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A Review of Aflatoxin M_1, Milk, and Milk Products

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1. Introduction

Aflatoxins are a group of closely related heterocyclic compounds produced predominantly by two filamentous fungi, *Aspergillus flavus* and *Aspergillus parasiticus*. Recent studies have shown that some *A. nominus* and *A. tamarii* strains are also aflatoxin producing, of which *A. nominus* is phenotypically similar to *A. flavus* (Kurtzman et al, 1987; Goto et al., 1997). Ito et al. (2001) isolated one more strain, *A. pseudotamarii*, which can produce aflatoxin. These fungi belong to the class Hyphomycetes, subdivision Deuteromycotina and family Aspergillaceae. They contaminate a vast array of food and agricultural commodities. Aspergillus species are capable of growing on a variety of substrates and under a variety of environmental conditions. Therefore, most foods are susceptible to aflatoxigenic fungi at some stage of production, processing, transportation, and storage. The outbreak of aflatoxicosis (famous as Turkey "X" disease) in England in 1960 caused the death of a large population of livestock (Blount, 1961) and led to the discovery of aflatoxin in groundnut meal contaminated by *A. flavus* (Hesseltine, 1979). Subsequently, aflatoxins were found in other feeds, especially maize (Chakrabarty, 1981) and cottonseed meal (Sharma et al., 1994). Aflatoxin M_1 (AFM_1) in milk and milk products is considered to pose certain hygienic risks for human health. Mammals that ingest aflatoxin B_1 contaminated diets eliminate into milk amounts of the principal 4-hydroxylated metabolite known as "milk toxin" or aflatoxin M_1 (Figure. 1). The economic impacts attributed to aflatoxin are incurred directly by loss in crops, livestock, and dairy and indirectly by a recurring expenditure in quality-control programs, research and education, lower foreign exchange earnings, and increased storage and packaging costs of vulnerable commodities. The potential hazard of aflatoxins to human health has led to worldwide monitoring programs for the toxin in various commodities as well as regulatory actions by nearly all countries.

2. Formation, toxicity, and regulation of aflatoxin M_1

Aflatoxin B_1 (AFB_1) is metabolized by the hepatic microsomal mixed-function oxidase system, but it also can undergo several metabolic conversations depending upon species (Marsi et al., 1974). The amounts of aflatoxin M_1 (AFM_1) excreted in milk as a percentage of AFB_1 averages 1-2%, varying from animal to animal, from day to day and from one milking to the other. The AFM_1 could be detected in milk 12-24 h after the first AFB_1 ingestion, reaching a high level after a few days. When the intake of AFB_1 is finished, the AFM_1

concentration in the milk decreases to an undetectable level after 72 h (van Egmond 1989). Battocone et al. (2003) observed that there was a linear relationship between AFB_1 dose and excretion of AFM_1 into ewes' milk.

International Agency for Research on Cancer (IARC, 1993) classified AFB_1 and AFM_1 as class 1 and 2B (or probable) human carcinogens, respectively. Lafont et al. (1989) observed a high genotoxic activity of AFM_1, although it was lower than that of AFB_1. The aflatoxins show both acute and chronic toxicity, and one of the outstanding features in the toxicology of the aflatoxins is the wide variation in response amongst different species of animals and even between the male and female of the same species. It can be seen that for some animals, such as the rat, aflatoxins are very carcinogenic, and yet in other species, it is difficult to demonstrate carcinogenicity. This considerable variation in biological response arises from the requirement that the mold metabolite itself has to be metabolized in order that a toxic response occurs, and the metabolites responsible for acute toxicity differ from those reponsible for carcinogenicity. There are several metabolic reactions (Fig. 1), such as the demethylation to aflatoxin P_1 and hydration to aflatoxin B_{2a}, which may lead to a decrease in toxicity.

