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Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to Aflatoxin B_1 as undesirable substance in animal feed

(Request N° EFSA-Q-2003-035)

(adopted on 3 February 2004)

SUMMARY

Aflatoxins are secondary metabolites of moulds, contaminating diverse food and feed materials. In consideration of the carcinogenic properties of aflatoxin B_1, human exposure should be reduced to levels as low as reasonable achievable. Current EU legislation addresses various susceptible plant-derived food commodities as well as milk. Milk is contaminated with the hydroxy-metabolite aflatoxin M_1, following exposure of lactating animals to aflatoxin B_1 present in feedstuffs. As aflatoxin M_1 has toxicological properties comparable to those of aflatoxin B_1, albeit a lower carcinogenic potency, maximum levels for aflatoxin M_1 have been set for consumable milk at 0.05 µg/kg, and 0.025 µg/kg for infant formulae, respectively, aiming to reduce human exposure to the lowest achievable level. Model calculations on the carry-over of aflatoxins present in feedstuff into milk revealed that under circumstantial maximum exposure from feed materials (albeit in compliance with the levels set for feed materials), milk obtained from high-yielding dairy cows and other milk producing animals, including small ruminants, buffalo and camels, might contain aflatoxin M_1 levels exceeding the present statutory limits. Surveys conducted by various EU member states, however, revealed a very low incidence of such milk samples, not complying with the current EU regulations. Taking into account the present agricultural practice and the possibility of aflatoxin B_1 to be present also in staple feeds grown in Europe, monitoring activity towards aflatoxin M_1 contamination of milk should be intensified and expanded to consumable milk from animal species other than dairy cows.

KEY WORDS
Aflatoxins, aflatoxin B_1, aflatoxin M_1, carry-over, dairy cattle, sheep, goats, buffalos, camels

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BACKGROUND

I. General Background

Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed[1] replaces since 1 August 2003 Council Directive 1999/29/EC of 22 April 1999 on the undesirable substances and products in animal nutrition[2].

The main modifications can be summarised as follows

- extension of the scope of the Directive to include the possibility of establishing maximum limits for undesirable substances in feed additives.
- deletion of the existing possibility to dilute contaminated feed materials instead of decontamination or destruction (introduction of the principle of non-dilution).
- deletion of the possibility for derogation of the maximum limits for particular local reasons.
- introduction the possibility of the establishment of an action threshold triggering an investigation to identify the source of contamination ("early warning system") and to take measures to reduce or eliminate the contamination ("pro-active approach").

In particular the introduction of the principle of non-dilution is an important and far-reaching measure. In order to protect public and animal health, it is important that the overall contamination of the food and feed chain is reduced to a level as low as reasonably achievable providing a high level of public health and animal health protection. The deletion of the possibility of dilution is a powerful mean to stimulate all operators throughout the chain to apply the necessary prevention measures to avoid contamination as much as possible. The prohibition of dilution accompanied with the necessary control measures will effectively contribute to safer feed.
During the discussions in view of the adoption of Directive 2002/32/EC the Commission made the commitment to review the provisions laid down in Annex I on the basis of updated scientific risk assessments and taking into account the prohibition of any dilution of contaminated non-complying products intended for animal feed. The Commission has therefore requested the Scientific Committee on Animal Nutrition (SCAN) in March

[1] OJ L140, 30.5.2002, p. 10
[2] OJ L 115, 4.5.1999, p. 32

2001 to provide these updated scientific risk assessments in order to enable the Commission to finalise this review as soon as possible (Question 121 on undesirable substances in feed)[3].

It is worthwhile to note that Council Directive 1999/29/EC is a legal consolidation of Council Directive 74/63/EEC of 17 December 1973 on the undesirable substances in animal nutrition[4], which has been frequently and substantially amended. Consequently, several of the provisions of the Annex to Directive 2002/32/EC date back from 1973.

The opinion on undesirable substances in feed, adopted by SCAN on 20 February 2003 and updated on 25 April 2003[5] provides a comprehensive overview on the possible risks for animal and public health as the consequence of the presence of undesirable substances in animal feed.

On the basis of this opinion, some provisional amendments are proposed to the Annex of Directive 2002/32/EC in order to guarantee the supply of some essential, valuable feed materials as the level of an undesirable substance in some feed materials, due to normal background contamination, is in the range of or exceeds the maximum level laid down in the Annex I of Directive 2002/32/EC. Also some inconsistencies in the provisions of the Annex have been observed.

It was nevertheless acknowledged by SCAN itself for several undesirable substances and by the Standing Committee on the Food Chain and Animal Health that additional detailed risks assessments are necessary to enable a complete review of the provisions in the Annex.

[3] Summary record of the 135th SCAN Plenary meeting, Brussels, 21-22 March 2001, point 8 – New questions (http://europa.eu.int/comm/food/fs/sc/scan/out61_en.pdf)
[4] OJ L 38, 11.2.1974, p. 31
[5] Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, adopted on 20 February 2003, updated on 25 April 2003 (http://europa.eu.int/comm/food/fs/sc/scan/out126_bis_en.pdf)

II. Specific Background

Among the aflatoxins (B_1, B_2, G_1 and G_2) aflatoxin B_1 is the most toxic, both for humans and animals, and is a potent carcinogen. Its metabolite aflatoxin M1 appears in milk and milk products as a direct intake of aflatoxin B_1- contaminated feed.

