Aspergillus flavus
and other aflatoxin-producing moulds

Phylum: Ascomycota
Class: Eurotiomycetes
Order: *Eurotiales*
Family: *Trichocomaceae*

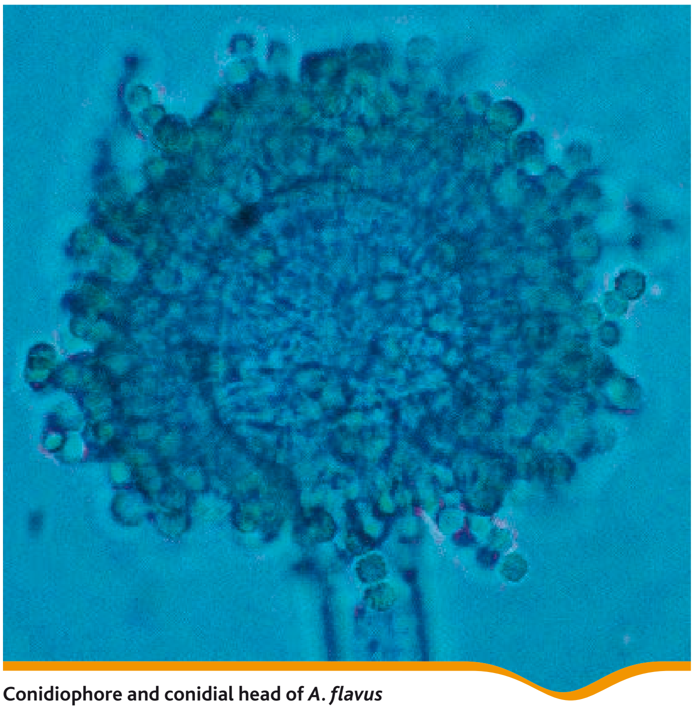
anses
French agency for food, environmental and occupational health & safety

Aflatoxin B_1 (AFB_1) is considered to be one of the most powerful natural genotoxic carcinogens. Its target organ is the liver.

Aspergillus flavus is the principal aflatoxin-producing species (only group B). *A. parasiticus* and *A. nomius* also produce group G aflatoxins, but these particular microscopic fungi (moulds) are only found very rarely in food.

Aflatoxins are compounds with a very low molecular weight. AFB_1 ($C_{17}H_{12}O_6$) and AFB_2 ($C_{17}H_{14}O_6$), whose respective molar masses are 312 and 314 g/mol, exhibit blue fluorescence under ultraviolet light. AFG_1 ($C_{17}H_{12}O_7$) and AFG_2 ($C_{17}H_{14}O_7$), whose respective molar masses are 328 and 330 g/mol, exhibit green fluorescence.

When absorbed by dairy cows or other ruminant mammals (e.g. goats, sheep, buffalos, camels), AFB_1 is partially metabolised and then excreted in the milk in the form of AFM_1 ($C_{17}H_{12}O_7$). It has a molar mass of 328 g/mol and exhibits blue-mauve fluorescence.



Conidiophore and conidial head of *A. flavus*

Characteristics and sources of *Aspergillus flavus*

Main microbiological characteristics

Its conidiophores are formed of roughened stipes measuring from 400 µm to 1 mm or more in length and terminating in a spherical vesicle of 20 to 50 µm in diameter, fertile on more than three quarters of its surface, and carrying both metulae and phialides of 7 to 10 µm long (Figure 1). The conidia are slightly roughened and spherical, 3 to 5 µm in diameter.

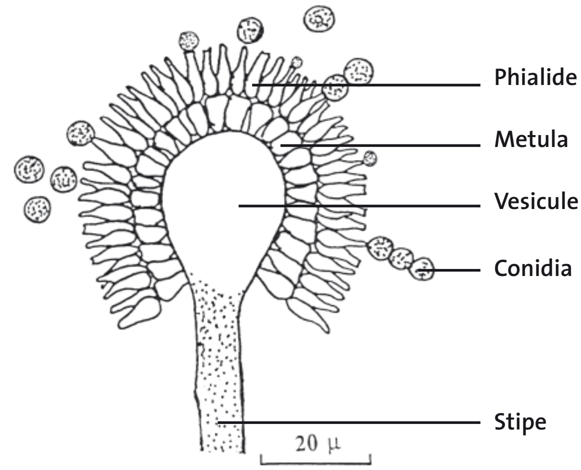


Figure 1. Microscopic view of *Aspergillus flavus*

Table 1. Growth characteristics and toxinogenicity of *Aspergillus flavus*

Growth	Min	Opt	Max
Temperature (°C)	10-12	33	43 - 48
pH	2.1	7.5	11.2
a_w	0.78-0.84	0.97	/
Toxinogenicity	**Min**	**Opt**	**Max**
Temperature (°C)	13	16 - 31	37
a_w	0.82	0.95-0.99	/