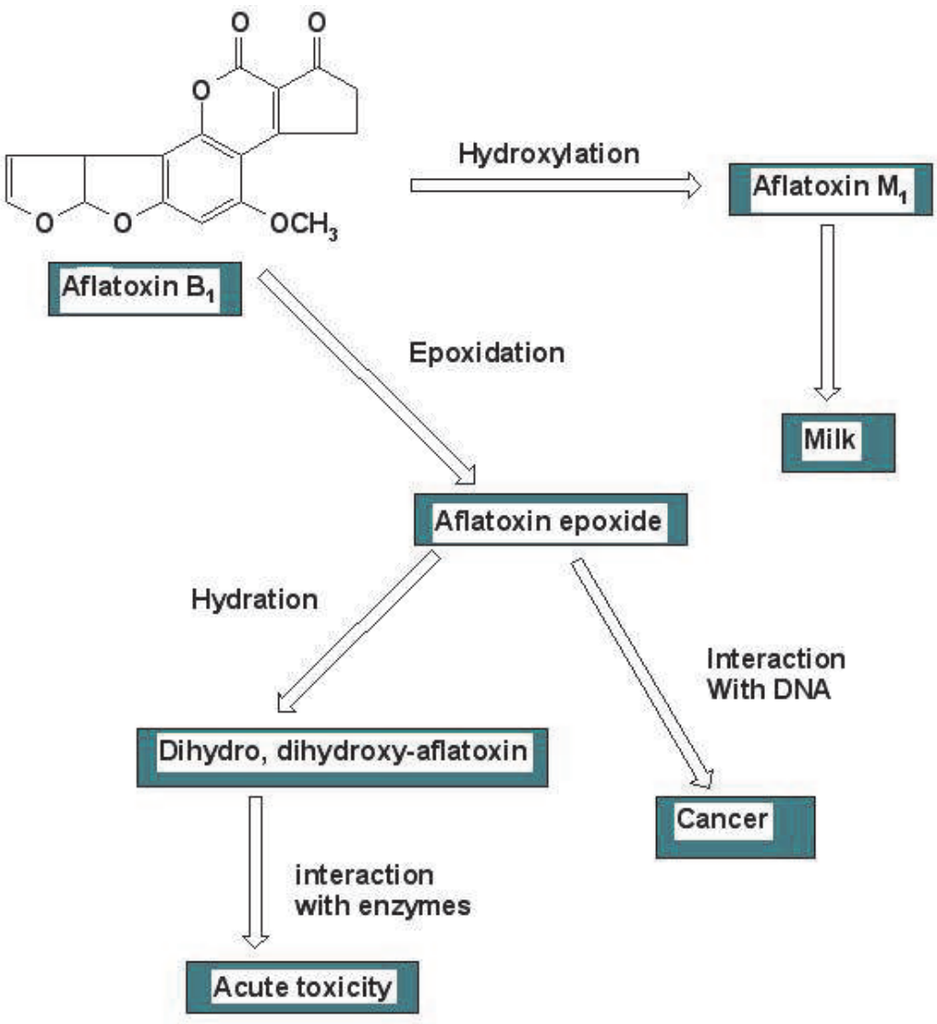

Fig. 1. Some metabolic products from AFB_1

It seems that milk has the greatest demonstrated potential for introducing aflatoxin residues from edible animal tissues into human diet. Aflatoxins are one of the major etiological factors in the development of hepatocellular carcinoma (IARC, 2002), and more recently associations between childhood aflatoxin exposure and both growth faltering (Gong et al., 2004) have been reported. Moreover, as milk is the main nutrient for growing young, whose vulnerability is notable and potentially more sensitive than that of adults, the occurrence of AFM_1 in human breast milk, commercially available milk, and milk products is one of the most serious problems of food hygiene.

In order to decrease aflatoxins risk nearly all developed countries are of the maximum permissible levels of AFB_1 in foods and feeds as well as the levels of AFM_1 in milk and milk products. Currently the limits of AFM_1 are highly variable (Table 1), depending upon the degree of development and economic standing of the countries. Some European Community and Codex Alimentarius prescribe that the maximum level of AFM_1 in liquid milk and dried or processed milk products should not exceed 50 ng/kg (Codex Alimentarius Commissions, 2001). However, according to US regulations the level of AFM_1 in milk should not be higher than 500 ng/kg (Stoloff et al., 1991). There are thus differences in maximum permissible limit of AFM_1 in various countries.

Region	Maximum Acceptabe Level (ng/l)	Type
European Union	50	Milk
Austria	50	Milk
Argentina	50	Milk
Bulgaria	500	Milk
Germany	50	Milk
Australia	20	Children's milk
Sweden	50	Liquid milk products
Netherlands	20	Butter
Switzerland	50	Milk and milk products
	250	Cheese
Belgium	50	Milk
USA	50	Milk
Czech Republic	100	Children's milk
	500	Adult's milk
Serbia	500	Milk
Iran	50	Raw, Pasteurized, and UHT milk
	200	Cheese
	20	Butter
France	30	Children's milk< 3 years
	50	Adult's milk
Turkey	50	Milk and milk products
	250	Cheese
Brazil	500	Milk