SCAN concluded[6] that the current EU legislation on aflatoxin B_1 in feed is stringent, detailed and effective in terms of human and animal health protection. There are no scientific reasons for its revision.

However, with the entry into force of Directive 2002/32/EC on 1 August 2003 feed materials such as groundnut, copra, palm-kernel, cotton seed, babassu, maize and products derived from the processing thereof, have to comply with the level of 20 µg/kg. The possibility to use under strict conditions these feed materials containing aflatoxin B_1 levels up to 200 µg/kg, has been deleted from 1 August 2003 onwards. It can be observed that some feed materials regularly exceed the 20 µg/kg aflatoxin B_1 level which will result that significant amounts of some of these feed materials can only be used for animal feed after an effective detoxification treatment.

On the other hand it can be observed that current feeding practices include the more frequent use of these feed materials directly on the farm, which could imply additional risks for elevated levels of aflatoxin M_1 in the case of dairy animals.

Although it is generally acknowledged that the current legislation is sufficient to guarantee that the aflatoxin M_1 level in the overall milk supply is below 0.05 µg/kg[7], some experts are of the opinion that the current levels in EU legislation for aflatoxin B_1 in complete feeds and complementary feeds for dairy animals/for animals in lactation do not provide sufficient guarantees, particularly in the case of high yielding dairy cattle, that the produced milk will comply with the EU legislation on aflatoxin M_1 in milk at farm level. According to these experts, the current maximum levels for aflatoxin B_1 in complementary feed and complete feed should be lowered.

[6] Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, point 7.6. Conclusions and recommendations.
[7] EU maximum level established by Commission regulation (EC) No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs (OJ L 77, 16.3.2001, p. 1)

According to the definitions of "food" and "placing on the market" laid down in Regulation (EC) No 178/2002 of the European parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety[8], milk should already comply with EU legislation at farm level.

Possible modifications to the EU-legislation on aflatoxin B_1 in animal feed can only be done after the availability of a detailed risk assessment addressing in particular the quantitative correlation between the levels of aflatoxin B_1 in feed and the resulting levels of the metabolite aflatoxin M_1 in milk for human consumption in particular in the case of intensive farming making use of high yielding dairy animals, in particular cattle.

TERMS OF REFERENCE

The European Commission requests the EFSA to provide a detailed scientific opinion on the presence of aflatoxin B_1 in animal feed.

This detailed scientific opinion should comprise

* the determination of the exposure levels (daily exposure) of aflatoxin B_1 for dairy animals in particular dairy cattle above which the level of transfer/carry over of aflatoxin B_1 from the feed to the milk, results in unacceptable levels of aflatoxin M_1 in the milk in view of providing a high level of public health protection

* the quantitative correlation between the levels of presence of aflatoxin B_1 in animal feed and the resulting levels of aflatoxin M_1 in milk for human consumption especially addressing intensive livestock production making use of high yielding dairy animals in particular cattle.

* identification of feed materials which could be considered as sources of contamination by aflatoxin B_1 and the characterisation, insofar as possible, of the distribution of levels of contamination

[8] OJ L 31, 1.2.2002, p.1

* identification of eventual gaps in the available data which need to be filled in order to complete the evaluation.

Insofar relevant, the opinion could also in addition comprise the

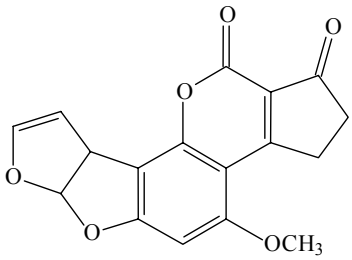
* determination of the toxic exposure levels (daily exposure) of aflatoxin B_1 for the different animal species of relevance (difference in sensitivity between animal species) above which
  - signs of toxicity can be observed (animal health / impact on animal health) or
  - the level of transfer/carry over of aflatoxin B_1 from the feed to the products of animal origin other than milk results in unacceptable levels of aflatoxin B1 or a toxic metabolite thereof in the products of animal origin other than milk in view of providing a high level of public health protection.

