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## **AFLATOXIN M1 OCCURRENCE IN DAIRY PRODUCTS WORLDWIDE: SUMMARY OF LITERATURE REVIEW AND POLICY IMPLICATIONS**

By

Felicia Wu and Nikita Saha Turna



## **Food Security Policy *Research Papers***

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## **ABSTRACT**

Research has suggested that mammals that consume aflatoxin B1 (AFB1, the most common and toxic of the aflatoxins) secrete a metabolite of AFB1 in their milk referred as aflatoxin M1 (AFM1). In lieu of fully understanding the human health risks and thus designing food safety standards appropriately, regulatory agencies have set standards for AFM1 in milk and dairy products simply based upon taking the existing AFB1 standard (in a given nation), and dividing by a factor that roughly estimates how much AFM1 is produced in milk when “parent” aflatoxin is present in dairy animals’ diets. But as more and more nations are suffering economic losses due to AFM1 in dairy products exceeding allowable limits – particularly the European Union limit of 0.05 µg/kg (which is extremely difficult to meet) – there is need to better characterize the true human health risk of this chemical in our daily diets, to inform policy decision-making and public health officials on the true nature of the risk.

This information is urgently needed because of recent discoveries of putative associations between aflatoxin and stunting, as well as the lack of general (public and policy-making as well as non-specialized scientists) knowledge of the difference between the toxicity of AFB1 and AFM1. There is a critical need to compare both the toxicity of and the exposure to AFM1 with AFB1 to be able to judge relative risks. It is also important to understand whether AFM1, like AFB1, has a toxicological interaction with HBV infection to increase liver cancer risk and to compare exposure patterns to these two chemicals. In several Feed the Future countries, high AFM1 contamination levels have been detected in milk. Unfortunately, people have assumed AFM1 is just as toxic as AFB1. Consequently, newspaper headlines have warned people to avoid drinking locally produced milk and this has set back early childhood milk-based nutrition interventions and paralyzed the dairy industry in Ethiopia, for example. Similar headlines have been published in other countries like Kenya.

Research is urgently needed to inform policy makers about the true and relative risk of AFM1 in milk. Towards this goal, this study represents the first (of two) stages of work in a global risk assessment of aflatoxin M1 (AFM1). Authors of this study conducted a literature review on the health effects of AFM1 through toxicological and limited human studies. In addition to this, they also found substantial occurrence data for AFM1 in milk and dairy products worldwide. This paper presents the findings of this desk review and makes policy recommendations for reducing AFM1 exposure in human populations worldwide.

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## INTRODUCTION

Shortly after aflatoxin – a potent human liver carcinogen – was discovered in peanuts and maize in the early 1960s (Kensler et al. 2011), it was discovered that mammals that consume aflatoxin B1 (AFB1, the most common and toxic of the aflatoxins) secrete a metabolite of AFB1 in their milk: **aflatoxin M1 (AFM1)**. While the human health effects of AFB1 are relatively well characterized (Wu et al. 2014) - including liver cancer and acute liver toxicity, and associations to child growth impairment and immune system dysfunction - the health effects of AFM1 are much less well-understood. However, given the prevalence of dairy food consumption around the world (milk, butter, cheese, yogurt, ice cream, etc.), it is critical to understand how AFM1 in dairy products could affect human health.

Yet thus far, in lieu of fully understanding the human health risks and thus designing food safety standards appropriately, regulatory agencies have set existing standards for AFM1 in milk and dairy products simply based upon taking the existing AFB1 standard (in a given nation), and dividing by a factor that roughly estimates how much AFM1 is produced in milk when “parent” aflatoxin is present in dairy animals’ diets (FAO 2004). For example: the US Food and Drug Administration has set the AFM1 standard in US dairy products at 0.5 µg/kg (sometimes referred to as parts per billion, or ppb), because its total aflatoxin action level is 20 µg/kg; and an animal that consumes aflatoxin in feed converts it to AFM1 at a rate of about 2.5%. But as more and more nations are suffering economic losses due to AFM1 in dairy products exceeding allowable limits – particularly the European Union limit of 0.05 µg/kg (extremely difficult to meet) – we must better characterize the true human health risk of this chemical in our daily diets, to inform policy decision-making and public health officials on the true nature of the risk. This information is urgently needed because of recent discoveries of putative associations between aflatoxin and stunting, as well as the lack of general (public and policy-making as well as non-specialized scientists) knowledge of the difference between the toxicity of AFB1 and AFM1. There is a critical need to compare both the toxicity of and the exposure to AFM1 with AFB1 to be able to judge relative risks. It is also important to understand whether AFM1, like AFB1, has a toxicological interaction with HBV infection to increase liver cancer risk and to compare exposure patterns to these two chemicals.

