Ochratoxin A (OTA) - producing *Aspergilli* and *Penicillia*

Phylum: *Deuteromycota*
Class: *Hyphomycetes*
Order: Hyphomycetales
Family: *Moniliaceae*

Moulds

The principal species producing OTA, a mycotoxin with a nephrotoxic effect, are: *Aspergillus carbonarius* and similar species (*A. steynii* and *A. westerdijkiae*) which belong to the group of black *Aspergilli*, *A. ochraceus*, *Penicillium verrucosum* (formerly *P. viridicatum*) and another similar species (*P. nordicum*).

Pure OTA, whose empirical formula is $C_{20}H_{18}ClNO_6$, is a white crystalline solid, with a molar mass of 403.8 g/mol. It is a weak organic acid with a pKa of 7.1, which is stable in storage as a result of its structure.

Characteristics and sources of ochratoxin A-producing *Aspergilli* and *Penicillia*

Main microbiological characteristics

Aspergillus carbonarius

The conidiophores are formed of smooth stipes 2 to 3 µm in length, the vesicles are spherical with a diameter of 60 to 90 µm, the metulae 12 to 18 µm long, the phialides 9 to 15 µm long, the conidia are spherical and 6 to 8 µm in diameter, black and rough (and sometimes covered with spikes) in a very characteristic manner.

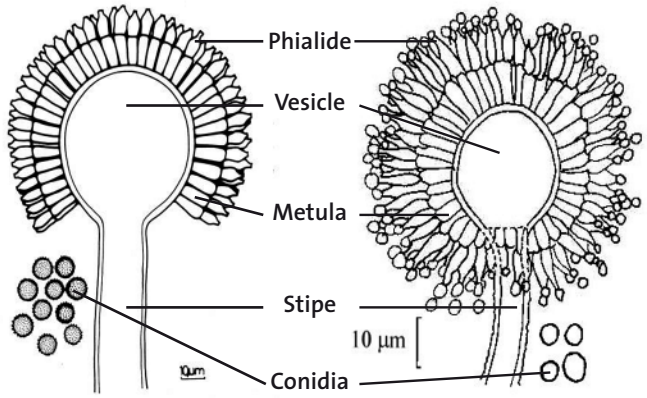
Aspergillus ochraceus

The conidiophores are formed of slightly roughened stipes (yellowish cells) 1 to 1.5 µm in length, the vesicles are spherical with a diameter of 25 to 50 µm, the metulae are 15 to 20 µm long, the phialides 9 to 12 µm long, and the conidia are smooth, spherical and 2.5 to 3.5 µm in diameter, smooth or slightly roughened.

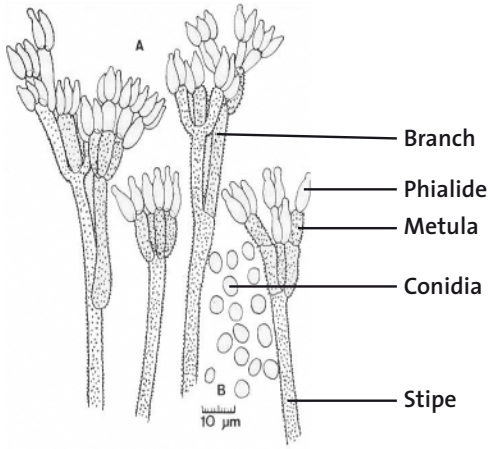
Penicillium verrucosum

The conidiophores have characteristic roughened stipes 200 to 500 µm in length and terminating in brushes, some isolates having three or four verticils and others two or three verticils with 1 or 2 ramifications per stipe, the metulae are 7 to 15 µm long, the flask-shaped phialides 7 to 9 µm long, and the conidia are smooth, spherical and 2.5 to 3.5 µm in diameter, or more rarely sub-spherical to ellipsoidal and 3 to 3.5 µm in length.

Deuteromycetes responsible for contaminations multiply through a vegetative cycle in the environment and propagate by means of conidia (asexual forms) produced in the conidiophores.



Aspergillus carbonarius *Aspergillus ochraceus*



Penicillium verrucosum

Data sheet on foodborne biological hazards
April 2012

Sources of the hazard

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

OTA is considered to be a "storage mycotoxin" for cereals and a "field mycotoxin" for grapevines. It is produced in cold and temperate climates by *P. verrucosum* (cereals) and *A. carbonarius* (grapevines) and in tropical and warm regions by *A. ochraceus* (raw coffee, cocoa, oilseed and protein crops). As the mould develops on the outside of the grain, the outer husk of cereals (the bran) is more contaminated with OTA. OTA is produced between three days and one week after the start of germination of the fungal spores, depending on humidity.

OTA can accumulate in animals consuming contaminated feed.

Transmission routes

Humans are exposed by consuming foods contaminated with OTA.

Recommendations for primary production

Preventive measures could only be applied at the source as it is almost impossible to detoxify foods contaminated with OTA.

- **Grapevines: appropriate crop protection programmes against mould on the grapes.**
- **Seeds and grains: post-harvest storage in accordance with storage strategies as recommended in good hygiene practice guides:**
  - **lower the temperature and the humidity of grain in storage;**
  - **ensure that storage areas are sheltered against external sources of damp (rain or run-off);**
  - **guard against infestation by insects or rodents in storage depots, etc.**
- **Dried fruits, nuts and legumes: keep produce and areas for storage, handling or food-processing free from damp.**

(1) International Agency for Research on Cancer.

(2) Relationship between the dose 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.

Foodborne human disease

Nature of toxic effects

The main effects of OTA are nephrotoxic. In humans, OTA is thus associated with a kidney disease known as Endemic Balkan Nephropathy (EBN). It is also thought to have immunotoxic and neurotoxic effects. Furthermore, OTA is classified by the IARC (1) in category 2B (possibly carcinogenic to humans) on the basis of the carcinogenic effects observed undeniably in rodents and possibly in humans.