ASSESSMENT

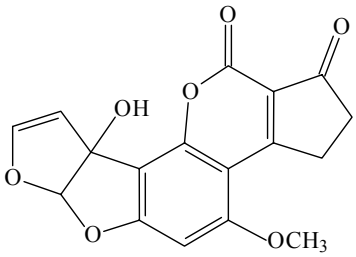
1. Introduction

Aflatoxins are a group of naturally occurring toxic secondary metabolites produced primarily by two species of the ubiquitous fungus *Aspergillus*: *A. parasiticus* and *A. flavus*. *A. parasiticus* is well adapted to a soil environment. *A. flavus* is more adapted to the aerial parts of plants (leaves, flowers). Aflatoxins are produced pre- and post-harvest under certain conditions of temperature, water activity and availability of nutrients, and can be found in agricultural products from tropical and subtropical regions. Primary contamination of agricultural crops grown in Europe has been generally considered unlikely for many years. However, aflatoxin formation was observed in Europe in products treated with organic acid to improve storability (Pettersson *et al*., 1989). Moreover, very recently aflatoxin contamination has been also reported to occur in maize, grown in Italy. High temperatures, drought and strong insect damage in some of the warmer provinces of the Po valley were conducive for *A. flavus* growth and subsequent production of aflatoxins on maize (Pietri and Diaz, 2003). Thus, although exposure to aflatoxins is generally considered to occur mainly from imported materials, contamination of European agricultural products cannot be entirely excluded.

Among the naturally occurring aflatoxins (B_1, B_2, G_1 and G_2), aflatoxin B_1 is the most important compound with respect to both, prevalence and toxicity for man and animals. Following ingestion with feed aflatoxin B_1 is metabolised in the liver, resulting in various metabolites, including the endo- and exo-epoxides of aflatoxin B_1, the hydroxy-metabolites aflatoxin M_1, aflatoxin M_2 and aflatoxin M_4, and aflatoxin P_1 and Q_1, as well as conjugated metabolites (for review see Gorelick, 1990, McLean and Dutton, 1995). The most important metabolite in animals is aflatoxin M_1, the 4-hydroxy metabolite of aflatoxin B_1 (see figure 1).



aflatoxin B_1



aflatoxin M_1

Figure 1: Chemical structures of aflatoxin B_1 and M_1

In addition to aflatoxin M_1 other aflatoxin metabolites are excreted with milk, including aflatoxin M_2 (the analogous metabolite of aflatoxin B_2) and aflatoxin M_4, another hydroxy- metabolite of aflatoxin B_1. Both, aflatoxin M_2 and M_4, occur in milk at much lower concentrations compared to aflatoxin M_1 and thus are considered as of less public health significance.

Aflatoxin B_1 is considered to be a human carcinogen (classified by the International Agency for Research in Cancer (IARC), in group 1), and clearly genotoxic. The risk for primary liver cancer is considerably increased in geographical regions with a high prevalence of carriers of the HbsAG (hepatitis B serum antigen) (for review see JECFA, 1998, Henry *et al.*, 1999, Henry *et al.*, 2001). Other diseases possibly related to human exposure to aflatoxin B_1 include toxic hepatitis and liver fibrosis, stunted growth in children, and Reye's syndrome (Gong *et al*, 2002).

For aflatoxin M_1 the information on possible adverse health effects on humans is scarce. The limited experimental animal studies carried out to determine toxicity and carcinogenicity of aflatoxin M_1 seem to indicate that aflatoxin M_1 has a hepatotoxic and a hepatocarcinogenic potential. The acute toxicity of aflatoxin M_1 seems to be similar or slightly less than that of aflatoxin B_1 but its carcinogenic potency is probably one or even two orders of magnitude lower than that of aflatoxin B_1 (Henry *et al*, 2001). It should be noted that aflatoxin M_1 is not only found in dairy milk, but also in breast milk of nursing mothers. Using aflatoxin M_1 as possible marker of exposure to aflatoxin B_1, El-Sayed *et al.* (2002) reported from Egypt a mean level of 0.3 $\pm$ 0.5 µg/L in breast milk of nursing mothers, and a corresponding mean blood level of 1.2 µg/L.

2. Analytical methods

For the monitoring of the presence of aflatoxins in food and feed materials, various validated methods of analysis exist. For the determination of aflatoxin B_1 these methods are based on solid phase extraction (SPE) cleanup in combination with liquid chromatography, and on immuno affinity (IA) cleanup in combination with liquid chromatography (Stroka *et al*, 2004). The SPE-based method has been validated - among others - for the determination of aflatoxin B_1 in feedstuffs at levels ranging from 8 -14 µg/kg, the IA-based method has been validated at levels ranging from 1-5 µg/kg. For aflatoxin M_1 in milk a method of analysis is available based on IA cleanup in combination with liquid chromatography (Dragacci *et al*, 2001). This method has been validated for the determination of aflatoxin M_1 in milk at levels ranging from 0.02 - 0.1 µg/L. These methods have demonstrably good performance characteristics at the low statutory limits and can be used to generate reliable surveillance data.

Certified reference materials are available for aflatoxin B_1 (several peanut meals and mixed feedstuffs), and for aflatoxin M_1 (several milk powders). The reference materials consist of naturally contaminated materials at levels of regulatory interest (Josephs *et al*., 2004). Through adequate mixing of blank and contaminated reference materials at certain ratios, reliable reference materials for analytical quality assurance can be obtained even at lower levels. The certified reference materials are available through the European Commission's Joint Research Centre/Institute for Reference Materials and Measurements (see http://www.irmm.jrc.be). These reference materials together with validated methods

and the access to proficiency testing provide a sound quality assurance framework for measurements of aflatoxin B_1 and aflatoxin M_1.

