Mycotoxins: Quality Management, Prevention, Metabolism, Toxicity and Biomonitoring

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1. Introduction

When fungi grow on a living organism or on stored food material that we consume, they may produce harmful metabolites that diffuse into their food (Garcia et al., 2009; Kabak and Dobson, 2009). It is believed that fungi evolved these metabolites as a means of protecting their food supply by preventing other organisms from eating it. These metabolites are referred to as mycotoxins, which literally mean "fungus poisons". Fungi that produce mycotoxins do not have to be present to do harm. When a fungus grows grains in storage, the environment may become unsuitable for the fungus and it dies. Although the fungus dies, during the growth stage, if it produces mycotoxins, this can poison the grains (Fokunang et al., 2006). The effects of poisoning by mycotoxin are referred to as mycotoxicoses. The knowledge that mycotoxicoses is the result of fungal actions was a relatively, recent discovery (Lackner et al., 2009). This is understandable since illnesses in this case are due to consumption of mycotoxins that has been released by the fungus and is not directly caused by the fungus (Coppock and Jacobsen, 2009).

1.1 The mycotoxin system

The mycotoxin system as shown in figure 1 may be considered in terms of four interacting subsystems namely; toxicology, metabolism, health, productivity and wealth. After exposure through ingestion, inhalation or skin contact, the toxicity of a mycotoxin is determined by a sequence of events such as metabolism, involving the administration, absorption, transformation, pharmacokinetics, molecular interactions, distribution, and excretion of the toxin and its metabolites (Fokunang et al., 2006). In turn, the toxicity of the mycotoxin will be manifested by its effect on the health and productivity of crops, human efforts and agricultural and livestock products**.**

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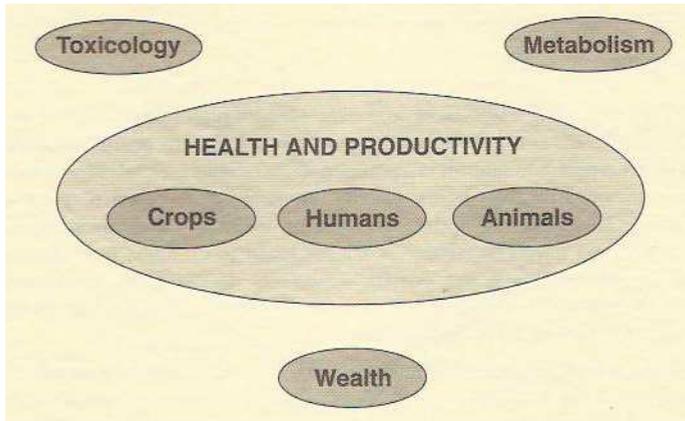
Fig. 1. The Mycotoxins system

1.2 The spoilage system

Biodeterioration is the net result of many interacting spoilage agents which may be widely described as biological, chemical, physical, micro-environmental and macro-environmental (Figure 2). However, the relative impact of these agents will often be mainly determined by the nature and extent of human intervention, occurring within the socio-economic system (Coker 1998). The factors that contribute to bio deterioration within an ecosystem are mainly moisture, temperature and pests status (Christensen, 1975; Hussein and Brasel 2001).
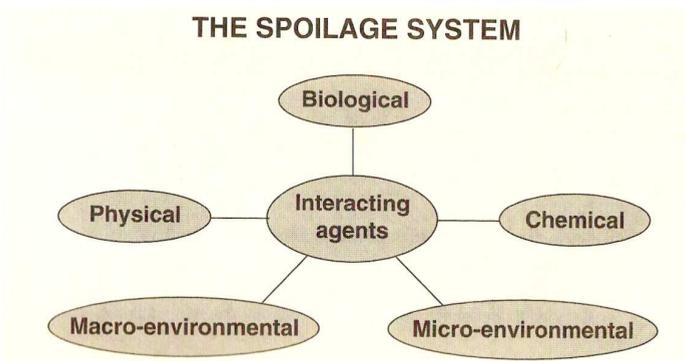

Fig. 2. The mycotoxin spoilage system

1.3 Mycotoxins of economic importance

The moulds and mycotoxins which are now considered to be of world-wide importance are shown in figure 3. Some mycotoxins are however of regional importance as illustrated in table 1. Regional myxcotoxins are specific to certain regions and the occurrence is link to climatic changes. The important mycotoxins have shown the capacity to have a significant impact upon human health and animal productivity in a wider distribution in a number of countries, most especially in the warm humid countries in sub-saharan Africa and Asia (Fokunang et al., 2006; Khlangwiset and Wu 2010).