Table 1. Maximum acceptable level of AFM_1 in some regions

Regulatory limits seem to be a practical compromise between the need to have carcinogen-free commodities and the economic consequences of setting regulatory limits (Pohland and Yess, 1992). However, Stoloff et al. (1991) observed that as to aflatoxins there was little scientific basis, or the existing scientific information was not used in setting legal limits in most countries. Thus, even the low regulatory limits set by countries could not prevent chronic effects of aflatoxins, due to continued exposure to subacute levels of aflatoxins.
Because of the following reasons, it seems that monitoring and preventive program are the most effective strategies to decrease the risk of exposure to both human and animals:

1. Evaluation of human exposure levels and health risk based on animal toxicological research
2. Difficulties in assessing dietary intake
3. Decontamination and remove mycotoxins from human and animal diets

2.1 AFM_1 in milk

Milk, as a liquid, is a highly variable product that rapidly loses its quality and spoils if not to be treated. Since milk may be processed in numerous ways, the effects of storage and processing on stability and distribution of AFM_1 are of great concern.
Kiermier and meshaley (1977) reported the effect of cold treatments. They observed that detectable AFM_1 decreased by 11 to 25% after 3 days at 5°C, 40% after 4 days at 0°C, and 80% after 6 days at 0°C. Whereas, McKinney et al. (1973) revealed that freezing at -18°C for 30 days resulted in an apparent loss of 14%, with 85% lost after 53 days. Stoloff et al. (1981) suggested less degradation of AFM_1 at -18°C with insignificant loss after 53 days. As to the effect of heating contradictory data have been reported. Kiermeier and Mashaley (1977), reported that various heat–time treatments caused reductions in the AFM_1 concentrations of milks between 12% and 40%. Choudhary et al. (1998) studied the effect of various heat-treatments on AFM_1 content of cow's milk and reported that sterilization of milk at 121 °C for 15 min caused 12.21% degradation of AFM_1, whereas boiling decreased AFM_1 by 14.50%. They concluded that destruction of AFM_1 depends on time and temperature combination of the heat treatment applied. In an investigation Conducted by Bakirci (2001), it was observed that pasteurization caused a decrease in the level of AFM_1 at the rate of 7.62%. Deveci (2007) showed that pasteurization can partially reduce the amount of AFM_1 in milk. However, some reports showing that aflatoxins are stable during heat-treatments such as pasteurization and sterilization (Van-Egmond et al. 1977; Wiseman and Marth, 1983; Yousef and Marth, 1989; Govaris et al. 2001) were also published. Fluctuation in data reported in literature could be attributed to the wide range of temperature, different analytical methods, and employment of both naturally and artificially contaminated milk.
AFM_1 distribution in milk is not homogeneous. Cream separation can affect AFM_1 distribution, since 80% is partitioned in the skim milk portion (Grant and Carlson, 1971) because of AFM_1 binding to casein (Brackett, 19982a). An amount of 30% of AFM_1 is indeed estimated to be associated with the nonfat milk solids and in particular with casein. According to Van Egmond and Paulsch (1986) the behavior of AFM_1 in processes which involve fat separation may be explained by its semipolar character, leading to predominance in the nonfat fraction.
Contradictory data have been reported on the influence of milk concentration on AFM_1. Kiermeier (1973) reported no losses of AFM_1, whereas some authors observed losses ranging from 60 to 75% following milk concentration (Moreau, 1976; Purchase, I. F. H., 1973). Data from the studies on the occurrence of AFM_1 in milk since the 1990s are reported in (Table 2.)

