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Review article

Update of survey, regulation and toxic effects of mycotoxins in Europe

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Abstract

The most frequent toxigenic fungi in Europe are *Aspergillus*, *Penicillium* and *Fusarium* species. They produce aflatoxin B1 transformed into aflatoxin M1 found in the milk, as well as Ochratoxins and Zearalenone, Fumonisin B1, T-2 toxin, HT-2 toxin and deoxynivalenol (vomitoxin), which are of increasing concern in human health. These mycotoxins are under continuous survey in Europe, but the regulatory aspects still need to be set up and/or harmonised at European level. They are found in foodstuffs and are not destroyed by normal industrial processing or cooking since they are heat-stable. Some of their metabolites are still toxic and may be involved in human diseases. Their toxic effects (liver, kidney and hematopoetic toxicity, immune toxicity, reproduction toxicity, foetal toxicity and teratogenicity, and mainly carcinogenicity) are mostly known in experimental models, the extrapolation to humans being always inaccurate. The inaccuracy of extrapolation to humans may be explained by the lack of adequate food consumption data, lack of knowledge about relative health risks associated with specifically proposed limits and by the possibility of synergism with other mycotoxins present in the same food commodities. Other pathological causes are viral hepatitis, immune or hormonal deficiencies or organ dysfunction. Even when a specific biomarker of a given mycotoxin is identified in humans, it remains difficult to establish the relation with a given illness, because of genetic polymorphism and the possible beneficial influence of diet, and because other environmental toxicants may well interfere. The acceptable daily intake limits are mostly based on animal data and may be too high, due to the differences in the sensitivity of different animal species. The prevention involves first reduction of mycotoxin levels in foodstuffs and further increasing the intake of diet components such as vitamins, antioxidants and substances known to prevent carcinogenesis. © 2002 Published by Elsevier Science Ireland Ltd.

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1. Toxins of *Fusarium*

A variety of *Fusarium* fungi, which are common soil fungi, produce a number of different mycotoxins of the class of trichothecenes: T-2 toxin, HT-2 toxin, deoxynivalenol (DON) and nivalenol and some other toxins zearalenone and fumonisins. The *Fusarium* fungi are probably the most prevalent toxin-producing fungi of the northern temperate regions and are commonly found on cereals grown in the temperate regions of America, Europe and Asia. *Fusarium* toxins have been

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shown to cause a variety of toxic effects in both experimental animals and livestock. On some occasions, toxins produced by *Fusarium* species have also been suspected to have caused toxicity in humans.

1.1. Fumonisins

The only fungi that produce significant quantities of fumonisins are *Fusarium verticillioides* (Sacc.) Nirenberg (= *F. moniliforme* (Sheldon)) and the related *F. proliferatum* (Matsushima) Nirenberg.

Fumonisins occur infrequently in foodstuffs, such as sorghum, asparagus, rice, beer and mung beans. Fumonisin B1 is the diester of propane-1,2,3-tricarboxylic acid.

In long-term feeding studies, purified fumonisin B1 caused both liver and kidney tumours in rodents. The NOEL for renal cancer in Fisher 344N rats is 0.67 mg/kg of body weight per day and the NOEL for renal toxicity is 0.2 mg/kg of body weight (bw) per day. The NOEL for liver cancer in male BD IX rats is 0.8 mg/kg of body weight per day and the NOEL in feed-restricted female B6C3F1 mice is 1.9 mg/kg of body weight per day.

Several biochemical modes of action have been postulated to explain the induction of disease in animals by fumonisins. Three hypotheses involve disruption of lipid metabolism as the initial step. The first proposed mechanism involves disruption of sphingolipid metabolism through inhibition of ceramide synthase. The demonstrated consequences of inhibition of this enzyme in liver and kidney are changes in all the major pools of sphingolipids, including increased concentrations of free sphingoid bases and free sphingoid-base metabolites and decreased biosynthesis of ceramide and other sphingolipids containing ceramide. Glycerophospholipid metabolism is also affected. Clear evidence of fumonisin-induced disruption of sphingolipid metabolism has been obtained in all target tissues except brain, and in all species tested (Riley et al., 1993; Gelderbrom et al., 1992).