In several Feed the Future countries, high AFM1 contamination levels have been detected in milk. Unfortunately, people have assumed AFM1 is just as toxic as AFB1. Consequently, newspaper headlines have warned people to avoid drinking locally produced milk and this has set back early childhood milk-based nutrition interventions and paralyzed the dairy industry in Ethiopia, for example. Similar headlines have been published in other countries like Kenya. Research is urgently needed to inform policy makers about the true and relative risk of AFM1 in milk.

This study represents the first (of two) stages of work in a global risk assessment of aflatoxin M1 (AFM1). We conducted a literature review on the health effects of AFM1 through toxicological and limited human studies. In addition to this, we also found substantial occurrence data for AFM1 in milk and dairy products worldwide. We present our findings in this paper and present policy recommendations for reducing AFM1 exposure in human populations worldwide.

This study represents the first (of two) stages of work proposed in a global risk assessment of aflatoxin M1 (AFM1). With joint support from the Food Security Policy Innovation Lab and the

Livestock Systems Research Innovation Lab we conducted a literature review on the health effects of AFM1 through toxicological and limited human studies. In addition to this, we also found substantial occurrence data for AFM1 in milk and dairy products worldwide. We present our findings in this paper and make policy recommendations for reducing AFM1 exposure in human populations worldwide.

## OBJECTIVES

While our overall objective for this work is a comprehensive **quantitative risk assessment** of AFM1's adverse health effects through dairy consumption worldwide, the portion that FSPIL has funded is the first step of that risk assessment: hazard identification, identifying which human health effects are linked to the toxin / chemical / microbe at hand, through a thorough literature review and applying weight-of-evidence practices. In addition, we began the first stage of the exposure assessment, reviewing and compiling occurrence data on AFM1 concentrations in milk from various dairy animals, as well as multiple dairy products, from all over the world. Below we present these findings.

## HAZARD IDENTIFICATION: HEALTH EFFECTS OF AFLATOXIN M1

Based on the Bradford Hill Criteria (1965), which are a set of nine criteria that determine whether the evidence is strong or weak linking a substance to particular health effects, we found that the major health effects of concern regarding AFM1 exposure are the potential for liver cancer (hepatocellular carcinoma, HCC), and immunological effects. These effects were found in animal studies and human studies (to a limited extent for cancer), the exposure preceded the adverse effect, there was a dose-response relationship between dose of toxin and adverse effect, and there was biological plausibility. For other health effects that have been linked with AFM1, such as child stunting, there is significantly less convincing evidence (e.g., that exposure precedes the adverse effect, and evidence of a dose-response relationship).

### AFM1-Associated Liver Cancer

It is well known that AFB1 causes liver cancer; indeed, is the most potent naturally occurring human liver carcinogen known (Kensler et al. 2011, Wu et al. 2014). However, less well known is whether AFM1 causes cancer. Several studies in human populations and one *in vitro* study indicate a plausible link between AFM1 exposure and increased risk of cancer. We did not include studies that claimed that such a link existed (and/or made calculations based on that assumption), but did not provide evidence of the link between agent and disease.

**Human studies.** AFM1 was initially classified as possibly carcinogenic to humans (Group 2B) by IARC (IARC, 1993), but later reclassified as group 1 human carcinogen (IARC, 2002). In 1997, a study in Taiwan found a dose-response relationship between urinary AFM1 levels and the risk of hepatocellular carcinoma in chronic hepatitis B virus patients (Yu et al., 1997). Another study published in 1999 also supported these results, where the authors followed 145 patients with chronic HBV for ten years (Sun et al., 1999). In this study they evaluated if AFB1 exposure with concomitant presence of hepatitis C virus (HCV) was associated with increased HCC risk, and



also measured the patients' urinary AFM1. The results indicated 3.3-fold increased risk of HCC in patients with detectable AFM1 (> 3.6ng/L) in urine. AFM1 was detected in 54% of the 145 HBV patients. A study in Egypt investigated AFM1 levels in urine and serum of cirrhosis and HCC patients (Mokhles et al., 2007). The results demonstrated higher AFM1 levels in serum and urine of cirrhotic patients than HCC patients and controls.

