

A food toxicological contemplation of mycotoxins

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Summary

Mycotoxins are the products of the endogenous metabolism of moulds that can infest crops and already lead to adverse health effects in mammals and humans at low concentrations after the consumption of contaminated feed and food, respectively. In the following report, the main mycotoxins detected in temperate zones, namely aflatoxins, ochratoxin A, Fusarium mycotoxins (zearalenone, trichothecenes and fumonisins), ergot alkaloids, patulin and citrinin, are briefly introduced. In this context, their underlying mechanisms of toxicity, their main toxic effects in humans and animals as well as the actual burden of foods with aflatoxins, ochratoxin A and Fusarium mycotoxins in Germany are described.

Keywords: Moulds, mycotoxins, aflatoxins, ochratoxin A, Fusarium toxins

Introduction

Products of the endogenous metabolism of moulds which can be harmful to humans and other living organisms, are grouped under the generic term „mycotoxins“. Mycotoxins can be formed by outdoor moulds (e. g. *Fusarium* spp.), which can infest the crops already in the field, as well as by indoor moulds (e. g. *Aspergillus* spp., *Penicillium* spp.), which can infest the harvested crops if they are stored inappropriately (i. e. too high storage humidity, too long intervals between the harvesting and the drying step or insufficient airing). Once they have been released by moulds, they may con-

taminate food items of plant and animal origin (♦ Figure 1).

In this contribution, information regarding the most relevant mycotoxins from a food toxicological point of view, namely aflatoxins, ochratoxin A, *Fusarium* mycotoxins, ergot alkaloids, patulin und citrinin, is summarized.

Aflatoxins

Aflatoxins are products of the endogenous metabolism of the moulds *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus oryzae*, which pose a great problem in tropical and subtropical regions of the world. Consequently, aflatoxins are mainly detected in imported products such as nuts (pistachios, peanuts, hazelnuts), dried fruit (figs) and spices (pepper, paprika powder). Aflatoxin B₁, aflatoxin B₂, aflatoxin G₁ and aflatoxin G₂ as well as aflatoxin M₁ and aflatoxin M₂ (♦ Figure 2), which are mainly detected in milk and milk products, belong to the group of the aflatoxins.

Metabolism

In order to become toxic, aflatoxin B₁ must be metabolically activated (♦ Figure 3). Aflatoxin B₁ is first metabolized to the aflatoxin B₁-8,9-epoxide in a cytochrome P450-catalyzed reaction. Thereafter, the epoxide reacts with the N⁷-position of guanine residues of the DNA and leads to the formation of the aflatoxin B₁-N⁷-guanine adduct. This adduct is chemically unstable and can be converted to the so-called aflatoxin B₁-formamidopyrimidine adduct by the opening of the imidazole ring or can decompose by depurination and lead to an apurinic site in DNA.

In both cases, DNA replication leads to gene mutations, whereby aflatoxin B₁ is a much stronger mutagen than aflatoxin G₁ and aflatoxin M₁. In contrast, the aflatoxins B₂, G₂ und M₂, which cannot be epoxidized, are hardly genotoxic. Aflatoxin B₁ also exhibits a pronounced cytotoxicity which is in part due to the significant formation of covalent protein adducts. In this case again it is the aflatoxin B₁-8,9-epoxide that plays a central role: It is first metabolized to the corresponding dihydrodiol and then to the corresponding dialdehyde (♦ Figure 3). The dialdehyde in turn can easily react with the ε-amino groups of lysine.

Toxicity

In countries, in which the contamination of the food chain with aflatoxins is very high, so-called aflatoxicoses may develop. They are characterized by nausea, vomiting, abdominal pain and gastrointestinal

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bleeding, icterus, spasms, coma, pulmonary and cerebral oedema formation as well as necroses and fatty degeneration of the liver, kidneys and heart.