3. Current legislation

Within the EU, diverse detailed and harmonized regulations for aflatoxins in food exist, and maximum levels have been set for aflatoxin B_1 and for total aflatoxins (aflatoxin B_1, aflatoxin B_2, aflatoxin G_1, aflatoxin G_2) in cereals, grains, groundnuts, dried fruit and spices, as well as for aflatoxin M_1 in milk and milk-products (for details see Commission Regulation 2003/2174/EC amending Commission Regulation 2001/466/EC). According to a recent review conducted by RIVM on behalf of the FAO (FAO 2004) approximately 60 countries have set specific limits for aflatoxin M_1. The EU, the new Member States, and the EFTA countries generally apply a maximum level of 0.05 µg aflatoxin M_1/kg milk. Some countries in Africa, Asia and Latin America also propose this level. In contrast, the USA as well as some European and several Asian countries accept a maximum level of 0.5 µg/kg aflatoxin M_1 in milk, which is also the harmonized MERCOSUR limit applied in Latin America. The 0.5 µg/kg limit for aflatoxin M_1 has also been adopted by the Codex Alimentarius (Codex Alimentarius, 2001). Thus, the maximum permitted level of aflatoxin M_1 in milk in the EU (Commission Regulation 2003/2174/EC) is among the lowest in the world, and is based on the ALARA (*As Low As Reasonably Achievable*) principle.

Considering the carry over into milk and the established adverse effects on animal health, approximately 45 countries have set specific levels for aflatoxin B_1 in feed for dairy animals (for details see FAO/WHO report 2004, and Van Egmond and Jonker, 2003). To support compliance with the maximum levels in milk intended for human consumption, stringent maximum levels were also set in the EU for feedstuffs which might be consumed by dairy cows (Directive 2002/32/EC). A limit of 0.005 mg/kg feed for dairy cattle is applied in the EU countries and in the new member states as well as in EFTA countries, but only in few countries outside Europe. This level is below the no-effect level in target animals.

4. Occurrence of aflatoxin B_1 in feed materials in Europe

The EU Directive 2002/32/EC concerning undesirable substances in animal feed identifies 'groundnut, copra, palm kernel, cottonseed, babassu, maize and products derived from processing thereof' as specific named feed components, and therefore for these materials there is a presumption of their having a potential for aflatoxin contamination, and a specific limit is accordingly applied.

In the UK between 1987 and 1990, 607 samples of feedstuff raw materials and compound feeds were analysed for aflatoxin B_1 (Ministry of Agriculture, Fisheries and Food, 1993). None of the samples contained aflatoxin B_1 above statutory limits with the highest aflatoxin B_1 concentrations being found in the raw materials. In the 1987/1988 survey (Ministry of Agriculture, Fisheries and Food, 1993), six sunflower seed samples contained 21-30 µg/kg of aflatoxin B_1, and four rice-based samples contained between 10-19 µg/kg. In the 1989/1990 survey (Ministry of Agriculture, Fisheries and Food, 1993), four cottonseed samples from Argentina contained 23-26 µg/kg, one sample of palm kernel from Nigeria contained 22 µg/kg, one sample of sunflower from India contained 24 µg/kg and three samples of rice/rice bran from India and Pakistan contained 10-17 µg/kg of aflatoxin B_1. The highest levels of contamination of feed components with aflatoxin B_1 were found in raw materials imported from India, other parts of Asia and South America (Ministry of Agriculture, Fisheries and Food, 1993). In 1992, a further 330 raw ingredients used for animal feed were analysed for a number of mycotoxins (Scudamore *et al.*, 1997), with the following components found to be contaminated with aflatoxin B_1: palm kernel (1-11 µg/kg); sunflower cake (1-15 µg/kg), maize gluten (1-47 µg/kg); maize germ (1-17 µg/kg); cottonseed 5-20 µg/kg); rice bran (1-13 µg/kg) and soy bean meal (1-4 µg/kg). No samples of peas, beans or manioc were found to be contaminated. In 1999 out of 139 samples of raw maize (destined for processing for either human consumption or animal feed) monitored for aflatoxin B_1 at UK entry ports or at large mills, 11 samples were found to have aflatoxin B_1 levels above 2 µg/kg (Ministry of Agriculture, Fisheries and Food, UK, 1999).

In Germany from January to August 2000, some 90 single feed and 53 mixed dairy concentrates were analysed for aflatoxin B_1 together with 3618 samples of tank milk being examined for aflatoxin M_1 (Blüthgen and Ubben, 2000). The single feeds contained <0.3-3.4 µg/kg and the mixed dairy concentrates contained 0.1-1.4 µg/kg of aflatoxin B_1.

Extracted copra, peanut cake, sunflower cakes and corn gluten were considered to be the most important carriers of aflatoxin B_1.

Eighty samples of animal feed of different origins collected from factories in Portugal were screened for aflatoxin B_1 (Martins and Martins, 1999). 36 samples were found to contain levels varying between 0.1-16 µg/kg.