Year	Region	Milk type	Samples	+ samples	Range(ng/liter)	Reference
1991	Kazakhstan	C	*	0	NA	Nikov et al.
1992	Cuba	C	85	22	>500	Margolles et al.
1993	Japan	C	37	0	NA	Tabata et al.
1994	USA	D	10	4	95	Kawamura et al.
	China	D	28	21	102.8	
	Italy	D	14	0	NA	
	New Zealand	D	3	0	NA	
	Poland	D	3	1	85	
1995	India	R	504	89	100-3.500	Rajan et al.
1996	Italy	UHT	161	125	<1- 23.5	Galvano et al.
		D	92	49	<1- 79.6	
1998	Kuwait	C	9	5	*	Srivastava et al
		R	7	5	*	
1999	Portugal	R	31	25	*	Martins
1999	Portugal	UHT	70	60	*	Martins
1999	Argentina	R	56	6	12-30	Lopez et al.
		PW	5	4	10-14	
		P	16	8	10-17	
1999-2000	Iran	R	186	119	≤10-410	Ghiasian et al.
2001	Turkey	R	90	79	12.5-123.6	Bakirci
2001	Iran	R	111	85	15-280	Kamkar
2002-2003	Brazil	R	22	13	*	Shundo and Sabino
2002-2003	Brazil	P	43	32	*	Shundo and Sabino
2002-2003	Brazil	UHT	34	34	*	Shundo and Sabino
2002	Greece	R	54	*	*	Kaniou-Grigoriadou et al.
2003	Iran	P	624	624	*	Alborzi et al.
2004	Turkey	P	3	2	*	Gurbay et al.
		UHT	24	14	*	
2004	Iran	R	319	172	15.4[a]	Tajkarimi et al
2003-2004	Iran	R	98	*	53[a]	Tajkarimi et al.
2003-2004	Italy	R	208	36	5-36.1	Virdis et al.
*	Turkey	P	85	75	*	Celik et al.
2004-2005	Italy	R	344	5	*	Decastelli et al
2004-2005	Brazil	P	12	7	11-161	Oliveira and Ferraz
		UHT	12	10	11-161	
		PW	12	8	11-161	

Year	Region	Milk type	Samples	+ samples	Range(ng/liter)	Reference
2005	Pakistan	R	168	168	10-700	Hussain and Anwar
2005	Iran	P	128	128	31-113	Oveisi et al.
2005-2007	Kuwait	R	177	176	4.9-67.8	Dashti et al.
2006	Iran	R	*	*	43-59	Mohammadi et al.
2006	Iran	P	110	110	8-89	Karimi et al.
2006	Iran	PW	42	42	51-914	Kamkar
2006	Iran	P	*	*	178.8-253.5	Sefidgar et al.
2006-2007	Iran	R	240	226	12.56[a]	Mohammadian et al.
		P	32	31	12.43[a]	
2007	Iran	P	*	*	23.22[a]	Mohammadi et al.
2007	Iran	UHT	*	*	19.53[a]	Mohammadi et al.
2007	Pakistan	B	55	19	13[a]	Hussain et al.
		C	40	15	14[a]	
		G	30	6	2[a]	
		S	24	4	2[a]	
		Ca	20	0	0	
2008	Iran	P	50	50	*	Movassagh Ghazani
		UHT	49	49	*	
2007-2008	Serbia	C	3	*	10-50	Polovinski-Horvatović et al.
2007-2008	Iran	UHT	210	116	8-249	Heshmati and Milani
2007-2008	Iran	C	75	59	60.1[a]	Rahimi et al.
		B	75	29	31.9[a]	
		Ca	40	5	19.0[a]	
		S	51	19	28.1[a]	
		G	60	19	30.1[a]	
2008	Spain	R	72	68	9.69[a]	Cano-Sancho et al.
2008	Iran	C	88	74	13-394	Fallah et al.
		G	65	28	13-55	
		S	72	43	15-102	
2009	Iran	P	91	66	13-250	Fallah
2009	Croatia	R	61	*	0.6-58.7	Bilandzic et al.
2009	Sudan	R	44	42	220-6900	Elzupir and Elhussein

P: pasteurized; D: Dry milk; PW: Powdered Milk; R: Raw Milk; C: Cow milk; B: Buffalo milk; S: Sheep milk; G: Goat milk; Ca: Camel milk; NA: Not applicable

* Not reported

[a] Average of contamination

Table 2. Occurrence and content of AFM_1 in milk samples

Many authors showed that Seasonal effect influences concentration of aflatoxin M_1. They reported higher concentration of AFM_1 in cold seasons as compared to hot seasons (Applebaum et al., 1982; Blanco et al., 1988b; Hussain and Anwar, 2008; Tajkarimi et al. 2008; Fallah, 2010, Bilandzic et al., 2010), the reason being in winters mostly milking animals are fed with compound feeds and thus concentration of aflatoxin B1 increases which in turn enhances AFM_1 concentration in milk. Moreover, temperature and moisture contents also affect the presence of aflatoxin B_1 in feeds. *A. flavus* and *A. parasiticus* can easily grow in feeds having moisture between 13% and 18% and environmental moisture between 50% and 60%, furthermore, they can produce toxin (Jay, 1992). Another reason of low AFM_1 level in summer may be attributed to out-pasturing of milking cattle.