Fig. 3. Mycotoxins of worldwide importance (Coker, 1999)

Fungal species	Mycotoxin products	Mycotoxicosis	Reference
Claviceps fusiformis	clavinet alkaloids	Ergotism	Cocker, 1999, Ding et al., 2006
C. purpurea	Ergotamine alkaloids	Ergotism	Anderson, & Conning, 1993
C. paspali	paspalinine	Paspalum staggers	Van Egmond. 1989 ; Fokunang et al., 2006
Diplodia maydis	diplodiatoxin	Diplodiosis	Desjardins et al 1997 ; Friesen et al., 2008
Phomopsis leptostromiformis	phomopsin	Lupinosis	Takayuki, and Bjeldanes, 1993; Hussein et al., 2001
Balasia spp	alkaloids	Fescue foot	Bowman, & Rand. 1990; Kiso et al., 2004
Stachybotrys atra	satratoxins	Stachybotryotoxicosis	Bresinky and Besl, 1990; Purzycki & Shain 2010.
Rhizoctonia legumicola	slaframine	Slobber syndrome	Pitt 1996; Wu et al., 2008.
Acremonium loliae	lolitrem	Ryegrass staggers	Cocker et al. 1999
Pithomyces chartarum	sporidesmin	Facial eczema	Lacey, 1991; O'Brian et al 2007

Table 1. Fungi species and mycotoxins of regional importance (Fokunang et al., 2006

2. Mycotoxin fungi

2.1 *Aspergillus flavus*

A. flavus is not a single species, but a "species complex", made up of eleven species that are known to occur in many kinds of plant materials, including stored grains (Christensen, 1975; Meggs, 2009). One of the species in the complex, *A. oryzae* has long been used in the Orient to prepare various kinds of food products, such as sake, tofu and soy sauce, which in turn are used in the United States (Pitt & Miscamble, 1995; Shuaib at al., 2010).

What was determined in early research of aflatoxins is that the condition which allows for growth of *A. flavus* and aflatoxins is very narrow (Klich, 2009). *A. flavus* hardly invades stored grains alone, that is as a pure culture (Purzcki and Shain 2010). Various other species of fungi will normally grow on a substrate prior to invasion by *A. flavus*, such as. *A. glaucus* and *Candida pseudotropicalis (Khlangwiset and Wu, 2010)*. In a preinvaded substrate, regardless of how dense the *A. flavus* invasion may be, aflatoxin will not form. Thus, in order for aflatoxin formation to occur in say a storage bin full of peanuts, *A. flavus* must be growing alone and the peanuts cannot have been previously or simultaneously invaded by other fungi, an occurrence that is rare (Lugauskas and Stakeniene; Magan et al., 2010). In the case of the Turkey-X disease, the peanuts that were responsible for the aflatoxin poisoning were from South America, where the process used to harvest and dry the peanuts was responsible for providing an environment that allowed for growth of *A. flavus* and aflatoxin (Edlayne et al., 2009). *Aspergillus flavus* does not normally contaminate grains and other crops while they are still in the field. It is only after the grains are harvested and stored does *A. flavus*, as well as other so-called "storage fungi" that have a low moisture requirement,

can the grain be invaded (He and Zhou, 2008). Although conditions favourable for growth of the *A. flavus* and production of aflatoxin is narrow, the fungus is common and widespread in nature. Under warm humid condition *A flavus* can invade stored gains such as corn as shown in figure 4. It can be found growing on various decaying vegetation where it may heat up the substrate to as high as 113-122°F as it consumes the material (Hedyati et al., 2007; Lee, 2009).

Fig. 4. *Aspergillus flavus* infestation on corn *Zea mays* (Hedyati et al., 2007)

The term aflatoxins was derived in the early 1960s when the death of thousands of turkeys (Turkey X' disease) ducklings and other domestic animals was attributed to the presence of *A. flavus* toxins in groundnut meals imported from South America (Nageswara et al., 2002). The amount of aflatoxin formed differs as to the substrate on which it is growing. Although the mycelial mass may be the same in each substrate, the aflatoxin produced would be far greater in peanuts than in say soybeans, where relatively very little would be produced. The growth of A. flavus producing aflatoxin in peanuts is shown in figure 5.

