



### AFLATOXINS - ANALYSIS AND RISK MANAGEMENT

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#### ABSTRACT

**Objective:** The purpose of this study is to analyze and assess the risk associated with the presence of aflatoxins in food and feed. Aflatoxins are produced by *Aspergillus flavus* and *Aspergillus parasiticus* and they are distributed worldwide. **Conclusions:** Aflatoxins are a potential danger for both animal and human health. Attention should be paid to the potentially dangerous synergistic or additive effects on animal health arising from the presence of more than one mycotoxin in feed. Given the ubiquity of mycotoxins in the world, it is also necessary to develop an effective program of risk management, which will be crucial to reduce costs and economic losses in farms associated with the risk of pollution.

**Keywords:** Aflatoxins, feed, carcinogenic effect, risk assessment and management

#### INTRODUCTION

Aflatoxins are carcinogenic metabolites of microscopic fungi *Aspergillus flavus* и *Aspergillus parasiticus* [4] and they are produced during growth, harvesting and storage of cereals and e distributed worldwide [18]. Four types of aflatoxins found in natural conditions: B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> и G<sub>2</sub>, the family of aflatoxins includes about 17 compounds [4].

##### Objective

The purpose of this study is to analyze and assess the risk associated with the presence of aflatoxins in food and feed.

##### Distribution

According to the report of the EFSA [8] in Italy during the period 1982-2007, were analyzed 3607 samples of maize to aflatoxins, and in 2003 the higher level were recorded at 155 ppb. In Turkey during the period 1982-2006, there were a total of 423 maize samples tested for the presence of corn aflatoxins as the maximum level was found in 2006 – 432 ppb.

In 1998 proved contamination of maize in the southeastern part of the United State, aflatoxin contamination reached 1500 ppb (5 times the 300 ppb – the highest margin in feed set by U.S. Food and Drug Administration [10].

In Kenya in 2004-2005 has been demonstrated in human acute aflatoxicosis [26], after consumption of maize contaminated with aflatoxins, causing death by over 150 people [3, 19].

In India, China and Thailand are cases of human aflatoxicosis [2], these countries represent geographic areas where climatic conditions allow the development of fungi and aflatoxins formation, while at the same risk of chronic exposure to aflatoxins [2, 34, 35].

European Authority for Food Safety (EFSA) on the basis of Eurostat data and data on climate published scientific report on "Modelling, predicting and mapping the emergence of aflatoxins in cereals in the EU due to climate change", it is model used to predict the development of *Aspergillus flavus* in maize, wheat and rice for the period 2011-2012, according to the EFSA report at risk of exposure to aflatoxins are Spain, Italy and Greece and Portugal, France, Bulgaria and Romania have been established as being at increased risk of occurrence of aflatoxins. EFSA opines that produce maps on the distribution of aflatoxins in Europe will be helpful to take preventive measures and prevent the accumulation of excessive amounts of aflatoxin B<sub>1</sub> in raw grain [24, 33].

In the European Union has established a rapid alert system for food and feed (Rapid Alert System for Food and Feed - RASFF), in order to prevent human and animals for adverse effects or potential risks arising from food, feed and materials intended for contact with food by providing rapid information exchange between Bulgaria, member states and the European Commission. RASFF members are 33 countries in Europe (including Bulgaria). In recent years, reduction of the number of all the signals (notifications) for aflatoxins in food and feed. In 2011 the number of notifications received 585: cereal products – 13; feed – 119; fruits and vegetables – 78; herbs and spices – 51; nuts and seeds – 320, others – 4. In 2012 – appear cases of aflatoxin M<sub>1</sub> in milk – 5 notifications [20].

#### MECHANISM OF ACTION

The primary route of entry of aflatoxins into the body is through the digestive system. Through the blood and lymph aflatoxins penetrate internal organs and tissues in the liver implement the biotransformation of aflatoxins, which aflatoxin B<sub>1</sub> is metabolized by the cytochrome P 450 enzyme system to the major carcinogenic metabolite AFB<sub>1-8,9</sub> epoxide (AFBO) [11] or less mutagenic forms such AFM<sub>1</sub>, Q<sub>1</sub>, P<sub>1</sub> [7, 25].

