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Carcinogen Role of Food by Mycotoxins and Knowledge Gap

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1. Introduction

In today's world health and safety are among the basic human needs. Ensuring food safety has been a major focus of international and national action over the last decades.

Both, microbiological and chemical risks are of concern. The "World Health Organization" (WHO) has identified as significant sources of food-borne diseases contamination of food and feed by mycotoxins (toxic metabolites of molds) and the contamination of fishery products by phycotoxins (toxins produced by algae).

Despite public health and prevention managers have paid particular attention to mycotoxins, in several areas of the world they are still an important food safety issue (Fig 1).

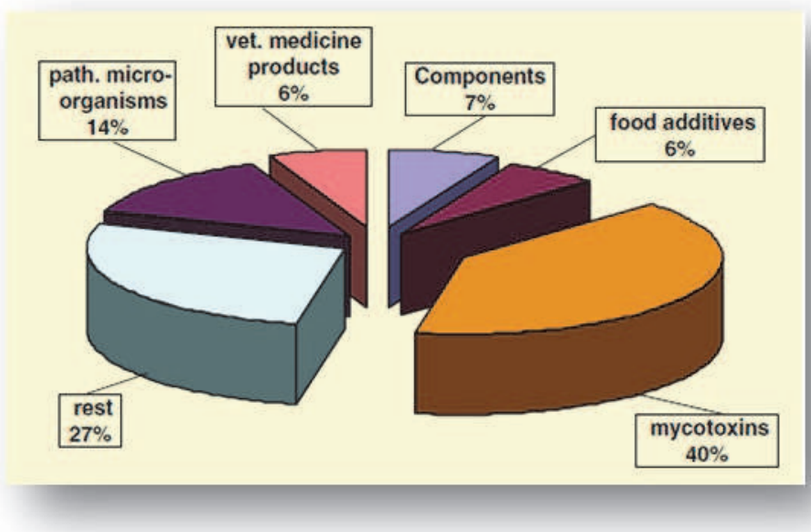


Fig. 1. Notifications for food and feed in 2005 (from EU rapid alert system, by European Commission 2006).

Mycotoxins can cause diseases in humans, crops and animals that have led many Countries to establish limits on mycotoxins in food and feed to safeguard people's health, as well as the economical interests of producers and traders.

The first limits for mycotoxins were set in the late 1960s for the aflatoxins. Already approximately 100 Countries in the world have developed specific limits for mycotoxins in foodstuffs and feedstuffs and their number continues to grow (Van Egmond & Jonker, 2004; WHO, 2002a).

1.1 General information on mycotoxins

The natural fungal flora associated with foods is dominated by four genera: Aspergillus, Fusarium, Penicillium and Claviceps.

The chemical structures of mycotoxins produced by these fungi are very diverse, as are the mycotoxicoses they can cause.

The term *mycotoxin* was coined in 1962 after an unexplained die-off of about 100,000 turkeys.

It was then discovered that the mysterious turkey's disease was tied to a feed essentially composed of peanuts contaminated by secondary metabolites of Aspergillus flavus or aflatoxins (Bennett & Klich, 2003).

Mycotoxins are invisible, odourless molecules, and cannot be detected by taste (Binder, 2007).

It is difficult to define mycotoxin in a few words. It is a natural low-molecular-weight molecule; it is a secondary metabolite produced by molds that has adverse effects even at low concentrations on the health of humans, animals, and crops. Those metabolites constitute a toxigenically and chemically heterogeneous class.

Mycotoxins are classified from the a chemical viewpoint into: cyclopeptides, polycetoacids, terpenes, and nitrogenous metabolites, depending on their biological origin and structure (Bhat et al., 2010).

They are mainly produced by the filamentous structure of molds mycelia. They have no biochemical significance for the growth and development of the fungus itself. Over 400 mycotoxins have been isolated and chemically characterized, though research has focused on those forms causing significant harm to humans, animals and crops (Hussein & Brasel, 2001; Zain, 2011). Aflatoxins (AFLs), Ochratoxins A (OTA), trichothecenes as Vomitoxin (DON), Zearelenone (ZEA), Fumonisins B1 and B2 (FUMO B1, FUMO B2), tremorgenic toxins, and ergot alkaloids are the most important mycotoxins if we consider their effects on human health.

It is very important to note that some molds are capable of producing more than one mycotoxin and some mycotoxins are produced by more than one species of mold.

The presence of mycotoxins in cereals, wine, beef, pork and oil seed and other food products has been accepted as natural in many of the EU countries, in the U.S.A, in Canada, Russia, and in most of the Asian Countries (Bhat et al., 2010; Van Egmond & Jonker, 2004).

Mycotoxins are not destroyed by cooking and by normal industrial food processing since they all are heat-stable.

Exposure to mycotoxins may result in a variety of illnesses that fall under the heading of mycotoxicosis, from ingestion or in occupational circumstances from dermal and inhalation exposure (Jarvis & Miller, 2005; Li et al., 2011; Straus, 2011).

Human exposure to mycotoxins may occur at all levels of the food chain: via consumption of plant-derived products contaminated with toxins (cereal grains, coffee, oil seeds, spices, fruit juices, and beverages as wine and beer), or even from carrying-over of mycotoxins and their metabolites (e.g. aflatoxin M1) in milk, meat, and other products of animal origin contaminated because of using feeds with mycotoxins (Bhat et al., 2010; CAST, 2003), or by exposure to air and dust containing toxins (Jarvis, 2002;). In recent times, in actual fact, concerns have been raised about exposures to mycotoxins in indoor environments as a damp houses and buildings (Straus, 2011).

In Ferrante and colleagues study (2007) was carried out a monitoring of wheat samples because it is the main ingredient of bread and pasta, basic aliments, and quantitatively substantial of Mediterranean diet. Wheat samples, from various areas of East Sicily, was analyzed for carcinogenic micotoxins (AFLs, OTA, ZEA). The results show that all samples collected do not contained AFLs whereas OTA and ZEA have been found in the samples to medium concentrations of 0.01 mg/kg and 0.108 mg/kg, respectively. Instead all samples contained OTA and ZEA. So this study confirmed the previous findings of the related studies present in literature.

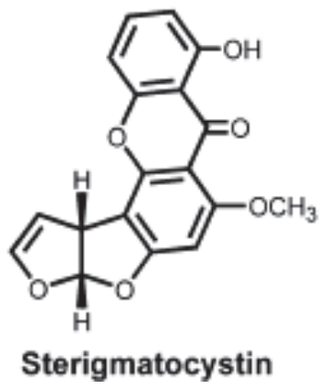
The main mycotoxins that contaminate food rarely are found in indoor environments.

Indeed, Penicillium and Aspergillus are the main contaminants of both food and damp buildings, though in the latter they produce different mycotoxins.

The genera found in outdoor air, as Aspergillus and Penicillium, are not associated with human or animal toxicosis.

The molds associated with buildings comprise a narrow group of species that grow thanks to the nutrients present in building materials and to the available water (Jarvis & Miller, 2005; Straus, 2011).

A. versicolor, that is often encountered in damp buildings, typically produces *Sterigmatocystin*, a class 2B carcinogen (IARC, 1993; Jarvis & Miller, 2005).



Sterigmatocystin

Fig. 2. Molecular formula of Sterigmatocystin.

The most frequent toxigenic fungi in Europe are Aspergillus, Penicillium and Fusarium (Bhat et al., 2010; Creppy, 2002), while the prevalence of specific toxins around the world is as follows: AFLs in Africa and in the Asian continent; AFLs and FUMO in Australia; AFLs, OTA,

ZEA and DON in North America; AFLs , FUMO, OTA, DON and T-2 toxin in South America; ZEA and DON in the Eastern European Countries; OTA, ZEA and DON in the Western European regions (Bhat et al., 2010; Van Egmond & Jonker, 2004; WHO, 2002a). However, with the open global trade these mycotoxins might also be detected in all areas of the world.