A longitudinal study evaluated the relationship between the onset of primary liver cancer (PLC) and AFM1 exposure in 515 patients with chronic hepatitis (Lu et al., 2010). The results showed increase in PLC year incidence with higher urinary levels of AFM1. The urine excretion of AFM1 was also associated with abnormal liver function. AFM1 levels in urine and serum alpha-fetoprotein (tumor biomarker) levels were evaluated in a study containing 58 textile workers and 64 controls (Saad-Hussein et al., 2013). The results indicated that AFM1 levels in urine were increased in the pre-spinning, spinning and weaving (textile processes) sub-groups, suggesting an increased risk to develop HCC.

***In vitro study.*** It is worth noting that the human studies, although providing strong suggestive evidence, do not directly assess toxicological mechanisms for AFM1 causing HCC. However, one *in vitro* study performed in a hepatoblastoma (liver tumor) cell line (HepG2) elucidates this mechanism. Marchese et al. (2018) showed that the molecular mechanism by which AFM1 may be biotransformed by cytochrome P450 enzymes to an epoxide form that binds to DNA is very similar to that for AFB1. Namely, the P450 enzymes that in other contexts detoxify environmental contaminants to which humans are exposed transform AFB1 and AFM1 to a carcinogenic form (through the exo-epoxide), which then directly causes DNA damage. Although the cancer potency may be lower, AFM1 has been shown to form DNA adducts that could – over time and with chronic exposure – increase the risk for liver cancer.

### **AFM1-Associated Immunological Dysfunction**

Over the last several decades, animal and then human studies have found evidence linking the parent compound AFB1 to immunotoxicity; we are currently preparing a review paper on this topic. Interestingly, several animal and cell line studies have also shown a link between AFM1 exposure and immunotoxicological effects. The immune system is very complicated, and the studies linking AFM1 to impaired immunity span multiple immunological health endpoints: from adverse effects to the spleen (a key organ in human and animal immunity), to immune cell dysfunction (e.g., Jurkat cells), to changes in expression of various immune substances such as cytokines, caspases, and P450 enzymes.

***In vivo studies.*** A recent study investigated the effects of AFM1 on the immune system of mice (Shirani et al., 2019). The spleen weight was found to be reduced in mice exposed to AFM1 compared to a negative control group. In the mouse group exposed to AFM1, the proliferation of splenocytes in response to phytohemagglutinin-A was reduced, IFN- $\gamma$  was decreased, IL-10 was increased, expression of miR-155 was reduced and phosphatidylinositol-3, 4, 5-tri-sphosphate 5-phosphatase 1 (Ship1) and suppressor of cytokine signaling 1 (Socs1) were significantly upregulated in T cells from spleens - all contributing to immunotoxicity. AFM1 (3.5 mg/kg) and combination of AFB1 (0.5 mg/kg) and AFM1 (3.5 mg/kg) were found to activate oxidative stress and cause renal damage in HEK 293 cells model and CD-1 mouse model (Li et al., 2018). Another

mouse study found AFM<sub>1</sub> to increase DNA fragmentation, downregulate caspase-3, caspase-9, CYP3A13, Bax and p53 expressions and upregulate TNF- $\alpha$  and Bcl-2 expressions and their target proteins which may induce disorders in intestinal function due to alterations in DNA fragmentation and genes expressions (Jebali et al., 2018).