There are several ways in which AFBO can be metabolized and have resulted in the development of cancer, toxicity or removed from the body (Fig.1 [32, 36]). AFBO has the ability to bind covalently with cellular macromolecules (proteins and DNA) [9], forming an AFB<sub>1</sub>- guanine and AFB<sub>1</sub> – albumin [23], resulting in the formation of gene mutations and cancer development. The formation of AFB<sub>1</sub> - DNA guanine in liver is critical for the carcinogenic effect of AFB<sub>1</sub>, as a result of mutations in key genes [13]. One such mutation found in the human p53 gene at codon 249. Studies show that AFBO causes transition from guanine to thymine at 3 nucleotide codon mutation making it a "hot spot." This mutation occurs with greater frequency among patients with hepatocellular carcinoma in areas with high exposure to aflatoxins [12]. Each year there have been 550,000 to 600,000 new cases of the disease [31], with 83% mortality was observed in East Asia and Africa [14, 28], the majority of patients with chronic hepatitis B and C infection [17].

Currently, there are modern analytical methods for determination of aflatoxin characterized by high accuracy and precision [27]. They are related to the use of specific antibodies and their involvement in enzyme-linked immunosorbent analysis [29] and the use of immunoaffinity columns for the purification of the sample (HPLC).

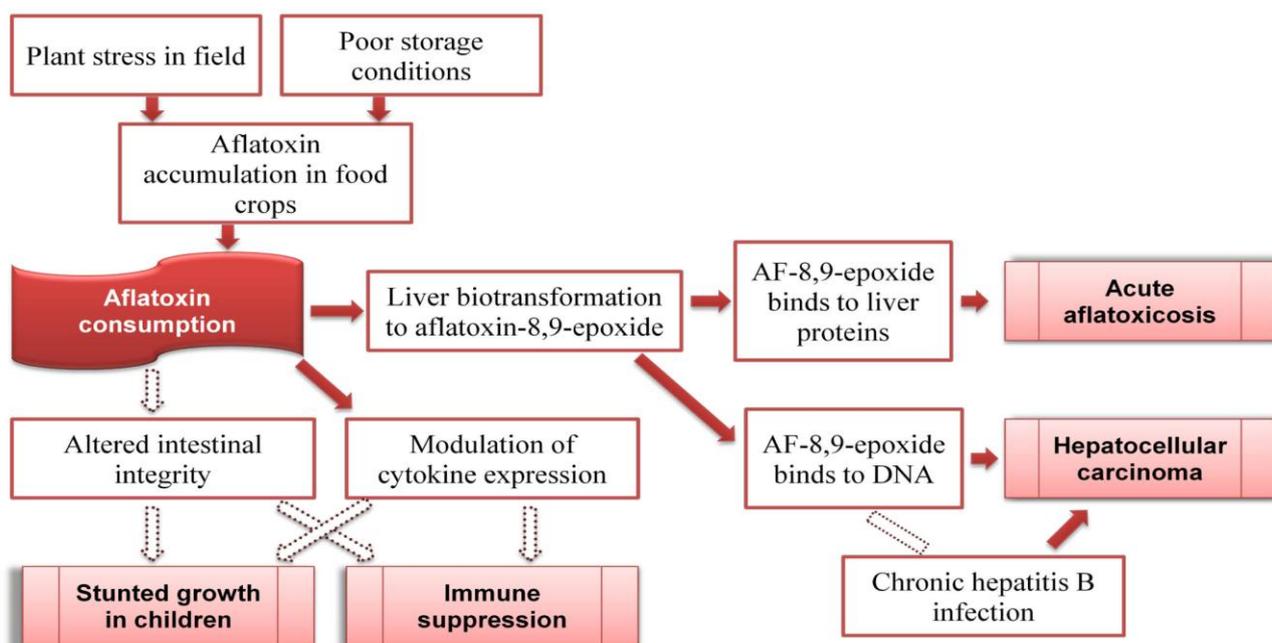


Figure 1: Mechanism of action of aflatoxins [32]

Source: Wu, 2010 [32] and Bbosa G.S., Kitya D., Lubega A., Ogwal-Okeng J., William W. Anokbonggo and David B. Kyegombe Review of the Biological and Health Effects of Aflatoxins on Body Organs and Body Systems Agricultural and Biological Sciences » "Aflatoxins - Recent Advances and Future Prospects", book edited by Mehdi Razzaghi-Abyaneh, ISBN 978-953-51-0904-4, Published: January 23, 2013 under CC BY 3.0 license. [36].

A major problem in proving aflatoxins is that they are not homogeneously distributed in foods, both animals and humans. Exposure to aflatoxins depends both on the quantity in different foods, and the manner of their admission. There are large national and regional differences in food intake, so that the exposure assessment is specific to each country and an obstacle to its uniformity.

