Aflatoxin B1 (AFB1) 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. AFB1 (C17H12O6) and AFB2 (C17H14O6), whose respective molar masses are 312 and 314 g/mol, exhibit blue fluorescence under ultraviolet light. AFG1 (C17H12O7) and AFG2 (C17H14O7), 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), AFB1 is partially metabolised and then excreted in the milk in the form of AFM1 (C17H12O7). It has a molar mass of 328 g/mol and exhibits blue-mauve fluorescence.

**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.

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

<table>
<thead>
<tr>
<th>Growth</th>
<th>Min</th>
<th>Opt</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>10-12</td>
<td>33</td>
<td>43-48</td>
</tr>
<tr>
<td>pH</td>
<td>2.1</td>
<td>7.5</td>
<td>11.2</td>
</tr>
<tr>
<td>aw</td>
<td>0.78-0.84</td>
<td>0.97</td>
<td>/</td>
</tr>
<tr>
<td>Toxinogenicity</td>
<td>Min</td>
<td>Opt</td>
<td>Max</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>13</td>
<td>16-31</td>
<td>37</td>
</tr>
<tr>
<td>aw</td>
<td>0.82</td>
<td>0.95-0.99</td>
<td>/</td>
</tr>
</tbody>
</table>

Figure 1. Microscopic view of Aspergillus flavus

Conidiophore and conidial head of A. flavus

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). 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.

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 Reasonably 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 AFB1. In Latin America, the prevalence of primary liver cancer and infection by the hepatitis B virus is low, whereas exposure to AFB1 is high. JECFA 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 AFB2, AFG1, AFG2 and AFM1 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 AFB1, unusual for this latitude and revealed by the presence of AFB1 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.

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(6) classified AFB1, as belonging to group 1 (carcinogenic to humans), AFM1 to group 2B (possibly carcinogenic to humans) and AFG1 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 AFB1 into AFB, 8,9-epoxide, characterised by a short shelf life, but highly reactive. AFB, 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.

[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.
Inactivation treatment for fungal spores and aflatoxins

The conidia of aflatoxin-producing moulds are susceptible to fungicidal chemical disinfectants authorised in the agri-food 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: \(D_{45\text{C}} = 160 \text{ h}\), \(D_{42\text{C}} = 16 \text{ h}\), \(D_{49\text{C}} = 40-45 \text{ min}\) and \(D_{52\text{C}} = 1 \text{ min}\), 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 (AFB1), 286-289°C (AFB2), 244-246°C (AFG1) and 299°C (AFG2). 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 cake. Detoxification processes for cake cake with ammonia associated with formaldehyde can eliminate up to 95% of the initial level of AFB1.

Monitoring in foods

European Directive 2002/32/EC\(^8\) (and its amendments) sets maximum authorised levels of AFB1, in substances intended for animal feed, in order to limit the levels of AFB1, 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:

- AFB1: 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;
- AFB1, AFB2, AFG1, AFG2: 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;
- AFB1: 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\(^11\)\(^12\) for the sampling and analysis of samples taken from foods.


References and links

General reference

- ANSES: [http://www.anses.fr/PN4701.htm](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.

Domestic hygiene

Recommendations for consumers

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

Useful links

- ANSES: [http://www.anses.fr/PN4701.htm](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.

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 cake.