***In vitro studies.*** Cytotoxicity of AFM<sub>1</sub> was examined alone and in combinations with ochratoxin A (OTA), zearalenone and  $\alpha$ -zearalenol on human intestinal Caco-2 cells (the cells that line the intestine and colon and affect permeability; Gao et al., 2016). AFM<sub>1</sub> and OTA were found to be more cytotoxic than other toxins and AFM<sub>1</sub> cytotoxicity was found to be increased in presence of other mycotoxins in this study. Another *in vitro* study showed that AFM<sub>1</sub> (0.12 and 12 $\mu$ M) individually or in combination with OTA increased epithelial permeability, reduced tight junction (TJ) proteins which regulate the barrier permeability and integrity of the intestine (Gao et al., 2017), which affects immunity by the increased risk of infectious agents escaping into the bloodstream. The effects of AFB<sub>1</sub> and AFM<sub>1</sub> on immune function were investigated using a lymphoblastoid Jurkat T-cell line (a type of human T-cell line) (Luongo et al., 2014). Results showed both AFB<sub>1</sub> and AFM<sub>1</sub> to significantly decrease Jurkat cell proliferation. Another study investigated the cytotoxic effects of AFB<sub>1</sub> and AFM<sub>1</sub> on Caco-2 cells by treating both undifferentiated (UC) and differentiated (DC) cells with AFB<sub>1</sub> and AFM<sub>1</sub> at various concentrations (Zhang et al., 2015). Results of this study indicated that AFB<sub>1</sub> and AFM<sub>1</sub> significantly inhibit UC and DC cell growth, increase lactate dehydrogenase levels and cause genetic damage in a time and dose dependent manner which might be due to generation of intracellular reactive oxygen species leading to membrane damage and breakage in DNA strands.

## **Summary of Hazard Identification**

Taken together, there appears to be evidence that the metabolite of aflatoxin B<sub>1</sub>, aflatoxin M<sub>1</sub>, may cause adverse health effects in terms of increasing cancer risk and immune system dysfunction. However, at the time, it is unclear what the *extent* is to which AFM<sub>1</sub> causes or contributes to these effects. In the next phase of our research, we will attempt to elucidate this, using existing dose-response studies on the topics (for both cancer and immunotoxicological endpoints). Nonetheless, because hazards have been identified associated with AFM<sub>1</sub>, exposure assessment is critical.

## **OCCURRENCE OF AFLATOXIN M<sub>1</sub> IN MILK AND DAIRY PRODUCTS**

The first step in human exposure assessment of chemicals and toxins is to assess the occurrence of the chemical or toxin in the relevant environmental medium. In our case, AFM<sub>1</sub> is the toxin of concern that humans encounter most commonly through milk and dairy products (it is also in human breastmilk, but for a relatively more limited period than these other sources throughout a lifetime). Therefore, we have searched the literature and found as much of the AFM<sub>1</sub> occurrence data as is possible at this time; for different countries, dairy animal species, milk of various types (e.g., raw, homogenized, pasteurized, buttermilk), and dairy products of various types (feta cheese, cream cheese, butter, yogurt, etc.). These results are reported in Tables 1 to 4.

We have organized these tables according to the type of product or species, the proportion of samples from the particular country and/or species testing positively for AFM1, the AFM1 concentrations in those dairy samples, and the associated study references.

## POLICY IMPLICATIONS

Similar to its parent compound aflatoxin B1, **aflatoxin M1** has shown the potential to cause cancer - through direct DNA damage after it has been biotransformed by liver enzymes - as well as adverse effects to the immune system. Although AFM1's potency may be considerably lower than that of AFB1 in inducing liver cancer or immunological effects (as AFM1 is itself one metabolite of AFB1 after biotransformation by the same liver enzymes), it is not known just how much the potencies differ. The International Agency for Research on Cancer has variously classified AFM1 as either a Group 1 known human carcinogen, or a Group 2B possible human carcinogen – both of which indicate potential cause for concern.

Moreover, our compilation of AFM1 occurrence data shows that AFM1 is widespread worldwide in milk from multiple dairy animals; as well as from dairy products such as cheese, butter, and yogurt. Although the majority of the reported milk and dairy product samples worldwide had AFM1 levels that were below the US FDA action level for AFM1 of 0.5 ppb, or 0.5 µg/kg (µg/L), there are three remaining concerns:

1. The majority of these milk and dairy product samples would NOT meet the European Union maximum tolerable limit for AFM1 of 0.05 µg/kg (ten times stricter than the FDA action level). This could mean substantial economic losses for nations attempting to export milk and dairy products to, or within, the EU.
2. For populations worldwide that consume large quantities of milk and/or dairy products, AFM1 exposures may reach levels that could cause concern for human health. In particular, infants and children who may consume more of these products may be more heavily exposed.
3. The African nations for which AFM1 occurrence data were available appear to have the highest AFM1 levels worldwide in their milk and dairy products: far higher than the FDA action level. These nations include Nigeria, Burundi, and Democratic Republic of the Congo. Two other nations with very high AFM1 levels in dairy products are Jordan and Syria. If populations in these nations consume high levels of dairy products, AFM1 could pose significant health concerns.