Assessment of exposure to aflatoxins can be further specified by providing potential changes in their levels in foods actually consumed by include information on the production and processing (industrial or domestic). Exposure assessment can be based on the average intake of aflatoxins from the general population of individuals with a value at the 90th percentile of the general population, or to evaluate only those individuals who actually consume food contaminated with aflatoxins. Exposure can be made for different age groups or target, depending on the scenario of the study (i.e. sharp intake compared with chronic intake). The exposure varies depending on the age of the subjects included in the study. Small children, in general, have a much higher risk (%) as compared to adults, for consumption of products containing aflatoxins or their metabolites - milk (up to seven times) and peanut oil (four times). Assessment of exposure to aflatoxins and their metabolites may be based on the measurement of specific biomarkers, taking calculated on the basis of pharmacokinetic interactions [22].

It is necessary to pay attention to the potential danger to human health arising from the presence of aflatoxins in foodstuffs of animal origin. In the presence of high levels of aflatoxin in feed could lead to the development of the illness or death of productive animals (acute or chronic aflatoxicosis). At lower levels in feed, aflatoxins may not significantly affect the animal organism, but the presence of residues pose a danger to human health. It was found that the risk to the person associated with indirect exposure to aflatoxins found in food products of animal origin is usually slightly

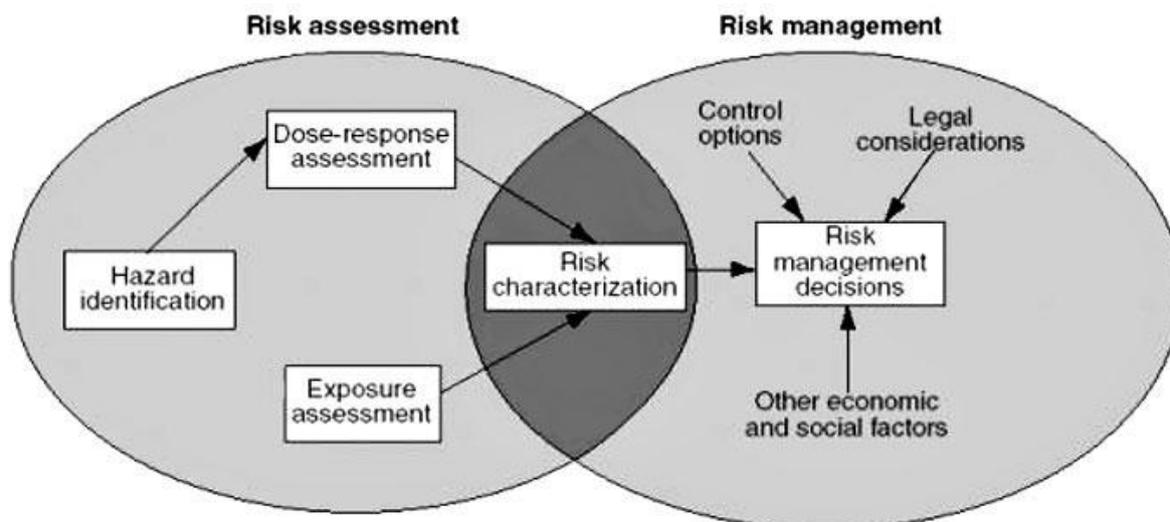
lower than the direct exposure of cereals or other foods containing aflatoxin [15, 16].

Risk characterization is qualitative and/or quantitative evaluation, including accompanying uncertainty and the burden and the likely occurrence or absence of known or potential adverse health effects on the population at risk of exposure to aflatoxins. Risk characterization is qualitative and/or quantitative evaluation, including accompanying uncertainty and the burden and the likely occurrence or absence of known or potential adverse health effects on the population at risk of exposure to aflatoxins.

Risk characterization is based on three main components: hazard identification, hazard characterization and exposure assessment. Risk characterization can also be establishing levels of daily exposure in which the risk is negligible lifetime (i.e. exposure should be below tolerable daily intake - TDI or another measure of safe dose). The latter determination can be relevant, taking into account the uncertainties (Fig. 2, [37]).

For substances which TDI cannot determine the safety margin between human exposure and adverse effects in animal species can be used as an indicator of the likelihood of human diseases, which can be used to manage the risk.

Along with the average population, the risk characterization for aflatoxins should also consider those groups that are most vulnerable to exposure as children (due to their lower body weight), and other groups that may be a difference in bioavailability, metabolism or genetic predisposition, and the elderly. In this respect, the adequacy of the safety factor of ten times in order to eliminate the differences in sensitivity between individuals arising from variability should be examined and assessed separately. Detailed risk assessments in relation to aflatoxins are periodically reviewed to receipt of new information in terms of exposure and the mechanism of action [5].



