processed food with extended shelf life [11]. Consequently, continuous monitoring of AFM1 in milk and dairy products is essential, as is the monitoring of AFB1 in feed. To effectively manage AFB1 levels in feed, it is crucial to control processing conditions by maintaining low temperatures and proper drying during production to inhibit mold growth. Additionally, implementing suitable storage practices, such as using airtight containers and maintaining low humidity levels, can further reduce the risk of mycotoxin contamination. Regular monitoring and timely testing of feed samples are vital to ensure compliance with safety standards and prevent AFB1 contamination [12]. As Mediterranean countries have large populations of non-cow milk-producing animals, it is considered essential to intensify their monitoring in terms of AFM1 presence. Additionally, milk is an essential food for human beings of all ages due to its important role in the human diet and its high nutritional value, and that is why it holds an important economic role [13]. The presence of AFM1 incurs economic damages due to production losses [14]. In Croatia, for example, extremely elevated concentrations of AFM1 (2013) in cow milk raised concerns and resulted in increased additional measures to control AFM1 in milk and AFB1 contamination in feedstuffs [15,16]. Increased awareness of milk producers and consumers about the toxicity of aflatoxins is required in order to reduce the potential health risks and economic loss. As the presence of AFM1 in milk and dairy products is strongly related to the occurrence of AFB1 in animal feeds, and consequently, this is affected by a series of parameters, such as the climatic conditions [17], it is important not to underestimate or neglect the specific hazard.

According to EFSA, Mediterranean countries are considered at high risk for AFB1 and, consequently, AFM1 presence [18]. AFM1's carcinogenicity mainly occurs in the liver by affecting the repressive P53 gene and mutual effects of DNA and RNA, as well as genetic destruction effects in humans [19,20]. Through the years, there has been a correlation between aflatoxin and other negative health effects besides cancer, such as child growth impairment, innate and adaptive immune suppression, and acute toxicosis when the uptake dose is high [21–25]. The abovementioned highlights the importance of examining the two significant indicators, the Estimated Daily Intake (EDI) and Hazard Index (HI) in various aged groups.

In light of these facts, this study aimed to assess the occurrence of AFM1 in milk and dairy products over the past two decades in high-risk Mediterranean countries through a systematic literature review. The study also sought to highlight the associated consumer risks by calculating the Estimated Daily Intake (EDI) and Hazard Index (HI) of AFM1 for different age groups and dairy products. Although several review papers have been published in recent years addressing AFM1 in dairy products and the risks associated with its presence and intake [10,26], none have provided a comprehensive comparison of AFM1 data in milk and dairy products across all Mediterranean countries.

2. Materials and Methods

2.1. Search Strategy and Data Sources

Three reviewers (EM, EG, and GT) searched the electronic databases (Scopus, PubMed, and Google Scholar) for peer-reviewed articles published between 2000 and 2020, using the following key words: AFM1, Occurrence, Mediterranean, Raw milk, Milk, Thermally Processed milk, Dairy Products, Milk Products, Pasteurized milk, Yoghurt, Cheese, UHT. Studies identified from searching electronic databases were combined, duplicates were removed, and papers were screened for relevance to the review based on information

contained in the title and abstract. Abstracts were screened by another reviewer (AM), and potentially eligible papers were identified.

2.2. Inclusion/Exclusion Criteria

Studies were included if (A) they were performed in Mediterranean Countries, (B) they included quantitative results, (C) they were published in English, (D) they examined the detection of AFM1 in milk and milk products, and (E) they were conducted between 2000 to 2020. Studies were excluded if they were review articles.

2.3. Study Selection and Data Extraction

The Systematic Review follows the general approach of PRISMA 2020 statement. The full text of references identified as potentially relevant was obtained, and papers were included by applying the inclusion criteria. The number of papers retrieved was 596. Of those, 198 were duplicates and were removed, and 275 papers were excluded based on the criteria used. The number of studies finally included in this systematic review was 123 (Figure 1) [27].

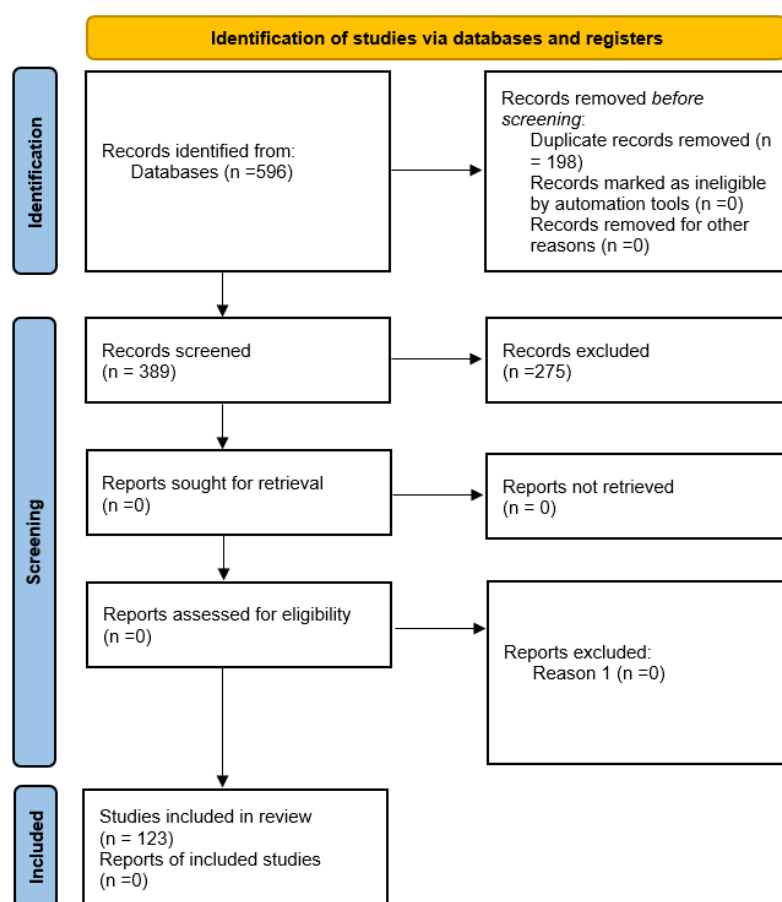


Figure 1. Flowchart of methodology.

2.4. Exposure Assessment

The Estimated Daily Intake (EDI) was calculated for infants, toddlers, children, and adults according to the following equation [28]:

$$\text{EDI (ng/kgbw/day)} = (\text{the concentration of AFM1}) \times (\text{daily consumption})/(\text{bw}),$$

where bw is body weight (kg), depending on the age group, i.e., 5 kg for infants, 12 kg for toddlers, 26.1 kg for children, 52.6 kg for adolescents, and 70 kg for adults [29]; the daily

milk intake, to be 200 mL for infants and 250 mL for older individuals; as well as a standard portion size of dairy products at 100 g.

Moreover, the Hazard Index (HI) was further calculated in order to estimate the exposure of humans at different ages to AFM1 based on nutritional data, using the equation [30]:

$$HI = \text{Estimated Daily Intake of AFM1} / \text{Tolerable Daily Intake for AFM1},$$

where Tolerable Daily Intake (TDI) for AFM1 is 0.2 ng/kg bw/day as suggested by Kaur et al. [31].

3. Results and Discussion

From the 123 papers included in this survey, 49 studies were from Europe, more specifically, 5 were from Croatia, 17 were from Italy, 1 was from France, 2 were from Spain, 2 were from Portugal, 1 was from Slovenia, 5 were from Greece, 9 were from Serbia, 2 were from Kosovo, 1 was from Bosnia and Herzegovina, 2 were from North Macedonia, 1 was from Romania and 1 was from Albania. Additionally, there were 3 from Morocco, 14 from Egypt, 2 from Algeria, 2 from Libya, 2 from Iran, and 4 from Lebanon, while the majority of the studies of AFM1 presence in milk and dairy products took place in Turkey (47 studies). All the relevant references of the studies included have been noted in the following tables.

In order to assess the data retrieved, we categorized AFM1 occurrence data as follows: occurrence of AFM1 in raw milk, thermally processed milk, and dairy products.