The second proposed biochemical mechanism involves disruption of fatty acid and glycerophospholipid metabolism.

Fumonisins also affect sites of cellular regulation that are apparently independent of the disruption of lipid metabolism, induction of oxidative stress and modulation of gene expression (Abado-Becognee et al., 1998; Mobio et al., 2000).

Since the proposed biochemical mechanisms of action involve alterations in de novo biosynthetic pathways, nutritional factors could play an important role in determining the potency of fumonisin B1 and the observed toxicological effects in rodents. No fumonisin was shown unequivocally to be genotoxic. No DNA adducts of fumonisin B1 have been found.

Consumption of mouldy sorghum or maize containing up to 64 mg/kg fumonisin B1 was associated with an outbreak of human disease in India involving gastrointestinal symptoms. The grain was also reported to be contaminated with other toxigenic fungi.

A provisional maximum is fixed for tolerable daily intake (PMTDI) for fumonisins B1, B2 and B3 alone or in combination, of 2 μg/kg of body weight per day on the basis of the NOEL of 0.2 mg/kg of body weight per day and a safety factor of 100. All the estimates of intake of fumonisin B1 based on the available data on national consumption were well below the group PMTDI. This remains true even when intake estimates for fumonisin B1 are increased by 40% to account for the presence of fumonisins B2 and B3 (Table 1 and Table 2).

1.2. Other fusarium toxins: trichothecenes

1.2.1. Deoxynivalenol

Deoxynivalenol (DON, vomitoxin) is a mycotoxin of the type B trichothecenes, which are epoxy-sesquiterpenoids. Deoxynivalenol occurs predominantly in grains such as wheat, barley, and maize and less often in oats, rice, rye, sorghum and triticale. The occurrence of deoxynivalenol is associated primarily with *Fusarium graminearum* (*Gibberella zeae*) and *F. culmorum*, both of which are important plant pathogens, causing *Fusarium* head blight in wheat. The incidence of *Fusarium* head blight is most affected by the timing of rainfall rather than by the amount at the time of flowering.

Table 1
Estimates of intake of fumonisin B1 based on international data

	Intake (μg/kg of body weight per day) (based on GEMS/food diets)				
	Africa	Middle East	Latin America	Far East	Europe
Mean	2.4	1.1	1.0	0.7	0.2
90th percentile	7.3	3.3	2.9	2.1	0.6

Deoxynivalenol may have adverse health effects after acute, short-term, or long-term administration. After acute administration, deoxynivalenol produces two characteristic toxicological effects: decrease in feed consumption (anorexia) and emesis (vomiting). The NOEL is 0.1 mg/kg of body weight per day.

In 1993, IARC placed deoxynivalenol in Group 3, not classifiable as to its carcinogenicity to humans (IARC, 1993).

The results of two studies in mice suggested that deoxynivalenol could suppress host resistance to *Listeria monocytogenes* and *Salmonella enteritidis*, with a NOEL of 0.25 mg/kg of body weight per day, and a LOEL of 0.12 mg/kg of body weight per day, respectively. Antibody responses were also affected by deoxynivalenol, the NOEL being 1 mg/kg of body weight per day in mice. A global effect of trichothecenes on platelet precursors has been evaluated by Froquet et al. (2000), characterised by a high toxicity at low concentrations. In pigs given naturally contaminated feed the NOEL was 0.08 mg/kg of body weight per day.

Many outbreaks of acute human disease involving nausea, vomiting, gastrointestinal upset, dizziness, diarrhoea and headache have occurred in Asia. These outbreaks have been attributed to consumption of *Fusarium*-contaminated grains and, more recently, to the presence of deoxynivalenol at reported concentrations of 3–93 mg/kg in grain for human consumption.

In two studies, none of the health effects described above were observed after consumption of grain containing deoxynivalenol at 0.02–3.5 mg/kg. Most of the studies on acute effects in humans were population-based or ecological studies. Thin-layer chromatography and enzyme-linked immunosorbent assay (ELISA) methods are also good means for screening of deoxynivalenol.

