

Cancer Association of South Africa (CANSA)

Fact Sheet and Position Statement on Aflatoxins in Agricultural Products Intended for Human Consumption

Introduction

Aflatoxin is a naturally occurring mycotoxin produced by two types of mould: *Aspergillus flavus* and *Aspergillus parasiticus*. *Aspergillus flavus* is common and widespread in nature and is most often found when certain grains are grown under stressful conditions such as drought. The mould occurs in soil, decaying vegetation, hay, and grains undergoing microbiological deterioration and invades all types of organic substrates whenever and wherever the conditions are favourable for its growth. Favourable conditions include high moisture content and high temperature. At least 13 different types of aflatoxin are produced in nature with aflatoxin B₁ considered as the most toxic. While the presence of *Aspergillus flavus* does not always indicate harmful levels of aflatoxin, it does mean that the potential for aflatoxin production is present.

[Picture Credit: Aflatoxin in Maize]



Aflatoxin is a toxin produced by mould that can damage the liver and may lead to liver cancer (hepatocellular carcinoma) as well as other health related problems. Aflatoxins cause cancer in some animals as well.

The fungi that produce aflatoxin grow on crops such as peanuts and wheat, maize, beans and rice. Aflatoxin is a problem particularly in undeveloped and developing countries.



Aflatoxins contaminate many African dietary staples such as maize, groundnuts, rice, and cassava, particularly under certain conditions: dry weather near crop maturity, high moisture during harvest, inadequate drying and storage of crops.

[Picture Credit: Ground Nuts]

Countries in latitudes between 40°N and 40°S - which includes all of Africa - are susceptible to aflatoxin contamination. Aflatoxin contamination of key staples - maize, groundnuts and sorghum - occurs above safe levels in many African countries.

[Picture Credit: Sorghum]



Prevalence data from Africa suggests that aflatoxin contamination in maize, groundnuts and sorghum is higher than the European Union aflatoxin standard (4 ppb) and that of USA (20 ppb) in many countries. However, even aflatoxin exposure at low levels can result in measurable human health impacts.

Aflatoxin contamination in sub-Saharan Africa - aflatoxin contamination is a growing threat to trade, food and health security in sub-Saharan Africa, where smallholder farmers are challenged by food production and now climate change. Sub-Saharan Africa is annually losing more than 450 million dollars in trade revenue of major staples, particularly maize, and groundnuts as a result of contamination from aflatoxins. Africa is at risk of toxins which are linked to suppressed immunity, liver cancer in humans and stunting in children. UNICEF says 40% of children in sub-Saharan Africa are stunted or have low height for their age which can be associated with impaired brain development.

Researchers say high temperatures and drought conditions favour the growth of fungus, while poor farming practises and food insecurity status of many people in sub-Saharan Africa increase their exposure to aflatoxin contamination. In addition high soil moisture content at harvest attributed to off-season rains as a result of climate variability increases contamination.

Aflatoxins can occur in foods, such as groundnuts, treenuts, maize, rice, figs and other dried foods, spices and crude vegetable oils, and cocoa beans, as a result of fungal contamination before and after harvest.

Dhakal, A. & Sbar, E. 2020.