In Poland some 1120 samples of oats, wheat, rye, barley and maize delivered for processing into mixed feeds were collected from 1975-1979 and some 625 samples of commercial mixed feeds and concentrates were collected in 1976 (Juszkiewicz and Piskorska-Pliszcynska, 1992). Aflatoxin B_1 was not found in any of the samples of barley, wheat, rye or oats, but was detected in 4% of the maize samples. Protein concentrates were found to contain aflatoxin B_1 from 5 to 500 µg/kg but with the more heavily contaminated samples being destined for pig rations.

Although much of the above mentioned surveillance data on animal feed is not very recent, there is no evidence to suggest that the situation has changed in the past few years with respect to occurrence of aflatoxins in feed or feed components. Supporting evidence regarding contamination of feed can be obtained from aflatoxin M_1 surveillance data (see below).

5. Feed materials at risk for high contamination

With respect to imported feeds, extracted copra, peanut cake, sunflower cakes and corn gluten were considered to be the most important carriers of aflatoxin B_1 (Blüthgen and Ubben, 2000). Scudamore *et al.* (1997) identified palm kernel, sunflower cake, maize gluten, maize germ, cottonseed, rice bran and soy beans to be contaminated with aflatoxin B_1. The likelihood for contamination of the above-mentioned feed materials relates also to the geographic origin.

With respect to feed materials originating from Europe, only few data are available, as aflatoxin formation was previously considered to occur mainly in geographic regions with a tropical or subtropical climate. However, recently some reports have challenged this general assumption. In an Italian study (Vallone and Dragoni, 1997) conducted over a 4-month period, samples taken from a corn silage trench were found to have levels of aflatoxin B_1 ranging from 25 to 40 µg/kg. This aflatoxin formation seems to be related to

the process of ensiling, where under unfavourable circumstances high temperature can develop followed by mould growth and subsequent toxin formation. When formic acid was used as an antifungal compound on stored grain, levels of > 400 µg/kg of aflatoxin B_1 were subsequently detected (Pettersson *et al*., 1989) but not when propionic acid was used. In consideration of these findings, the use of formic acid for this purpose has been discouraged in the EU since 1999. This measure may not be applied in the new Member States and outside the EU.

In a very recent study (Pietri and Diaz, 2003), the formation of aflatoxin B_1 has been observed in samples of maize, originating from the Po valley in Italy. High temperatures, drought and strong insect damage were conductive for *A. flavus* to grow and to produce aflatoxins. Subsequently, milk samples taken at the farm level in that region in early autumn 2003 exceeded the 0.05 µg/kg limit as a result of incorporating this locally grown contaminated maize into the ration of dairy cows.

In conclusion, the overall assumptions that aflatoxins occur only in imported feeding stuffs, used in the concentrates for dairy cattle, and that subsequently a strict monitoring of imported feed materials would provide sufficient protection with respect to animal health and towards undesirable aflatoxin M_1 concentrations in milk, is challenged by these recent findings. Moreover, previous evidence (Veldman *et al.*, 1992) already suggested, that high yielding dairy cows might represent a category of animals in which a higher carry over rate of aflatoxin M_1 into milk, may result in milk concentrations at the level of individual animals, or farms that exceed the current EU limits.

6. Adverse effects on animal health

Aflatoxin B_1 is a strong acute toxin in various animal species (Eaton and Groopman, 1994). The principal target organ is the liver, were the reactive aflatoxin 8,9-epoxides induce hepatocellular damage (a detailed evaluation of the cellular toxicity is presented by JECFA, 1998). The observable differences in the susceptibility of individual animal species are related to the rate of epoxide formation and the relative activity of phase II metabolism, yielding non-toxic conjugates. Bovine species are generally less sensitive compared to non-ruminants because aflatoxins are partly degraded by the forestomach flora. Clinical signs in animals, associated with aflatoxin exposure consist of anorexia, icterus, depression, weight loss, nasal discharge, gastrointestinal affections, haemorrhages, ascitis and pulmonary oedema.

In cattle, clinical signs occurred after exposure to concentrations of 1.5 – 2.23 mg/kg feed, and in small ruminants after exposure to > 50 mg/kg feed (Miller and Wilson, 1994). Post-mortem examination of exposed animals revealed liver cell damage (centrolobular necrosis) and bile duct proliferation as well as kidney lesions. Blood biochemical parameters were altered reflecting the degree of liver damage. Decreasing milk production of exposed animals, and a photosensitising effect can precede gross clinical signs of intoxication (Miller and Wilson, 1994). As mentioned above, the low susceptibility to aflatoxins can be explained by pre-systemic elimination by the rumen flora of cattle.

Considering these findings, it can be concluded that at the given permissible level of 0.020 mg aflatoxin B_1/kg in feed materials (Directive 2002/32/EC) no acute adverse health effects in dairy cattle and other ruminants are to be expected.

Experimental and field studies indicated that long term exposure to relatively low concentrations of aflatoxins, however, may result in hepatic fibrosis and liver cell tumours. Hepatic carcinomas could be induced in birds, trout, pigs, and sheep. However, tumour formation in connection with Aflatoxin B_1 contaminated feed has not been reported in animals under European farm conditions. Therefore, tumour formation in farm animals is not considered to be a relevant endpoint of toxicity.