Data sheet on foodborne biological hazards
April 2012

Sources of the hazard

Aspergillus flavus is ubiquitous (found in vegetation, water, soil, etc.). The conidia are mainly dispersed in the environment by air, but also by water, animals and humans.

Aflatoxins are produced in the field or during storage, mainly in regions with a subtropical or mediterranean climate, but also in temperate regions during particularly hot and dry seasons.

A. flavus is responsible for contaminating cereals (principally maize and maize-based products), oil-seed and cattle cake, nuts (such as groundnuts and pistachios), all sorts of spices, dried fruit (such as figs), coffee, cocoa beans and dairy products (AFM_1). Contamination and the growth of aflatoxin-producing moulds are facilitated on seeds and fruit damages (impact, attack by insects, etc.).

Several cases of acute aflatoxicosis have been described on stock farms, especially pig farms. The animals usually died within a few hours following severe haemorrhage. In poultry, the chronic form of the intoxication is the most frequent. The symptoms are reduced performance associated with bleeding and discoloured flesh.

Transmission routes

Humans are exposed to foodborne aflatoxins when ingesting foods contaminated by aflatoxins.

In addition to exposure to foodborne toxins, humans can become exposed to the conidia of *A. flavus* by the pulmonary route (inhalation). The conidia are one of the principal allergens of bronchial aspergillosis in humans and are responsible for pulmonary infections in immunocompromised patients.

Recommendations for primary production

Measures to prevent the hazard at the source are the only possible solution, as it is almost impossible to detoxify foods contaminated by aflatoxins.

- **Respect good farming practice (avoid damage to fruit and seeds, attacks by insects, etc.) to avoid introducing the hazard.**
- **Respect good practice when storing cereals, especially keeping product into dry environment to prevent any change in initial levels of aflatoxins.**

Foodborne human disease

Nature of toxic effects

In 1993, the IARC [(1)] classified AFB_1 as belonging to group 1 (carcinogenic to humans), AFM1 to group 2B (possibly carcinogenic to humans) and AFG_1 to group 3 (not classifiable as to its carcinogenicity to humans).

Most epidemiological studies show a correlation between chronic exposure to aflatoxin through diet and prevalence of primary liver cancer. This relationship is modulated however by other aggravating factors, such as infection by the hepatitis B virus. The genotoxicity of aflatoxins is due to the metabolism of AFB_1 into AFB_1 8,9-epoxide, characterised by a short shelf life, but highly reactive. AFB_1 8,9-epoxide is considered to be the principal genotoxic metabolite binding to the DNA.

In cases of acute intoxication, the typical but non-specific clinical symptoms include jaundice, depression, anorexia and diarrhoea. Mortality reached 25% during intoxications in India in 1975 and 40% in eastern Kenya in 2004. Two human syndromes, of indefinite aetiology, have been linked to the ingestion of food contaminated by aflatoxins: kwashiorkor, associating hypoalbuminaemia and immunosuppression, and Reye's syndrome, associating encephalopathy and degeneration of the viscera.

Dose-effect [(2)] and dose-response [(3)] relationships

A dose-response relationship for aflatoxins has been established for humans. JECFA [(4)] and the SCF [(5)] have not set a Daily Tolerable Intake (DTI) for aflatoxins. As there is no threshold for the genotoxic carcinogenic effects of these substances, the only realistic approach is to reduce exposure to the lowest level possible based on the ALARA principle (As Low As Reasonnably Achievable). However, on the basis of epidemiological data, JECFA (1999) has calculated that, for Europe, the ingestion of 1 ng of aflatoxins per kg of bodyweight per day over an entire lifetime leads to an increase in the incidence of liver cancer of 0.013 cases per year per 100,000 people.