It is too difficult to compare the data from the literature due to wide differences between and within the countries related to feeding, animal and environmental factors, extraction and analysis procedures, and regulatory limits for aflatoxins in feeds and milk. However, in recent years the incident of AFM_1 contamination seems to have been balanced on the one hand by increasing precision of extraction and analysis procedures and on the other hand the setting of stricter regulatory limits for alatoxins in feeds and milk (Galvano et al., 1996). Today the high efficiency of immuno-enzymatic extraction and the accuracy of analytical methodology and equipment, such as high pressure liquid chromatography and fluorescence detectors, allow detection limits to decrease, improving the percentage of positive samples. Furthermore, in recent years attention to the concern of aflatoxins in feeds as well as in milk has increased in most of the developed countries.

2.2 AFM_1 in cheese

Occurrence of aflatoxin in cheese can be owing to three possible causes:

1. AFM_1 present in raw milk as a consequence of carry over of AFB_1 from contaminated animal feed to milk
2. Synthesis of aflatoxin (B1, B2, G1, and G2) by fungi that grow on
3. cheese (although the low level of carbohydrate does not make it a very suitable substrate)
4. The use of powdered milk contaminated with AFM1 for cheese production

Contrasting data have been reported on the influence of cheese preparation on AFM_1 recovery. Studies performed in the early years showed variable losses of AFM_1 during cheese production: 65%, 47%, <20% and <15% according to Purchase et al. (1972), McKinney et al. (1973), Grant and Carlson (1971) and Stubblefield and Shannon (1974), respectively. In contrast, later investigations of several authors (Brackett and Marth, 1982b; Brackett and Marth, 1982c; Munksgaard et al., 1987; Van Egmond and Paulsch, 1986; Bakirci, 2001, Govaris et al. 2001; Deveci, 2007) reported increases in AFM_1 concentration in cheese as a function of cheese type, technologies, and the amount of water eliminated during processing. For example, Mohammadi et al. (2008) investigated some factors, which are involved in the process of making Iranian white brine cheese. They reported that some factors such as renneting temperature, press time, and saturated brine pH affected the amount of water eliminated and in turn the content of AFM_1 in the cheese curds. However, many results have been drawn from experiments in which the processed milk contained the toxin at high levels, which seldom appear in the practice. Therefore, additional investigations should verify the influence of cheese making on AFM_1 occurrence to avoid uncertainty in actual practice when the concentration of the toxin in the processed milk is at around the maximum permissible level of 0.05 mg/kg that is frequently recorded in monitoring programmes.

The increase in AFM_1 concentration in cheese has been ascribed to the affinity of AFM_1 for casein (Allcroft and Carnagham, 1363; Applebaum et al., 1982; Brackett and Marth, 1982a; Grant and Carlson, 1971). Brackett and Marth (1982a) suggested that since is possible to extract AFM_1, it must not be covalently bound but linked by hydrophilic interactions hydrophobic areas of the casein. According to Dosako et al. (1980), AFM_1 is a water-soluble component and due to the hydrophobic sides of the casein molecule, AFM_1 has affinity to casein of milk. Therefore, they defined a factor named "Enrichment Factor" (EF) for cheeses. Further surveys should be done to find as for cheese manufacture influences on AFM_1 distribution.

Some tests heve been carried out on several kinds of cheeses as to overall stability of AFM_1 during ripening and storage. Fremy et al. (1990) and Dragacci et al. (1995) reported that the concentrations of AFM_1 in Camembert cheese were higher at the beginning than at the later time of ripening. These results were in agreement with studies by Govaris et al. (2001). Such results however, conflict with reports of earlier studies that indicate different behaviour of AFM_1 in various other types of cheeses. Thus, in Camembert and Tilsit (Kiermeier and Buchner 1977), Cheddar (Brackett and Marth 1982b) and Brick (Brackett et al., 1982) cheeses stored for 3, 14 and 6.5 months, respectively, the concentration of the toxin increased during the early stage of their ripening to decrease thereafter to reach about its initial concentration at the beginning of ripening. On the other hand, the concentration of AFM_1 in Parmesan cheese started high at the beginning of the ripening period, decreased until about the fifth month and then slowly increased up to the tenth month of storage (Brackett and Marth 1982d). In contrast, the AFM_1 content of Mozzarella remained almost constant during storage of 4.5 months (Brackett and Marth, 1982d). Additionally, studies by Deveci (2007), and Huseyin Oruc et al. (2007) showed that the amount of AFM_1 in white pickled and Kashar cheeses did not significantly affect over the storage. Kaniou-Grigoriadou et al. (2005) found that the final ripened cheese was free of aflatoxin M_1.