Aflatoxins are known to impair the cellular and humoral immune system, rendering animals more susceptible to bacterial, viral, fungal and parasitic infections. This immunosuppressive effect impairs also acquired resistance following vaccination, and may occur at a sub-clinical level of intoxication. Whereas acute clinical intoxications are rarely seen under the conditions of modern agricultural practise, sub-optimal weight gain, lower milk and egg production, as well as an increased susceptibility towards infectious diseases may lead to considerable economic losses in animal production due to aflatoxin exposure (Shane, 1994). The Panel concluded that for these effects, a no-effect level could not be defined from the available data. However, the Panel noted that the margin between toxic doses (> 1.5 mg/kg feed) and the statutory limit (0.020 mg/kg feed) of at least 75-fold would provide adequate protection from these effects.

7. Aflatoxin M_1 in dairy milk

In ruminants, a considerable part of the ingested aflatoxin B_1 is degraded in the rumen and does not reach systemic circulation. The absorbed fraction of aflatoxin B_1 is extensively metabolised in the liver, resulting predominantly in aflatoxin M_1, which enters the systemic circulation or is conjugated to glucuronic acid, and subsequently excreted via bile. Circulating aflatoxin M_1 can be excreted via the kidneys or appears in milk. Previously, the excreted amount of aflatoxin M_1 in the milk of dairy cows was estimated to represent 1-2 % of the ingested aflatoxin B_1 (Van Egmond, 1989). This carry-over rate, however, can vary in individual animals, from day to day and from one milking to the next as it is influenced by various (patho-)physiological factors, including the feeding regime, health status and individual biotransformation capacity, and finally by the actual milk production. For high yielding dairy cows with a production of up to 40 litres of milk per day, carry-over percentages as high as 6.2 % have been reported (Veldman *et al*, 1992) (see for details chapter 7).

However, recent data on aflatoxin M_1 in milk samples as collated in Table 1 and originating from different EU Member States show that the prevalence of aflatoxin M_1 contaminated samples seems to be very low. From the summed data (11,831 samples), the incidence of occurrence of samples above the statutory limit of 0.05 μg/kg was only 0.06%. Most of the data are based on bulked milk samples. Data from individual farms (280 samples) show a higher incidence of occurrence of samples above the statutory limit (1.8%). It is interesting to note that a ewes' milk sample was amongst those found to contain aflatoxin M_1 levels above the statutory limit. The Panel noted that the tight restrictions on controlling aflatoxin B_1 in feed intended for dairy cattle may not be applied in the same way for feedstuffs intended for ewes.

As mentioned in chapter 5, recent information from Italy has indicated that in two regions the prevalence of aflatoxin M_1 in milk samples above the statutory limit is increased as a result of incorporating home-grown maize into the animal feed (6% of tested samples in the first half of 2003, rising to 7.8% in July/October 2003) (RASFF News Notification 03/87) 2003).

Table 1: Aflatoxin M_1 in dairy milk (μg/kg of milk): Results of surveys conducted by EU member states

Country	Survey Year	Total no. samples	No. from individual farms	Aflatoxin M_1 in range µg/kg			Ref.
				<0.01	0.01-0.05	>0.05	
UK	2001	100	50	97	3	0	Food Standards Agency, 2001
Portugal	1999	102	31	43	57	2[a]	Martins & Martins, 2000
Spain	2000/2001	92	92	89	3	0	Rodriguez *et al.*, 2003
Italy	1996	161	0	148	13	0	Galvano *et al.*, 2001
Greece	1999/2000	166	52	92	71	3[b]	Roussi *et al.*, 2002
Greece	2000/2001	132	55	80	50	2[c]	Roussi *et al.*, 2002
Germany	1999	6537	unknown	6325	211	0	NN
Germany	2000	3618	0	3614	4	0	Blüthgen and Ubben, 2000
Cyprus	1992-2003	270	unknown	244	26	0	Ioannou *et al*., 1999
Austria	1999	20	unknown	20	0	0	NN
Finland	1999	296	unknown	295	1	0	NN
France	1999	234	unknown	234[d]	0	0	NN
Ireland	1999	62	unknown	60[e]	0	0	NN
NL	1999	30	unknown	25	5	0	NN
Sweden	1999	11	unknown	11	0	0	NN

[a] UHT whole milk and UHT semi-skimmed samples

[b] Raw dairy and concentrated milk

[c] Raw dairy and raw sheep milk

[d] Not detectable with a limit of detection of 0.03 µg/kg

[e] Not detectable with a limit of detection of 0.02 µg/kg

NN Data provided to the European Commission by EU Member States

8. Aflatoxin B_1 carry over rates and milk concentrations in high yielding cows

Initially, the carry over rate for aflatoxins from contaminated feeds into milk of dairy cows was considered to average 1 – 2%, as mentioned above. In high yielding cows, however, changes in the plasma - milk barrier and the consumption of significantly higher amounts of concentrated feeds might result in a higher carry over rate of aflatoxin M_1 into milk. This hypothesis was supported by Veldmann *et al.* (1992) who reported a percentage of up to 6.2% of the given dose of aflatoxin B_1 being excreted in milk. Therefore, with the aim of estimating the theoretical possibility of undesirable high aflatoxin M_1 levels in milk exceeding the set maximum concentration of 0.05 μg/kg, the worst case situation was modelled assuming