According to the 2nd French Total Diet Study (TDS 2, ANSES 2011), depending on where the upper and lower hypothetical boundaries on contamination data were placed, mean exposure to all aflatoxins were respectively 0.0019 and 0.89 ng/kg of bw/day. The number of theoretical extra cases of liver cancer per year in the adult French population, related to this exposure, would seem to be very low (< 0.07% for the upper bound, or 5.2 cases) compared to the number of cases of liver cancer estimated in France for 2010 (InVS).

Epidemiology

The most recent recognised case of acute intoxication took place from April to September 2004 in provinces in central and eastern Kenyan, during which 341 cases were diagnosed leading to 123 deaths.

Most epidemiological studies supporting the link between aflatoxin and liver cancer were made in Southeast Asia, China, and West and Equatorial Africa, all of them parts of the world with a high prevalence of the hepatitis B virus and AFB_1. In Latin America, the prevalence of primary liver cancer and infection by the hepatitis B virus is low, whereas exposure to AFB_1 is high. JEFCA has recommended that new epidemiological studies be undertaken in regions deemed to be at risk, and also, for certain countries, vaccination campaigns against the hepatitis B virus. When these studies will be achieved, the risk of aflatoxin to humans can be reassessed. In parts of Africa, impaired growth and altered immune parameters have also been observed in children.

Role of foods

Main foods to consider

In 2007 and 2008, in Europe, a large majority of notifications by the Rapid Alert System for Food and Feed (RASFF) concerned aflatoxins, detected mainly in nuts but also in decreasing order in cereals.

During the EAT 2 survey, the aflatoxins AFB_2, AFG_1, AFG_2 and AFM_1 were not detected or quantified in foods consumed in France. AFB1 was only detected or quantified in 0.4% of foods, and only in dark chocolate. Exposure to aflatoxins is equivalent to that estimated during the 1st Total Diet Study (TDS 1, INRA 2004).

Levels of aflatoxins in foods are dependent on changes in weather conditions over the seasons and from year to year. For example, maize harvested in 2003 (a heatwave summer) in a southern European country, showed contamination by AFB_1, unusual for this latitude and revealed by the presence of AFM_1 in cow's milk. A similar case (same cereal and same country) occurred in 2005. A survey carried out in the United States (seven Midwest States) in 1988, which was also an unusually hot, dry year for the region, showed that 8% of maize harvested in this region contained aflatoxins.

(1) International Agency for Research on Cancer.

(2) Relationship between the dose (the quantity of microbial cells ingested during a meal) and the effect on an individual.

(3) For a given effect, the relationship between the dose and the response, i.e., the probability of this effect appearing in the population.

(4) Joint FAO/WHO Expert Committee on Food Additives.

(5) EU Scientific Committee on Food.

Inactivation treatment for fungal spores and aflatoxins

The conidia of aflatoxin-producing moulds are susceptible to fungicidal chemical disinfectants authorised in the agri-foods industry, subject to observing the recommended usage practices.

The conidia of *A. flavus* are susceptible to heat. The most reliable D values[(6)] at a neutral pH and strong aw are: $D_{45°C}$ = 160 h; $D_{50°C}$ = 16 h, $D_{52°C}$ = 40-45 min and $D_{60°C}$ = 1 min, with a z value[(7)] varying between 3.3 and 4.1°C.

Aflatoxins are highly soluble in water, insoluble in non-polar solvents and highly soluble in medium-polar solvents such as chloroform and methanol. Their melting points are 268-269°C (AFB_1), 286-289°C (AFB_2), 244-246°C (AFG_1), 230°C (AFG_2) and 299°C (AFM_1). Thermal treatments (sterilisation, pasteurisation, freezing) or drying (dehydration, lyophilisation), with the exception of roasting, have little effect on aflatoxins. Even roasting groundnuts only brings about a reduction of 50 to 80% of the initial level of aflatoxins. During processes for oil extraction, aflatoxins are mostly found in the resulting cattle cake. Detoxification processes for cattle cake with ammonia associated with formaldehyde can eliminate up to 95% of the initial level of AFB_1.

Monitoring in foods

European Directive 2002/32/EC[(8)] (and its amendments) sets maximum authorised levels of AFB_1 in substances intended for animal feed, in order to limit the levels of AFM_1 in milk.