Fig. 3. Distibution of mycotoxins around the world. (AFLAs: Aflatoxins; FB: Fumonisins; OTA: Ochratoxin A; ZEA: Zearalenone; T-2: T-2 toxin).

At this stage, in Europe mycotoxins are controlled, but the regulatory policies still need to be standardized in the various European Countries.

Factors contributing to the presence or production of mycotoxins in foods or feeds include storage, environmental and ecological conditions, but also farming activities, such as drying, handling, packaging and transport, contribute to molds growth and to increase the production of mycotoxins when good practices are not followed (Zain, 2011).

Researchers have found a variety of factors operating interdependently in the production of mycotoxins. Those factors have been categorized as physical, chemical, and biological.

Physical factors include environmental characteristics as temperature, relative humidity, and insect infestation.

Chemical factors include the use of fungicides or fertilizers; biological factors, instead, depend on the interactions between the colonizing toxigenic fungi and the substrate, in fact some plant species are more susceptible to colonization while environmental conditions may increase the vulnerability of others that are more resistant (Zain, 2011).

2. Health effects from dietary exposure to mycotoxins

Concern about mycotoxins is based on well-documented human mycotoxicosis such as ergotism in Europe, alimentary toxic aleukia (ATA) in Russia, acute aflatoxicosis in South and Est Asia, and human primary liver cancer in Africa and South Est Asia.

OTA, instead, is suspected to be a causal factor of Balkan Endemic Nephropathy (BEN) in Yugoslavia and *chronic interstitial nephritis* (CIN) in North Africa.

More epidemics, associated with economic losses and war, have occurred in Russia (1924 and 1944), Ireland (1929), France (1953) and Ethiopia (1978) (Murphy, 2006).

The first episodes of lethal disease in humans caused by ATA have been in Russia, followed by other Countries.

This devastating disease, often fatal, is characterized by necrotizing, hemorrhagic and central nervous system effects and has been identified as a toxic manifestation of grain contamination by mold.

Retrospectively, it has been proven to be caused by; toxins, a metabolite of *Fusarium sporotrichioides*, the most common fungus isolated from contaminated grains incriminated in this russian disease (Richard, 2007; Li et al., 2011).

Still today, especially in developing Countries, mycotoxicoses represent a serious problem, difficult to prevent.

In Africa, many acute diseases are associated with mycotoxins, like aflatoxic hepatitis in Kenya and the vascular ergotism in Ethiopia. From studies carried out on people in Kenya, Mozambique and Swaziland, higher levels of AFLAs were found in food and these were correlated with levels detected in human fluids as urine and blood (Sibanda et al., 1997).

It is hard to define mycotoxins, as well as to classify them. The classification schemes tend to reflect the training of the categorizing person (Zain, 2011).

Mycotoxins cause various and powerful deleterious effects on human health, as some of them are carcinogenic, mutagenic, teratogenic, estrogenic, hemorrhagic, immunotoxic, nefrotoxic, hepatotoxic, dermotoxic and neurotoxic (see Table. 1).

AFLs are important causal factors of liver cancer while FUMO and OTA are suspected to have an important role in the etiology of esophageal cancer and in the famous BEN *(Balkan Endemic Nephrotoxicity)* respectively (Tab. 2.).

The impact of mycotoxins on health depends on various factors, as the fungus species, the daily intake of the mycotoxins consumed and their concentration, the toxicity of the compound, their mechanisms of action, the body weight of the individual, the presence of other synergic or protective elements present in the food, the metabolism and defense mechanisms of the host (Hussein & Brasel, 2001; Stayn, 1995).

Their toxic effects, however, are mostly proven in experimental models.

The inaccuracy of extrapolation of results for humans is an important point for research; it may be explained by the lack of adequate food consumption data, by the gap of knowledge about health risks associated with the actual proposed limits and by the big possibility of synergism or additive toxicity with other mycotoxins present in the same food (Creppy, 2002).

Genera	Mycotoxins	Biological effect
Aspergillus		
	Aflatoxin B1	Carcinogenic, mutagenic, immunotoxic, hepatotoxic
	Aflatoxin G1	Carcinogenic, mutagenic, immunotoxic, hepatotoxic
	Aflatoxin M1	Carcinogenic, mutagenic, immunotoxic, hepatotoxic
	Ochratoxin A	Carcinogenic, teratogenic, immunotoxic, nefrotoxic
	Sterigmatocystin	Carcinogenic , mutagenic, teratogenic
	Cyclopiazonic acid	Mutagenic, neurotoxic
Penicillium		
	Patulin	Genotoxic, mutagenic
	Ochratoxin A	Carcinogenic, teratogenic, immunotoxic, nefrotoxic,
	Citrinin	Nefrotoxic
	Penitrem A	Neurotoxic
	Cyclopiazonic acid	Mutagenic, neurotoxic
Fusarium		
	Vomitoxin	Hemorrhagic, dermotoxic
	Nivalenol	Hemorrhagic, dermotoxic
	Zearalenone	Estrogenic, dermotoxic
	T-2 toxin	Hemorrhagic, dermotoxic
	Diacetoxyscirpenol	Hemorrhagic
	Fumonisins	Carcinogenic
	Moniliformin	Hemorrhagic
	Tenuazonic acid	Hemorrhagic
Alternaria		
	Alternariol	Foetotoxic, teratogenic, hemorrhagic
	Alternariol methyl ether	Foetotoxic, teratogenic, hemorrhagic
Claviceps		
	Ergot alkaloids	Neurotoxic

Table 1. Some mycotoxins and their biological effects on human health.

Mycotoxins	Desease
Aflatoxins	Aflatoxicosis
	Hepatocarcinogenicity
	Encephalophaty
	Reye's sindrome
Ochratoxin A	Balcan Endemic Nephropathy (BEN)
	Kidney tumors
Sterigmatocystin	Pulmonary tumours
	Hepatocarcinogenicity
	Urinary tract tumors
	Kidney tumors
Fumonisins	Esophageal cancer
	Hepatocarcinogenicity
	Pulmonary edema
	Leukoencephalomalacia
	Hepatotoxicity
	Nephrotoxicity
Zearalenone	Cervical cancer

Table 2. List of mycotoxins and relative pathologies.

2.1 Carcinogenic mycotoxins and their mechanisms of action

2.1.1 Aflatoxins

The main aflatoxins include aflatoxins B1, B2, G1 and G2 (see Fig. 4) produced by *Aspergillus flavus* and *A. parasiticus* that contaminate plants and plant products.

Among all aflatoxins, the Aflatoxin B1 (AFB1) is the most potent hepatocarcinogenic substance known; recently, after a thorough risk evaluation, it has been proven to be also genotoxic (Van Egmond & Jonker, 2004; Zain, 2011).

In 1993, the WHO-International Agency for Research on Cancer (WHO-IARC, 1993 a,b) evaluated the carcinogenic potential of Aflatoxins and they were classified as carcinogenic for humans (Group 1). In particular, studies have shown that AFB1 may act in synergy with hepatitis B virus in human hepatocellular carcinoma.

For this type of carcinogen, it is generally felt that there is no threshold dose below which no tumor formation would occur. In other words, only a zero level of exposure will result in no risk.

Aflatoxins are known to bind DNA and induce mutagenic and carcinogenic effects in rats. Especially the AFB1 is bioactivated from its original form to a mutagenic and carcinogenic metabolite (Hussein & Brasel, 2001).