Deoxynivalenol was found to be a frequent contaminant of a large number of samples of cereal grains such as wheat (57% positive), maize (41% positive), oats (68% positive), barley (59% positive), rye (49% positive) and rice (27% positive). It was also detected in buckwheat, popcorn, sorghum, and triticale and in some processed food products such as wheat flour, bread, breakfast cereals, noodles, baby and infant foods, and cooked pancakes in Europe and northern America. In addition, it has been reported in barley products, malt and beer. The mean concentrations in data sets in which samples containing deoxynivalenol were found were 4–9000 μg/kg for barley, 3–3700 μg/kg for maize, 4–760 μg/kg for oats, 6–5100 μg/kg for rice, 13–240 μg/kg for rye and 1–5700 μg/kg for wheat.

The results of a 2-year feeding study in mice did not suggest that deoxynivalenol presents a car-

Table 2
Some national estimates of intake of fumonisin B1 in Europe and in the world

Country	Intake (μg/kg of body weight per day)	
	Mean or median	90th percentile
Argentina	0.2	na
Canada	0.02	0.08
Netherlands	0.06, 1.0[a]	na
Switzerland	0.03	na
United Kingdom	0.03	0.1
United States	0.08	na

na = not reported or calculated.

[a] The first value is for whole population, the second for regular maize eaters.

cinogenic hazard. Estimation of the dietary intake of deoxynivalenol on the basis of the single weighted mean concentrations and the GEMS/food regional diets resulted in values that exceeded the PMTDI for four of the five regional diets.

1.2.2. T-2 and HT-2 toxins

These toxins are type A trichothecene mycotoxins, which are closely related epoxy, sesquiterpenoids. Surveys have revealed the presence of T-2 and HT-2 toxins in grains such as wheat, maize, oats, barley, rice, beans, and soya beans as well as in some cereal-based products. T-2 and HT-2 toxins have been reported to be produced by *Fusarium sporotrichioides*, *F. poae*, *F. equiseti*, and *F. acuminatum*. The most important producer is *Fusarium sporotrichioides*, a saprophyte (i.e. not pathogenic to plants) with growth at −2–35 °C and only at high water activities (above 0.88).

T-2 toxin is a potent inhibitor of protein synthesis both in vivo and in vitro. The effective concentration for protein inhibition in vitro is lower than the effective concentrations for all other effects that have been demonstrated.

The immune system is a primary target of T-2 toxin, and the effects include changes in leukocyte counts, delayed hypersensitivity, depletion of selective blood cell progenitors, depressed antibody formation, allograft rejection, and a blastogenic response to lectins.

Outbreaks of acute poisoning in which the effects reported included nausea, vomiting, pharyngeal irritation, abdominal pain and distension, diarrhoea, bloody stools, dizziness and chills.

The disease was lethal in a high proportion of cases. In investigations conducted, cultures implicated in the outbreak were shown to produce T-2 toxin. Data on the concentrations of T-2 and HT-2 toxins in food commodities were submitted by Brazil, China, Finland, Germany, Norway, Sweden and the United Kingdom. The mean concentrations of data sets where positive samples of HT-2 were found were 0.4–15 μg/kg in barley, 2.4–14 μg/kg in maize, 3.7–20 μg/kg in oats, 26–100 μg/kg in rice, 0.03 μg/kg in rye and 0.2–20 μg/kg in wheat.

Physical, chemical and biological methods have been used to decontaminate grain containing trichothecenes, but few studies are available on any reduction in the concentrations of T-2 or HT-2 toxins. Thermal processing is usually ineffective. The toxic effects of T-2 toxin and its metabolite HT-2 toxin could not be differentiated, and the toxicity of T-2 toxin in vivo might be due at least partly to HT-2 toxin. Hence HT-2 toxin was included in the PMTDI, resulting in a group PMTDI of 60 ng/kg of body weight per day for T-2 and HT-2 toxins, alone or in combination.