3.1. Occurrence Levels of AFM1 in Raw Milk

In Table 1, AFM1 occurrence data in raw milk are presented in detail. The raw milk samples that were used for the studies were from cow, sheep, goat, camel, buffalo, and donkey, and in some studies, it was not clearly declared. Regarding milk's origin, cow milk was the most frequently tested (Table 1). According to our results, the detection frequency of AFM1 in the milk of different animal species was in the following order: camel > goat > cow > buffalo > sheep > donkey. More than 40% of all the samples tested, irrespective of their type, were found to be contaminated with AFM1. To specify, 61% of the analysed camel milk samples were detected as positive for AFM1 and represented the highest occurrence of AFM1 during the study period [32–34], followed by goat milk at approximately 50% [15,33–38]. Cow's milk has traditionally been the main focus of testing as it represents the higher production globally. According to FAO [39], whole fresh cow milk represents 82.7% of global milk production, followed by milk from buffaloes (13.3%), goats (2.3%), sheep (1.3%), and camels (0.4%). The difference between milk types may result from the feeding systems and farm management practices used [40], as well as genetic variation among species, which can affect AFM1 excretion rates in milk [26].

Table 1. Data on the presence of AFM1 in raw milk in Mediterranean countries during the period 2000–2020.

Year	Country	Milk Type	Aflatoxin Detection (%)	Aflatoxin M1 Range (ng/kg)	Aflatoxin M1 >50 ng/kg EU Limit (%)	Method	LOD	References
2001	Turkey	n/a	87.8	n/a	44.3	TLC-UV	12.5 ^a	[41]
2001	Albania	C	29.2	0.05–85.0	8.3	TLC	50.0 ^a	[42]
2002	Greece	C, S, G	C: 73.3 S: 66.7 G: 40.0	5.0–55.0	3.3	HPLC-FD	5.0 ^a	[43]
2003	Italy	C	95.5	n/a	8.4	HPLC-FD	42.8 ^b	[44]

Table 1. Cont.

Year	Country	Milk Type	Aflatoxin Detection (%)	Aflatoxin M1 Range (ng/kg)	Aflatoxin M1 >50 ng/kg EU Limit (%)	Method	LOD	References
2004	Libya	C	71.4	0.0–3.1	0.0	HPLC-FD	n/a	[45]
2005	Greece	S	0.5	0.1–18.2	0.0	ELISA	5.0 ^a	[46]
2006	Italy	S	81.0	2.0–108.0	1.6	HPLC-FD	25.0 ^a	[47]
2006	Italy	C, S, G	44.9	n/a	6.0	HPLC-FD ELISA	2.0 ^a 5.0 ^a	[48]
2007	France	n/a	3.4	5.0–26.0	0.0	HPLC-FD	8.0 ^a	[49]
2009	Turkey	C	33.3	5.4–300.2	8.3	ELISA	5.0 ^a	[50]
2009	Egypt	C, G, B, CA	100.0	5.0–250.0	CA: 20.0 G: 26.0 C: 34.0 B: 48.0	ELISA	10.0 ^a	[33]
2009	Italy	C	31.5	15.5 ± 9.8 (winter) 16.5 ± 6.9 (summer)	56.0	ELISA	5.0 ^a	[35]
2010	Croatia	C	100.0	35.8–58.6 (winter) 11.6–14.9 (summer)	1.6	ELISA	1.1 ^a	[51]
2010	Turkey	C	81.7	0.0–150.0	22.5	HPLC-FD	n/a	[52]
2010	Turkey	C	100.0	25.0 ± 3.0	0.0	ELISA	n/a	[53]
2011	Lebanon	C	73.6	2.6–126.0	27.3	ELISA	5.0 ^a	[3]
2012	Morocco	C	27.0	10.0–100.0	8.0	HPLC-FD	25.0 ^a	[54]
2013	Turkey	B, C	C: 0.0 B: 27.0	n/a	n/a	LC-MS/MS	8.0 ^a	[55]
2013	Greece	S, G	18.4	5.0–148.9	1.7	ELISA	5.0 ^a	[56]
2013	Italy	B, S, G, C	48.0	5.0–16.0	0.0	HPLC-FD	3.0 ^a	[35]
2014	Croatia	C, G, S, D	100.0	C: 3.7–162.3 G: 2.8–40.8 S: 2.1–5.9 D: 3.4–10.4	C: 6.7 G: 0.0 S: 0.0 D: 0.0	ELISA	23.2 ^a	[15]
2014	Turkey	C	30.1	3.3–150.0	17.0	HPLC-FD	21.0 ^b	[57]
2014	Italy	S, G	S: 4.6 G: 13.6	S: 12.6 ± 14.1 G: 47.2 ± 19.6	S: 0.19 G: 2.3	HPLC-FD	n/a	[58]
2015	Serbia	n/a	n/a	358.0 (winter) 375.0 (spring) 29.0 (summer) 103.0 (autumn)	56.3	ELISA	5.0 ^b	[59]
2015	Croatia	C	n/a	5.9–26.6	78.0	ELISA	22.2 ^b	[60]
2015	Serbia	C	n/a	5.0–1250.0	78.0	LC-MS/MS	20.0 ^b	[61]
2015	Algeria	C	5	9.0–103	0.0	HPLC-FD	8.0 ^a	[62]
2015	Italy	C	n/a	5.0–25.0	0.0	ELISA	5.0 ^b	[63]
2015	Egypt	G, CA	G: 54 CA: 18	G: 20.0–150.0 CA: 30.0–90.0	G: 8.0 CA: 4.0	ELISA	5.0 ^a	[34]

Table 1. Cont.

Year	Country	Milk Type	Aflatoxin Detection (%)	Aflatoxin M1 Range (ng/kg)	Aflatoxin M1 >50 ng/kg EU Limit (%)	Method	LOD	References
2015	Greece	D	0.0	0.0	0.0	ELISA	n/a	[64]
2015	Turkey	C	85.0	6.6–77.0	12.0	ELISA	5.0 ^a	[65]
2015	Egypt	n/a	73.0	0.1–0.7	95.0	ELISA	n/a	[11]
2016	Italy	C	2.2	18.0 ± 2.0 and 208.0 ± 2.0	0.5	ELISA HPLC-FD	5.0 ^b 2.0 ^b	[66]
2016	Kosovo	n/a	2.8	5.2–26.6	2.7	ELISA	9.0 ^b	[67]
2016	Croatia	C, G, S	n/a	C: 0.9–85.4 G: 2.0–18.6 S: 2.3–11.2	n/a	ELISA	22.2 ^b	[37]
2016	North Macedonia	n/a	n/a	n/a	2.9	ELISA HPLC-FD	5.0 ^b n/a	[68]
2016	Turkey	C	21.1	n/a	16.0	HPLC-FD	n/a	[69]
2016	Serbia	n/a	n/a	4.5–39.8	0.0	HPLC-FD	25.0 ^a	[70]
2016	Egypt	n/a	95.6	0.1–0.04	0.0	ELISA	5.0 ^a	[71]
2016	Egypt	n/a	83.3	0.0–698.3	52.6	HPLC-FD	n/a	[72]
2016	Egypt	n/a	60.0	13.0–125.0	66.7	HPLC-FD	n/a	[73]
2016	Egypt	n/a	30.0	0.2–0.6	30.0	HPLC-FD	2.0 ^a	[74]
2016	Egypt	C	16.7	21.0–95.0	8.4	HPLC-FD	n/a	[75]
2017	Italy	B, C	B: 7.2 C: 12.3	n/a	B: 0.0 C: 0.2	ELISA HPLC-FD	4.0 ^b 4.0 ^b	[76]
2017	Greece	D	13.9	5.0–26.5	0.0	ELISA	5.0 ^a	[77]
2017	Serbia	n/a	34.4	n/a	73.3	ELISA	5.0 ^b	[78]
2017	Libya	n/a	95.0	n.d–10.9	0.0	ELISA	n/a	[79]
2017	Serbia	n/a	n/a	n/a	29.3	n/a	n/a	[80]
2017	Egypt	n/a	33.3	6.4–70.0	13.3	ELISA	n/a	[81]
2017	Egypt	C, S, CA	C: 62.5 S: 62.5 CA: 65.0	C: 30.1 ± 4.9 S: 28.2 ± 3.0 CA: 12.4 ± 2.7	C: 20.0 S: 8.0 CA: 3.9	ELISA	n/a	[32]
2017	North Macedonia	C	11.1	2.0–1003.0	9.7	ELISA	5.0 ^a	[82]
2018	Spain	C	n/a	0.1–0.5	0.0	LC-MS/MS	25.0 ^a	[83]
2018	Italy	C, S, D	C: 12.9 S: 5.0 D: 0.0	8.0–150.0	S: 1.5	ELISA	5.0 ^a	[84]
2018	Turkey	n/a	62.5	3.0–47.8	0.0	HPLC-FD	1.0 ^a	[85]
2018	Serbia	n/a	80.9	5.0–5078.0	49.1	HPLC-FD	5.0 ^b	[86]
2018	Romania	n/a	28.0	10.0–89.0	1.3	ELISA	n/a	[87]
2018	Turkey	n/a	100	0.1–10.1	0.0	ELISA	2.0 ^a	[88]
2019	Turkey	n/a	n/a	5.1–78.7	3.3	ELISA	5.0 ^a	[89]
2019	Egypt	n/a	49.0	53.0–207.0	n/a	HPLC-UV	50.0 ^a	[90]

Table 1. Cont.