- occurrence of aflatoxin B_1 consistently at the maximum permissible level in feed concentrates
- the maximum concentrate intake of a high yielding dairy cow
- the contamination of the basic feedstuffs used in the rations of dairy cows at the maximum permissible level

Using these parameters, calculations were performed for the major milk producing animal species, comprising not only dairy cattle, but also small ruminants like sheep and goats, as well camels and buffalos, which might also deliver milk to European consumers. Animal feed consumption data for all these species were based on averages as reported previously (see reports of the Scientific Committee on Animal Nutrition). Data of these calculations are summarized in table 2.

Table 2: Estimated concentrations of aflatoxin M_1 in milk of various animal species considering a carry-over rate of either 6% (reported level for high yielding cows) or 2% (assumed average level).

Species	Case	Milk kg/d	Total feed intake kgDM/d	Compl. feeds in kgDM/d	Feed mat. in kgDM/d	Compl. feeds AFB_1 µg/kg	Feed mat. AFB_1 µg/kg	AFB_1 intake µg/d	Carry over	AFM_1 µg/kg milk
Cattle	A	50	26.0	19.5	6.5	5.0	20.0	227.5	0.06	0.27
	B	25	17.5	7.0	11.5	5.0	20.0	265.0	0.02	0.21
	C	25	17.5	7.0	11.5	5.0	0.0	35.0	0.02	0.03
Sheep	A	4	4.5	3.3	1.2	5.0	20.0	40.5	0.06	0.61
	B	2	3.0	1.0	2.0	5.0	20.0	15.0	0.02	0.45
	C	2	3.0	1.0	2.0	5.0	0.0	5.0	0.02	0.05
Goat	A	6	6.0	4.0	2.0	5.0	20.0	60.0	0.06	0.60
	B	3	4.0	1.5	2.5	5.0	20.0	57.5	0.02	0.38
	C	3	4.0	1.5	2.5	5.0	0.0	7.5	0.02	0.05
Camel	A	20	15.0	10.0	5.0	5.0	20.0	150.0	0.06	0.45
	B	10	10.0	3.0	7.0	5.0	20.0	155.0	0.02	0.31
	C	10	10.0	3.0	7.0	5.0	0.0	15.0	0.02	0.03
Buffalo	A	10	12.0	2.0	10.0	5.0	20.0	210.0	0.06	1.26
	B	5	10.0	1.0	9.0	5.0	20.0	185.0	0.02	0.740
	C	5	10.0	1.0	9.0	5.0	0.0	5.0	0.02	0.02

Case A represents a high performance animal exposed to the maximum level of aflatoxin B_1 in complementary feedstuffs and feed materials, according to Directive 2002/32/EC. The carry-over rate is set at 6 %.

Case B represents an animal with an average performance exposed to the maximum level of aflatoxin B_1 in complementary feedstuffs and feed materials according to Directive 2002/32/EC. The carry over-rate is set at 2 %

Case C represents an animal with average performance exposed to the maximum level of aflatoxin B_1 in complementary feedstuffs according to Directive 2002/32/EC but without additional exposure to aflatoxin B1 via feed materials. The carry over-rate is set at 2 %.

Data presented in table 2 demonstrates that under worst-case conditions, i.e. in cases A and B, the current maximum permissible level of aflatoxin M_1 in milk of 0.05 µg/kg, might be exceeded in all species. It should be emphasized that these calculations

represent a worst-case situation, thus neglecting many basic principles of nutrient balance and energy supplies for the mentioned animals species.

Previously, Pettersson (1998) presented a comparable model calculation using all published data since 1985 (10 observations form 5 controlled experiments) on the carry over of aflatoxin into milk of cows, fed complementary feedstuffs containing aflatoxin concentration around the maximum permitted levels. The carry over rate could be expressed by the following equation ($r^2 = 0.915$) (figure 2):

$$\text{Aflatoxin } M_1 \text{ (ng/kg milk)} = 10.95 + 0.787 \text{ x } (\mu\text{g aflatoxin } B_1 \text{ intake per day})$$

Expanding the data analysis to all trials in which daily feeding contained less than 150 µg/kg aflatoxin B_1 (in total 21 observations form 6 individual studies), but neglecting individual milk yields, a lower regression coefficient was found ($r^2 = 0.417$).