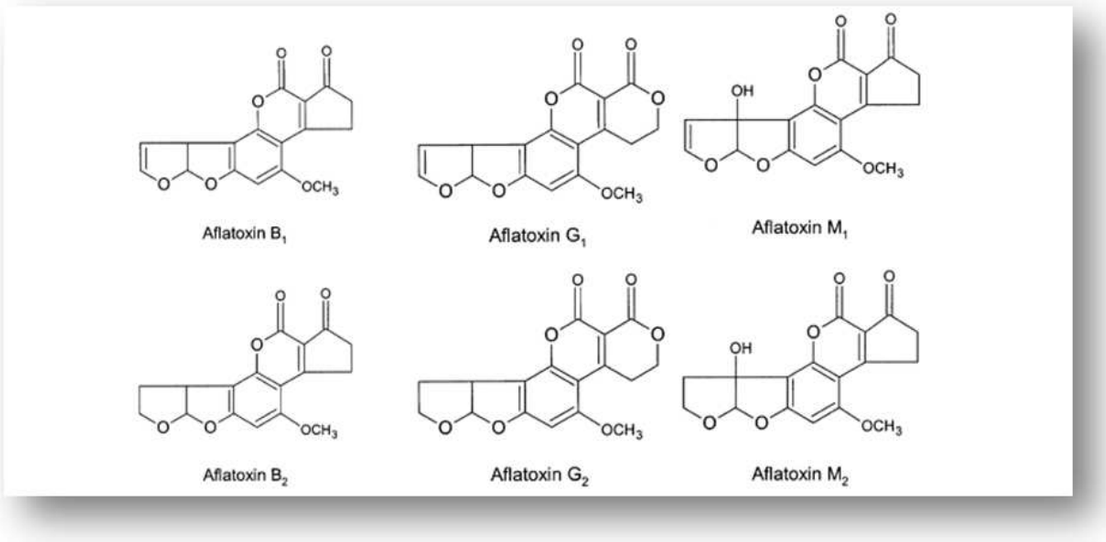


Fig. 4. Molecular formula of Aflatoxins and their metabolites.

Aflatoxins have been found also in tissues of children suffering from Kwashiorkor and Reye's syndrome, that has led to the hypothesis that they could also be causal factors for these diseases.

Reye's syndrome, in particular, is characterized by liver and kidney enlargement and cerebral edema (Hussein & Brasel, 2001).

Aflatoxins M1 (AFM1) and M2 (AFM2) are the hydroxylated metabolites of aflatoxin B1 (AFB1) and B2 (AFB2) respectively and may be found in milk products obtained from livestock that have ingested contaminated feed.

Aflatoxins can be present in cows milk, pork meat or chicken eggs if those animals were exposed to aflatoxins in their feed. The main sources of aflatoxins in feeds are peanuts, meal maize and cottonseed meal (Creppy, 2002; McLean & Dutton, 1995; Richard, 2007).

The concentration of AFB1 in feed can be strongly reduced thanks to good manufacturing and storage practices. If those preventive measures fail, however, AFB1 can be reduced in feed by blending (or diluition) or by physical or chemical treatments. Physical treatments include heat, microwaves, gamma-rays, X-rays and ultra-violet light (Creppy, 2002).

About 0.3–6.2% of AFB1 from animal feed is transformed into AFM1 in milk. The toxicity of AFM1 is about one order of magnitude less than that of AFB1 (Creppy, 2002; Van Egmond & Jonker, 2004 ; Voss et al., 2002).

AFM1 is a possible carcinogenic to humans.

In literature are not yet known specific exposure biomarkers for aflatoxins.

The AFM1 intake 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 three son per day for the Middle Eastern diet and 0.1 ng/person per day for the African diet (Creppy, 2002).

Aflatoxins have been regulated with limits in many Countries of the world; for more detailed information see section 6.

2.1.2 Ochratoxin A

OTA or (N-[(3R)-(5-chloro-8-hydroxy-3-methyl-1-oxo-7-isochromanyl)-carbonyl]-l-phenylalanin) (Fig. 5), is a mycotoxin produced by several strains of Aspergillus and Penicillium species, and it is generally associated with a variety of products, such as cereals, coffee beans, cocoa beans, and dried fruit (Abrunhosa et al., 2010; Aragua´s et al., 2005; Juan et. al., 2008).

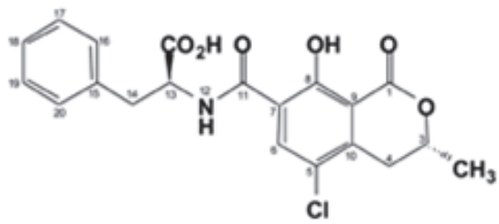


Fig. 5. Chemical structure of OTA.

OTA consists of a dihydroisocoumarin subunit, which is linked to phenylalanine through a peptide bond.

OTA toxicity has been intensively studied (Mally & Dekant, 2009; Abrunhosa et al., 2010); the kidney is the target of OTA toxicity in all animal species exposed to this mycotoxin.

Dietary human exposure to OTA is been suspected to be involved in BEN, a progressive kidney disease associated with an increased risk for the development of urothelial cancers, that occur in some rural areas of Bosnia, Bulgaria, Croatia, Romania, and Serbia.

OTA has a very high affinity to plasma proteins, as 99.9% of the circulating OTA is bound to plasma proteins.

OTA is poorly metabolized and slowly excreted with plasma half-life of 230 hrs in humans (Mally & Dekant, 2009).

Inappropriate farm management practices have been associated with higher OTA amounts. It is also known that meat may contain OTA through secondary contamination and carry-over (Pfohl-Leszkowicz & Manderville, 2007).

Studies have not completely identified so far the mechanism nor the extent of the carcinogenic potential of OTA in humans; therefore, OTA is classified by IARC as possible carcinogen (Group 2B) (Abrunhosa et al., 2010).

The provisional tolerable weekly intake (PTWI) for OTA established by the Joint FAO/WHO Expert Committee on Food Additives and Contaminants is 100 ng/kg bw/week (Juan et al., 2008; Kabak, 2009; Pfohl-Leszkowicz & Manderville 2007).

Some scientist have shown that the DNA lesions (on dG exclusively) induced by OTA in vivo exposure were no longer repaired in case of repeated exposure; these lesions could then be proposed as a marker of exposure in populations at risk (Creppy, 2002; Abrunhosa et al., 2010).

Prevention of growth of *A. ochraceus* consists in application of standard practices as rapidly and thoroughly drying the grains and also fumigation, aeration and cooling, sealed storage and controlled atmosphere.

An important preventive factor for correlated pathologies is the diet rich in antioxidants, vitamins, amino acids and aspartame. In fact positive and protective effects of aspartame in kidney and brain have been reported. Actually aspartame is used for prevention of OTA genotoxicity in kidneys (Creppy, 2002).

Several studies are available on OTA-induced nephrotoxicty and renal tumor formation.

The OTA mechanisms of action are divided into direct (covalent DNA adduction) and indirect (oxidative DNA damage) (Pfohl-Leszkowicz & Manderville, 2007; Mally & Dekant, 2009; Reddy & Bhoola, 2010).

Oxidative DNA damage induced by direct oxidation of DNA constituents or through oxidative stress has been implicated in renal tumor formation and it represents the most accepted theory in current literature (Mally & Dekant, 2009; Abrunhosa et al., 2010).

However, currently the OTA carcinogenicity pathway is unknown because it has not yet been observed sperimentally.

Several pathways of kidney tumorigenicity by ochratoxin have been proposed by scientists:

- DNA-adduct formation
- Oxidative stress
- Cell proliferation
- Modulation of apoptosis
- Alteration of gene expression

(Kamp et al., 2005; Kuan et al., 2008; Mally & Dekant, 2009 ; Reddy & Bhoola, 2010).