**Fig. 2 Risk assessment and risk management**

Source: Courtesy United States Environmental Protection Agency, Office of Research and Development [37]

In terms of mycotoxins, there is a variety of risk management to ensure food safety. They range from the prevention of mold growth and the establishment of regulatory constraints, a shift to alternative uses. All these features are associated with huge economic costs.

One of the obstacles to harmonization in the field of trade refers to the establishment of maximum residue limits (MRLs,) in relation to

different types of mycotoxins. Regulations are in over 100 countries on 13 kinds of mycotoxins. In Europe, thirty-nine states have some regulations (99% of the region's population). In EU harmonized limits for aflatoxins as a place restriction on aflatoxin B1 in feed. Directive 1999/29/EC is replaced by Directive 2002/32/EC of the European Parliament and the Council on undesirable substances in animal feed, being the maximum acceptable concentrations of aflatoxin B1 in feed materials, complete and complementary feeding stuffs (Annex 1) [8]

**Table 1: Maximum aflatoxin B1 (mg/kg) in feed [1]**

Undesirable substances	Products intended for animal feed	Aflatoxin B1 Maximum content in mg/ kg (ppm) relative to a feed with a moisture content of 12%
Aflatoxin B <sub>1</sub>	All feed materials	0,02
	Complete feeding stuffs for cattle, sheep and goats except:	0,02
	- Complete feed for dairy animals	0,005
	- Complete feeding stuffs for calves and lambs	0,01
	Complete feed for pigs and poultry (except young animals)	0,02
	Other complete feeding stuffs	0,01
	Supplementary feed for cattle, sheep and goats (except complementary foods for dairy animals, calves and lambs)	0,02
	Complementary feed for pigs and poultry (except young animals)	0,02
	Other complementary feed	0,005

Commission Regulation 165/2010 are set maximum concentration limits for aflatoxin B1, a sum of B1, B2, G1 and G2, aflatoxin M1 in foods for people.

MRLs now usually based on scientific assessments. They cannot be exceeded in goods traded on the market, but the levels of mycotoxins slightly above these levels can be tolerated on the basis accident (chance). A large percentage of incidences of aflatoxins are well below the MRL. Exports of goods is an ongoing process, so it is not possible levels should be based on the weighted average of the actual levels, adjusted for several goods, or to revise the permissible levels for different products based on annual data. In the final assessments of risk assessment of exposure to aflatoxins can be based on actual residue levels (not

MRL), a worst case scenario. The concern of regulators is that if allowed higher soynosti for MRL, it will be an acceptable level for the industry and there would be a change in the upward direction to reach that level that this will lead to increased exposure [30].

Carcinogens for which no thresholds of toxicity are not permitted as food additives. When these chemicals cannot be completely avoided (as in the case of certain mycotoxins), using different approaches. A mathematical model is used in most of which it is assumed that the effects of low doses are linear, in order to extrapolate the possibility of side effects at lower doses (Fig. 3-SCMK [6]).

Dose corresponding risk level  $10^{-5}$  or  $10^{-6}$ , in some jurisdictions, e was assessed as presenting negligible risk. According to other

jurisdictions, the most appropriate method for regulating the genotoxic carcinogens or even genotoxic agents (eg, patulin) that the carcinogenic potential has not been proven to determine the levels that are "the lowest reasonably possible point" is (ALAR-"as low as is reasonably achievable") or as low as technologically achievable [30]

Alternative regulation can be based on biological factors such as mode of action and the burden of proof, which is certified by the severity of the resulting injury. This information can be combined with assessment of tumor potential, as is the magnitude in the TD05. Separating TD05 of uncertainty factor 5000 value was obtained which is equivalent to the level of risk of 1:100 000 and

the resulting values for safe levels are similar to those obtained using a linearized model for low doses [15, 16].

A new concept is to use a Benchmark dose (BMD). This approach finds application in both threshold and non-threshold at toxicologically compounds. Indicator BMD (Fig. 4) is defined as a dose that corresponds to a given change in adverse reaction in comparison to unexposed individuals in response and less than 95% confidence limit, called indicator of dose level. BMDL = Benchmark dose (BMD) is modeled to 10% above the critical risk. The approach is an alternative to the NOAEL - an approach that has been used for many years in a dose-response relationship. BMDL10 = 95% lower confidence limit of BMD for an additional 10% risk of a critical effect [33].