Because of its extremely strong mutagenicity and cytotoxicity (see above) aflatoxin B₁ is the aflatoxin with the greatest carcinogenic potential and induces liver tumours in experimental animals. The International Agency for Research on Cancer (Lyon, France) which is part of the World Health Organization (WHO), classified aflatoxin B₁ as a human carcinogen a decade ago [1]. The chronic exposure of humans to aflatoxin B₁ also leads to the induction of liver carcinomas. Moreover, it has been known for a number of years that the risk to develop liver tumours increases by about a factor of 60 if humans are chronically exposed to aflatoxins through contaminated food and at the same time are chronically infected with hepatitis B or C viruses. It has also been pointed out that a relationship between the strong contamination of the food chain with aflatoxins and the occurrence of the so-called Reye syndrome (an acute encephalopathy in combination with a fatty degeneration of the liver), most notably in tropical countries, may exist.

Ochratoxin A

Ochratoxins are produced by various *Aspergillus* and *Penicillium* species, among others by *Aspergillus ochraceus*, *Aspergillus carbonarius* and *Penicillium verrucosum*, and chemically represent amide derivatives of L-phenylalanine (♦Figure 4).

The most frequently formed and at the same time the most toxic ochratoxin is ochratoxin A. It contaminates grains and grain products, legumes, coffee beans, beer, wine, raisins, grape juice as well as meat products and is mainly nephrotoxic. It has been known for a long time that because of its structural homology to the amino acid L-phenylalanine ochratoxin A is able to inhibit competitively the phenylalanine-tRNA-synthase. However, the above-mentioned enzyme is only inhibited in the presence of very high concentrations of ochratoxin A, which in no case are achieved if humans are exposed to ochratoxin A-contaminated food items. Hence, the inhibition of the phenylalanine-tRNA-synthase does not explain the observed renal toxicity.

Ochratoxin A induces kidney tumours in rats. However, the mechanism(s) that lead(s) to the forma-

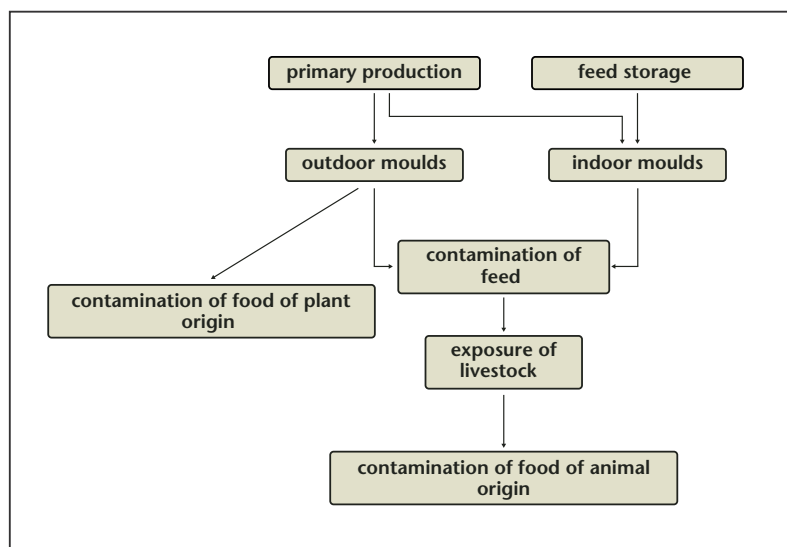


Fig. 1: Contamination of food items of plant and animal origin with toxins from outdoor and indoor moulds

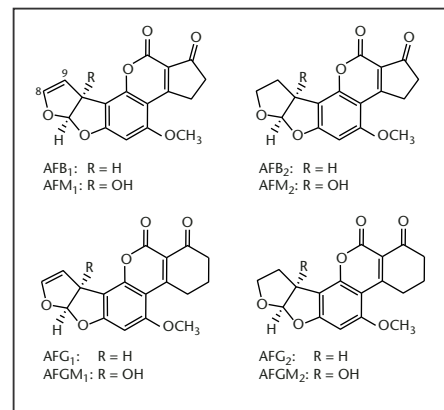


Fig. 2: Structural formulae of the most important aflatoxins in food

AFB_{1/2} = aflatoxin B_{1/2}, AFM_{1/2} = aflatoxin M_{1/2}, AFG_{1/2} = aflatoxin G_{1/2}, AFGM_{1/2} = aflatoxin GM_{1/2}