Fig. 5. Stored peanuts infected by *A flavus* producing aflatoxin (Kios et al., 2004)

Other seeds of cereal crops, wheat, corn, barley, oats and sorghum are also generally of low-aflatoxin-risk (Nageswararao et al 2002). Weather and climate were also contributing factors. The amount of toxin produced vary with the isolate of *A. flavus*. That is different sources of *A. flavus* will produce different amounts of aflatoxins. Some isolates of *A. flavus* may not even form aflatoxin (Fuchs et al., 1991; Awad et al., 2010). Although the aflatoxins are the major toxins associated with mycotoxicosis, another mycotoxin called cyclopiazonic acid (figure 6), has been associated in the aetiology of Turkey X disease (Bradburn et al., 1994; Kios et al., 2004).
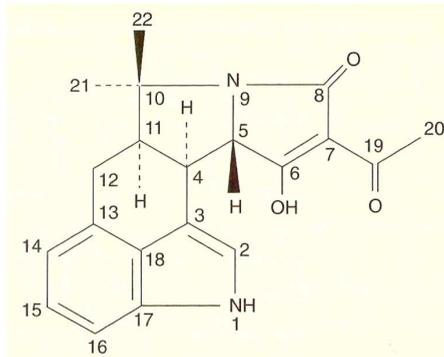

Fig. 6. Cyclopiazonic acid associated in the aetiology of Turkey X disease (Bradburn et al., 1994)

2.1.1 Aflatoxin toxic effects

Studies by Christensen (1975), over a period of several years, examined 100 different samples of black pepper from all over the world. In dilution cultures of these samples, the number of fungus colonies in whole or ground black pepper averaged 52,000 per gram/black pepper and the upper range was over half a million per gram (Coker, 1998). These colonies were mostly of *A. flavus*, *A. ochraceus* and *A. versicolor*. All three species are known to be aflatoxin producers. Some samples of ground pepper were caked lightly with fungus mycelium when first opened in the laboratory and with time, a number of these became solidly caked with mycelium (Jalili et al., 2011).

How heavily contaminated is 52,000 to 500,000 colonies of fungi, per gram? Let's make a comparison for what is acceptable levels of fungal colonies isolated in other food products at the time Christensen published his results. Wheat, for example, that is intended for milling into flour seldom contains any more than a few thousand colonies of fungi per gram of grain. If barley has as many as 10,000 colonies of the same kind of fungi per gram as in black pepper, it would be rejected for malting in beer making. If breakfast cereals or bread were as contaminated as black peppers, they would have so musty an odour and taste that they would be too revolting to eat. Apparently, the natural spicy odour and flavour of black, as well as white pepper are potent enough to conceal the taste and odour of these fungi.

2.1.2 Mycotoxins of other fungal species of *Aspergillus*, *Penicillium* and *Fusarium*

2.1.2.1 *Aspergillus ochraceus* and ochratoxin

Aspergillus ochraceus is also a species complex, and consist of nine species. These species are common in soil, decaying vegetation, and in stored seeds and grains undergoing microbial

deterioration. However, this fungus is seldom isolated from more than a small percentage of seeds or grains that are undergoing microbiological deterioration in storage because it is evidently not a good competitor, as is also the case with *A. flavus*. This is a general rule, but *A. ochraceus* has been isolated from 40% or more of surface-disinfected kernels of corns from bins in which deterioration was in progress. It has also been the major organism in some lots of whole black pepper (Desjardins and Hohm, 1997; Chang et al., 2011).

Production of ochratoxin, by *A. ochraceus*, was first described in South Africa (Christensen 1975), where it was isolated along with a number of other fungi. In experiments done with this isolate, the LD50 (the single dose that will kill 50 percent of the individual animals tested) of ochratoxin for rats is 22mg/kg (= 22 milligrams of the toxin per kilogram of body weight of the rat), but a lesser amount will result in severe liver damage. A single dose of 12.5 mg/kg (=12.5 milligrams of the toxin per kilogram of body weight of the rat) was administered to pregnant rats on the tenth day of gestation, and of the 88 foetuses involved, 72, or 81.8% died or were resorbed (Coker 1998; He & Zhou 2010). Ducklings seem to be equally sensitive to ochratoxin as they are to aflatoxin (Ates et al., 2011).

Another fungus, *Penicillium viridicatum*, can also produce ochratoxin, and is relatively common in stored corn and is a more common producer of ochratoxin than *A. ochraceus* (Blumenthal, 2004).