1.2.3. Zearalenone

A PMTDI for zearalenone (ZEA) of 0.5 μg/kg of body weight is now established, based on the NOEL of 40 μg/kg of body weight per day obtained in a 15-day study in pigs.

ZEA and some of its metabolites have been shown to competitively bind to oestrogen receptors. The relative binding affinities to the rat uterine cytoplasmic receptor for ZEA and derivatives are, in decreasing order, α-zearalanol > α-zearalenol > β-zearalanol > ZEA > β-zearalenol (Kuiper-Goodman et al., 1987; Eriksen and Alexander, 1998).

1.2.3.1. Acute toxicity. ZEA shows low acute toxicity after oral administration in mice, rats and guinea pigs (oral LD50 values of >4000 up to >20 000 mg/kg bw) (Kuiper-Goodman et al., 1987; JECFA, 2000). It is more toxic by i.p. injection. The NOEL in pigs is 40 μg/kg of body weight per day and in rat 100 μg/kg of body weight (Kuiper-Goodman et al., 1987; JECFA, 2000).

1.2.3.2. Chronic toxicity and carcinogenicity. B6C3F1 mice were fed diets containing 0, 50 or 100 mg/kg ZEA for 103 weeks (males: 0, 8 or 17, females: 0, 9 or 18 mg/kg bw per day). Hepatocellular adenomas were found in 8, 6 and 14% and 0, 4 and 14% in males and females, respectively. The increase was statistically significant in the high-dose females. However, the incidence of pituitary carcinomas in treated and control animals was not significantly different statistically (NTP, 1982). A NOEL of 0.1 mg/kg of body weight per

day can be taken from this study (Becci et al., 1982). Zearalenone and derivatives are classified by IARC, in Group 3 (IARC, 1999) (not classifiable as to their carcinogenicity to humans).

1.2.3.3. Genotoxicity. ZEA did not induce mutations in *S. typhimurium* (Ames test) or mitotic crossing over in *S. cerevisiae*. It induced sister chromatid exchanges (SCE), chromosomal aberrations and polyploidy in Chinese hamster ovary cells in vitro in the absence of exogenous metabolic activation. It also induced SOS repair in bacteria (Ghedira-Chekir et al., 1998, 1999).

1.2.3.4. DNA-adduct formation. In female BalbC mice, treated i.p. with a single dose of ZEA (2 mg/kg bw), 12–15 different DNA-adducts were found in the kidney and liver. DNA-adducts in the liver and the kidney were detected by ^{32}P-post-labelling. Co-administration of α-tocopherol (4 mg/kg bw) significantly decreased DNA-adduct formation (Grosse et al., 1997; Ghedira-Chekir et al., 1999).

1.2.3.5. Immunotoxicity. Several alterations of immunological parameters were found at high ZEA concentrations in vitro (inhibition of mitogen-stimulated lymphocyte proliferation, increases IL-2 and IL-5 production) (Eriksen and Alexander, 1998; JECFA, 2000).

1.2.3.6. Reproductive and developmental toxicity. ZEA causes alterations in the reproductive tract of laboratory animals (mice, rat, guinea-pigs, hamsters, rabbits) and domestic animals. Various estrogenic effects, e.g. decreased fertility, increased embryolethal resorptions, reduced litter size, changed weight of adrenal, thyroid and pituitary glands and change in serum levels of progesterone and estradiol have been observed but no teratogenic effects were found in mice, rats, guinea pigs and rabbits (Kuiper-Goodman et al., 1987; Bacha et al., 1993; Maaroufi et al., 1996; JECFA, 2000). Pigs and sheep appear to be more sensitive than rodents. A NOEL of 40 μg/kg of body weight per day can be concluded based on this study.

1.2.3.7. Effects in humans. ZEA was measured in endometrial tissue from 49 women. There were 27 endometrial adenocarcinomas, 11 endometrial hyperplasias and 11 normal proliferative endometria with ZEA values of 47.8 ± 6.5, 167 ± 17.7 ng/ml and below detection limit in the groups, respectively. In 8 cases of hyperplastic and 5 cases of neoplastic endometrial tissue, ZEA was not detected (Tomaszewski et al., 1998).