“Aflatoxins are metabolites produced by toxigenic strains of molds, mainly *Aspergillus flavus* and *A. parasiticus*, which grow in soil, hay, decaying vegetation, and grains. Aflatoxin toxicity occurs due to acute or chronic exposure to aflatoxin. The term "aflatoxin" is derived from the name of *Aspergillus flavus*. It was named around 1960 after its discovery as the source of a disease in turkey called "turkey X disease" in turkeys fed rations of peanuts and cottonseed. Aflatoxins form one of the major groupings of mycotoxins. Aflatoxin is produced by fungal action during production, harvest, storage, and processing of food and feed. The U.S. Food and Drug Administration (FDA) considers it to be an unavoidable contaminant of foods. Aflatoxin toxicity has been well established in both humans and animals. Aflatoxin exposure can cause nausea, vomiting, abdominal pain, convulsions acutely, and its chronic exposure can also lead to various complications like hepatotoxicity, immunotoxicity, and teratogenicity. Aflatoxin is one of the major causes of hepatocellular carcinoma in developing countries. There are different types of aflatoxin. Aflatoxin B1 (AFB1) and aflatoxin B2 (AFB2) are produced by both *A. flavus* and *A. parasiticus*, and AFB1 is believed to be the most potent among all aflatoxins. Aflatoxin M1 (AFM1) is found in the fermentation broth of *A. parasiticus*, but it and aflatoxin M2 are also developed when an infected liver metabolizes AFB1 and AFB2. AFM1 can be transmitted by milk. AFB1 and AFM1 have been classified as group 1 and group 2B human carcinogens by the International Agency for Research on Cancer (IARC).”

Caceres, I., Khoury, A.A., Khoury, R.E., Lorber, S., Oswald, I.P., Khoury, A.E., Atoui, A., Puel, O. & Bailly, J.D. 2020.

“The study of fungal species evolved radically with the development of molecular techniques and produced new evidence to understand specific fungal mechanisms such as the production of toxic secondary metabolites. Taking advantage of these technologies to improve food safety, the molecular study of toxinogenic species can help elucidate the mechanisms underlying toxin production and enable the development of new effective strategies to control fungal toxicity. Numerous studies have been made on genes involved in aflatoxin B₁ (AFB₁) production, one of the most hazardous carcinogenic toxins for humans and animals. The current review presents the roles of these different genes and their possible impact on AFB₁ production. We focus on the toxinogenic strains *Aspergillus flavus* and *A. parasiticus*, primary contaminants and major producers of AFB₁ in crops. However, genetic reports on *A. nidulans* are also included because of the capacity of this fungus to produce sterigmatocystin, the penultimate stable metabolite during AFB₁ production. The aim of this review is to provide a general overview of the AFB₁ enzymatic biosynthesis pathway and its link with the genes belonging to the AFB₁ cluster. It also aims to illustrate the role of global environmental factors on aflatoxin production and the recent data that demonstrate an interconnection between genes regulated by these environmental signals and aflatoxin biosynthetic pathway.”

Major Types of Aflatoxins and Their Metabolites

At least 14 different aflatoxins are produced in nature. Aflatoxin B₁ is considered the most toxic and is produced by both *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin M₁ is present in the fermentation broth of *Aspergillus parasiticus*, but it and aflatoxin M₂ are also produced when an infected liver metabolises aflatoxin B₁ and B₂.

The different major types of aflatoxins are:

- Aflatoxin B₁ and B₂, produced by *Aspergillus flavus* and *A. parasiticus*
- Aflatoxin G₁ and G₂, produced by *Aspergillus parasiticus*
- Aflatoxin M₁, metabolite of aflatoxin B₁ in humans and animals (exposure in nanogram levels may come from a mother's milk)
- Aflatoxin M₂, metabolite of aflatoxin B₂ in milk of cattle fed on contaminated foods
- Aflatoxicol
- Aflatoxin Q₁ (AFQ₁), major metabolite of AFB₁ in *in vitro* liver preparations of other higher vertebrates

Aflatoxins B₁, B₂, G₁, and G₂ are mycotoxins that may be produced by three moulds of the *Aspergillus* species: *A. flavus*, *A. parasiticus* and *A. nomius*, which contaminate plants and plant products. Aflatoxins M₁ and M₂, the hydroxylated metabolites of aflatoxin B₁ and B₂, may be found in milk or milk products obtained from livestock that has ingested contaminated feed.

[Picture Credit: Mycotoxins]



Of these six aflatoxins, aflatoxin B₁ is the most frequent one present in contaminated samples and aflatoxins B₂, G₁ and G₂ are generally not reported in the absence of aflatoxin B₁. Most of the toxicological data relate to aflatoxin B₁. Dietary intake of aflatoxins arises mainly from contamination of maize and groundnuts and their products.