2.1.2.2 *Aspergillus versicolor* and sterigmatocystin

This species is another storage fungus. However, it is never found as the only fungus or as the predominating fungus in deteriorating cereals. Normally, by the time a grain sample has become very mouldy, *A. versicolor*, along with other *Aspergillus* species and usually other filamentous fungi and yeasts as well. Some of the black pepper mentioned earlier, as being decayed by fungi, was very heavily invaded by *A. versicolor*, but not by this fungus exclusively. This species, under the right conditions, produces sterigmatocystin, a toxic compound given the name because the fungus once was called *Sterigmatocystis*. The toxin is known to cause lung, liver and kidney tumours in laboratory animals and has been implicated as the cause of disease in calves that have consumed feed heavily invaded by *A. versicolor (Ben-Ami et al., 2011)..* Experiments carried out in which the fungus were grown, on feed that was fed to calves, produced symptoms of the disease in the calves. However, tests were not done to detect the toxin in the calves. The toxin has also been detected in mouldy coffee beans in Africa, but no evidence indicates that even if these beans were used to brew coffee that the toxin would be in the drink.

2.1.2.3 *Aspergillus fumigatus* and fumagillin

This particular species is known to be an animal pathogen. Infection occurs through inhalation of spores and affects the lungs. Infection may also occur in eggs and the foetuses of cows. However, it also produces a metabolic product that may be considered a toxin or an antibiotic. This species differs from the others that we have discussed in that it is said to be thermophilic, that is, it is found in substrate where there are extremely high temperatures, up to 122°F (=50°C). This species is usually found on material that is in the advanced stages of decomposition in which the substrate temperature has been significantly raised by microbial decomposition (Edward, 2009). Under the proper conditions, *A. fumigatus* produces fumagillin. This compound is used as an amoebicide that is, as a means to rid the body of amoebae that are human pathogens and has been used effectively in honey bees as well. However, the correct dosage of this compound is critical. A little bit more than you need to get rid of the amoebae and you will be getting rid of the patient as well (Fokunang et al., 2006).

2.2 The genus *Fusarium*

Species of Fusarium are widespread in nature as saprobes in decaying vegetation and as parasites on all parts of plants (Harris et al., 1999; Walters et al., 2010). Many cause diseases of economically important plants. For this reason, there has been a great deal of research carried out in this genus by both plant pathologist and mycologist. However, there are a number of species that produce mycotoxins, mostly trichothecenes and zearalenone. We will discuss a few common examples.

2.2.1 *Fusarium tricinctum*

The effects of the first trichothecene toxin, T-2, documented was in the 1940s where it was associated with an outbreak of alimentary toxic aleukia (ATA). At its peak, in 1944, the population in the Orenbury District and other districts of the then USSR suffered enormous casualties, more than 10 percent of the population was affected and many fatalities occurred. The term *alimentary toxic* refers to the toxin being consumed in foods and *aleukia* refers to the reduced number of leucocytes or white blood cells in the affected person. Other symptoms included bleeding from nose and throat, multiple, subcutaneous haemorrhages (IARC, 1993; Bily et al., 2004).

The infected food in this case was millet, which made up a great part of the diet of the people in the region, and at times, during WWII, it was not uncommon to allow the millet to be left standing in the fields over winter because bad weather in the fall prevented its harvest at the proper time. During the late winter and early spring the millet would become infected with a variety of fungi, including *F. tricinctum*, and when the people gathered and ate this fungus, many came down with what was diagnosed as ATA. Thousands were affected, and many died. Locally, Joffe, a plant pathologist determined the outbreak of ATA was caused by consumption of a toxin, present in the millet, which had been contaminated by *F. tricinctum* (Biley et al., 2004). This was a remarkable conclusion since this was 20 years before aflatoxin was discovered. However, Joffe did not isolate or identify the toxin involved and as a result his work remained unknown until about 1965 when he presented a summary of his research at a symposium on mycotoxins. The mycotoxin involved was later given the common name T-2, and classified as one of several trichothecenes. Fed orally to rats, it has an LD50 of 3.8mg/kg, which is lower than that of aflatoxin, but still toxic enough.

2.2.2 *Fusarium graminearum* production

Corn is a stable in many countries and is used as a major ingredient in preparation of food for pigs and other domestic animals. Like many other grains, the kernels can be infected with fungi before and after harvest, and can affect the nutritional value of corn as food or feed. If the weather is rainy and the ears of corn are maturing in late summer and early fall, *F. graminearum* may infect only a few to a third of the kernels (Bennett et al., 1988; Cheng et al., 2011). Whatever amount of the ear is infected, all the kernels in that portion becomes heavily infected and decayed by the fungus. This fungus-infected corn is unattractive to pigs, as well as other animals, and they refuse to it. For this reason, this phenomenon has been called a *refusal factor*.