Increased incidence of early telearche has been reported from the southeast region of Hungary. ZEA was found in concentrations from 18.9–103.5 μg/ml in serum sampled from the patients and ZEA was also present in samples of surplus food collected from the patients (Szuetz et al., 1997).

In conclusion and concerning zearalenone, hepatocellular adenomas and pituitary tumours were observed in long-term studies of carcinogenicity in mice. However, these tumours were observed only at doses greatly in excess of the concentrations that have hormonal effects, i.e. at levels of 8–9 mg/kg of body weight or more. Thus it can be concluded that these tumours are a consequence of the estrogenic effects of ZEA. Human adenocarcinomas and endometrial hyperplasia found in ZEA contaminated women are still under investigation.

2. Toxins of *Aspergillus*

2.1. Aflatoxin B1 and its metabolite Aflatoxin M1

Aflatoxins may be produced by three species of *Aspergillus*—*A. flavus*, *A. parasiticus*, and the rare *A. nomius*—which contaminate plants and plant products. *A. flavus* produces only B aflatoxins, while the other two species produce both B and G aflatoxins. Aflatoxins M1 and M2 are the hydroxylated metabolites of aflatoxin B1 and B2 and may be found in milk products obtained from livestock that have ingested contaminated feed. The main sources of aflatoxins in feeds are peanut meal maize and cottonseed meal. Aflatoxin B1 (AFB1) is the most potent hepatocarcinogen known in mammals, the risk assessment of which

Table 3
Cancer risks associated with proposed maximum levels of aflatoxin M1 in milk and with the weighted mean for the European regional diet for populations with a consistently high intake but with low, medium or high prevalence of HBsAg+

Proposed maximum level of aflatoxin M1 in milk	HBsAg+ (%)	Average potency[a]	Aflatoxin M1 intake (ng/person per day)	Aflatoxin M1 intake (ng/kg of body weight per day)[b]	Additional cancer cases/year per 1×10^6
0.05	1	0.0013	15	0.25	3.2
	5	0.0025	15	0.25	6
	25	0.0083	15	0.25	20
0.5	1	0.0013	150	2.5	32
	5	0.0025	150	2.5	60
	25	0.0083	150	2.5	200
Weighted mean of 0.023	1	0.0013	6.8	0.11	1.5
	5	0.0025	6.8	0.11	2.8
	25	0.0083	6.8	0.11	9.4

[a] Potency in units of cancers/year per 100 000/ng per kg of body weight per day.
[b] Assuming a body weight of 60 kg.

is very well established. The most threatening aspect of AFB1 contamination is now related to aflatoxin M1 (Table 3). About 0.3–6.2% of AFB1 in animal feed is transformed to aflatoxin M1 in milk. Aflatoxin M1 is produced by metabolism of AFB1. Maximum levels of 0.05 and 0.5 μg/kg are found in milk. The toxicity of aflatoxin M1 is about one order of magnitude less than that of AFB1 (Table 3 and Table 4).

The metabolism of AFB1 to the epoxide and to aflatoxin M1 can be blocked in vitro (human hepatocytes) and in vivo (rats) by treatment with oltipraz, an antischistosomal drug, which blocks the formation of the epoxide and induces the major aflatoxin detoxification enzyme, glutathione-S-transferase (GST). Oltipraz is being tested in phase I and II clinical trials in China in the prevention of liver cancer. The results of these studies will be useful for clarifying the metabolism and mode of action of aflatoxins in humans.

Studies in which the recently developed biomarkers of exposure (aflatoxin–albumin adducts in serum, aflatoxin N7 guanine adducts in urine, aflatoxin M1 metabolite in urine or patterns of p53 gene mutations) are still underway. Screening tests for aflatoxin M1 in milk and milk products include radioimmunoassay and ELISA methods. Both biomarkers and aflatoxin M1 assay may be used to assess human exposure.