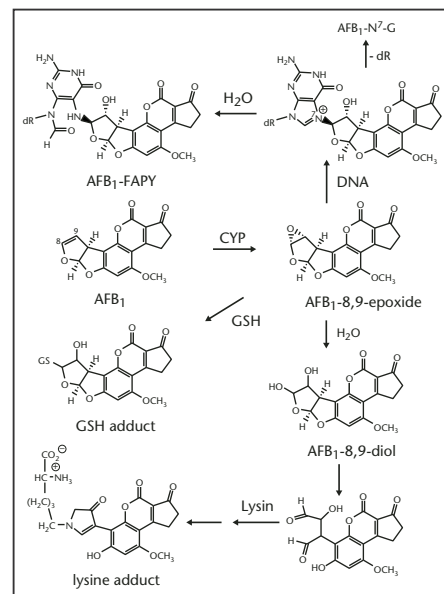


Fig. 3: Metabolic activation of aflatoxin B₁ to aflatoxin B₁-8,9-epoxide and formation of covalent adducts with DNA and proteins

AFB₁ = aflatoxin B₁; AFB₁-FAPY = aflatoxin B₁-formamidopyrimidine adduct; AFB₁-N⁷-G = aflatoxin B₁-N⁷-guanine adduct; CYP = cytochrome P450; dR = deoxyribose; GSH = glutathione

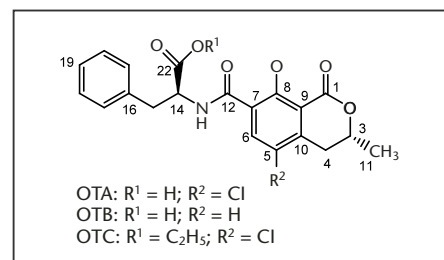


Fig. 4: Structural formulae of the most important ochratoxins
OTA/B/C = ochratoxin A/B/C

tion of kidney tumours have been controversially discussed up to the present time. Although there were early indications that DNA adducts are formed in the kidneys of ochratoxin A-treated rats, it is not clear whether these are oxidative DNA lesions or covalent adducts of DNA bases with the mycotoxin or its metabolites.

A kidney disease first described in the 1950s, the so-called „Balkan endemic nephropathy“ [2], was linked for a long time to a chronic ochratoxin A exposure. Symptoms of the „Balkan endemic nephropathy“ are:

- degeneration of the kidney tubuli
- body shrinkage and weight loss
- kidney failure
- development of kidney and bladder tumours

Recent studies show that the „Balkan endemic nephropathy“ is most probably caused by eating bread that is contaminated with the toxin aristocholic acid, which derives from the birthwort (*Aristolochia clematitis*) [3, 4]. On the one hand, the farmers in some rural areas of the Balkan could not afford to buy expensive herbicides, so that weeds could not be decimated in the crop fields. On the

other hand, no efficient crop clean-up took place in the flour mills.

Despite efforts to reduce the amount of ochratoxin A in food items, a certain degree of contamination seems to be unavoidable at the moment. The tolerable weekly intake amounts to 120 ng/kg body weight. In the case of European adult consumers, the weekly exposure at the moment lies within the range of 15–60 ng ochratoxin A/kg body weight.

Fusarium toxins

Filamentous fungi of the large *Fusarium* genus are able to grow in regions with moderate as well as low temperatures and are therefore widely distributed, also across Europe. The main toxins produced by the different *Fusarium* species are zearalenone, trichothecenes and fumonisins.

Zearalenone

Zearalenone contaminates grains and grain products, mainly maize and maize products, but also barley, oat, wheat, sorghum and millet. Toxin production can occur in the field before harvesting as well as during the storage of cereals. Because of its structure, zearalenone can bind

to estrogen receptors in the uterus, hypothalamus and pituitary gland. The affinity of zearalenone to the estrogen receptors α and β is about one tenth to one twentieth of the affinity of the endogenous estrogen 17 β -estradiol towards the estrogen receptors α and β . In this way zearalenone can lead to marked estrogenic effects.

In pigs, zearalenone is reduced to α -zearalenol which induces an even stronger estrogenic response than zearalenone itself. The pronounced reduction of zearalenone to α -zearalenol seems to explain why pigs are particularly susceptible to zearalenone-contaminated feed. In this animal species zearalenone leads to swelling of the female genitals and to a reduced fertility. In a carcinogenicity study an increased incidence of liver and pituitary adenomas in female mice was observed, whereby only the number of liver adenomas was significantly enhanced from a statistical point of view [5]. In the past it was hypothesized that zearalenone was responsible for the massive occurrence of premature symptoms of puberty in very young girls for example in Puerto Rico and Hungary. However, in these cases one cannot discard that the girls could have taken up other plant estrogens.