Year	Country	Milk Type	Aflatoxin Detection (%)	Aflatoxin M1 Range (ng/kg)	Aflatoxin M1 >50 ng/kg EU Limit (%)	Method	LOD	References
2019	Egypt	C, B, S, G	C: 100.0 B: 40.0 S: 33.3 G: 26.7	C: 146.1–8547.7 B: 187.3–4646.8 S: 531.6–7776.1 G: 139.5–2715.7	100	ELISA	4.0 ^a	[38]
2019	Kosovo	C	41.7	4.0–833.0	15.4	ELISA	4.0 ^a	[91]
2019	Bosnia & Herzegovina	C	0	max: 32.0	0.0	ELISA	10.0 ^b	[92]
2020	Lebanon	C	n/a	11.0–440.0	28.0	HPLC-FD	3.2 ^a	[93]
2020	Egypt		20.0	n/a	95.45	TLC-UV	12.0 ^a	[94]
2020	Italy	D	89.0	12.5–125.0	0.0	HPLC-FD	4.2 ^a	[95]
2020	Algeria	C	46.4	95.6–557.2	1.2	ELISA	89.0 ^a	[96]

Milk type: C: cow; S: sheep; G: goat; CA: camel; B: buffalo; D: donkey. n/a: not available; TLC: Thin-Layer Chromatography; HPLC-FD: High-Performance Liquid Chromatography-Fluorescence Detector; LC-MS/MS: Liquid Chromatography with tandem Mass Spectrometry. ^a: ng/L ^b: ng/kg.

Based on the review results, Serbia exhibited the highest incidence of samples exceeding the EU regulatory limit at 37.7%, followed by Egypt and Lebanon at 35.2% and 27.7%, respectively. Serbia, even though it does not have a coastline in the Mediterranean, is often included on the list of Mediterranean countries, as such classification is mostly based on the geographical, economic, geopolitical, historical, ethnic, and cultural ties to the region as a whole and additionally on other factors, such as climate and flora [97]. It is worth noting that milk samples from countries such as Greece, Italy, Algeria, Romania, Bosnia and Herzegovina exhibited percentages of AFM1 exceeding European limits below 2%, while samples from Libya, France, Lebanon, and Spain did not surpass the specified limits. The observed variation was expected as the occurrence is strongly related to the climatic conditions. Many studies support that the climate severely affects the presence or no of AFB1 in seeds. The two most important factors that affect the mycotoxigenic moulds, including *Aspergillus* species, are (1) water availability and (2) temperature [98]. Magan et al. [17] indicate that warmer tropical climates and longer periods of drought stress would have a significant impact on mycotoxin's persistence and appearance in crops, seeds, etc. Other studies reported higher concentrations of AFM1 in cold seasons as compared to hot seasons [60,99]. *A. flavus* and *A. parasiticus* can easily grow in feeds with moisture between 13% and 18% and environmental moisture between 50% and 60%; furthermore, they can produce toxins [100]. At the same time, this relation of AFM1 occurrence with climatic conditions could possibly be used in predictive models for AFM1 that may assist farmers and the dairy industry in implementing precautionary measures and consequently better control AFM1 presence at the end products. In that sense, predictive models have been proposed and implemented for other products, such as pistachio nuts [101] and maize (AFLA-maize) [102], and it is expected to be expanded in the dairy sector as well. Even though milk producers globally aim effectively to control AFM1 concentration in raw milk and its products, it is apparent that it is not always achievable [103]. It is suggested that the most effective way to prevent the AFM1 contamination of milk is to reduce AFB1 in feed with better technical and more hygienic storage and harvesting. Recently, several alternative ways have been proposed. For instance, clay additives that have been used to pelletize animal feeds and as an anti-caking agent in order to improve the flow characteristics possess an added benefit, as they were also found to reduce the detrimental effects of aflatoxins and other mycotoxins [104]. Another technique studied for the reduction of AFB1 in animal feed is the usage of gaseous ozone treatment for the reduction in

micro-organisms and AFB1 levels in poultry feed, conducted by Torlak et al. [105]. Their results indicate that the use of ozone gas can be applied as an effective treatment in animal feed for microbial reduction and AFB1 degradation. It is of special interest to evaluate these approaches and start implementing them widely. No single strategy offers the perfect solution for the control of AFM1. Actually, a combination of methods is needed, such as a predictive model of monitoring AFB1, good agricultural practices, increasing farmer awareness, pre-harvest and post-harvest management of feed crops, as well as physical or chemical decontamination of feed and milk [67,97,106].

The results overall represent that EU regulations managed at a certain point to effectively control the problem of AFM1 in raw milk in EU countries, while in other countries, there seems to be a need for effective precautionary measures, possibly even adopting a stricter regulatory limit for AFM1 [107,108]. The Rapid Alert System for Food and Feed (RASFF) notifications in the study period highlight AFM1 as a serious hazard for food and feed in the EU, mainly notified after EU border rejections [109].

3.2. *Thermally Processed Milk Detection Levels of AFM1*

Table 2 shows the detection of AFM1 in thermally processed milk, namely pasteurized, powder, UHT, infant formula, concentrated, evaporated, reconstituted, skimmed, and semi-skimmed, while in some studies, the type of milk was not declared. Notably, almost every year, studies were published with a high frequency of AFM1 positive samples, while some studies reached even 100% positive samples, namely pasteurized milk in Iran [4], UHT milk in Turkey [110], and pasteurized milk in Morocco [111]. As AFM1 is thermally stable, the results were to be expected given the fact of the high occurrence noted in raw milk data. Serbia, Lebanon, Turkey, Iran, and Egypt exhibited an elevated prevalence of AFM1 in processed milk, with respective rates of 41.5%, 36.5%, 34.8%, 26%, and 18.3%, something that correlates with the relative findings on raw milk. A main consideration point is the fact that there is uncertainty about the AFM1 levels after pasteurization, as mentioned by others [67,93,112], which suggests that more research in this field is required due to the lack of solid evidence. Some reports support that aflatoxins are stable during heating treatments such as pasteurization and sterilization [113–116], while others support that pasteurization can partially reduce the amount of AFM1 in milk [117]. Diversity in the data reported in the literature could be attributed to the wide range of temperature–time combinations applied in milk processing, the different analytical methods (ELISA, HPLC, and TLC) used for AFM1 quantification, and the analysis of both naturally and artificially contaminated milk [10] used in the research so far. According to the World Health Organization (WHO), in order to reduce AFM1 occurrence and its risks, extensive scientific information about the possible AFM1 concentration following milk processing is totally necessary [118]. It is considered essential to assess the effect of various processes in AFM1 contamination of milk and employ ways for decontamination [119] if possible, such as the use of bentonite (clay particle), which has been effective in reducing AFM1 contamination in milk to levels below the European tolerable limits [120], and biological methods that are based on the action of micro-organisms such as yeasts, moulds, bacteria and algae on mycotoxins, through competition for nutrients and space that have been studied as an alternative for the AFM1 decontamination [121]. Furthermore, the application of membrane filters may also be evaluated as a safe strategy to remove the AFM1-bound dead microbial cells from decontaminated milk [122]. According to thermal process sources, it does not have much effect on aflatoxin contamination (WHO). The lack of information and uncertainty about the effect of milk pasteurization makes difficult the decision on the methods for decontamination [122–124].