Regulation (EC) no.1881/2006[(9)] as amended sets the maximum levels of aflatoxins not to be exceeded in food products for human consumption. These maximum levels are as follows:

- AFB_1: 2, 5 or 8 µg/kg for groundnuts, other seeds and dried fruits depending on the stage of development; 2 to 5 µg/kg for cereals depending on the product and its stage of processing; 5 µg/kg for certain spices; and 0.1 µg/kg for cereal-based preparations for very young children;
- AFB_1, AFB_2, AFG_1, AFG_2: 4, 10 or 15 µg/kg for groundnuts, other seeds and dried fruits depending on the stage of processing; 4 to 10 µg/kg for cereals, depending on the product and its stage of processing; and 10 µg/kg for certain spices;
- AFM_1: 0.05 µg/kg for milk; and 0.025 µg/kg for formulae for very young children.

Regulation (EU) no.178/2010[(10)] completes the previous Regulation (EC) no.401/2006[(11)] for the sampling and analysis of samples taken from foods.

There are standardised methods for determining the levels of aflatoxins potentially present in different food matrices. For instance, the NF EN ISO 17375:2006 standard describes a method for assaying AFB_1 in animal feed. The NF EN ISO 16050:2011 standard describes a method for assaying AFB_1 and determining overall levels of aflatoxins B_1, B_2, G_1 and G_2 contained in cereals, nuts and products derived from them. The ISO 14675:2003[(12)] and ISO 14501:2007[(13)] standards describe methods for assaying AFM_1 contained in milk, dairy products or powdered milk.

Recommendations to operators

- **Respect the regulations in force, which set maximum levels of aflatoxins not to be exceeded in foods for human and animal consumption.**
- **Respect good storage practice (see Recommendations for primary production).**
- **Respect good hygiene practice when preserving or preparing food.**
- **Use detoxification processes for potentially contaminated cattle cake.**

Domestic hygiene

Recommendations for consumers

- **Store concerned foodstuffs (cereals, nuts, dried fruits and spices) in a dry place.**

References and links

General reference

- AFSSA, 2009. Final report of the risk assessment related to the presence of mycotoxins in human and animal food chains
http://www.anses.fr/Documents/RCCP-Ra-Mycotoxines2009.pdf
- ANSES, 2011. French national surveillance study on dietary exposure to chemical substances – 2nd Total Diet Study 2006-2010 (EAT 2). Volume 1: Inorganic contaminants, minerals, persistent organic pollutants, mycotoxins and phyto-oestrogens.
http://www.anses.fr/Documents/PASER2006sa0361Ra1.pdf
- INRA, 2004. French Total Diet Study (TDS 1). Mycotoxins, minerals and trace elements. 68 pages.
http://www.anses.fr/Documents/RapportEAT1EN.pdf
- InVS, 2010. Projections de l'incidence et de la mortalité par cancer en France en 2010.
http://www.invs.sante.fr/applications/cancers/projections2010/donnees_generales.htm
- RASFF Report, 2008. Rapid Alert System for Food and Feed. Annual Report 2008. European Commission. 56p.
http://ec.europa.eu/food/food/rapidalert/report2008_en.pdf

Useful links

- ANSES: http://www.anses.fr/PN4701.htm
- European Reference Laboratory for mycotoxins: Joint Research Centre for the European Commission - Geel, Belgium.
- National Reference Laboratory (NRL) for mycotoxins (group B3d according to Annex I of Directive 96/23/EC of the Council): ANSES, Maisons-Alfort Laboratory for Food Safety.
- National Reference Centre (NRC) Mycoses and Antifungal Agents: Molecular Mycology Unit - Institut Pasteur, Paris.

(6) D is the time necessary, at a given temperature T, to divide by 10 the initial population of a given microbiological hazard.

(7) z is the increase in temperature (°C) corresponding to variation by a factor of 10 of the decimal reduction time.

(8) Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed.

(9) Commission Regulation (EC) No.1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

(10) Commission Regulation (EU) No 178/2010 of 2 March 2010 amending Regulation (EC) No 401/2006 as regards groundnuts (peanuts), other oilseeds, tree nuts, apricot kernels, liquorice and vegetable oil.

(11) Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs.

(12) Milk and milk products - Guidelines for a standardized description of competitive enzyme immunoassays - determination of aflatoxin M1.

(13) Milk and milk powder - Determination of aflatoxin M1 content -- Clean-up by immunoaffinity chromatography and determination by high-performance liquid chromatography.