Trichothecenes

Trichothecenes constitute the biggest group of Fusarium toxins. The most important compounds in this group are T2 toxin, deoxynivalenol und nivalenol (♦ Figure 5). They mainly contaminate grains (among others oat, wheat and maize) as well as grain products, but also potatoes in the field or during their storage.

They are strongly cytotoxic and do so by binding to the 60S subunit of ribosomes, thereby inhibiting protein biosynthesis and inducing apoptosis in a number of different organs. In animal experiments they particularly led to hemato- and immunotoxicity, but also to neuro-, embryo- and fetotoxicity (e. g. reduced litter

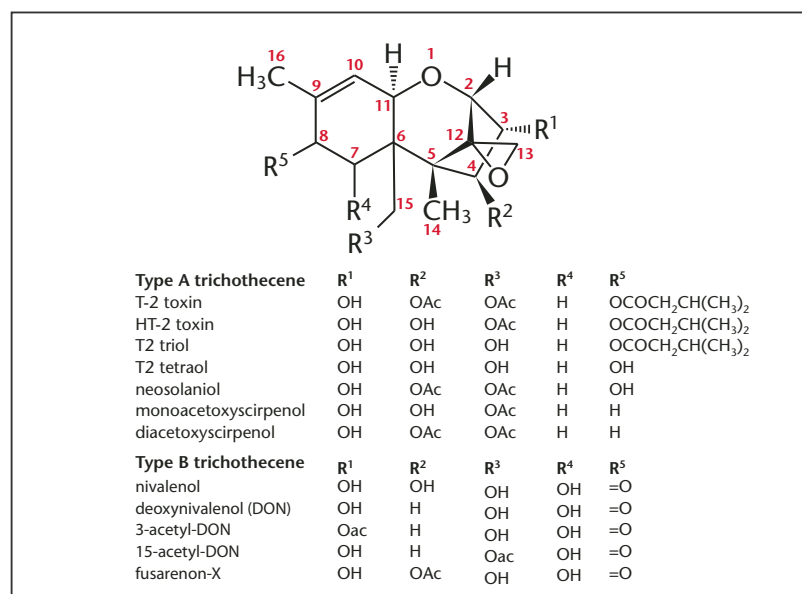


Fig. 5: Structural formulae of important trichothecenes

size and induction of malformations in rodents). In the case of deoxynivalenol, also known as vomitoxin, the most pronounced symptoms of the intoxication are nausea and vomiting. Due to this, deoxynivalenol often leads to a reduced feed intake in pigs and rodents.

If trichothecenes are taken up orally, a strong mucosal irritation in the oral cavity and intestine (nausea, vomiting, bloody diarrhea) is observed in humans. If they are taken up topically (i.e. through the skin), they result in a strong irritation and inflammation of the outer skin. During and after the Second World War, a mass disease, which was named alimentary toxic aleukia, was observed in the former Soviet Union [6]. Symptoms of this disease were:

- vomiting, abdominal pain, diarrhea
- leucopenia
- bone marrow aplasia
- high susceptibility to infections
- fever
- bleeding from the nose, throat and gums as a consequence of necroses in the oral cavity and oesophagus

The consumption of mouldy, wintered grains contaminated with trichothecenes (mainly T2 toxin) was assumed to be the cause of the above-mentioned disease.