Table 2. AFM1 detection in different types of dairy products in Mediterranean countries during the period 2000–2020.

Year	Country	Process Type	Aflatoxin Detection (%)	Aflatoxin M1 Range (ng/kg)	Aflatoxin M1 >50 ng/kg EU Limit (%)	Method	LOD (ng/L) ^a or (ng/kg) ^b	References
2000	Portugal	UHT	84.2	5.0–50.0	2.9	HPLC-FD	5.0 ^a	[125]
2002	Greece	P, UHT, C	P: 85.4 UHT: 82.3 C: 93.3	n/a	P: 0.0 UHT: 0.0 n/a	HPLC-FD	5.0 ^a	[43]
2005	Turkey	P	88.2	n/a	64.0	ELISA	n/a	[126]
2005	Turkey	Po	n/a	0.0–705.0	n/a	n/a	n/a	[127]
2006	Turkey	I	59.3	10.0–200.0	1.0	HPLC-FD	10.0 ^a	[128]
2006	Turkey	UHT	58.1	108.0	47.0	ELISA	10.0 ^a	[129]
2007	Morocco	P	88.8	1.0–117.0	7.4	HPLC-FD	1.0 ^a	[112]
2007	Italy	P, UHT	n/a	n/a	0.6	HPLC-FD	2.0 ^b	[130]
2007	Iran	P, I	96.3	P: 31.0–113.0 I: 1.0–14.0	P: 78.0 I: 0.0	ELISA	5.0 ^b	[131]
2008	Turkey	Po	62.5	n/a	45.0	ELISA	1.0 ^b	[132]
2008	Turkey	UHT	67.0	10.0–630.0	31.0	ELISA	10.0 ^a	[133]
2010	Spain	UHT	94.4	9.3 ± 2.6	0.0	ELISA	5.0 ^b	[134]
2010	Turkey	P	n/a	25.0 ± 3.0	n/a	ELISA	n/a	[53]
2011	Lebanon	P, Po	P: 68.0 Po: 35.7	P: 3.3–84.4 Po: 9.2–16.5	42.0	ELISA	5.0 ^a	[3]
2012	Iran	P	100.0	0.5–9.8	0.0	HPLC-FD	25 ^a	[4]
2013	Italy	UHT	42.0	5.0–16.0	0.0	HPLC-FD	5.0 ^a	[36]
2014	Turkey	I	38.1	5.5–20.1	0.0	ELISA	5.0 ^a	[135]
2014	Turkey	UHT	73.2	6.4–71.3	7.3	ELISA	2.0 ^a	[136]
2015	Algeria	P, R	P: 29.0 R: 0.0	9.0–103.0	P: 7.0 R: 0.0	HPLC-FD	2.5 ^a	[62]
2015	Serbia	n/a	n/a	Po: 847.0	32.3	ELISA	5.0 ^b	[61]
2015	Serbia	P, I	72.8	20.0–320.0	75.0	HPLC-FD	3.0 ^b	[137]
2015	Turkey	UHT	75.6	0.4 and 26.6	0.0	ELISA	5.0 ^a	[138]
2015	Egypt	UHT	50.0	0.0–0.1	60.0	ELISA	n/a	[11]
2016	Kosovo	UHT	2.6	7.2 and 9.9	n/a	ELISA	9.0 ^b	[67]
2016	Croatia	UHT	n/a	2.3–21.4	n/a	ELISA	22.2 ^b	[37]
2016	Italy	UHT	60.0	9.0 and 26.0	0.0	HPLC-FD	8.0 ^a	[139]
2016	Egypt	P	80.0	13.0–21.0	0.0	HPLC-FD	n/a	[74]
2017	Italy	P	71.4	n/a	0.0	LC-MS/MS	n/a	[140]
2017	Libya	UHT	75–100	0.0–160.0	17.0	ELISA	5.0 ^a	[79]
2017	Egypt	P	40.0	25.6–73.6	13.3	ELISA	n/a	[81]
2018	Turkey	UHT, P, Skimmed, Semi-Skimmed	n/a	UHT: 9.3 ± 4.6 P: 6.3 ± 3.8 Skimmed: 6.8 ± 3.6	n/a	ELISA	2 ^b	[141]

Table 2. Cont.

Year	Country	Process Type	Aflatoxin Detection (%)	Aflatoxin M1 Range (ng/kg)	Aflatoxin M1 >50 ng/kg EU Limit (%)	Method	LOD (ng/L) ^a or (ng/kg) ^b	References
2019	Lebanon	I	88.0	20.1 ± 1.3	13.0	ELISA	5.0 ^a	[142]
2019	Turkey	UHT	30.8	4.3–127.4	45.8	ELISA	5.0 ^a	[143]
2019	Turkey	UHT	UHT:100.0	UHT: 26.0–80.0	UHT: 53.8	ELISA	n/a	[110]
2020	Egypt	UHT	0.0	0.0	0.0	HPLC-FD	2.0 ^a	[94]
2020	Morocco	P, UHT	P: 100.0 UHT: 35.0	P: 25.5 ± 12.1 UHT: 14.7 ± 10.2	0.0	HPLC-FD	5.0 ^b	[111]
2020	Lebanon	P, UHT	n/a	13.0–219.0	54.5	HPLC-FD	3.2 ^a	[93]

Process type: P: Pasteurized; Po: Powder; UHT: Ultra-High Temperature; I: Infant; C: Concentrated; R: Re-constituted. n/a: not available; HPLC-FD: High-Performance Liquid Chromatography-Fluorescence Detector; LC-MS/MS: Liquid Chromatography with tandem Mass Spectrometry. ^a: ng/L ^b: ng/kg.

3.3. Dairy Products Occurrence Levels

Table 3 also provides comprehensive data on the presence of AFM1 in dairy products. The dairy products included in the studies were cheese (homemade, industrial, karish, mozzarella, akawi, roumy, ayran, domiati, mish, rash, zabady, cube), yoghurt, ice-cream, butter, dairy dessert, trachana, cream, kaymak, and in some studies, the type of product was not declared. Turkey and Egypt have the highest proportion, after Libya. Many studies indicate the presence of AFM1 at rather high concentrations in dairy products [11,126,144,145]. According to our results, the mean frequency of AFM1 in different dairy products was in the following order: cheese > butter > trachanas > yoghurt > dairy dessert > ice cream. It is noteworthy that among all the dairy product samples, mozzarella cheese showed the highest rate, exceeding the permissible limit. There are some factors that may affect the AFM1 levels in each type of cheese [145]. Some of them are the type of milk (milking animal, country's geographical origin, and seasonal conditions), degree of milk contamination (milk quality), cheese manufacture, ripening, and storage conditions (salt concentration, relative humidity, the content of water eliminated during processing, renneting temperature, cut size, pressing time, pH of cheese and brine), in addition to the analytical methods used in toxin quantification [111,146–154]. With regards to the relation of AFM1 and cheese or other dairy products, it is important to highlight that several reports indicate that cream separation can affect AFM1 concentration in the end product since 80% is partitioned in the skim milk and 30% is associated with the non-fat milk solids, particularly casein [46]. The behaviour of AFM1 in processes that involve fat separation may be explained by its semi-polar character: AFM1 is a water-soluble compound binding with the hydrophobic sides of casein, thus leading to predominance in the non-fat fraction. According to Battacone et al. [152], AFM1's concentration is higher in curd than it is in milk. Some studies also support that the increased levels of AFM1 in cheeses are due to the AFM1 association with casein [155,156]. The ripening and storage time of cheese has been revealed to not affect the AFM1 levels in some studies [157–159]. On the contrary, in some other investigations, ripening and storage increased the AFM1 levels [160,161], while some other studies noticed that the AFM1 concentration in some types of cheese decreases during storage due to the toxin's degradation over time [162,163]. The same uncertainty was noted in yoghurt samples as well, where researchers have diverse opinions on the effect of the processing on AFM1 values. Studies on AFM1 contamination of yoghurt are very limited and rather contradictory. Some researchers [164–166] indicate no change, while others report that after fermentation, AFM1 levels in yoghurt samples showed a significant decrease compared with those initially added to milk [167]. According to Govaris et al. [167], this decrease in AFM1 levels might be attributed to factors such as low pH, the formation of organic acids or other fermentation by-products, or even the presence of lactic acid bacteria. The low pH

during fermentation alters the structure of milk proteins such as caseins, leading to the formation of yoghurt coagulum. The change in casein structure during yoghurt production may affect the association of AFM1 with this protein [155,156], causing adsorption or occlusion of the toxin in the precipitate.