South African Regulations Governing Tolerance for Fungus-Produced Toxins in Foodstuffs

The Minister of Health has, in terms of Section 15(1) of the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No 54 of 1972), made the following Regulations:

For the purposes of Section 29 (1)(b)(i) of the Act, in so far as it is applied to and is applicable to foodstuffs, the following foodstuffs are hereby deemed to be contaminated, impure or decayed –

- (a) Peanuts intended for further processing, which contain more than 15 µg/kg [15 ppb] of aflatoxin (total);
 - (b) All foodstuffs, ready for human consumption, which contain more than 10 µg/kg [10 ppb] of aflatoxin, of which aflatoxin B₁ is more than 5 µg/kg [5ppb];
 - (c) Milk containing more than 0.05 µg/l of aflatoxin M₁
- (Regulations Governing Tolerance for Fungus-Produced Toxins in Foodstuffs).

The European Food Safety Authority (EFSA) and Safe Levels of Aflatoxins

In 2007 EFSA's Scientific Panel on Contaminants in the Food Chain (CONTAM) provided risk managers with the scientific basis necessary to decide on the proposal of the *Codex Alimentarius* on setting maximum levels of aflatoxins in ready-to-eat almonds, hazelnuts and pistachios higher than those currently in place in Europe.

In an opinion adopted in January 2007, the CONTAM Panel concluded that increasing the current EU maximum levels of 4 µg/kg [4 ppb] total aflatoxins in these three nuts to 8 or 10 µg/kg total aflatoxins would have minor effects on the estimated dietary exposure, cancer risk and calculated margin of exposure. The Panel also concluded that exposure to aflatoxins from all food sources should be kept as low as reasonably achievable because aflatoxins are genotoxic and carcinogenic.

Moreover, the data indicated that the reduction of total dietary exposure to aflatoxins could be achieved by reducing the number of highly contaminated foods reaching the market and reducing exposure from contaminated food sources other than almonds, hazelnuts and pistachios.

In June 2009 the European Commission asked EFSA to assess the effect on public health of an increase of the maximum level for total aflatoxins from 4 µg/kg [4 ppb] to 10 µg/kg [10 ppb] allowed for tree nuts other than almonds, hazelnuts and pistachios (e.g. Brazil nuts and cashews). This would facilitate the enforcement of the maximum levels, in particular as regards mixtures of nuts.

The Panel concluded that public health would not be adversely affected by increasing the levels for total aflatoxins from 4 µg/kg to 8 or 10 µg/kg for all tree nuts. However, the Panel reiterated its previous conclusions regarding the importance of reducing the number of highly contaminated foods reaching the market.

In order to estimate human exposure in these two assessments, EFSA took into consideration occurrence data submitted by 20 Member States and third parties in 2006, as well as food consumption data obtained from the GEMS/Food Consumption Clusters Diets of the World Health Organisation, based on data of the Food and Agriculture Organisation.

In June 2009 EFSA launched a call for proposals to study the potential increase in aflatoxin B₁ in cereals in the EU as a result of climate change. The project will gather and analyse data on aflatoxin B₁ in order to build predictive models, define scenarios and create maps highlighting potential future contamination of cereal crops. The results will help to inform any future work in this area by EFSA and give an indication of potential emerging food contamination by mycotoxins in the EU due to climate change.

Aflatoxins and Human Health

Humans are exposed to aflatoxins by consuming foods contaminated with products of fungal growth. Such exposure is difficult to avoid because fungal growth in foods is not easy to prevent. Even though heavily contaminated food supplies are not permitted in the market place in developed countries, concern still remains for the possible adverse effects resulting from long-term exposure to low levels of aflatoxins in the food supply.

Aspergillus flavus seen under an electron microscope.