These different profiles of AFM_1 in various cheese products may be the result of several factors such as heat treatment (Brackett and Marth 1982b, Yousef and Marth 1989), proteolysis (Brackett and Marth 1982b, d, Brackett et al. 1982, Yousef and Marth 1989), exposure of contaminated milk to light (Yousef and Marth 1989), and especially to an inadequate method of analysis (Yousef and Marth 1989). Some results of studies on the behaviour of AFM_1 during cheese ripening seem to represent changes in the recovery of toxin by the method during the different phases of the study rather than real changes in the level of AFM_1 in cheese (Brackett and Marth 1982a).

Several investigations on the partitioning of AFM_1 during cheese production staring with different milk contamination levels reported a wide range of distribution of AFM_1 between whey and curd. Some authors observed that half or more of the AFM_1 was in the way: 50%, 50%, 66%, 100%, 60%, and 53-58% according to Grant and Carlson (1971), Stubblefield and Shannon (1974), Blanco et al. (1988a), Purchase et al. (1972), Lopez. et al. (2000), and Huseyin Oruc et al. (2007), respectively. In contrast, others reported that most of AFM_1 was with curd: ranging from 66% to 72%, from 73% to 77%, 80%, 100%, and 59.1% according to Marshaley et al. (1986), El Deeb et al. (1992), Mckinney et al. (1973), Allcroft and Carnaghan (1963), and Deveci (2007), respectively. Kaniou-Grigoriadou et al. (2005) observed that enrichment factor in the production of Feta cheese made from naturally contaminated milk ranged between 4.3 and 5.6. Kamakar et al. (2008) showed that the mean concentration of toxin in curd and cheese was 3.12 and 3.65-fold more than that in whey and 1.68 and 1.80 fold more than that in cheese milk, respectively.

It is thought that since AFM_1 is a semi-polar component, it has less affinity to serum protein (Applebaum et al., 1982). Regarding the affinity of AFM_1 with proteins, Recently, Barbiroli et al. (2007) indicated that there is no simple physical method to remove AFM_1 from ovine and caprine milk. Neither ultrafiltration, nor acidic or enzymatic treatments were able to influence the toxin's interaction with casein or whey proteins. Only the combined action of heat and low pH (as used in ricotta cheese production) was able to denature whey proteins to a point where they lost their AFM_1-binding capacity.
According to Blanco et al. (1988a) these contrasting results can be attributed to different factors such as extraction techniques, methodology, type and degree of milk contamination, differences in milk quality, expression of the results, the presence of a small portion of curd in whey which could influence AFM_1 concentration, and the cheese manufacture process.

Year	Region	Samples	+ samples	Range(ng/kg)	Refernce
1990	Syria	*	0	NA	Haydar et al.
1991	Kazakhestan	*	*	*	Nikov et al.
1993	Japan	37	0	NA	Tabata et al.
1995	Japan	41	0	NA	Taguchi et al.
1995	Spain	35	16	20-200	Jose Barios et al.
2001-2002	Turkey	600	30	100-800	Yaroglu et al.
2003-2004	Iran	80	66	150-2410	Kamkar
2004	Italy	41	4	79.5-389	Virdis et al.
2005	Turkey	100	99	0-4100	Tekinsen and Ucar
2005-2007	Kuwait	40	32	23.8-452	Dashti et al.
2008	Spain	72	0	-	Cano-Sancho et al
2008	Iran	75	49	30-313	Fallah et al.
2009	Iran	72	59	30-1200	Fallah

NA: Not Applicable
*: Not Reported

Table 3. Occurrence and content of AFM_1 in cheese samples

The incidence of positive cheese samples for AFM_1 (Table 3) seem to be widely variable. Taguchi et al. (1995) found no positive samples in imported cheese in Japan. Virdis et al. (2008) detected few positive samples, whereas Tekinsen and Ucar (2008) observed a high incidence of positive samples. As regards the contamination level, several authors (Kamkar, 2006; Fallah, 2010) found a maximum contamination level over 1000 ng of AFM_1 per kg. This latter contamination level could be hazardous.