EFSA has recently established a tolerable weekly intake (TWI) of 120 ng/kg of body weight for OTA. While the exposure of the general population in Europe is below this TWI, additional data regarding OTA-exposure of infants and children are considered necessary to account for their specific dietary preferences and needs (EFSA, 2006; Kabak, 2009; Mally & Dekant, 2009).

2.1.3 Sterigmatocystin

Sterigmatocystin, or (3a*R*,12c*S*)-8-hydroxy-6-methoxy-3a,12c-dihydro-7*H*-furo[3',2':4,5]furo[2,3-*c*]xanthen-7-one (Fig. 6) is a toxic metabolite closely related to the aflatoxins structure and consists of a xanthone nucleus attached to a bifuran structure. The IARC have included the sterigmatocystin in group 2B, which means it is a likely carcinogenic to humans.

Sterigmatocystin is generally produced by the fungi *Aspergillus nidulans* and *A. versicolor* (Veršilovskis & De Saeger, 2010).

It has been found in mouldy grain, green coffee beans and cheese although current information on its occurrence in foods is again incomplete and poor.

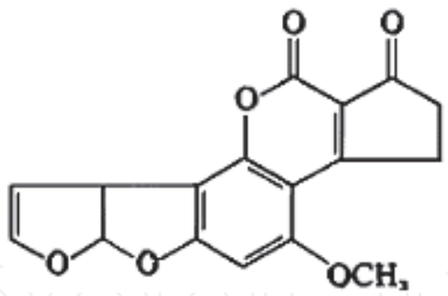


Fig. 6. Chemical structure of Sterigmatocystin

It appears to occur much less frequently than the aflatoxins perhaps because the analytical methods for its determination have not been as sensitive, therefore it is possible that small concentrations of Sterigmatocystin in food commodities may at times not have been detected. Sterigmatocystin is considered a potent carcinogen, mutagen and teratogen toxin. A number of closely related compounds such as o-methyl Sterigmatocystin are known and some may occur naturally.

Effects of chronic exposure that have been reported include induction of hepatomas in rats, pulmonary tumours in mice, renal lesions and alterations in the liver and kidneys of African monkeys. Rats fed with 5-10 mg/kg of sterigmatocystin for two years showed a 90% incidence of liver tumours.

The acute toxicity, carcinogenicity and metabolism of Sterigmatocystin have been compared to those of aflatoxin and several other hepatotoxic mycotoxins.

Contamination by Sterigmatocystin usually occurs in wheat, maize, animal feed, hard cheese, pecan nuts and green coffee beans but also in cereals, grain products, fruits and marmalade, dried meat products and grapefruit juice.

Relatively high levels of sterigmatocystin have been formed in bread, cured ham and salami after inoculation with *A. versicolor*. No country has legislation for sterigmatocystin; however, it is important to emphasize that the natural occurrence of this mycotoxin appears to be infrequent, though only a limited number of surveys have been carried out (Noda et al., 1981; Stich & Laishes, 2006; Veršilovskis & De Saeger, 2010).

2.1.4 Fumonisin

Fusarium verticillioides, *F. moniliforme* and *F. proliferatum* are the fungi that produce significant quantities of fumonisins (Fig. 7 and Fig. 8). Today at least 15 related fumonisin compounds have been identified but the fumonisin B1 or diester of propane-1,2,3-tricarboxylic acid (FB1) (Fig. 6) and fumonisin B2 (FB2) (Fig. 7) are the predominant forms. The fumonisins are highly water-soluble because they lack an aromatic structure.

Fumonisins occur in maize and corn and infrequently in foodstuffs such as sorghum, asparagus, rice, beer and beans (Creppy, 2002; Murphy, 2006; Zain, 2011).

The IARC has classified Fumonisins as possible human carcinogens (Class 2B). Consumption of fumonisin has been associated with elevated human esophageal cancer incidence in various parts of Africa, China, Central America, and among the black population in Charleston, South Carolina, USA (Hussein & Brasel, 2001; Murphy et al., 2006).

FB1 is responsible for leukoencephalomalacia (necrotic lesions in the brain) in horses and pulmonary edema in swine. Hepatotoxicity and nephrotoxicity effects have also been reported in connection with chronic dietary exposure to fumonisin. Fumonisins are also a real risk factor in neural tube and related teratogenic defects because they reduce folate uptake (Zain, 2011).

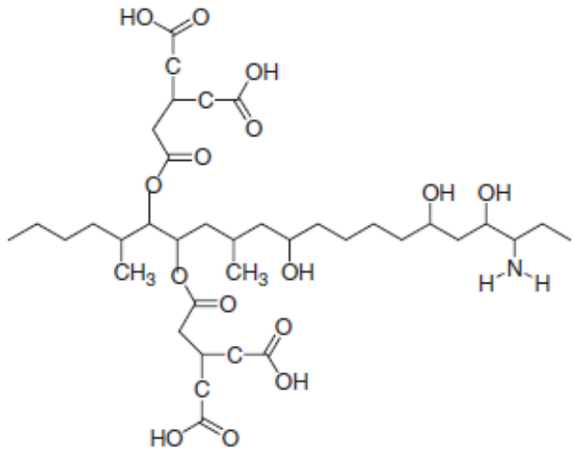


Fig. 7. Chemical structure of FMB1

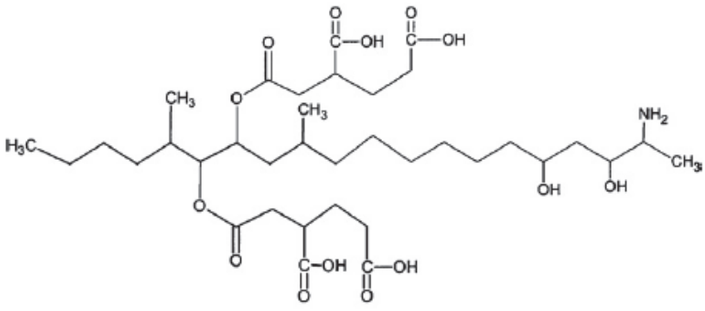


Fig. 8. Chemical structure of FMB2.

Fumonisins are known to disrupt sphingolipid synthesis and concentrations; thus, the altered plasma sphingosine/sphinganine ratios is an important biomarker of fumonisin dietary exposure (Murphy et al., 2006).

Many biochemical pathways have been postulated to explain the induction of illnesses by fumonisins. Several hypotheses are proposed and all are related to disruption of lipid metabolism as the initial step.

Some researchers have proposed that the first mechanism involves disruption of sphingolipid metabolism through inhibition of ceramide synthase. Glycerophospholipid metabolism is also affected (Voss et al., 2002).

A second theory proposes a biochemical mechanism that involves the fatty acid disruption, Glycerophospholipid metabolism, the relative induction of oxidative stress but also an incorrect modulation of gene expression.

Fumonisin has been unequivocally shown to be genotoxic, in fact DNA adducts of fumonisin B1 have been found.

A provisional maximum of tolerable daily intake (PMTDI) for FB1, FB2 and FB3, individually or in combination, is fixed at 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 (Creppy, 2002).

2.1.5 Zearalenone

Zearalenone (Fig. 9) is a mycotoxin produced by *F. graminearum* and other *Fusarium* molds as *F. culmorum, F. cerealis, F. equiseti,* and *F. semitectum,* using corn, wheat, barley, oats and sorghum as substrates. *Fusarium* infects cereals in the field. Toxin production occurs before harvesting, but also post harvest if the crop is not handled and dried properly (see section 6).