Fumonisin

Fumonisin (♦ Figure 6), which almost exclusively contaminate maize, interfere with the biosynthesis of sphingolipids. These molecules are needed in all eukaryotic cells to build up cell structures and to regulate a number of different cell functions. Due to a fumonisin-induced inhibition of ceramide synthase (sphinganine *N*-acyl transferase), free sphinganine accumulates in the liver and kidneys of rodents and thereafter damages these organs. Because of the missing sphingolipids, the folic acid transporter, among other transporters, is impaired in mice, so that the placenta and the embryo take up

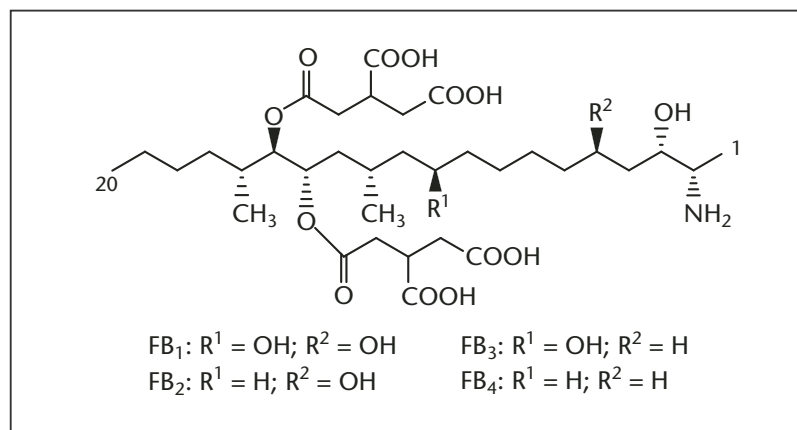


Fig. 6: Structural formulae of important fumonisins
 $\text{FB}_{1/2/3/4}$ = Fumonisin B₁/B₂/B₃/B₄

less folic acid and neural tube defects, leading to an „open spine“, occur.

Fumonisin induce a deadly disease in horses, the so-called equine leukoencephalomalacia. In sick animals a massive liquefactive necrosis of the subcortical white matter of one or both cerebral hemispheres, accompanied by oedema formation in the immediate surroundings of the necrotic areas, is observed. In 1989, thousands of pigs died in the USA after feeding them with maize that was highly contaminated with fumonisins, whereby the cause of death was pulmonary oedema. In rats and mice, fumonisins induce liver and kidney tumours.

Epidemiologic studies in South Africa, the People's Republic of China, Iran, Kenya, Brazil and the USA suggest that there might be a correlation between the contamination of food with fumonisins and the incidence of oesophageal cancer.

However, a prospective study was not able to demonstrate a correlation between sphingolipid plasma levels as a biomarker of exposure towards fumonisins and the frequency of oesophageal cancer in humans [7]. Furthermore, in the past an increased incidence of neural tube defects in babies at the border between Mexico and the USA, in China and in South Africa, regions in which maize was highly contaminated with fumonisins, was observed.

Ergot alkaloids

The fungus *Claviceps purpurea*, known as „ergot“, grows parasitically on cereal plants, mainly rye, in humid years. Up to the present time, more than 30 ergot alkaloids have been identified, all of which are lysergic acid or clavine derivatives (♦ Overview 1).

Symptoms of an acute intoxication with ergot alkaloids are sensation of

Lysergic acid amide alkaloids	ergine, ergometrine
Lysergic acid peptide alkaloids	ergotamine group (e. g. ergotamine, ergosine, ergosecaline) ergotoline group (e. g. α - and β -ergocryptine, ergocornine, ergocristine) ergoxine group (e. g. ergostine)
Clavine alkaloids	agroclavine, chanoclavine

Overview 1: Ergot alkaloids

thirst, abdominal pain, nausea, headache, paraesthesias, seizures and death due to respiratory paralysis or circulatory collapse. Uterine bleeding and abortions have often been described. Mass intoxications due to the consumption of rye bread made from ergot-infected grain occurred in Europe since the 9th century. In this regard, two forms of the chronic intoxication were described:

- gangrenous ergotism (burning pain, vascular spasms, loss of limbs and death)
- convulsive ergotism (headache, nausea, seizures, painful muscle contractions and psychoses)

The complex effects of ergot are mainly due to its high content of lysergic acid derivatives, which show structural similarities to the neurotransmitters noradrenaline, dopamine and serotonin (♦ Figure 7). The vasoconstrictory effect of ergotamine is used in human medicine to treat acute migraine attacks as well as postpartum to decrease uterine bleeding.