2.3 AFM_1 in yogurt

Several studies have been conducted regarding the effect of yogurt manufacturing on AFM_1 content. Some authors reported no influence on aflatoxin M_1 content (Blanco et al. 1993; Stoloff, 1980; Stoloff et al. 1981; Van Egmond, 1983; Van Egmond and Paulsch, 1986). In contrast, Munksgaard et al. (1987) and Bakirci (2001) detected variable increases of AFM_1 content in yogurt related to the milk. The effect of fermentation was assessed by Govaris et al. (2002). They reported that AFM_1 levels in all yoghurt samples showed a significant decrease from those initially present in milk. This decrease in AFM_1 was attributed to factors such as low pH, formation of organic acids or other fermentation by-products, or even to the

presence of lactic acid bacteria. The low pH during fermentation alters the structure of milk proteins such as the caseins leading to formation of yoghurt coagulum. The change in casein structure during yoghurt production may affect the association of AFM_1 with this protein (Brackett and Marth 1982) causing adsorption or occlusion of the toxin in the precipitate.

As to AFM_1 stability over storage of yogurt, Van Egmond et al. (1977) observed no reduction of AFM_1 in yogurt stored for 7 days at 7 ºC. Megalla and Hafez (1982) observed complete transformation AFB_1 in its hydroxy derivative AFB_2A caused by the acids present in yogurt. Whereas, Rasic et al. (1991) revealed a high reduction (up to 97%) of AFM_1 in yogurt and acidified milk. El Deeb et al. (1992) observed that enzymatic, microbial, and particularly acid coagulation caused degradation of AFM_1 in buffalo milk. Maryamma et al. (1990) reported a high reduction of AFM_1 in fermented goat milk. As a result of Study by Govaris et al. (2002), during refrigerated storage, AFM_1 was rather more stable in the yoghurts with pH 4.6 than with pH 4.0. The percentage loss of the initial amount of AFM_1 in milk was estimated at about 13 and 22% by the end of the fermentation, and 16 and 34% by the end of storage for yoghurts with pHs 4.6 and 4.0, respectively.

Since it is known that exposure of the aflatoxin molecule to strong acid, such as trifluoracetic acid, can cause its acid-catalyzed hydration, leading, for example, from AFB_1 to AFB_2A (Cohen and Lapointe, 1981), but not its degradation or neutralization, the effect of the weak acidity of yogurt on aflatoxin should be more investigated.

Some investigations have been conducted related to the effect of aflatoxin on nutritive properties of yogurt. El Deeb et al. (1989) observed some negative effects of AFM_1 on Lactobacillus bulgaricus (cell wall thickening and shortening of cell chain lenghth) and *Staphylococcus thermophilus* (cell wall thickening and cell shape changing from coccoid to oval). Rasic et al. (1991) found that *S. thermophilus* was affected by the presence of in milk during fermentation of yoghurt, exhibiting longer cell chains in the contaminated than in the uncontaminated yoghurt samples. Similarly, Govaris et al. (2002) observed that the growth rate of *S. Thermophilus* and curdling time were affected by the higher level and not by the lower level of AFM_1. Unlike cheese and milk samples, the presence of AFM_1 in yogurt has not frequently been studied. Thus, more investigations are needed because:

1. currently, human consumption of yogurt has greatly increased
2. there are contradictory data on AFM_1 stability over manufacture and storage in the literature
3. The presence of aflatoxin in yogurt could reduce the nutritional values of its consumption.

2.4 AFM_1 in other milk products

Many other milk products such as cream, butter, ice cream may contain AFM_1. The presence of AFM_1 in these products has rarely been investigated and could be of interesting aspects for study. Some surveys conducted on the occurrence of AFM_1 in milk products are reported in (Table. 4). In a study by Bakirci (2001), the levels of AFM_1 in the products made from contaminated milk namely butter, butter milk, cream, skim milk was investigated. The mean AFM_1 level found in cream samples was 64.4% of AFM_1 concentration of bulk-tank milk. Whereas, mean AFM_1 level of skim milks was 3% higher than those of bulk-tank milk. These values were close to the results given by Van Egmond et al. (1977), and lower than the values given by Wiseman et al. (1983). Levels of AFM_1 in butter samples in the study were less, and they were as 33.80% of AFM_1 amounts of bulk-tank milk. Mean AFM_1 levels obtained from buttermilk samples were similar to those of bulk-tank milk (mean 83% of it).