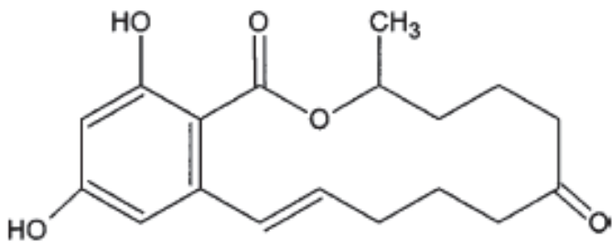


Fig. 9. Chemical structure of ZEA

Among the human population, children are the most affected due to consumption of ZEA-contaminated foods (mainly cereals and cereal-based food products) (Massart & Saggese, 2010). This toxin is worldwide distributed and can contaminate most of the cereals like barley, maize, oats, sorghum, millet, rice, soybeans but also wheat and bread. ZEA has been implicated in several incidents of early puberty, as it is suspected to be the causative agent in an epidemic of early puberty changes in young children in Puerto Rico.

ZEA is a non-steroidal compound that exhibits oestrogen-like activity in some farm animals (e.g. cattle, sheep and pigs) and it is a 6-(10-hydroxy-6-oxo-trans-1-undecenyl)-b-resorcylic acid l-lactone.

Altogether ZEA may have a species-dependent carcinogenic effect possibly secondary to the hormonal effect (Massart & Saggese, 2010); however, no data about formation of DNA-adducts in humans are actually available.

ZEA and some of its metabolites have been shown to competitively bind to oestrogen receptors (α-zearalenol and β-zearalenol); all of them have been included by IARC in Group 3, as not classifiable as carcinogens to humans.

Human adenocarcinomas and endometrial hyperplasia found in ZEA contaminated women are still under investigation (Bhat et al., 2010; Codex Alimentarius Commission, 2000; Creppy, 2002; Massart & Saggese, 2010; Zain, 2011).

No international homogeneous maximum limit exists in foodstuff for ZEA. Eight countries of the World have specific regulations for ZEA ranging from 30 to 1000 μg/kg.

The JECFA has established a provisional maximum tolerable daily intake for ZEA to be 0.5 µg/kg of body weight (JECFA, 2000).

The Canadian daily intake of ZEA from maize and maize-based cereals has been estimated to be 0.005 - 0.087 µg/kg b.w. for 12 - 19 year-old males, the highest consumption group. An additional intake from popcorn was estimated to be 0.001 - 0.023 µg/kg b.w.

3. Combined toxic effects of mycotoxins

It has been known for many years that several products derived from plants contaminated by fungi during plant growth or during harvest and storage of the food item, can contain more types of mycotoxins; as a consequence, food intake results in a simultaneous exposure to a mix of mycotoxins; for example, often citrinin and ochratoxin A are found together (see Table 3).

There are several combinations of mycotoxins that frequently occur as verified by specific monitoring programs. These combinations are summarized in Table 3.

Mycotoxins	References
OTA and Citrinin	Pohland et al., 1992; Vrabcheva et al., 2000; Pfohl-Leszkowicz et al. 2007
OTA and ZEA	Halabi et al. 1998
AFB1 , FB1 and ZEA	Oliveira et al., 2006
OTA and AFB1	Sedmikova et al., 2001
FB1 and Moniliformin	Gutema et al., 2000
DON, NIV, T2, HT-2	Eskola et al., 2001
DON, NIV, T2, HT-2, ZEA	Tanaka et al., 2010

Table 3. Combinations of mycotoxins in food.

When mycotoxins are of similar structure and of the same species or of the same family, it is likely to expect similar mechanism of action, therefore mycotoxins are likely to exert additive effects. However, there are relatively poor information on the interaction between mycotoxins that occur at the same time and the consequences for animal and human health.

The available data in literature show that adequate studies to establish antagonistic, additive or synergistic effects after combined exposure to mycotoxins are rare and some of them are difficult to interpret.

The few studies conducted on the mycotoxins co-presence have shown that combined exposure to several mycotoxins generally results in an additive toxic effect with a few exceptions. The synergic capacity is relatively small for NIV, DON, T-2, ZEA and FB1.

The interaction between NIV and T-2 has been seen as synergistic because the effect of T-2 is potentiated in the presence of high levels of NIV (Berthiller et al., 2009; Bouslimi et al., 2008; Speijers & Speijers, 2004; Tammer et al., 2007).

It would be proper for future research to try to understand how mycotoxins can interact and thus interfere between them.

4. Ghost mycotoxins

Mycotoxins may also occur in conjugated forms such as:

- soluble or masked mycotoxins
- mycotoxins incorporated into/associated with/attached or bound to macromolecules.

Conjugated mycotoxins can be produced after metabolization by living plants, fungi and mammals, after food processing or because of some cooking methods.

It has been shown that wheat enzymatically produces deoxynivalenol 3-glucoside (D3G) from DON to defend itself from the very same toxin attack.

During storage, on the contrary, the plant product generally has a low metabolic activity and its enzymatic protections may not be as active.

Mycotoxins can be altered by subsequent food processing and most frequently through conjugation. The reliable analytical methods, measurement standards and occurrence and toxicity for this forms of mycotoxins are still lacking.

So, the risk assessment from exposure of consumers to mycotoxins should also take into account the presence of those forms (Berthiller et al., 2009; Bouslimi et al., 2008; Tammer et al., 2007).

4.1 Fungal conjugates

Some mycotoxin conjugates can be excreted directly by fungi, although examples are rare. This is the case of the well known 3-acetyl deoxynivalenol (3ADON) and 15-acetyl deoxynivalenol (15ADON), that can be found in cereals contaminated by *Fusarium*. Both substances are biosynthetic precursors of DON.

Some strains of *F. sambucinum* and *F. sporotrichoides* produce compounds such as 4-propionyl HT-2 toxin, 8-nhexanoyl neosolaniol, 8-butyryl neosolaniol, 8-isobuturyl neosolaniol, and 8-pentenoyl neosolaniol in hydroponics culture.

Zearalenone 4-sulfate (Z4S) was found to be a natural *Fusarium* metabolite.

Similarly, *Rhizopus arrhizus* is capable of catalysing sulfation of ZEA to Z4S. (Berthiller et al., 2009; Tammer et al., 2007).

4.2 Plant conjugates

Plants protect themselves from xenobiotic compounds like mycotoxins by converting them into more polar metabolites.

It remains unclear, however, whether glycosylated mycotoxins are less toxic than the storage forms or they are part of the mechanism of the plant self-protection.

In wheat and maize cell suspension cultures OTA is transformed into ochratoxin α and methylester-ochratoxin A (two isomers of hydroxyochratoxin A) as well as into their glucosides and methyl esters.

Fumonisin conjugates were long believed to occur only after food processing but recently it has been shown that bound fumonisins could also be found in unprocessed maize.

The exact chemical nature of these naturally occurring hidden forms is still unknown. In particular, it has to be understood whether conjugates are formed by the plant from non covalent interactions, e.g. with starch or proteins.

4.3 Food-processing conjugates

Food processing, especially heating and fermentation, can potentially alter some mycotoxins. D3G in fact was detected in malt and beer made from barley naturally contaminated with Fusarium, but also DON conjugates with higher masses as diglucosides and triglucosides have been detected in beer. Generally, very little is known regarding bound mycotoxins.

Depending on the type of linkage to proteins, starch, pectins, hemicellulose, cellulose and lignin, it is possible that at least some of the bound mycotoxins could become bioavailable again in the digestive tract of humans and animals.

Conjugation does not occur only during food processing; an excessive heat can also alter mycotoxins structure considerably; in fact, although mycotoxins are very stable, thermal degradation products of NIV and DON have been found in food and feed.

Fumonisins conjugates with sugars, amino acids and proteins are known to occur in food and feed. Heating FB1 with reducing sugars can yield N-(carboxymethyl) FB1, even in corn products.

Protected lysine and cysteine methyl esters react with FB1 demonstrating that free groups as thiol or amino groups of proteins are likely to react with the mycotoxins. Furthermore, it has been proven that thermal food processing influences the chemical structure and toxicity of fumonisins (Berthiller et al., 2009; Bouslimi et al., 2008; Speijers & Speijers, 2004; Tammer et al., 2007).