Table 3. AFM1 detection in different types of dairy products in Mediterranean countries during the period 2000–2020.

Year	Country	Dairy Product Type	Aflatoxin Detection (%)	Aflatoxin M1 Range (ng/kg)	Aflatoxin M1 >50 ng/kg EU Limit (%)	Method	LOD (ng/L) ^a or (ng/kg) ^b	References
2001	Turkey	C	89.5	0.0–810.0	0.0	ELISA	100.0 ^b	[168]
2001	Italy	Y	61.0	1.0–32.1	0.0	HPLC-FD	1.0 ^a	[169]
2003	Turkey	C	100.0	131.1–152.4	100.0	ELISA	100.0 ^b	[170]
2004	Turkey	C	44.4	7.0–202.0	n/a	TLC-UV	n/a	[171]
2004	Portugal	Y	18.8	19.0–98.0	6.0	HPLC-FD	10.0 ^b	[172]
2004	Libya	C	75.0	110.0–520.0	100.0	HPLC-FD	n/a	[45]
2005	Turkey	C	100.0	100.0–5200.0	60.0	HPLC-FD	100.0 ^b	[173]
2005	Turkey	C	100.0	51.0–115.0	100.0	ELISA	n/a	[174]
2005	Greece	C	0.0	0.0	0.0	ELISA	50.0 ^b	[46]
2006	Turkey	Y	58.8	0.0–150.0	24.0	ELISA	n/a	[1]
2006	Turkey	C	100.0	250.0–4100.0	100.0	ELISA	50.0 ^b	[175]
2008	Italy	C	16.6	50.0–250.0	79.0	ELISA	37.0 ^b	[161]
2008	Turkey	C	82.6	50.0–690.0	27.3	ELISA	50.0 ^b	[133]
2008	Slovenia	C	15.0	51.0–223.0	10.0	ELISA	50.0 ^b	[176]
2008	Turkey	C	71.4	50.0–400.0	38.1	ELISA	50.0 ^b	[177]
2009	Turkey	C	82.4	52.0–860.0	100.0	ELISA	50.0 ^b	[178]
2009	Turkey	C	60.0	16.0–1043.0	13.3	ELISA	50.0 ^b	[5]
2010	Spain	C, Y	C: 0.0 Y: 2.8	C: 0.0–12.5 Y: 8.4–18.04	1.0	ELISA	25.0 ^b	[134]
2010	Turkey	C	100.0	19.0–27.0	0.0	ELISA	50.0 ^b	[53]
2010	Turkey	C	51.3	52.0–2520.0	100.0	ELISA	50.0 ^b	[179]
2011	Turkey	Y, C	Y: 87.5 C: 90.0	Y: 10.0–475.0 C: 6.0–264.0	Y: 20.0 C: 13.6	ELISA	5.0 ^b	[180]
2011	Turkey	I	26.1	6.1–32.2	0.0	ELISA	5.0 ^a	[181]
2011	Turkey	C, Y, DD	C: 63.0 Y: 56.0 DD: 52.0	C: 12.0–378.0 Y: 2.5–78.0 DD: 1.5–80.0	C: 5.0 Y: 14.0 DD: 10.0	ELISA	50.0 ^b and 5.0 ^b	[182]
2011	Turkey	C	28.3	70.6–771.0	10.2	ELISA	50.0 ^b	[183]
2012	Lebanon	C	67.6	5.0–315.0	17.3	ELISA	50.0 ^a	[184]
2013	Egypt	C	46.7	0.1–59.6	3.3	HPLC-FD	50.0 ^a	[185]
2013	Italy	C	42.0	5.0–18.0	0.0	ELISA	10.0 ^a	[36]
2013	Turkey	C, Y	C: 43.0 Y: 100.0	C: 9.0–487.0 Y: 125.0–269.0	C: 8.0 Y: 100.0	ELISA	50.0 ^b and 5.0 ^b	[186]

Table 3. Cont.

Year	Country	Dairy Product Type	Aflatoxin Detection (%)	Aflatoxin M1 Range (ng/kg)	Aflatoxin M1 >50 ng/kg EU Limit (%)	Method	LOD (ng/L) ^a or (ng/kg) ^b	References
2014	Turkey	C	98.0	15.0–3774.0	11.1	HPLC-FD	10.0 ^b	[187]
2014	Turkey	T	52.5	0.5–36.6	0.0	HPLC-FD	0.5 ^b	[188]
2014	Turkey	C Home, C Industrial	CI: 28.8 CH: 21.7	CI: 0.0–16.7 CH: 0.0–4.6	CI: 0.0 CH: 0.0	HPLC-FD	5.0 ^b	[189]
2014	Turkey	C, Y	100.0	C: 40.4–130.9 Y: 40.6–72.0	C: 10.0 Y: 10.0	ELISA	2.0 ^b	[190]
2014	Turkey	I	46.0	5.0–50.0	34.0	ELISA	5.0 ^b	[191]
2015	Serbia	n/a	n/a	n/a	37.8	n/a	n/a	[59]
2015	Serbia	C (white and hard)	n/a	n/a	13.0	LC-MS/MS	38.0 ^b and 6.0 ^b	[192]
2015	Turkey	C (kup)	41.7	16.0 and 136.0	20.0	HPLC-FD	6.2 ^a	[193]
2015	Egypt	Processed, Kariesh, Mozzarella, Akawi, Roumy Cheese, Yoghurt	P: 75.0 K: 100.0 M: 100.0 A: 80.0 RC: 75.0 Y: 100.0	P: 0.1–1.6 K: 0.3–1.6 M: 0.0–1.5 A: 0.6–1.6 RC: 0.5–0.1 Y: 0.0–0.8	P: 53.3 K: 85.0 M: 70.0 A: 100.0 RC: 86.6 Y: 100.0	ELISA	n/a	[11]
2016	Turkey	Y, Ayran	n/a	n/a	0.0	HPLC-FD	n/a	[70]
2016	Turkey	C	52.0	10.6–702.0	85.0	ELISA	5.0 ^b	[194]
2016	Egypt	C	C:51.1	5000.0–9700.0	0.0	ELISA	5.0 ^b	[60]
2016	Egypt	karish C, Y	K: 100.0 Y: 75.0	K: 32.7–856.1 Y: 31.5–66.1 (summer)	K: 83.3 Y: 100.0	HPLC-FD	n/a	[71]
2016	Egypt	C (roomie, karish)	RC: 80.0 KC: 60.0	RC: 10.0–21.0 KC: 45.0–200.0	RC: 0.0 KC: 50.0	Vicam-Fluorometry	n/a	[73]
2017	Egypt	B, Cream	B: 25.0 Cr: 25.0	B: 0.0 Cr: 0.1–0.5	B: 10.0 Cr: 25.0	HPLC-FD	5.0 ^a	[74]
2017	Egypt	Y, ProcC, Do-miati C	Y: 20.0 PrC: 40.0 DC: 46.7	Y: 3.3–11.4 PrC: 12.5–38.7 DC: 22.6–74.2	Y: 0.0 PrC: 0.0 DC: 13.3	ELISA	n/a	[81]
2017	Serbia	n/a	n/a	n/a	4.2	ELISA	n/a	[82]

Dairy Product Type: C: Cheese; Y: Yoghurt; I: Ice-cream; B: Butter; DD: Dairy Dessert; T: Trachana. n/a: not available; TLC: Thin-Layer Chromatography; HPLC-FD: High-Performance Liquid Chromatography-Fluorescence Detector; LC-MS/MS: Liquid Chromatography with tandem Mass Spectrometry. ^a: ng/L ^b: ng/kg.