The same results were reported by Grant and Carlson (1971). During butter processing, protein membrane around fat globules is broken down and serum phase is separated. Due to the chemical structure of AFM_1 and its affinity to casein, it adsorbs on this fraction of protein (Yousef & Marth, 1989), therefore, cream contained less AFM_1 than milk, and butter contained less amount of AFM_1 than cream. As a result of the associate effects of these factors, AFM_1 concentration occurs in lipid phase (like butter and cream) less than serum phase and protein fraction (Grant & Carlson, 1971).

Year	Region	type	Samples	+ samples	Range(ng/kg)	Refernce
2005	Turkey	Butter	92	92	10-7000	Tekinsen and Ucar
2005	Turkey	Butter	27	25	0-100	Aycicek et al.
2005	Iran	Infant formula	120	116	1-14	Oveisi et al.
		Milk-based cereal weaning food	80	72	3-35	
2005	Egypt	Beast milk	443	248	4.2- 889	Polychronaki et al.
2005-2007	Kuwait	Breast milk	12	5	8.83-15.2	Dashti et al.
2008		Kashk	125	53	28-291	Fallah et al.
		Doogh	136	25	13-53	
2009	Iran	Butter	31	8	13-26	Fallah
		Ice Cream	36	25	15-132	

Table 4. Occurrence of AFM_1 reported in some regions

AFM_1 is frequently observed in the aflatoxin exposed individuals and in the breast milk. AFM_1 toxicity in this respect is important as it is known that within aflatoxin exposed nursing mothers it can provide a source of aflatoxin exposure to the infant (El-Nezami, 1995). The occurrence of AFM_1 in breast milk has been investigated in some regions. There is increased awareness of the link between growth and health of the fetus and infant, and disease risk in later life. Long term pre and postnatal exposure to aflatoxins could be one of the factors contributing to growth faltering and/or the early onset of hepatocellular carcinoma (HCC) in countries with a high incidence of the disease. Additionally, the presence of other aflatoxins, B_1, B_2, G1, G_2 and M_2, has also been reported in breast milk (IARC, 1993). The identification and understanding of factors determining the presence of toxicants in human milk is important and may provide a strong basis for controlling the transfer of chemicals to the infants through breast milk.

3. Conclusion

At present, since it considers that there is not enough information to establish a reasonable exposure level, The World Health Organisation (WHO) recommends the reduction of AFM_1 consumption to a minimum so as to minimize AFM_1 potential risks. The regulatory limits are widely variable and there has been little scientific basis in their setting. Efforts should be made in attempting to provide further and extensive scientific information on human health

hazards related to low-level long term aflatoxin exposure and to standardize the already existing regulatory limits for aflatoxins.

Future studies should verify the effect of milk storage and processing on AFM_1 occurrence to avoid actual uncertainty. However, since it is generally assumed that neither storage nor processing determine reduction of AFM_1 content, further information on possible AFM_1 concentration following milk processing should be furnished.

The occurrence of AFM_1 in cow milk and milk products is widespread. For this reason, milk and milk products have to be controlled continuously by accurate and reliable analytical techniques for presence of AFM_1 contamination. It is also extremely important to maintain low levels of AFM_1 in the feeds of dairy animals. In order to achieve this, dairy cow feds should be kept away from contamination as much as possible. Therefore, animal feeds should be checked regularly for aflatoxin and, particularly important, storage conditions of feeds must be strictly controlled.

The occurrence of aflatoxin and their metabolites in human breast milk is of great concern. Since serious health hazards to the mother, fetus, and infant could occur. Therefore extensive and periodic surveys should be performed. Additionally, the incidence and occurrence of AFM_1 in dried milk infant formula should be more investigated.

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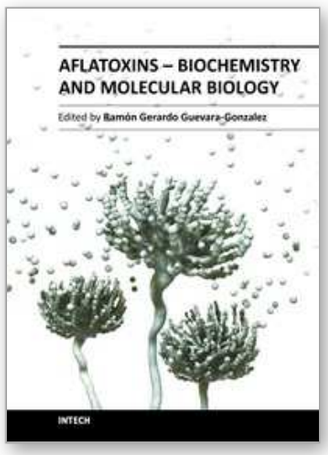

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Aflatoxins â€“ Biochemistry and Molecular Biology is a book that has been thought to present the most significant advances in these disciplines focused on the knowledge of such toxins. All authors, who supported the excellent work showed in every chapter of this book, are placed at the frontier of knowledge on this subject, thus, this book will be obligated reference to issue upon its publication. Finally, this book has been published in an attempt to present a written forum for researchers and teachers interested in the subject, having a current picture in this field of research about these interesting and intriguing toxins.

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