The contamination of grains with ergot is a problem at the present time, whereby the degree of contamination varies from year to year. Again and again flours with clearly higher ergot contents than the allowed 0.05 %, which correspond to about 1 mg total ergot alkaloids/kg grain, appear on the market. Pregnant women, unborn children and breast-fed babies are the groups at risk. Precautionary consumer protection demands that only grains, which are to the greatest possible extent ergot-free, should be industrially processed or directly reach the consumers.

Patulin

Patulin is produced by *Aspergillus clavatus*, *Aspergillus giganteus*, *Penicillium patulinum*, *Penicillium expansum* and *Penicillium urticae*. In the past, it was mainly detected in apple juices, but also in flour products and meat. It possesses a high affinity to thiol groups and can therefore inhibit for example membrane-bound ATPases. Following its oral uptake, mucosal irritations in the intestinal tract together with nausea, vomiting and diarrhea are observed. Although patulin is associated with a very low health risk when compared to aflatoxins, no mouldy apples should be used to produce apple juices.

Food	Mycotoxin	Number of samples	Percentage of samples with quantifiable levels [%]	Mean [μ g/kg type of product]
buckwheat grains	aflatoxin B ₁	12	16.7	1.78
buckwheat grains	sum of aflatoxine B ₁ , B ₂ , G ₁ and G ₂	12	16.7	2.14
peanut (roasted, with shell)	aflatoxin B ₁	133	2.3	0.126
peanut (roasted, with shell)	sum of aflatoxine B ₁ , B ₂ , G ₁ und G ₂	133	2.3	0.200
pumpkin seed	aflatoxin B ₁	117	1.7	0.006
pumpkin seed	sum of aflatoxine B ₁ , B ₂ , G ₁ und G ₂	117	2.6	0.017
almond (with shell, unroasted)	aflatoxin B ₁	99	5.1	0.116
almond (with shell, unroasted)	sum of aflatoxine B ₁ , B ₂ , G ₁ und G ₂	99	6.1	0.126
pepper (black, ground)	aflatoxin B ₁	109	25.7	0.158
pepper (black, ground)	sum of aflatoxine B ₁ , B ₂ , G ₁ und G ₂	109	27.5	0.180
sesame	aflatoxin B ₁	102	18.4	0.076
sesame	sum of aflatoxine B ₁ , B ₂ , G ₁ und G ₂	102	28.2	0.130
buckwheat grains	ochratoxin A	102	49.0	0.560
peanut (roasted, with shell)	ochratoxin A	123	48.8	1.44
pumpkin seed	ochratoxin A	111	14.4	0.066
almond (with shell, unroasted)	ochratoxin A	93	6.5	0.057
pepper (black, ground)	ochratoxin A	106	61.3	1.33
sesame	ochratoxin A	99	10.9	0.078
pils	ochratoxin A	93	60.2	0.018
wheat flour	ochratoxin A	118	30.5	0.123
rye flour	Summe T2-Toxin und HT2-Toxin	64	4.7	0.213
soy bean	sum of T2-toxin and HT2-toxin	48	0	
wheat flour	sum of T2-toxin and HT2-toxin	62	0	

Tab. 1: Aflatoxin, ochratoxin A, T2 toxin and HT2 toxin contents in foods according to Food Monitoring 2011 [8]

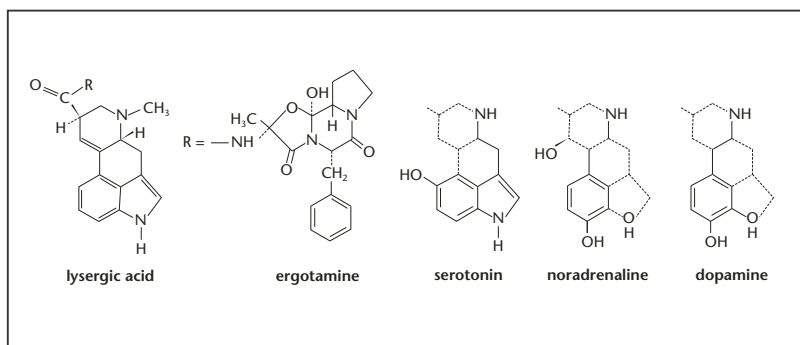


Fig. 7: Structural formula of lysergic acid and ergotamine and structural relationship with serotonin, noradrenaline and dopamine

Citrinin

Citrinin is formed by various different *Penicillium*, *Aspergillus* and *Monascus* species and is present in red yeast rice, a rice variety that is fermented by *Monascus* spp. In South-east Asia, red yeast rice is traditionally used as a natural food additive and in the USA it was marketed as a food additive with a cholesterol-lowering effect until 2000. Citrinin itself chronically damages the epithelial cells of the kidney tubuli, may induce kidney tumours and is mutagenic, aneuploidogenic and teratogenic.