3.4. Estimated Daily Intake (EDI) and Hazard Index (HI)

Tables 4 and 5 present the EDI and HI indicators, as calculated based on the data collected for the AFM1 detection in milk and dairy products in Mediterranean countries during the years included in this systematic review.

Table 4. Estimated Daily Intake (EDI) and Hazard Index (HI) for processed milk and dairy products consumption in Mediterranean countries during the period 2000–2020.

Year	Country	Based on the AFM1 Occurrence Data	Type of Product	EDI			HI		
				Age Groups			Age Groups		
				Children (36 Months–9 Years Old and 26.1 kg)	Adolescents (10–17 Years Old and 52.6 kg)	Adults (>18 Years Old and 70.0 kg)	Children (36 Months–9 Years Old and 26.1 kg)	Adolescents (10–17 Years Old and 52.6 kg)	Adults (>18 Years Old and 70.0 kg)
2000	Portugal	[125]	UHT	0.05–0.48	0.02–0.24	0.02–0.18	0.24–2.39	0.12–1.19	0.09–0.89
2002	Greece	[43]	P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			UHT	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2005	Turkey	[126]	P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2005	Turkey	[127]	Po	0.00–6.75	0.00–3.35	0.00–2.52	0.00–33.76	0.00–16.75	0.00–12.59
2006	Turkey	[129]	UHT	1.03	0.51	0.39	5.17	2.57	1.93
2007	Morocco	[112]	P	0.01–1.12	0.00–0.56	0.00–0.42	0.05–5.60	0.02–2.78	0.02–2.09
2007	Italy	[130]	P, UHT	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2007	Iran	[131]	P	0.30–1.08	0.15–0.54	0.11–0.40	1.48–5.41	0.74–2.69	0.55–2.02
2008	Turkey	[132]	Po	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2008	Turkey	[133]	UHT	0.10–6.03	0.05–2.99	0.04–2.25	0.48–30.17	0.24–14.97	0.18–11.25
2010	Spain	[134]	UHT	0.09 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	0.45 ± 0.12	0.22 ± 0.06	0.17 ± 0.05
2010	Turkey	[53]	P	0.24 ± 0.03	0.12 ± 0.01	0.09 ± 0.01	1.20 ± 0.14	0.59 ± 0.07	0.45 ± 0.02
2007	Morocco	[3]	P	0.03–0.81	0.02–0.40	0.01–0.30	0.16–4.04	0.08–2.01	0.06–1.51
			Po	0.09–0.16	0.04–0.08	0.03–0.06	0.44–0.79	0.22–0.39	0.16–0.29
2012	Iran	[4]	P	0.00–0.09	0.00–0.05	0.00–0.04	0.02–0.47	0.01–0.23	0.01–0.18
2013	Italy	[36]	UHT	0.05–0.15	0.02–0.08	0.02–0.06	0.24–0.77	0.12–0.38	0.09–0.29
2014	Turkey	[136]	UHT	0.06–0.68	0.03–0.34	0.02–0.25	0.31–3.41	0.15–1.69	0.11–1.27
2015	Algeria	[62]	P	0.09–0.99	0.04–0.49	0.03–0.37	0.43–4.93	0.21–2.45	0.16–1.84
			R	0.00	0.00	0.00	0.00	0.00	0.00
2015	Serbia	[61]	Po	8.11	4.03	3.03	40.57	20.13	15.13
2015	Serbia	[137]	P	0.19–3.07	0.10–1.52	0.07–1.14	0.96–15.33	0.48–7.60	0.36–5.71
2015	Turkey	[138]	UHT	0.00 and 0.25	0.00 and 0.13	0.00 and 0.10	0.02 and 1.27	0.01 and 0.63	0.01 and 0.48
2015	Egypt	[11]	UHT	0.00	0.00	0.00	0.00	0.00	0.00
2016	Kosovo	[67]	UHT	0.07 and 0.09	0.03 and 0.05	0.03 and 0.04	0.34 and 0.47	0.17 and 0.24	0.13 and 0.18
2016	Croatia	[37]	UHT	0.02–0.20	0.01–0.10	0.01–0.08	0.11–1.02	0.05–0.51	0.04–0.38
2016	Italy	[139]	UHT	0.09 and 0.25	0.04 and 0.12	0.03 and 0.09	0.43 and 1.25	0.21 and 0.62	0.16 and 0.46
2016	Egypt	[74]	P	0.12–0.20	0.06–0.10	0.05–0.08	0.62–1.01	0.31–0.50	0.23–0.38
2017	Italy	[140]	P	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2017	Libya	[79]	UHT	0.00–1.53	0.00–0.76	0.00–0.57	0.00–7.66	0.00–3.80	0.00–2.86
2017	Egypt	[81]	P	0.25–0.70	0.12–0.35	0.09–0.26	1.23–3.52	0.61–1.75	0.46–1.31
2018	Turkey	[141]	UHT	0.09 ± 0.04	0.04 ± 0.02	0.03 ± 0.02	0.45 ± 0.22	0.22 ± 0.11	0.17 ± 0.08
			P	0.06 ± 0.04	0.03 ± 0.02	0.02 ± 0.01	0.30 ± 0.18	0.15 ± 0.09	0.11 ± 0.07
			Skimmed	0.07 ± 0.03	0.03 ± 0.02	0.02 ± 0.01	0.33 ± 0.17	0.16 ± 0.09	0.12 ± 0.06
2019	Egypt	[94]	UHT	0.00	0.00	0.00	0.00	0.00	0.00
2019	Turkey	[143]	UHT	0.04–1.22	0.02–0.61	0.02–0.46	0.21–6.10	0.10–3.03	0.08–2.28
2019	Turkey	[110]	UHT	0.25–0.77	0.12–0.38	0.09–0.29	1.25–3.83	0.62–1.90	0.46–1.43
2020	Morocco	[111]	P	0.24 ± 0.12	0.12 ± 0.06	0.09 ± 0.04	1.22 ± 0.58	0.61 ± 0.29	0.46 ± 0.22
			UHT	0.14 ± 0.10	0.07 ± 0.05	0.05 ± 0.04	0.70 ± 0.49	0.35 ± 0.24	0.26 ± 0.18
2020	Lebanon	[93]	P, UHT	0.12–2.10	0.06–1.04	0.05–0.78	0.62–10.49	0.31–5.20	0.23–3.91
2001	Turkey	[168]	C	0.00–3.10	0.00–1.54	0.00–1.16	0.00–15.52	0.00–7.70	0.00–5.79
2001	Italy	[169]	Y	0.00–0.12	0.00–0.06	0.00–0.05	0.02–0.61	0.01–0.31	0.01–0.23
2003	Turkey	[170]	C	0.50–0.58	0.25–0.29	0.19–0.22	2.51–2.92	1.25–1.45	0.94–1.09

Table 4. Cont.