What can be done to reduce the risk? There are several possible solutions:

***Reduce the amount of the parent compound, AFB1, in dairy animal feed.*** Of all possible solutions, this one is likely to be the most practical and feasible. By reducing the amount of the parent compound (and most toxic of the aflatoxins) AFB1 in dairy animal feed, the dairy animals will metabolize less of it to the metabolite AFM1, which is then secreted in their milk. Although maize and peanuts are the major sources of aflatoxin in both animal feed and human food, aflatoxin can accumulate in most types of feeds if it is stored for longer than one month in warm and wet climates (typically between 30 degrees north and south latitude), where there is the

possibility for cross-contamination with other crops that may harbor *Aspergillus* fungi. Reducing AFB1 in animal feed, therefore, would entail moving away from maize and peanut sources, and ensuring that the animal feed is stored for not too long and in relatively cooler and drier conditions.

***Include additives in dairy animal feed that can adsorb the aflatoxin.*** Work has also been done to develop binders, or enterosorbents, that can be added to animal feed to “bind” the aflatoxin in the animals’ guts; so that they excrete it before it travels to the liver and is metabolized to AFM1. One example of this that has been tested extensively in both animal and human populations is NovaSil (Wang et al. 2017). Others are under development both in the public and industrial sectors. That would mean that - even if there were AFB1 present in the animal feed - the adsorbent feed additive would reduce the internal dose of aflatoxin for the animals, who would then produce less AFM1 in milk.

***Reduce the amount of AFM1 in the finished product through lactic acid fermentation.*** In a recent study, lactic acid fermentation – a common method to ferment a variety of traditional foods worldwide – was shown to reduce AFM1 levels from the original food to the finished food product (Ezekiel et al. 2019). Lactic acid fermentation is a key processing step in many popular dairy foods worldwide: yogurt, kefir, kishk, certain cheeses, and kumis (airag). In addition to the sensory changes - such as taste and texture - made to the milk to produce these foods, lactic acid fermentation has the benefit of reducing mycotoxin levels.

***Encourage reduction of dairy consumption in populations where AFM1 levels have been shown to be extremely high.*** This is a sub-optimal control strategy to reduce AFM1 exposure, because dairy products provide important nutrients in many populations worldwide; including overall caloric intake, fat, calcium, protein, and probiotics (in the case of fermented dairy foods). Therefore, this suggestion to reduce AFM1 exposure should only be made if the above strategies are not possible.

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**Table 1. AFM1 contamination in milk from different animals**

Country	Animal	% AF-positive samples	AFM1 concentration, ug/L	Reference
Croatia	Cow	100	0.003–0.162	Bilandzic et al., 2014
	Goat	100	0.003–0.04	
Iran	Cow	78.7	Mean: 0.0601 ± 0.0574 µg/L	Rahimi et al., 2010
	Water buffalo	38.7	Mean: 0.0319 ± 0.0246	
	Camel	12.5	Mean: 0.0190 ± 0.075	
	Sheep	37.3	Mean: 0.0281 ± 0.0137	
	Goat	31.7	Mean: 0.0301 ± 0.0183	
Italy	Cow	12.9		Cammilleri et al., 2019
	Sheep	5		
	Donkey	0		
Turkey	Buffalo	27	<0.008–0.032 µg/L	Kara and Ince, 2014
	Cow	0	<0.008 µg/L	