[Picture Credit: Cornell University]

Evidence of acute aflatoxicosis in humans has been reported from many parts of the world, namely the Third World Countries. The syndrome is characterised by vomiting, abdominal pain, pulmonary oedema, convulsions, coma, and death with cerebral oedema and fatty involvement of the liver, kidneys, and heart.

Conditions increasing the likelihood of acute aflatoxicosis in humans include limited availability of food, environmental conditions that favour fungal development in crops and commodities, and lack of regulatory systems for aflatoxin monitoring and control.



Because aflatoxins, especially aflatoxin B₁, are potent carcinogens in some animals, there is interest in the effects of long-term exposure to low levels of these important mycotoxins on humans. **In 1988, the IARC placed aflatoxin B₁ on the list of human carcinogens (Group 1).** This is supported by a number of epidemiological studies done in Asia and Africa that have demonstrated a **positive association between dietary aflatoxins and Liver Cancer (hepatocellular carcinoma).** Additionally, the expression of aflatoxin-related diseases in humans may be influenced by factors such as age, sex, nutritional status, and/or concurrent exposure to other causative agents such as viral hepatitis (HBV) or parasite infestation.

Other health effects of aflatoxins include:

- Deleterious effect on the reproductive system leading to infertility in humans exposed to chronic aflatoxin-contaminated foods - it has been reported that higher concentrations of aflatoxins occur in the semen of infertile men
- Immunosuppression - aflatoxin exposure has been shown to cause immune suppression, particularly in cell-mediated responses
- Maternal serum aflatoxin is a risk factor for jaundice in infants
- Significant associations or correlations between low birth weight and aflatoxins
- Acute exposure to aflatoxins as a cause of aflatoxicosis leading to haemorrhagic necrosis of the liver, bile duct proliferation, oedema, and lethargy
- Chronic exposure to aflatoxins leading to liver cancer
- Aflatoxins and their metabolites as well as the generated ROS have been reported to cause various cancers in different endocrine glands like pituitary gland, granulosa cell tumours of the ovary and adenomas and adenocarcinomas of the adrenal gland, kidneys, thyroid gland, ovaries, testes, thyroid gland, parathyroid glands and endocrine pancreas
- Co-occurrence of aflatoxins and hepatitis B virus (HBV) produces a synergistic effect which increases the relative risk of liver cancer
- Binding and interference with enzymes and substrates that are needed in the initiation, transcription and translation processes involved in protein synthesis
- In young children, aflatoxin exposure is associated with underweight and stunting
- Aflatoxins in the blood may promote transmission of HIV through the negative effects of aflatoxins on secretory IgA and selenium deficiency effects, viral replication, progression and general impairment of immunity
- Zinc and Vitamin A deficiency
- Increase in the risk of diarrhoea and pneumonia
- Disruption of mitochondrial functions in the various parts of the body that require production of energy in the form of ATP
- Acute dietary exposure to AFB1 has been implicated in epidemics of acute hepatic injury resulting in permanent liver damage
- Chronic forms of aflatoxicosis include teratogenic effects associated with congenital malformations, mutagenic effects where aflatoxins cause changes (mutations) in the genetic code, altering DNA and these changes can be chromosomal breaks, re-arrangement of chromosome pieces, gain or loss of entire chromosomes, or changes within a gene
- Aflatoxins have been reported to affect the various body organs like the liver, kidneys, lungs, brain, testes and many endocrine and exocrine organs, the heart, skeletal muscles and the different body systems
- Mycotoxins especially aflatoxins and its metabolites and other products such as the reactive oxygen species (ROS) like the AFB-8,9-epoxides may interfere with the normal functioning of the nerve cells by forming DNA adducts, protein adducts, oxidative stress factors, mitochondrial directed apoptosis of the nerve cells as well as inhibiting their synthesis of protein, RNA and DNA
- Aflatoxins also cause abnormalities in mitochondrial DNA, structure and function, including defective oxidative phosphorylation in brain cells
- People working in food industries as their occupational setting get exposed to aflatoxins especially AFB1 when they inhale aflatoxin-contaminated dusts like during grain shelling and processing and have been reported to have a higher incidences of upper respiratory tract and lung cancers
- Aflatoxins are reported to have serious acute effects on the cardiovascular systems including vascular fragility and haemorrhaging in tissues
- Aflatoxins and its metabolites as well as the generated reactive oxygen species(ROS) has been reported to have a deleterious effects on the bone and blood cells as well as induction of cancers