Year	Country	Based on the AFM1 Occurrence Data	Type of Product	EDI			HI		
				Age Groups			Age Groups		
				Children (36 Months–9 Years Old and 26.1 kg)	Adolescences (10–17 Years Old and 52.6 kg)	Adults (>18 Years Old and 70.0 kg)	Children (36 Months–9 Years Old and 26.1 kg)	Adolescences (10–17 Years Old and 52.6 kg)	Adults (>18 Years Old and 70.0 kg)
2004	Turkey	[171]	C	0.03–0.77	0.01–0.38	0.01–0.29	0.13–3.87	0.07–1.92	0.05–1.44
2004	Portugal	[172]	Y	0.07–0.38	0.04–0.19	0.03–0.14	0.36–1.88	0.18–0.93	0.14–0.70
2004	Libya	[45]	C	0.42–1.99	0.21–0.99	0.16–0.74	2.11–9.96	1.05–4.94	0.79–3.71
2005	Turkey	[173]	C	0.38–19.92	0.19–9.89	0.14–7.43	1.92–99.62	0.95–49.43	0.71–37.14
2005	Turkey	[174]	C	0.20–0.44	0.10–0.22	0.07–0.16	0.98–2.20	0.48–1.09	0.36–0.82
2005	Greece	[46]	C	0.00	0.00	0.00	0.00	0.00	0.00
2006	Turkey	[1]	Y	0.00–0.57	0.00–0.29	0.00–0.21	0.00–2.87	0.00–1.43	0.00–1.07
2006	Turkey	[175]	C	0.96–15.71	0.48–7.79	0.36–5.86	4.79–78.54	2.38–38.97	1.79–29.29
2008	Italy	[161]	C	0.19–0.96	0.10–0.48	0.07–0.36	0.96–4.79	0.48–2.38	0.36–1.79
2008	Turkey	[133]	C	0.19–2.64	0.10–1.31	0.07–0.99	0.96–13.22	0.48–6.56	0.36–4.93
2008	Slovenia	[176]	C	0.20–0.85	0.10–0.42	0.07–0.32	0.98–4.27	0.48–2.12	0.36–1.59
2008	Turkey	[177]	C	0.19–1.53	0.10–0.76	0.07–0.57	0.96–7.66	0.48–3.80	0.36–2.86
2009	Turkey	[178]	C	0.20–3.30	0.10–1.63	0.07–1.23	1.00–16.48	0.49–8.17	0.37–6.14
2009	Turkey	[5]	C	0.06–4.00	0.03–1.98	0.02–1.49	0.31–19.98	0.15–9.91	0.11–7.45
2010	Spain	[134]	C	0.00–0.05	0.00–0.02	0.00–0.02	0.00–0.24	0.00–0.12	0.00–0.09
			Y	0.03–0.07	0.02–0.03	0.01–0.03	0.16–0.35	0.08–0.17	0.06–0.13
2010	Turkey	[53]	C	0.07–0.10	0.04–0.05	0.03–0.04	0.36–0.52	0.18–0.26	0.14–0.19
2010	Turkey	[179]	C	0.20–9.66	0.10–4.79	0.07–3.60	1.00–48.28	0.49–23.95	0.37–18.00
2011	Turkey	[180]	Y	0.04–1.82	0.02–0.90	0.01–0.68	0.19–9.10	0.10–4.52	0.07–3.39
			C	0.02–1.01	0.01–0.50	0.01–0.38	0.11–5.06	0.06–2.51	0.04–1.89
2011	Turkey	[181]	I	0.02–0.12	0.01–0.06	0.01–0.05	0.12–0.62	0.06–0.31	0.04–0.23
2011	Turkey	[182]	C	0.05–1.45	0.02–0.72	0.02–0.54	0.23–7.24	0.11–3.59	0.09–2.70
			Y	0.01–0.30	0.00–0.15	0.00–0.11	0.05–1.49	0.02–0.74	0.02–0.56
			DD	0.01–0.31	0.00–0.15	0.00–0.11	0.03–1.53	0.01–0.76	0.01–0.57
2011	Turkey	[183]	C	0.27–2.95	0.13–1.47	0.10–1.10	1.35–14.77	0.67–7.33	0.50–5.51
2012	Lebanon	[184]	C	0.02–1.21	0.01–0.60	0.01–0.45	0.10–6.03	0.05–2.99	0.04–2.25
2013	Egypt	[185]	C	0.00–0.23	0.00–0.11	0.00–0.09	0.00–1.14	0.00–0.57	0.00–0.43
2013	Italy	[36]	C	0.02–0.07	0.01–0.03	0.01–0.03	0.10–0.34	0.05–0.17	0.04–0.13
2013	Turkey	[186]	C	0.03–1.87	0.02–0.93	0.01–0.70	0.17–9.33	0.09–4.63	0.06–3.48
			Y	0.48–1.03	0.24–0.51	0.18–0.38	2.39–5.15	1.19–2.56	0.89–1.92
2014	Turkey	[187]	C	0.06–14.46	0.03–7.17	0.02–5.39	0.29–72.30	0.14–35.87	0.11–26.96
2014	Turkey	[188]	T	0.00–0.14	0.00–0.07	0.00–0.05	0.01–0.70	0.00–0.35	0.00–0.26
2014	Turkey	[189]	CI	0.00–0.06	0.00–0.03	0.00–0.02	0.00–0.32	0.00–0.16	0.00–0.12
			CH	0.00–0.02	0.00–0.01	0.00–0.01	0.00–0.09	0.00–0.04	0.00–0.03
2014	Turkey	[190]	C	0.15–0.50	0.08–0.25	0.06–0.19	0.77–2.51	0.38–1.24	0.29–0.94
			Y	0.16–0.28	0.08–0.14	0.06–0.10	0.78–1.38	0.39–0.68	0.29–0.51
2014	Turkey	[191]	I	0.02–0.19	0.01–0.10	0.01–0.07	0.10–0.96	0.05–0.48	0.04–0.36
2015	Serbia	[59]	n/a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2015	Serbia	[192]	C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2015	Turkey	[193]	C	0.06 and 0.52	0.03 and 0.26	0.02 and 0.19	0.31 and 2.61	0.15 and 1.29	0.11 and 0.97
2015	Egypt	[11]	Processed C	0.00–0.01	0.00	0.00	0.00–0.03	0.00–0.02	0.00–0.01
			Kariesh C	0.00–0.01	0.00	0.00	0.01–0.03	0.00–0.02	0.00–0.01
			Mozzarella C	0.00–0.01	0.00	0.00	0.00–0.03	0.00–0.01	0.00–0.01
			Akawi C	0.00–0.01	0.00	0.00	0.01–0.03	0.01–0.02	0.00–0.01

Table 4. Cont.

Year	Country	Based on the AFM1 Occurrence Data	Type of Product	EDI			HI		
				Age Groups			Age Groups		
				Children (36 Months–9 Years Old and 26.1 kg)	Adolescences (10–17 Years Old and 52.6 kg)	Adults (>18 Years Old and 70.0 kg)	Children (36 Months–9 Years Old and 26.1 kg)	Adolescences (10–17 Years Old and 52.6 kg)	Adults (>18 Years Old and 70.0 kg)
			Roumy C	0.00	0.00	0.00	0.00–0.01	0.00	0.00
			Y	0.00	0.00	0.00	0.00–0.02	0.00–0.01	0.00–0.01
2016	Turkey	[69]	Y, Avran	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2016	Turkey	[194]	C	0.04–2.69	0.02–1.33	0.02–1.00	0.20–13.45	0.10–6.67	0.08–5.01
2016	Egypt	[71]	C	19.16–37.16	9.51–18.44	7.14–13.86	95.79–185.82	47.53–92.21	35.71–69.29
2016	Egypt	[72]	Karish C	0.13–3.28	0.06–1.63	0.05–1.22	0.63–16.40	0.31–8.14	0.23–6.12
			Y	0.12–0.25	0.06–0.13	0.05–0.09	0.60–1.27	0.30–0.63	0.23–0.47
2016	Egypt	[73]	Roomie C	0.04–0.08	0.02–0.04	0.01–0.03	0.19–0.40	0.10–0.20	0.07–0.15
			Karish C	0.17–0.77	0.09–0.38	0.06–0.29	0.86–3.83	0.43–1.90	0.32–1.43
2017	Egypt	[74]	B	0.00	0.00	0.00	0.00	0.00	0.00
			Cream	0.00	0.00	0.00	0.00–0.01	0.00	0.00
2017	Egypt	[81]	Y	0.01–0.04	0.01–0.02	0.00–0.02	0.06–0.22	0.03–0.11	0.02–0.08
			ProcC	0.05–0.15	0.02–0.07	0.02–0.06	0.24–0.74	0.12–0.37	0.09–0.28
			Domiat C	0.09–0.28	0.04–0.14	0.03–0.11	0.43–1.42	0.21–0.71	0.16–0.53
2017	Serbia	[82]	n/a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Process type: P: Pasteurized; Po: Powder, UHT: Ultra-High Temperature, I: Infant; C: Concentrated; R: reconstituted. Dairy Product Type: C: Cheese; Y: Yoghurt; I: Ice-cream; B: Butter; DD: Dairy Dessert; T: Trachana. n/a: not available; n.d.: not determined.

Table 5. Estimated Daily Intake (EDI) and Hazard Index (HI) for infant formula consumption according to data from studies during the period 2000–2020 in Mediterranean countries.