**Table 2. AFM<sub>1</sub> contamination in different types of bovine milk**

Country	Type of milk	% AFM <sub>1</sub> positive samples	Min-Max	Reference
Brazil	Pasteurized milk	95.2	0.01-0.2 µg/L	Shundo et al. (2009)
	UHT milk	76	0.008-0.215 µg/L	Iha et al., 2013
	Milk with additives	76	0.009-0.0061 µg/L	
	Powder milk	100	0.02-0.76 µg/L	Jager et al., 2013
	Fluid milk	40	0.009-0.069 µg/L	
China	Raw milk	4.64	<n.d – 0.06 µg/L	Li et al., 2018
	UHT milk	54.9	0.006-0.16 µg/L	Zheng et al., 2013
	Pasteurized milk	96.2	0.023-0.154 µg/L	
Croatia	Raw milk	2	0.006-0.027 µg/L	Bilandzic et al., 2015
Egypt	Raw milk	38	0.023-0.073 µg/L	Amer and Ibrahim, 2010
Greece	Pasteurized milk	85.4	Not reported	Roussi et al., 2002
	Raw milk	46.4	Not reported	Tsakiris et al., 2013
India	Pasteurized milk	87	0.063–1.012 µg/L	Rastogi et al. (2004)
	Raw milk	100	0.001–3.8 µg/L	Siddappa et al., 2012
	UHT milk	64.4	n.d-2.1 µg/L	
Iran	Pasteurized milk	67.10	0.0056-0.529 µg/L	Kamkar, 2006
	UHT milk	62.3	0.006-0.516 µg/L	Fallah, 2010
Italy	UHT milk	57.7	0.00071-0.0036 µg/L	Campone et al., 2018
	Pasteurized milk	99.5	0.005-0.03 µg/L	
Japan	Raw milk	100	0.00705–0.1298 µg/L	Nakajima et al. (2004)
Jordan	Buttermilk	100	7.97–2027.11 ng/kg	Omar, 2012
	Pasteurized milk	68	0.0033–0.084 µg/L	

Country	Type of milk	% AFM1 positive samples	Min-Max	Reference
Lebanon	Raw milk	73.6	0.0026-0.126 µg/L	Assem et al. (2011)
	Powder milk	35.7	0.0092–0.016 µg/L	
	Pasteurized milk	88.8	0.001-0.117 µg/L	
Morocco	Fresh milk	100	0.407–0.952 µg/L	Zinedine et al., 2007
Nigeria	Skimmed milk	100	0.248–2.510 µg/L	Susan et al., 2012
	Partially skimmed milk	100	0.139–1.238 µg/L	
	Raw milk	71	0.004-0.845 µg/L	
Pakistan	Fresh milk	91.7	0.02-3.09 µg/L	Iqbal and Asi 2013
	Raw milk	85	0.02-0.08 µg/L	Asghar et al., 2018
Palestine	UHT and pasteurized milk	27.5	0.007-0.07 µg/L	Al Zuheir and Omar, 2012
Portugal	Raw milk	99.4	n.d–0.069 µg/L	Duarte et al., 2013
Saudi Arabia	UHT milk	82	0.01-0.19 µg/L	Dashti et al., 2009
	Pasteurized milk	97.2	0.06-1.2 µg/L	Abdallah et al., 2012
Serbia	UHT milk	98.5	0.02-0.41 µg/L	Kos et al., 2014
	Organic milk	100	0.01-0.08 µg/L	
	Raw milk	100	0.08-1.2 µg/L	
	Heat-treated milk	32.6	0.09–0.145 µg/L	Tomasevic et al., 2015
Spain	Raw bulk milk	18.9	0.009 to 1.36 µg/L	Cano-Sancho et al., 2010
	Rural milk	85.6	n.d-0.2 µg/L	Rodríguez-Blanco et al., 2019
South Africa	Raw milk	48	0.002-0.08 µg/L	Mulunda and Mike, 2014

Country	Type of milk	% AFM1 positive samples	Min-Max	Reference
South Korea	Raw milk	95.5	0.22-6.9 µg/L	Lee et al., 2009
Sudan	Powder milk	100	0.01-0.85 µg/L	Elzupir and Elhussein , 2010
	Pasteurized milk	100	0.008-0.765 µg/L	Ali et al., 2014
Syria	Raw milk	83.8	0.026-2.007 ng/mL	Ghanem and Orfi, 2009
Taiwan	Raw milk	100	0.05-0.101 µg/L	Peng and Chen, 2009
Tanzania	UHT milk	58.1	n.d – 0.544 µg/L	Mohammed et al., 2016
Thailand	Fluid milk	86	0.001–0.030 µg/L	Ruangwises and Ruangwises, 2010
Turkey	Raw milk	21.1	0.011-0.1 µg/L	Unusan, 2006