4.4 Mammalian conjugates

Conjugates that arise from mammalian metabolization unlikely have a role in food or feedstuffs and are therefore not regarded as masked mycotoxins.

Mycotoxins are conjugated by humans and animals during metabolization in the liver and are excreted in urine. Serum albumin adducts of AFB1 can be formed by Patulin that has been shown to react with cellular nucleophiles such as proteins or glutathione. This is the most probable pathway of detoxification in man and animals after dietary exposure to patulin-contaminated apples.

The ability to form glutathione and cysteine conjugates has also been shown for ochratoxin A. The most common mycotoxin conjugation products in mammals are glucuronides, as found for the *Fusarium* mycotoxins DON and ZEA. Besides the unchanged mycotoxins, both deoxynivalenol glucuronides and zearalenone glucuronide were detected in the urine of exposed animals.

Some DON and ZEA sulfates are formed and excreted by animals too. These conjugates might be used as biomarkers; by doing so, also inhalation of mycotoxins (e.g. by grain workers or from indoor moulds) and the consumption of all bioavailable forms of

mycotoxins (including masked mycotoxins) might be monitored (Berthiller et al., 2009; Bouslimi et al., 2008; Speijers & Speijers, 2004; Tammer et al., 2007).

5. Mycotoxins control strategies, a possible form of prevention

Good agricultural practices (GAPs), good manufacturing practices (GMPs), HACCP (Hazard Analytical Critical Control Point), biological control measures and transgenic approaches, are actually the only realistic and possible form of primary prevention for mycotoxins flowering.

A control program for mycotoxins from the field to the table should include the HACCP approach, thus requiring an understanding of the interactions of the toxigenic fungi with crop plants, of the production and harvest methods for crops, of the production of livestock using grains and processed feeds, a thorough knowledge including diagnostic capabilities for mycotoxicoses, the development of new practices of foods processing for human consumption including storage and delivery (Jard et al., 2011).

A good protocol for mycotoxins checking is necessary to manage all control points and finally for being able to ensure to the consumer a food supply free from mycotoxins (Binder, 2007 ; Richard, 2007; Abrunhosa et al., 2010).

The results of HACCP show that the products obtained have an elevated hygienic quality, thus lowering the risk of contamination. The dried pasta, for example, is one of the foods most commonly consumed by Italians and Italy is leading the world ranking for the consumption of pasta. Also, pasta is conquering more and more important positions in the dietary habits of various peoples. The study of Ferrante and colleagues (2009) was to assess the presence of ZEA and OTA with the aim of identifying the consumer's risk.

The results of this study have showed OTA and Zea concentrations below the limit of methods in all types of dried pasta tested, also have showed that the HACCP system adopted by Italian food industries are adequate for preserve the food quality. This study moreover have showed in particular that the products for celiac consumers and the products made with whole wheat flour have an elevated hygienic quality.

The efficient storage systems can be also a good practice for reduction of contamination by mycotoxins.

One of the conservation techniques for food is the lyophilization. Ferrante and colleagues in 2006 carried out the study for verify presence of : tri-5 gene, ZEA, NIV and DON, in the primary products used for the preparation of lyophilized foodstuffs and in their final products (prontocrepes and prontocone).

The study have showed that the Tri-5 gene are always present in prontocrepes and always absent in prontocone products. With the air treatment a reduction of DON and NIV in both products has been obtained. The nitrogen treatment has had a single influence on the DON and in both final products, in which has had a reduction to 50 %. While, the NIV with the same treatment has reached the lowest percentage of reduction in both final products. The treatments affect relatively on the contamination of processed-foods that, therefore, in this case was due on the first quality of the primary products.

Populations residing in developed Countries are usually considered to be less exposed to mycotoxins than those in developing Countries; this might be attributed to various factors:

- execution and practice of modern food handling/preservation technology
- successful governmental regulation and commercial control over food quality and safety.

However, even monitoring and exercising of good agricultural and manufacturing practices (GAP and GMP) along with an effective HACCP approach might not completely avoid or eliminate mycotoxins in the food chain (Bhat, 2010; Jard et al., 2011; Zain, 2011).

5.1 Biological control

Various biocontrol strategies are possible for reducing the levels of mycotoxins in the crops, such as development of atoxigenic bio-control fungi that can out-compete their closely related, toxigenic strains in field environments.

It has been reported the existence of non-toxigenic strains of *A. flavus* and *A. parasiticus* that can reduce the post-harvest aflatoxin contamination by 95.9%.

The competitive use of biological agents such as *F. verticillioides* strains was observed to suppress the growth of fumonisin-producing fungi.

Control of fumonisins producing fungi by endophytic bacteria has also been reported and competitive exclusion was thought to be the mechanism involved.

It has also been reported that Pichia anomala, Pichia kluyveri and Hanseniaspora uvarum can inhibit in vitro OTA.

Furthermore, fungal strains of Trichoderma have been demonstrated to control pathogenic molds through mechanisms such as competition for nutrients and space, fungistasis, rhizosphere modification, mycoparasitism, biofertilization and the stimulation of plant-defense mechanisms (Bhat, 2010; Jard et al., 2011; Zain, 2011).

5.2 Chemical control

Appropriate use of pesticides during the production period could help in minimizing fungal infections or insect infestation of crops and consequently mycotoxins contamination; therefore, Fumonisins contamination could be reduced by application of fungicides that have been used for controlling Fusarium, such as prochloraz, propiconazole, epoxyconazole, tebuconazole, cyproconazole and azoxystrobin.

On the other hand, fungicides such as itraconazole and amphotericin B have been shown to effectively control the aflatoxin-producing Aspergillus species. However, use of fungicides is been discouraged because of economic, environment and severe food safety issues (Bhat, 2010; Jard et al., 2011; Zain, 2011).

5.3 Decontamination

Decontamination of food and feed from mycotoxins could be achieved through chemoprotection or enterosorption.

Chemoprotection from aflatoxins has been demonstrated with the use of a number of chemical compounds like oltipraz and chlorophylin or through dietary interventions based on broccoli sprouts and green tea that increase animal's detoxification processes or prevent the production of epoxide, that is known to cause chromosomal anomalies.

However, this intervention might not be sustainable in the long-term because it is expensive and shows some side effects.

Entersorption is based on the discovery of certain clay minerals, such as Novasil, that can selectively adsorb mycotoxins enough to prevent their absorption from the gastrointestinal tract .

There are different adsorption agents but their efficacy in preventing mycotoxicosis varies. Calcium montorillonites have proven to be the most highly selective and effective of enterosorbents. However, with enterosorption, there is a risk that non-specific adsorption agents may prevent uptake of micronutrients from the food (Bhat, 2010; Jard et al., 2011; Zain, 2011).

5.4 Breeding for resistance

Breeding for resistance is the most promising and encouraged long-term strategy for control of mycotoxins contamination in Africa. Sources of resistance to *A. flavus* and *Fusarium spp*. have been identified, particularly *F. verticillioides*.

Zain (2011) states that "Prototypes of genetically engineered crops have been developed that

a. contain genes for resistance to the phytotoxic effects of certain trichothecenes, thus helping reducing fungal virulence or
b. contain genes encoding fungal growth inhibitors for reducing fungal infection in the USA".

To devise effective strategies to control fungal infections and minimize mycotoxins production in host plants, a good knowledge of genetic variability and plants population structure is necessary (Kazan et. al., 2011; Jard et al., 2011; Zain, 2011).

6. Regulation of mycotoxins in foods and feeds

A series of studies on mycotoxins in food and feed have been carried out around the world in order to improve policy making.