Year	Country	Based on the AFM1 Occurrence Data	EDI		HI	
			Age Groups		Age Groups	
			Infants (0–11 Months and 5 kg)	Toddlers (12–35 Months and 12 kg)	Infants (0–11 Months and 5 kg)	Toddlers (12–35 Months and 12 kg)
2006	Turkey	[128]	0.40–8.00	0.17–3.33	2.00–40.00	0.83–16.67
2007	Iran	[131]	0.04–0.56	0.02–0.23	0.20–2.80	0.08–1.17
2014	Turkey	[135]	0.22–0.80	0.09–0.34	1.10–4.02	0.46–1.68
2015	Serbia	[137]	0.80–12.80	0.33–5.33	4.00–64.00	1.67–26.67
2019	Lebanon	[142]	0.80 ± 0.05	0.33 ± 0.02	4.00 ± 0.26	1.67 ± 0.11

When HI is less than 1, the consumers are deemed safe; if it is greater than 1, there may be warning signs of liver cancer [23,195]. Following the abovementioned statement and the results of the calculated HI in different age groups in countries and years for the consumption of dairy products included in this study, it is shown that in some Mediterranean countries, the EDI is high and the HI is much higher than 1, mostly in southern east Mediterranean countries. These differences may be attributed to geographical variations among countries, which in turn influence climate and dairy production practices. AFM1 contamination in milk and dairy products varies depending on the consumption of feed concentrates, the season, the environmental conditions, and farming systems [17,60].

Moreover, as presented, EDI and HI were calculated higher in cheese and other dairy products compared to milk. Given AFM1's affinity for casein, high concentrations in curd during cheese manufacturing are anticipated [196], with reports indicating AFM1 levels can be three-fold higher in soft cheeses like feta and five-fold higher in hard cheeses

compared to the milk used [178]. Some cheeses, such as Brick, Limburger, and Cheddar, maintain stable AFM1 levels during ripening, whereas others, like Karish, show a gradual decrease [197,198]. These variations can be attributed to differences in cheese manufacturing practices, types of cheese, ripening conditions, and analytical methods. Additionally, factors such as low pH, the presence of lactic acid bacteria, and the production of by-products may contribute to reducing AFM1 levels in dairy products and subsequently lower their intake [167].

Assessing our data collected from the studies included in the research and presented in the tables, it is apparent that the most burdened areas, according to the Maximum Hazard Index, are located in the southeastern Mediterranean. At the same time, it appears that among the dairy product types, cheese is the one with the greater risk load, followed by milk, as shown in Figure 2.

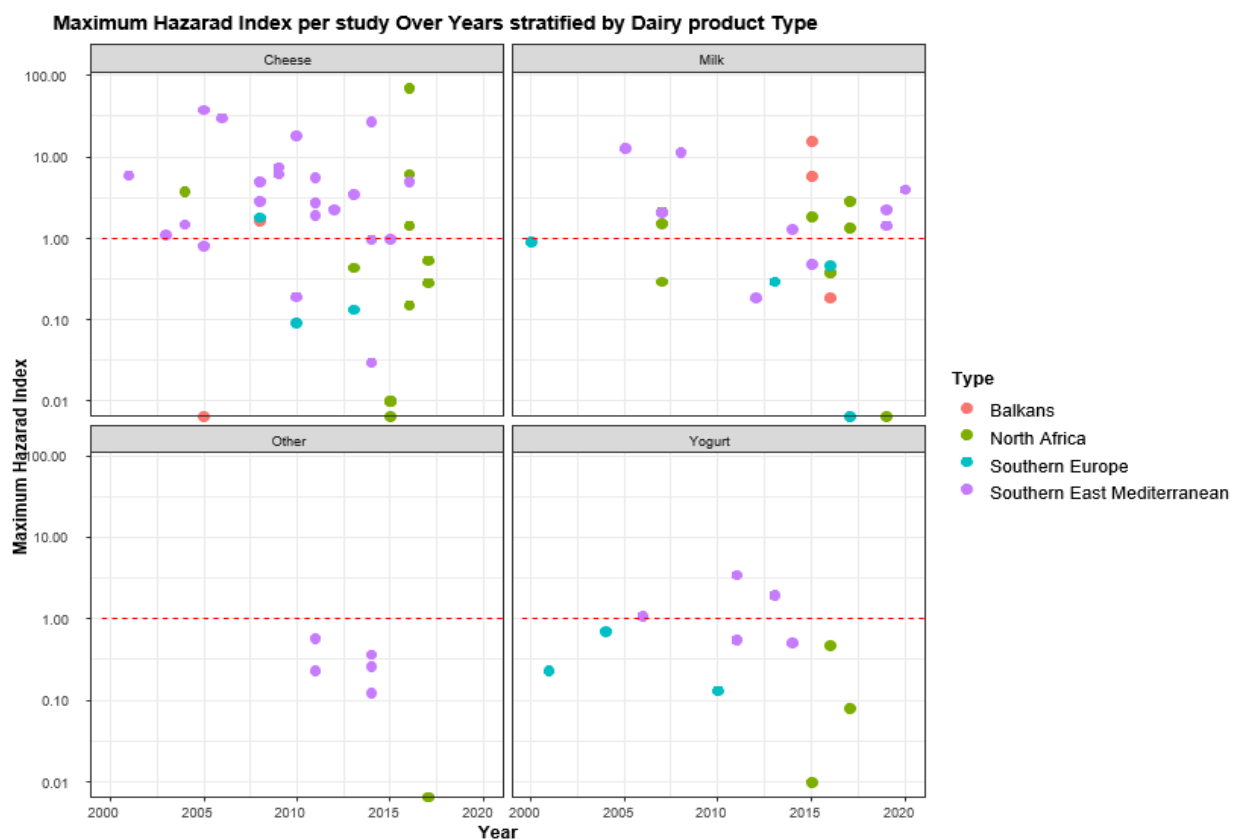


Figure 2. Maximum Hazard Index per study over years stratified by dairy product type and area.

Given the priority of human health, the daily intake of AFM1 is often estimated, particularly for infants, toddlers, and children. These age categories of consumers are generally considered to be more exposed to AFM1 due to their high milk consumption and rapid development [198]. Additionally, the low body weight of newborns and toddlers compared to adults results in a greater EDI of contaminants per kilogram of body mass. Therefore, it is crucial to prioritize the monitoring and regulation of AFM1 contamination in infant formula milk, not only in high-risk countries but globally.

Continuous surveillance of AFM1 levels is essential, particularly in nations identified as high-risk for AFM1 presence [199]. However, estimating the exposure likely to have a negative impact on human health is challenging due to the lack of an established acceptable daily intake for AFM1 by any country or international standard-setting organization, such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [200]. It is also important to note that exposure to AFM1 could be higher due to the concurrent consumption of milk and other dairy products, which may lead to cumulative intake and a higher HI.

Finally, the current survey, utilizing data from various studies employing diverse methodological analyses, validations, and types of dairy products, may entail a potential limitation. Nevertheless, the aggregated data from all Mediterranean countries still holds significant value in addressing the existing gap in the literature. With no doubt, ongoing research endeavours should explore the development of predictive models focused on evaluating human exposure, thereby ensuring the continual protection of public health.

4. Conclusions

This study provides a snapshot of AFM1 occurrence over 20 years in Mediterranean countries, acknowledging potential biases in the data reviewed. Despite this, the overall assessment is valuable for strategic planning to manage this significant public health hazard. Future research should address uncertainties related to milk storage and thermal processing, as neither reliably reduces AFM1 levels in dairy products. Continuous monitoring of dairy products using reliable analytical techniques is essential, alongside strict control of AFB1 in animal feed and its storage. An AFM1 observatory is needed to function as an alert system through systematic monitoring of AFB1 and AFM1 levels, coupled with climate data. This systematic review highlights that AFM1 remains a persistent hazard in the dairy sector, posing a severe risk to consumers. The evident lack of effective interventions throughout milk production necessitates action from both the scientific community and authorities. A fundamental approach involves consistent monitoring of AFM1 in milk and dairy products and AFB1 in feed, given the direct correlation between AFM1 in milk and AFB1 in feed. This systematic monitoring facilitates timely precautionary measures. Additionally, predictive models for AFB1 and AFM1 are crucial, offering valuable insights for farmers and dairy producers to protect consumer health.

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