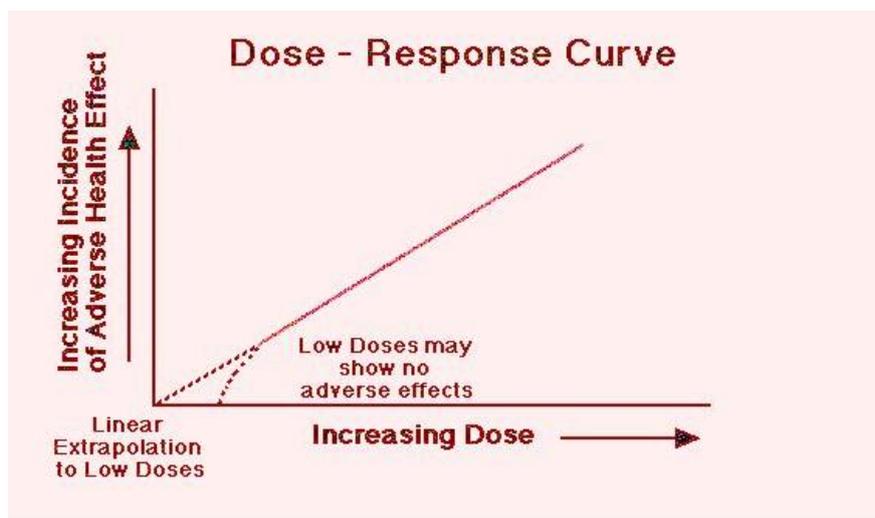


Figure 3: Extrapolation of dependence "dose-response" at low doses.

Source: CHM 110-Chemistry and Issues in the Environment [6]

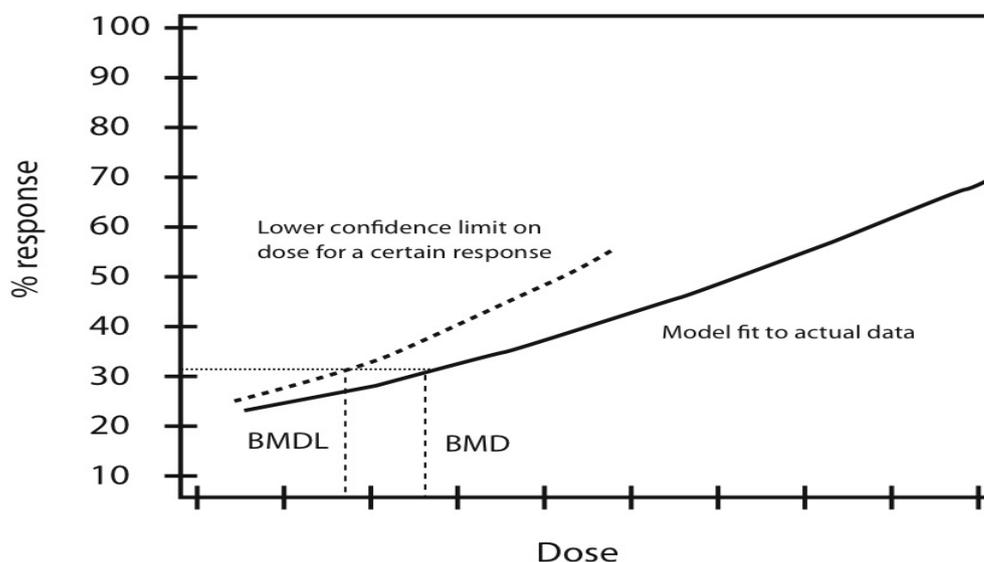


Figure 4: Benchmark dose (BMD).

Source: Pharmaceutical Safety Evaluation and Regulation  
[https://www.dlsweb.rmit.edu.au/set/LearningObjects/P3Project/module2/dr\\_ta\\_na.htm](https://www.dlsweb.rmit.edu.au/set/LearningObjects/P3Project/module2/dr_ta_na.htm) [38]

Other factors influencing legislation on aflatoxins are determining the TDI stage hazard assessment and the availability of appropriate methods of sampling and reliable methods for aflatoxin analysis.

For the purposes of risk management in the countries - members of the EU in 2010 published a guidance document for that authority shall, for the control of compliance with the legislation on aflatoxins for the official control of the EU.

## CONCLUSIONS

Aflatoxins are a potential danger for both animal and human health. Attention should be paid to the potentially dangerous synergistic or additive effects on animal health arising from the presence of more than one mycotoxin in feed. Given the ubiquity of mycotoxins in the world, it is also necessary to develop an effective program of risk management, which will be crucial to reduce costs and economic losses in farms associated with the risk of pollution (Regulation (EC) 178 / 2002) [21].

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