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on the haemopoietic system in bone marrow and lymphoid organs where blood, blood cells and blood components are produced

- Different parts of the nephron are exposed to aflatoxins especially the AFB₁ and its metabolites leading to nephrotoxicity before it is excreted in the urine
- Aflatoxin especially AFB has been reported to interfere with the functioning of the various endocrine gland by disrupting the enzymes and their substrates that are responsible for the synthesis of the various hormones
- There is also a problem of poverty resulting in food insecurity and thus consumption of harmful food unfit for humans

Chronic consumption of aflatoxin-contaminated foods is a common problem in both humans and animals worldwide especially in poor developing nations of south East Asia and sub-Saharan Africa where there is poor food harvesting, processing and storage of food and food products thus allowing the growth of mould on them. Aflatoxins, their metabolites, the aflatoxin-8,9-epoxide and the generated ROS causes deleterious effects on the various body organs and body systems including the development of cancers especially the liver cancer mainly due to AFB₁ exposure.

Aflatoxins are also responsible for the suppression of both the humoral and cell-mediated immunity and thus making individuals susceptible to infectious diseases. Aflatoxins also responsible for the malabsorption of various nutrients thus leading to nutritional deficiencies, impaired immune function, malnutrition and stunted growth and hence the development of kwashiorkor and marasmus in infants. Aflatoxins also can affect almost all of the different body systems and hence the health of the affected individuals, especially in poor developing nations of south East Asia and sub-Saharan Africa where there is poor food harvesting, processing and storage thus allowing the growth of mould on them.

McCullough, A.K. & Lloyd, R.S. 2019.

“Chronic dietary exposure to aflatoxin B₁ (AFB₁), concomitant with hepatitis B infection is associated with a significant increased risk for hepatocellular carcinomas (HCCs) in people living in Southeast Asia and sub-Saharan Africa. Human exposures to AFB₁ occur through the consumption of foods that are contaminated with pervasive molds, including *Aspergillus flavus*. Even though dietary exposures to aflatoxins constitute the second largest global environmental risk factor for cancer development, there are still significant questions concerning the molecular mechanisms driving carcinogenesis and what factors may modulate an individual's risk for HCC. The objective of this review is to summarize key discoveries that established the association of chronic inflammation (most commonly associated with hepatitis B viral (HBV) infection) and environmental exposures to aflatoxin with increased HCC risk. Special emphasis will be given to recent investigations that have: 1) refined the aflatoxin-associated mutagenic signature, 2) expanded the DNA repair mechanisms that limit mutagenesis via adduct removal prior to replication-induced mutagenesis, 3) implicated a specific DNA polymerase in the error-prone bypass and resulting mutagenesis, and 4) identified human polymorphic variants that may modulate individual susceptibility to aflatoxin-induced cancers. Collectively, these investigations revealed that specific sequence contexts are differentially resistant against, or prone to, aflatoxin-induced mutagenesis and that these associations are remarkably similar between in vitro and in vivo analyses. These recent investigations also established DNA polymerase ζ as the major polymerase that confers the G to T transversion signature. Additionally, although the nucleotide excision repair (NER) pathway has been previously shown to repair aflatoxin-induced DNA adducts, recent murine data demonstrated that NEIL1-initiated base excision repair was significantly more important than NER relative to the removal of the highly mutagenic AFB₁-Fapy-dG adducts. These data suggest that

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inactivating polymorphic variants of NEIL1 could be a potential driver of HCCs in aflatoxin-exposed populations.”