Contamination of foods with aflatoxins, ochratoxin A and *Fusarium* toxins

The content of aflatoxins, ochratoxin A and the *Fusarium* toxins T2 and HT2 were determined in a variety of food items in the context of the Report on Food Monitoring 2011 in Germany [8] (♦ Table 1).

The highest amounts of aflatoxins were detected in buckwheat grains, whereby an exceedance of the allowed maximum aflatoxin levels only occurred in one peanut sample from Taiwan and one buckwheat grain sample from Germany. The highest mean ochratoxin A contents among all food items analyzed were detected in peanuts. Moreover, markedly higher levels of ochratoxin A were measured in part in buckwheat grains than in wheat flour; in

three buckwheat grain samples from Germany and one from the People's Republic of China, the determined contents exceeded the maximum allowed levels of 3 µg/kg food. The *Fusarium* toxins T2 and HT2 were detected in 4.7 % of the rye flour samples analyzed. In contrast, no T2-toxin and HT2-toxin were detected in the soy bean and wheat flour samples analyzed in 2011.

In summary, the number of cases, in which the maximum allowed mycotoxin levels were exceeded, was very low in 2011. However, as very recently demonstrated by the detection of aflatoxin B₁ in forage maize from Serbia [9], the mycotoxin monitoring of foods and animal feed is still of great relevance.

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Conflict of Interest

The author declares no conflict of interest according to the guidelines of the International Committee of Medical Journal Editors.

References

1. International Agency for Research on Cancer (IARC). *Some traditional herbal medicines, some mycotoxins, naphthalene and styrene*. In:

International Agency for Research on Cancer (IARC). (Hg). Monographs on the Evaluation of Carcinogenic Risks to Humans. 82. Aufl., IARC Press, Lyon (2002), S. 171–293

2. Stefanovic V, Radovanovic Z (2008) Balkan endemic nephropathy and associated urothelial cancer. *Nat Clin Pract Urol* 5: 105–112
3. Grollman AP, Shibutani S, Moriya M et al. (2007) Aristolochic acid and the etiology of endemic (Balkan) nephropathy. *Proc Natl Acad Sci USA* 104: 12129–12134
4. Jelakovi B, Karanovi S, Vukovi-Lela I et al. (2012) Aristolactam–DNA adducts are a biomarker of environmental exposure to aristolochic acid. *Kidney Int* 81: 559–567
5. National Toxicology Program (1982) Carcinogenicity bioassay of zearalenone in F344/N rats and B6C3F1 mice. *Natl Toxicol Program Tech Rep Ser* 235: 1–155
6. Joffe AZ. *Fusarium poae* and *F. sporotrichioides* as principal causal agents of alimentary toxic aleukia. In: Wyllie TD, Moorhouse LG (Hg). *Mycotoxic fungi, mycotoxins, mycotoxicosis: an encyclopedia handbook*. 3. Aufl., Marcel Dekker, New York (1978), S. 21–86
7. Shephard GS, van der Westhuizen L, Sewram V (2007) Biomarkers of exposure to fumonisin mycotoxins: A review. *Food Addit Contam* 24: 1196–1201
8. Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (2011) *Berichte zur Lebensmittelsicherheit 2011*. URL: www.bvl.bund.de/SharedDocs/Downloads/01_Lebensmittel/01_lm_mon_dokumente/01_Monitoring_Berichte/lmm_bericht_2011.pdf?__blob=publicationFile&v=4 Zugriff 12.08.13
9. Niedersächsisches Ministerium für Ernährung, Landwirtschaft und Verbraucherschutz (2013) *Aflatoxin B₁ in Futtermais – Aktueller Sachstand*. URL: www.ml.niedersachsen.de/portal/live.php?navigation_id=27751&article_id=113258&psmand=7 Zugriff 12.08.13

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