**Table 3. AFM<sub>1</sub> in dairy products**

Country	Sample	% AF-positive samples	Min–Max (µg/kg)	Reference
<b>Brazil</b>	Cheese	30	0.091–0.3	Becker-Algeri et al., 2016
<b>Burundi</b>	Yogurt	100	8.2–63.2	Udomkun et al., 2018
<b>Democratic Republic of Congo</b>	Yogurt	67	4.8-26	
	Cheese	100	18.5-261.1	
<b>Greece</b>	Feta cheese	0	–	Becker-Algeri et al., 2016
<b>Iran</b>	White cheese	80	0.052-0.75	Fallah et al., 2009
	Cream cheese	72	0.058-0.79	
	Livan cheese	65	0.03-0.31	Fallah et al., 2011
	Cheese	53	0.082-1.25	Rahimi et al., 2009
	White cheese	60	0.041-0.37	Tavakoli et al., 2012
	Feta cheese	83	0.15-2.4	Kamkar, 2006
	Cheese	47.6	n.d-0.31	Mohajeri et al., 2013
	Yogurt	98.3	n.d-0.087	Issazadeh et al., 2012
<b>Kuwait</b>	White cheese	80	0.024–0.45	Dashti et al., 2009
<b>Lebanon</b>	Cheese	55.0	n.d-0.32	Elkak et al., 2012
	Yogurt	32.8		Khoury et al., 2011
<b>Libya</b>	Cheese	75	0.11-0.52	Elgarbi et al., 2004
<b>Pakistan</b>	White cheese	78	0.004-0.6	Iqbal and Asi, 2013
	Cream cheese	59	0.004–0.46	
	Butter	45	0.004–0.41	
	Yogurt	61	0.004-0.62	
	Sweets	97.1	n.d-1.5	Sadia et al., 2012
<b>Saudi Arabia</b>	Cheese	80	0.024–0.452	Dashti et al., 2009
<b>Serbia</b>	Milk products	38	0.27-0.95	Tomasevic et al., 2015
<b>Spain</b>	Yogurt	2.8	n.d-0.051	Cano-Sancho et al., 2010
	Cheese	0		
<b>Turkey</b>	Cheese	94	0.012–0.38	Ertas et al., 2011
	Yogurt	56	0.0025–0.078	
	Dairy dessert	52	0.0015–0.08	
	Butter	100	0.01-7.0	Tekinsen and Ucar, 2008
	Cream cheese	99	0-4.1	

Country	Sample	% AF-positive samples	Min–Max (µg/kg)	Reference
	Yogurt	88	0.01–0.48	Atasever et al., 2011
	Yogurt	3.3	0.024–0.028	Sahin et al., 2016

**Table 4. AFM1 contamination in milk from different regions of countries**

Country	Sample	Region	% AFM1 positive samples	Min-Max (µg/L)	Reference
Brazil (São Paulo state)	Raw milk	Bauru	72.9	0.013–0.708	Santili et al., 2015
		Araçatuba	56.3	0.012–0.725	
		Vale do Paraíba	27.5	0.014–0.224	
China	Raw milk	Heilongjiang, Jilin, and Liaoning provinces	0.3	n.d – 25.5 ng/L Mean: 20.7 ± 6.8	Li et al., 2018
		Gansu, Ningxia, and Shaanxi provinces	11.49	n.d- 55 ng/L Mean: 19.9 ± 21.6	
		Beijing, Hebei, and Henan provinces	1.56 (1 out of 64 samples)	40 ng/L	
		Anhui, Hubei, and Hunan provinces	9.38	n.d-60 ng/L Mean: 46.7 ± 10.4	
Iran	Raw milk	Kerman province	50	<0.01 to 0.41 µg/L	Rohani et al., 2011
	Raw milk and pasteurized milk	Fars province	55.56	0-0.0999 µg/L	Hashemi, 2016
Pakistan	Fresh milk	Karachi	91.7	0.02-3.09 µg/L	Asghar et al., 2018
	Raw and processed	Punjab province	93	0.006–0.554 µg/L	Ahmad et al., 2019