In the current state of knowledge, there is no evidence to confirm that any population group is particularly at risk.

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

No dose-effect or dose-response relationships have been determined in humans for OTA. Toxicity studies have been carried out in rats, pigs, poultry and domestic pets.

On the basis of a Lowest Observed Adverse Effect Level (LOAEL) of 8 µg/kg of body weight per day for early onset markers of renal toxicity in pigs (the most susceptible animal species) and a safety factor of 450 (taking into account uncertainty in the extrapolation of experimental results from animals to humans and interspecies variability), in 2006 EFSA proposed a Provisional Tolerable Weekly Intake (PTWI) of 120 ng/kg of body weight per week, which was renewed in 2010 on the basis of two new studies.

Epidemiology

Little epidemiological information is available.

Role of foods

Main foods to consider

Production of OTA has mainly been observed in the following products:

- cereals produced in temperate zones (*P. verrucosum*): wheat, maize, barley.
- wine, grape juice, raisins (*A. carbonarius*).
- dried fruit and nuts, coffee, liquorice (*A. ochraceus*, *A. steynii*, *A. westerdijkiae*).

The results of the 2nd French Total Diet Study (TDS 2; 2006-2010) on the exposure of the French population showed that the food groups with the highest levels are bread and dried bread products (0.13 µg/kg), pasta (0.1 µg/kg), rice and wheat (0.07 µg/kg), delicatessen meats (0.05 µg/kg) and breakfast cereals (0.03 µg/kg).

Table 1. **Characteristics of the growth and toxinogenesis of ochratoxin A-producing *Aspergilli* and *Penicillia***

	A. carbonarius			*A. ochraceus*			*P. verrucosum*		
Growth	**Min**	**Opt**	**Max**	**Min**	**Opt**	**Max**	**Min**	**Opt**	**Max**
Temperature	10	30	41	8	24 - 31	41	0	20	31
pH	2	5.6	10	2.2	5.6	10	2.1	5.6	10
a_w	0.85	0.96 - 0.99	/	0.77	0.95 – 0.99	/	0.80	0.95	/
% CO_2	/	/	/	/	/	80 %	/	/	/
Toxinogenesis	**Min**	**Opt**	**Max**	**Min**	**Opt**	**Max**	**Min**	**Opt**	**Max**
Temperature	/	15 - 30	/		25 - 30	/	0	20	31
pH	/	/	/		/	/	/	5.6	/
a_w	0.87	0.93 – 0.98	/	0.85	0.95	/	0.86	0.92	/
% CO_2	/	/	/		/	30 %	/	/	/

In both adults and children, bread and dried bread products were found to be the primary source of exposure to OTA (20-80%). In adults, alcoholic beverages were also found to be a leading contributor (23% for the lower bound, as a result of the quantification of OTA in wine).

The results of TDS 2 showed a mean reduction of exposure to OTA by a factor of 1.1 to 1.5 compared to estimates made during TDS 1 (2000-2004) (adults: 1.9 *vs* 2.2 ng/kg bw/d and children: 2.8 *vs* 4.1 ng/kg bw/d, respectively) with neither adults nor children exceeding the PTWI proposed by EFSA.

The reduction in exposure to OTA can be explained in part by the introduction in 2006 of regulations governing the maximum levels of certain mycotoxins in foods. Levels of OTA also depend on changing weather conditions over the seasons and in different years.

Inactivation treatments for fungal spores and OTA

The spores of ochratoxin A-producing moulds are susceptible to antifungal chemical disinfectants authorised in the agri-foods industry, subject to observing the recommended usage practices.

As OTA is highly stable under different thermal conditions (melting points of 90°C and 169°C), the physical and chemical treatments used in the food industry do not eliminate it.

Roasting coffee beans reduces OTA levels but there are no studies on the toxicity of molecules resulting from the degradation of OTA.

Monitoring in foods

European Commission Regulation (EC) No.1881/2006 sets maximum levels of OTA that must not be exceeded in foods intended for human consumption. This Regulation was amended by Regulation (EC) No.105/2010 to cover spices and liquorice.

Regulation (EC) No.178/2010 on the sampling and analysis of foods amended Regulation (EC) No.401/2006.

Recommendation 2006/576/EC concerns maximum levels not to be exceeded in products intended for animal feed, especially for pigs and poultry.

Recommendations to operators

- **Respect good storage practice (see Recommendations for primary production).**
- **Respect good hygiene practice when preserving or preparing food. Sorting operations to remove parts of the product contaminated by proven toxinogenic moulds is the best method for reducing levels of OTA.**

Masks should be worn during handling operations inside grain silos.

Domestic hygiene

Recommendations to consumers

- **Store the foodstuffs concerned (cereals, dried fruits, nuts and legumes) in a dry place.**
- **If food is visibly contaminated by mould, remove the affected part and surrounding areas if it is clearly limited; otherwise throw it away.**

References and links

- AFSSA, 2009. *Évaluation des risques liés à la présence de mycotoxines dans les chaînes alimentaires humaine et animale* [Risk assessment related to mycotoxins in human and animal food chains].
- ANSES, 2011. French national surveillance study on dietary exposure to chemical substances – 2nd Total Diet Study 2006-2010 (TDS 2). Volume 1: Inorganic contaminants, minerals, persistent organic pollutants, mycotoxins and phyto-oestrogens.
- INRA, 2004. French Total Diet Study (TDS 1). Mycotoxins, minerals and trace elements. 68 pages.
- MAFF UK, 1999. Survey of human exposure to ochratoxin A. *Food Surveillance Information Sheet* 172.