The weighted mean concentration of aflatoxin M1 in milk is 0.023 μg/kg in the European-type diet, 0.022 μg/kg in the Latin American diet, 0.36 μg/kg in the Far Eastern diet, 0.005 μg/kg in the Middle Eastern diet and 0.002 μg/kg in the African diet. These mean concentrations are based on a large number of milk samples analysed. The intake of aflatoxin M1 from milk is calculated to be 6.8 ng/person per day for the European diet, 3.5 ng/person per day for the Latin American diet, 12 ng/person per day for the Far Eastern diet, 0.7 ng/person per day for the Middle Eastern diet and 0.1 ng/person per day for the African diet.

The concentration of AFB1 in feed can be reduced by good manufacturing practice and good storage practices. If preventive measures fail, however, AFB1 can be reduced in feed by blending or by physical or chemical treatment. The physical treatments include heat, microwaves, gamma-rays, X-rays, ultra-violet light, and adsorption. Adsorption of aflatoxins onto hydrated sodium calcium aluminosilicate and other inert materials has been used in the animal feed industry in an attempt to reduce the aflatoxin M1 content of milk. The most successful chemical procedure for degrading aflatoxins in animal feed is ammoniation, which leads to decomposition of

Table 4
Maximum limits for mycotoxins in foods in various European countries and USA

Mycotoxin	Country	Maximum limit (μg/kg or μg/l)	Foods
Aflatoxin B1	Finland	2	All
	Germany	2	All
	The Netherlands	5	All
	Belgium	5	All
	Portugal	25	Peanuts
		5	Children's food
		20	Others
	Austria	1	All
		2	Cereals, nuts
	Switzerland	1	All
		2	Maize, cereals
	Spain	5	All
	Luxembourg	5	All
	Ireland	5	All
	Denmark	5	All
	Greece	5	All
Aflatoxin B1, B2, G1 and G2	Sweden	5	All
	Norway	5	Peanuts, Brazil nuts, buckwheat
	Finland	5	All
	Germany	4	All
		0.05	Enzymes and enzyme formulations
	Great Britain	4	Nuts and dried figs, Nuts and dried figs, on import before sorting
	France	10	All
	Italy	50	Peanuts
	Austria	5 (B2+G1+G2)	All
		0.02 (M1+B1+B2+G1+G2)	Children's food
	Switzerland	5 (B2+G1+G2)	All
		0.01	Baby food
	USA	20	All
	Belgium	5	Peanuts
	Bosnia	1 (B1+G1)	Cereals
		5	Beans
Aflatoxin M1	Sweden	0.050	Liquid milk products
	Austria	0.050	Milk
	Germany	0.050	Milk
	The Netherlands	0.050	Milk
		0.020	Butter
		0.200	Cheese
	Russia	0.5	
	Switzerland	0.020	Baby food
		0.050	Milk and milk products
		0.250	Cheese
	Belgium	0.050	Milk
	USA	0.50	Milk

Table 4 *(continued)*

Mycotoxin	Country	Maximum limit (μg/kg or μg/l)	Foods
	Czech Rep.	0.1	Children's milk
		0.5	Adult's milk
	France	0.03	Children's milk
		0.05	Adult's milk
	Bulgaria	0.5	
Deoxynivalenol	USA	1000 (monitoring)	Wheat
	Russia	1000	Cereals
	Austria	750	Wheat
Ochratoxin A	Romania	5	All
	Czech Rep.	1	Children's food
		20	
	Denmark	5	Cereals
		25	Pigs
	Austria	5	Cereals
	Switzerland	2	Cereals
	Greece	20	All
	France	5	All (proposal 4)
	The Netherlands	0	Cereals
Fumonisin B1 + B2	Switzerland	1000	Maize
Zearalenone	Romania	30	Cereals, vegetable oils
	Austria	60	Cereals
	France	200	Cereals, vegetable oils
	Russia	1000	Cereals, vegetable oils
T_2-toxin	Russia	100	All

95–98% of the AFB1, and this procedure is used in various countries.