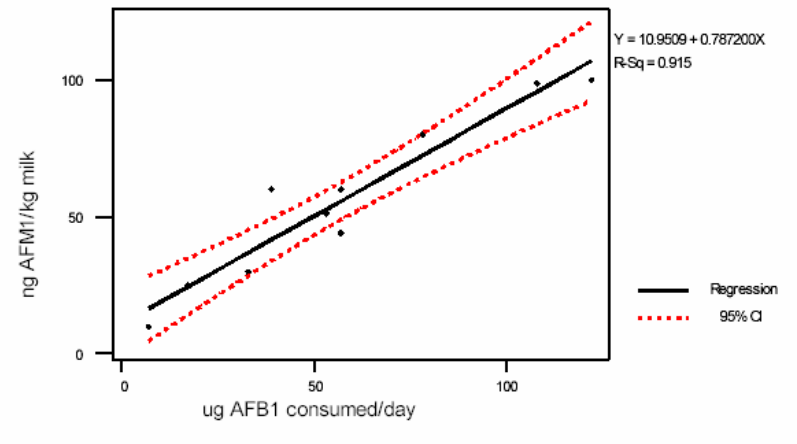


Figure 2: *Regression analysis of the carry over of aflatoxins into milk including all reported data obtained from studies with an aflatoxin B_1 intake of < 150 µg/day (with permission from Pettersson et al., 1998)*

From these calculations the Panel concluded, that the likelihood of aflatoxin M_1 contamination exceeding the current maximum level for milk is not very likely to occur in dairy cattle, but, in turn, cannot be entirely excluded. This means that at least theoretically a dairy farmer cannot rely in all cases on existing maximum levels for aflatoxin B_1 in feed materials to provide 100% assurance that milk from individual animals will comply with the statutory limit of 0.05µg/kg of aflatoxin M_1. The

probability of this occurring is remote, as indicated by the current surveys presented in Table 1, but nevertheless the possibility exists with high producing animals.

For the other animal species addressed, including sheep, goats, camels and buffalos, a more precise estimate of daily exposure is necessary, using contamination data from more commonly used feedstuffs for these species. In a generalized approach, concentrations of aflatoxin M_1 in milk of these species could exceed the statutory levels regularly. A more precise exposure assessment is also needed for goats, camels and buffalos because their milk and products thereof need not only to comply with the maximum level established for aflatoxin M_1 in milk, but also with the maximum level of 0.025 µg/kg of aflatoxin M_1 in infant formulae and follow on formulae.

CONCLUSIONS

The national surveys of animal feed components imported or traded in the EU, indicated a high degree a compliance with the existing legislation. This is confirmed by data on the occurrence of aflatoxin M_1 in dairy milk that shows an equally low level of exceedance (0.06%) of the statutory limit, even with milk from individual farms (1.8% exceedance). Consequently, the current maximum levels of aflatoxin B_1 in animal feeds not only provides adequate protection from adverse health effects in target animal species, but more importantly seem to successfully prevent undesirable concentrations of aflatoxin M_1 in milk. However, most of the monitored samples represented bulked milk, and data analysis also revealed a higher prevalence of positive samples at the individual farm level (amounting to 1.8% of the analysed farm samples).

As it was generally acknowledged that the risk for aflatoxin B_1 contamination is only high in geographical regions with a tropical or sub-tropical climate, the monitoring of feed ingredients for the presence of aflatoxin B_1 focussed, as of yet, on imported feeds, such as extracted copra, peanut cake, sunflower cakes, corn gluten, rice bran, cottonseed, palm kernel and soy beans as these seemed to be the most important carriers of aflatoxin B_1 . However, in 2003, Italy had to report for the first time an increase in the number of milk samples exceeding the statutory limit. Contamination rates above the legal limit amounted to 6% and 7.8%, respectively, in certain regions. These higher contamination rates were linked to a high contamination of locally grown maize that was used as animal feed.

Model calculations for a worse case situation, assuming exposure of dairy cattle to aflatoxin B_1 at the current EU permissible levels in feeds, indicated the possibility of milk contamination exceeding the maximum level. This is particularly the case if animals are exposed to aflatoxins not only via feed concentrates, but also from their basic feeds (roughage, silage). Moreover, milk from high yielding cows may contain Aflatoxin M_1 amounts exceeding the statutory levels, as these animals consume an unusually high amount of feed concentrates, and adaptive physiological alterations (modification of the blood-milk barrier at very high initial milk production) may increase the carry over rate to 6%. It needs to be emphasised, however, that this represents a worse case situation, and that practical experience indicates that this is a rarely occurring situation .

RECOMMENDATIONS

- Monitoring of aflatoxin B_1 in imported feedstuffs (concentrates) and aflatoxin M_1 in dairy milk, should be encouraged in all EU member states. In consideration of the recent data from Southern Europe, monitoring programmes for feed materials, especially maize and products thereof, should be extended to cover materials originating from those parts of Europe, where a subtropical climate and extensive agricultural practice favour fungal growth and subsequent formation of aflatoxins.

- Actual data on the carry-over rate of aflatoxins into milk should be generated in consideration of modern production systems, high performance animals, and the subsequent amendments in modern animal nutrition.

- Monitoring of milk samples should be expanded to milk and milk products from animal species others than dairy cows, as model calculations revealed a potential high risk of contamination.

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