Regulations relating to mycotoxins have been established in many Countries to protect consumers from the harmful effects of these compounds. In 2003 about 100 Countries (covering approximately 85% of the world's inhabitants) had already specific regulations or detailed guidelines on mycotoxins in food. Regulations concerned AFB1, AFB2, AFG1, AFG2 and AFM1, trichothecenes (Vomitoxin, diacetoxyscirpenol, T-2 toxin and HT-2 toxin), FB1, FB2, and FB3, agaric acid, ergot alkaloids, OTA, patulin, phomopsins and ZEA, leaving out sterigmatocystin.

Harmonized EU limits now exist for 40 mycotoxin–food combinations. The direct or indirect influence of European organizations and programs on the EU mycotoxins regulatory developments was significant.

The current maximum limits are more and more based on scientific evidence from authoritative agencies such as FAO/WHO, Joint Expert Committee on Food Additives of the United Nations (JECFA) and the European Food Safety Authority (EFSA) (Van Egmond & Jonker, 2004).

The FDA has action levels for aflatoxins but the European Community levels are more restrictive (Creppy, 2002; Mally & Dekant, 2009).

Efforts have continued internationally to establish better guidelines to control mycotoxins. FAO, for example, has worked with developing Countries (African and East-Asian Countries) to mitigate mycotoxins contamination in foods and feeds.

Many Countries have regulatory or guideline limits for OTA in foods but in the majority of cases OTA content limits have been established only for cereals and cereal products (see Tab. 5).

There are still some discussions in those organizations regarding the limit that should be established for OTA in some cereal-derived foods, in fact in some Countries there is no limit or it is not harmonized with the international level (see Tab. 5) (Araguás et al., 2005).

Limit of AFB1 on ppb (µg/kg)	Number of countries	Limit of AFB1, B2,G1,G2 on ppb (µg/kg)	Number of countries
25	1	35	2
20	3	30	3
15	2	20	17
10	5	15	8
5	21	10	8
2	29	5	3
1	1	4	29
20	3	3	1
		1	3
		0	2

Table 4. Worlwide limits for AFB1 and sum of AFB1, B2, G1, G2 in food.

Many Countries, even if industrialized, such as USA and Canada, still today lack of legal limit for AFB1, showing how the problem is unfortunately underestimated.

Furthermore, looking at Table 5, it is also evident that still today in many Countries of the world there are no limits for OTA, ZEA and Fumonisins, and that worldwide regulations for sterigmatocystin are completely lacking.

Several Countries, such as Bahamas, Bolivia, Burkina Faso, Cameroon, Ecuador, Ethiopia, Iraq, Myanmar, Nicaragua, Pakistan, Panama, Qatar, Trinitad and Tobago, Uganda, United Arab Emirates, Yemen, Zambia and Zimbawe, until 2003, hadn't yet established any regulations or limits for any mycotoxins, that means that the carcinogenic risk to which local populations are subjected is high.

Myctoxin	Country	Maximum limit (ppb)	Food
AFB1	EU*	2	Food, peanuts, shell fruits, dried fruits, cereals
		5	Unprocessed maize , spices and tea
		8	Unprocessed peanuts
		0.1	Cereal and other complementary foods for infants and small children
		0.1	Dietary foods for special medical purposes (specifically for infants)
		2	Maize, cereals
	Algeria	10	Peanuts, nuts, cereals.
	Armenia	5	All foods
	China	20	Maize and maize products, peanut and peanut products, peanut oil, irradiated peanut
		10	Rice, irradiated rice, edible vegetable oil
		5	Soya bean sauce, grain paste, vinegar, other grains, beans, fermented foods, fermented bean products, starch products, fermented wine, red rice, butter cake, pastry biscuit and bread
	Croatia	5	Cereals, beans, peanuts, coffee, tea
		30	Spices
	Cuba	5	Cereals, peanuts, cocoa mass
	Egypt	5	Peanuts and cereals
	Honduras	1	Maize
		10	Corn
	Iran	10	Barley
		5	Pistachio nuts, peanuts, walnuts, other nuts, edible seeds, dates, dried grapes (raisins and sultanas), figs and all dried fruits, maize, rice, wheat, legumes
		0.5	Baby food based on cereals with milk
		1	Baby food based on cereals without milk
	Israel	5	Nuts, peanuts, maize flour, figs and their products and other foods
	Japan	10	All foods
	Jordan	15	Almonds, cereals, maize, peanuts, pistachio nuts, pine nuts, rice
	Korea Rep.	10	Grains, soy-bean, peanuts, nuts, wheat and the products made from these by simple processing
	Malawi	5	Peanuts export
	Moldova Rep.	5	Cereals, legumes, flour, cocoa, nuts, coffee, sunflower, tea
	Morocco	10	Wheat bran and all foods
		5	Vegetable oils, cereals, wheat meal

Myctoxin	Country	Maximum limit (ppb)	Food
		1	Peanuts, pistachio nuts, almonds, vegetable oils in pasta, children foods
	Nepal	20	Cereals
	Nigeria	20	Foodstuffs
	Oman	10	Foodstuffs
	Russia	5	All foods
	Senegal	50	Peanut products
		300	Peanut products (feedstuff ingredients)
	South Africa	5	All foodstuffs
	Switzerland	1	All foods
	Tanzania Rep.	5	Cereals, oil seeds
	Tunisia	2	All foods
Σ (B1, B2, G1, G2)	EU*	4	Peanuts , dried fruits, cereals and related products intended for direct human consumption
		15	Unprocessed peanuts
		10	Unprocessed shell fruits, dried fruits
		10	Maize unprocessed, spices and thea.
	Algeria	20	Peanuts, nuts, cereals.
	Australia§	15	Peanuts, tree nuts
	Barbados	20	All foods
	Bosnia & Herzegovina	1&	Cereals
		5	Beans
	Canada	15	Nuts and nut products
	Chile	5	All foods
	Colombia	10	All foods
		20	Maize
	Costa Rica	35	Maize
	Croatia	3	Cocoa beans, almonds, flours, hazelnuts, walnuts
	Cuba	5	All foods
	Dominican Rep.	0&	Maize(products), groundnut, soya, tomato(products)
		20	Imported maize
	Egypt	10	Peanuts , cereals
		20	Corn
	Guatemala	20	Maize, kidney beans, rice, sorghum, groundnuts, and groundnut butter
	Honduras	1°	All foods
		0.01&	Baby food
	India	30	All foods
	Indonesia	20	Peanuts, coco nuts, spices, traditional drugs, erbs
	Iran	15	Pistachio nuts, peanuts, walnuts, other nuts, edible seeds, dates, dried grapes (raisins and sultanas), figs and all dried fruits,wheat

Myctoxin	Country	Maximum limit (ppb)	Food
		10	Legumes
		50	Barley
		30	Maize, rice
	Israel	15	Nuts, peanuts, maize flour, figs and their products and other foods
	Jamaica	20	Food, grains
	Jordan	30	Almonds, cereals, maize, peanuts, pistachio nuts, pine nuts, rice
	Kenya	20	Peanuts, vegetable oils
	Kuwait	0.2	Milk and milk products(except dried milk)
		0.05	Infant and children food
	Macedonia	1&	Wheat, maize, rice and cereals
		5&	Beans
	Malaysia	35	All foods
	MERCOSUR+	20	Peanuts, maize and products thereof
	Mexico	20	Cereals and products
		12	Corn flour for tortillas
	Mozambique	10	Peanut, maize, peanut butter, peanut milk
	Philippines	20	Nut products
	Salvador El	20	All foods
	Saudi Arabia	0.05	Infant and children food
	Singapore	5	Corn, nuts, and cereal products
	South Africa	10	All foodstuffs
	Switzerland	5°	All foods
	Taiwan	15	Peanut, corn, maize
		10	Rice, sorghum, legumes, nuts, wheat and barley, oats, edible oils and fats
	Tanzania Rep.	10	Cereals, oil seeds
	Thailand	20	All foods
	USA	20	All foods
	Venezuela	20	Corn, corn flour, peanuts, peanut butter
	Vietnam	10	Foodstuffs
AFM1	EU*	0.025	Newborn and baby food
		0.05	Milk and derivates
	Armenia	0.5	Milk
	Barbados	0.5	Milk
	Chile	0.05	Milk
	China	0.5	Milk and milk products
	Croatia	0.5	Milk and milk products
	Honduras	0.05	Milk and milk products
		0.02	Baby food
		0.25	Cheese
	India	30	Milk and milk products