2.2. Ochratoxin A

Ochratoxin A (OTA) is produced by *Penicillium verrucosum*, and by *Aspergillus ochraceus* together with a low percentage of isolates of the closely related *Aspergillus niger*. These three groups of species differ in their ecological niches, in the commodities affected, and in the frequency of occurrence in different geographical regions. *P. verrucosum* grows only at temperatures below 30 °C and down to 0.80 water activity. It is therefore found only in cool temperate regions and is the source of OTA in cereals and cereal products in Canada and Europe. As cereals are widely used in animal feeds in Europe, OTA may come through animal products in human diet (Table 4). OTA is absorbed from the gastrointestinal tract. Distribution in a number of species is via blood, mainly to the kidneys, lower concentrations being found in liver, muscle and fat. Transfer to milk has been demonstrated in rats, rabbits and humans, but little is transferred to the milk of ruminants owing to metabolism of OTA by the rumen microflora.

OTA has a long half-life in non-ruminant mammals, e.g. 24–39 h in mice, 55–120 h in rats, 72–120 h in pigs, 456–504 h in vervet monkeys (*Cercopithecus aethiops*) (Stander et al., 2001), 510 h in macaque and 840 h in a human volunteer. OTA is cytotoxic to human CFU-GM and BFU-E in vitro (Rio et al., 2000). The National Toxicology Program (USA) reported carcinogenesis studies in 1989 and noted the consistent presence and severity of karyomegaly in male and female rats and the aggressive nature of the renal tu-

mours in this study. However the biological and mechanistic significance of these observations was unclear.

The genotoxicity of OTA has been first demonstrated by Creppy et al. (1985) in vivo and in vitro with indications that the DNA lesions were repaired within some days for a single dose. Kane et al. (1986) have then shown that the DNA lesions induced by OTA in vivo were no longer repaired in case of repeated exposure. Putative DNA adducts were found consistently with a ^{32}P-postlabelling method in the kidneys of mice and rats dosed with OTA, but none of these adducts has been demonstrated to contain a fragment of OTA, (Pfohl-Leszkowicz et al., 1993, 1994; Creppy, 1999, 2000; Maaroufi et al., 1999). This has, however, been demonstrated in vitro by the group of Dirheimer in 2000 (Obrecht-Pflumio and Dirheimer, 2000). Adducts are formed on dG exclusively. This could be proposed as a marker of exposure in population at risk.

Ochratoxin A has been found in human blood samples, most notably in a number of countries in the cool temperate areas of the Northern hemisphere. However, no case of acute intoxication in humans has been reported. Ochratoxin A was found more frequently and at high average concentrations in blood samples obtained from people living in regions where a fatal human kidney disease (known as Balkan Endemic Nephropathy) occurs and is associated with an increased incidence of tumours of the upper urinary tract (Petkova-Bocharova et al., 1988). Nevertheless, similar average concentrations have been found in some other European countries where this disease is not observed. This could be due to difference in analytical methods and eating habits. The epidemiological and clinical data available do not provide a basis for calculating the likely carcinogenic potency in humans and it may be that other nephrotoxic agents are involved in the etiology of Balkan Endemic Nephropathy.

Prevention of the growth of *A. ochraceus*, which occurs primarily in stored foods, consists of the standard methods for preventing growth of any fungus in dried foods. The major commodities in which *A. ochraceus* may produce OTA are store grains. The traditional means of avoiding fungal growth in grains is to dry them rapidly and thoroughly and to keep them dry. To provide a water activity below 0.8 a reduction of moisture content in grains is necessary to prevent formation of OTA by *A. ochraceus*. Further effective approaches to grain storage include fumigation, aeration and cooling, sealed storage and controlled atmospheres, especially in tropical and subtropical regions, where insect damage is a major problem.

Important factors affecting body burdens and pathologies include the quality of the diet in providing antioxidants, vitamins and amino acids such as phenylalanine like in the sweetener aspartame. The beneficial effects of aspartame in the kidney and the brain have been reported (Baudrimont et al., 1997; Creppy et al., 1996; Belmadani et al., 1998). Aspartame actually prevents the genotoxicity of OTA as determined by DNA-adduct formation in animal tissues (Creppy, 2000), and karyomegaly mainly in kidney (Baudrimont et al., 2001).

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