CANSA’s Position on Aflatoxins in Agricultural Products for Human Consumption

The Cancer Association of South Africa (CANSA) is of the opinion that no level of aflatoxin exposure is considered safe for humans.

The Cancer Association of South Africa (CANSA) supports the maximum allowable levels of aflatoxins of the European Union which makes provision for 4ppb of aflatoxin in grains for human consumption and 10ppb for tree nuts, however, CANSA accepts the following levels set by the Directorate of Food Control, National Department of Health, as allowable levels:

For the purposes of Section 29 (1)(b)(i) of the Act, in so far as it is applied to, and is applicable to, foodstuffs, the following foodstuffs are hereby deemed to be contaminated, impure or decayed –

- a. Peanuts intended for further processing, which contain more than 15 µg/kg of aflatoxin (total);
- b. All foodstuffs, ready for human consumption, which contain more than 10 µg/kg of aflatoxin, of which aflatoxin B₁ is more than 5 µg/kg;
- c. Milk containing more than 0.05 µg/l of aflatoxin M₁

The position of CANSA is based on the following:

- Aflatoxin B₁ is carcinogenic to humans (Group 1)
- Aflatoxins have received greater attention than any other mycotoxins because of their demonstrated potent carcinogenic effect in susceptible laboratory animals and their acute toxicological effects in humans
- Intake of aflatoxin occurs mainly through consumption of maize and peanuts, which are dietary staples for many groups and individuals in South Africa, especially among lower income groups
- Aflatoxin M₁ concentrations in human urine and human breast milk have been correlated with dietary aflatoxin intake – therefore, there is no question about exposure of infants who are breastfed to aflatoxin M₁
- Hepatitis B infection may exacerbate the effects of aflatoxin exposure
- Other consequences of chronic exposure to aflatoxins include decreased immune and reproductive function in humans
- Children who are chronically exposed to aflatoxin may experience growth failure apart from the increased risk of liver cancer
- Infants may be exposed to aflatoxin through breast milk
- A Foetus may be exposed to aflatoxin during pregnancy if the mother consumes aflatoxins
- Individuals with a compromised immune system have an increased risk of the negative health effects of aflatoxin (e.g. HIV, Aids, Tuberculosis, Cancer)

According to the The **International Agency for Research on Cancer (IARC)**, the specialised cancer agency of the World Health Organization:

- Aflatoxins are carcinogenic to humans and animals
- There is sufficient evidence in humans for the carcinogenicity of aflatoxin B₁ as well as the negative health effects of other aflatoxins

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- Aflatoxin B₁ causes cancer of the liver (hepatocellular carcinoma)
- There is sufficient evidence for the carcinogenicity in experimental animals of naturally occurring mixtures of aflatoxins and of the individual aflatoxins B₁, G₁, and M₁
- There is limited evidence in experimental animals for the carcinogenicity of aflatoxin B₂
- There is strong evidence that the carcinogenicity of aflatoxins operates by a genotoxic mechanism of action that involves metabolic activation to a genotoxic epoxide metabolite, formation of DNA adducts, and modification of the TP53 gene. In human hepatocellular carcinoma from areas where exposure to aflatoxins is high, up to 50% of tumours have been shown to harbour a specific point mutation in the TP53 tumour-suppressor gene.

Medical Disclaimer

This Fact Sheet is intended to provide general information only and, as such, should not be considered as a substitute for advice, medically or otherwise, covering any specific situation. Users should seek appropriate advice before taking or refraining from taking any action in reliance on any information contained in this Fact Sheet. So far as permissible by law, the Cancer Association of South Africa (CANSA) does not accept any liability to any person (or his/her dependants/estate/heirs) relating to the use of any information contained in this Fact Sheet.

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