Myctoxin	Country	Maximum limit (ppb)	Food
	Indonesia	5	Milk, cheese
	Iran	0.02	Baby food based on cereals with milk
		0.5	Milk powder
		0.01	Milk powder for babies
		0.2	Cheese
		0.02	Butter, gee
	Israel	0.05	Milk and milk products
	Korea Rep.	0.5	Milk and milk products
	MERCOSUR+	0.5	Milk
		5	Milk powder
	Moldova Rep.	0.5	Milk, cottage cheese, butter
	Morocco	0.05	Milk and milk products
		0.03	Milk (product) for infants under 3 years
		0.5	Milk powder
		0.3	Milk powder for infants under 3 years
	Russia	0.5	Milk and milk products
	Singapore	0.5	Milk, cheese
	South Africa	0.05	Milk
	Switzerland	0.02	Newborn and baby food
		0.05	Milk and milk products
		0.250	Cheese
	Taiwan	0.5	Milk
		5	Milk powder
	USA	0.5	Milk
	Venezuela	0.5	Milk
		5	Milk powder
OTA	EU*	5	Unprocessed cereals
		3	Cereals and processed cereal-based food intended for direct human consumption
		10	Dried grapes (raisins and sultanas)
		5	Roasted coffee beans and ground roasted coffee, with the exception of instant coffee
		10	Instant coffee
		2	Wine (red, white and rosé) and other wine and / or other drinks based on grape must. Grape juice, based ingredients of grape juice
		2	Cocoa and chocolate powder
		0.5	Cereal and other complementary foods for infants and dietary foods for special medical purposes (specifically for infants)
		0.5	Chocolate and its derivatives
		0.2	Beer
		1	Pork and derivatives
	Cuba	5	Coffee, cereals
	Indonesia	Not detectable	Coffee

Myctoxin	Country	Maximum limit (ppb)	Food
	Iran	50	Barley, maize
		20	Legumes
		10	Dates, dried grapes (raisins and sultanas), figs and all dried fruits
		5	Rice, wheat
		1	Baby food based on cereals without milk
	Israel	50	Cereals, cereal products and other foods
	Morocco	30	Cereals
	Singapore	2.5	Cereal, raw coffee beans and roasted coffee beans
	Sudan	15	Wheat
	Switzerland	2	Cereals
FB1+ FB2	EU*	4000	Unprocessed maize
		1000	Maize intended for direct human consumption, maize-based foods for direct human consumption
		800	Maize-based breakfast cereals and maize-based snacks
		200	Processed maize-based foods and baby foods for infants and young children
	Cuba	1000	Maize, rice
	Switzerland	1000	Maize
	USA	2000	Unprocessed maize
ZEA	EU*	20	Processed maize-based foods for infants and young children
		100	Unprocessed cereals other than maize
		350	Unprocessed maize
		400	Refined maize oil
		75	Cereals intended for direct human consumption, cereal flour, bran and germ.
		50	Bread pastries, biscuits, cereal snacks and breakfast cereals, excluding maize-snacks and maize-based breakfast cereals
		100	Maize intended for direct human consumption, maize-based snacks and maize-based breakfast cereals
		100	Maize intended for direct human consumption
	Armenia	1000	All foods
	Chile	200	All foods
	Indonesia	Not detectable	Maize
	Iran	400	Barley

Myctoxin	Country	Maximum limit (ppb)	Food
		200	Maize, rice, wheat
	Moldova Rep.	1000	Wheat , barley, maize and their flour
	Morocco	200	Cereals and vegetable oils
	Russia	1000	Cereals and vegetable oils

Eu*= 27 countries (Austria, Bulgaria, Cyprus, Czech. Rep., Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, The Netherland, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Great Britain). Norway, Iceland and Liechtenstein to follow the limits of the European Union (harmonized standards).
° = Σ (B2, G1 and G2).
&= Σ (B1+G1).
§= All Australian regulations were harmonized with New Zealand.
+= MERCOSUR member states: Argentina, Brazil, Paraguay and Uruguay.

Table 5. Worldwide maximum limit of carcinogenic mycotoxins for food.

7. Conclusions and future research

Besides the demonstrated effects of mycotoxins on humans and animals, some important aspects of their toxicology and possible control mechanism are still unknown and unexplored. The occurrence of mycotoxins in the food chain is therefore an unavoidable and serious problem that the world is facing not without efforts nor difficulties.

The toxic effects of mycotoxins (on liver and kidney, hematopoietic toxicity, immune system toxicity, reproductive system toxicity, foetal toxicity and teratogenicity, and moreover carcinogenicity) are mostly known in experimental models but the extrapolation for humans is often inaccurate.

The inaccuracy of extrapolation for humans may be explained by the lack of adequate food consumption data and/or lack of knowledge about relative health risks associated with specifically proposed limits and finally by the gap of knowledge on the possibility of synergism with other mycotoxins present in the same food products.

Wide gaps still exist on the toxicological effects of feeding animals with mycotoxin-contaminated feeds.

Further research also needs to be focused on epidemiology of toxic effects, especially in humans.

Development of new genetically modified plants by the application of genetic engineering that might be resistant to fungal invasion might also prove today to be a good option for preventing growth of toxigenic fungi, though they leave open many question marks in any case.

Development of methods for simple, rapid and economic analysis chemical contaminants throughout the food chain. The researchers Ferrante and Oliveri Conti have carried out the development of a method for simultaneous determination of 13 mycotoxins and some their

metabolites in LC-MS tandem TQD in serum and urine of humans (Article awaiting acceptance by journal) after enzyme treatment of samples.

Even though research papers, reviews, monographs, and government reports are available on the contamination of food and feed by fungal toxins, nonetheless most of the information are restricted to only one type of mycotoxin (Bhat et al., 2010), and they do not take into account ghost mycotoxins.

Much still needs to be done, both at governmental and scientific level, in order to reach higher standards of protection for consumers. It is important to harmonize as soon as possible the maximum limits for food taken from Country to Country due to global trade, that increases the risks due to mycotoxins ingestion in the population of the importing Country.

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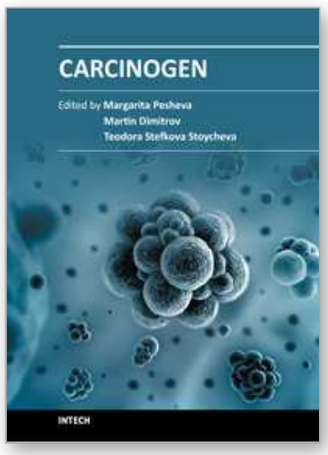

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During the last decades, cancer diseases have increased all over the world. The low quality of food and strong pollution of environment are the main prerequisites for carcinogenesis. The main problem for scientists is to find strategy for prevention of cancer diseases. Therefore, the information about the models for studying carcinogenesis and mutagens which appear during cooking, environmental pollutants, and tests for specific detection of carcinogens is particularly important. The book "Carcinogen" is intended for biologists, researchers, students in medical sciences and professionals interested in associated areas.

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