Regardless of what the composition of the rest of the feed, if it contains more than 5 percent of kernels with this refusal factor, the pigs will not eat it and weight loss will occur. They will starve rather than consume it. The infected corn contains an emetic compound produced by the fungus, and if this corn is consumed by pigs, they suffer prolonged

vomiting, after which they sensibly refuse to eat more of the corn. The toxin involved is deoxynivalenol (DON), also known as vomitoxin. The isolation and identification of this toxin has occurred only within the last 25 years (Bhat 2008).

Various methods have been tried to make the vomitoxin contaminated corn more acceptable to pigs. Among some of the means that have been tried are adding molasses to the feed to conceal whatever flavour or odour makes it unacceptable to the pigs, heating the feed, in hopes of destroying or inactivating whatever it is that is making the pig refuse to eat it, and composting it so that the heat will break down the toxin. However, none of these treatments have made the corn acceptable to pigs and are impractical (Bluhm et al., 2004).

The detection of infected corn or feed is also a problem. Since we are talking about mycotoxin here, the inability to isolate the causal agent, *F. graminearum*, is not evidence that the mycotoxin is absent. Long after a fungus has died off, mycotoxin secreted into the substrate, will still be present. The refusal of pigs to eat feed or corn is an indication that the refusal factor is present, but not necessarily conclusive. There are a number of reasons as to why pigs will refuse to eat. Pigs may be traumatized by being moved to a new pen, strange surroundings or even being offered different food. The only way that the toxin can be detected is to isolate, purify and identify it by spectrographic or other analysis (Taranu et al., 2011).

2.2.3 Importance of Trichothecenes as a biological weapon

2.2.3.1 Yellow rain

During the mid 1970s, when Vietnam was invading Laos, there were stories of "yellow rain" in areas where entire villages were killed. One eye witness account of such an event was told by a Hmong refugee, in Thailand. While tending his poppies, outside of his village, he and his family witnessed the bombing of their village by the Vietnamese, with a yellow powder that came down like yellow rain. Returning to the village, he found all of the animals and most of the people were dead. The bodies were bleeding from the nose and ears and their skin were blistered and yellowed. The few people left alive, when he arrived, were "jerking like fish when you take them out of the water". These people also eventually died. The witness took his family away from the village, but as they left they felt shortness of breath and sick to their stomach. This story is similar to other stories that were heard concerning yellow rain (Coppock and Jacobsen, 2009).

It was believed by the United States at that time that the Soviet Union was somehow involved in what occurred in the Hmong village, and medical teams were sent to investigate. However, because of the remoteness of these villages, news of such attacks normally took 4 to 6 weeks to reach someone who could notify the medical teams. By the time investigators reached a village, there was no evidence as to what happened. It would not be until 1980 that a Defense Department chemist recognized the symptoms described by victims of the bombing as similar to trichothecene mycotoxicosis. Samples from victims and from vegetation in the areas were tested and some were found to contain trichothecenes. With this information, President Ronald Reagan accused the Soviet Union of violating the Geneva Convention and Biological Weapons Convention, which of course they denied. However, these accusations would continue for three more years (Sudakin, 2003).

While the accusations and denials were aired, the media and scientific community gave a more critical examination of the yellow rain story. The analysis that demonstrated

Trichothecenes were being used was initially based on a single leaf, collected where one of the chemical attacks occurred. Subsequent specimens were collected later that also showed Trichothecenes were present, but the ratio of trichothecenes differed where it was found and was entirely absent in some samples. In addition, little fanfare was given to the over one hundred samples analyzed by the United States Army, which *did not find any indication of trichothecenes*. The eye witness accounts also came into question. Although it was implied that many villages were attacked with yellow rain, all of the witnesses were from a single refugee camp in Thailand, and even these accounts were thought to be unreliable (Kankunen et al., 2009). For example in relating a story of the bombing, one villager had initially said that 213 villagers were killed, but in a later retelling, there were only thirteen people killed and then forty.

Further erosion of the government's yellow rain story came about when a Yale University entomologist, whose expertise was in Southeast Asian bees, examined yellow rain samples and observed that they contained pollen from the native plants in the area. Based on the appearance of these samples, it was concluded that they were faeces of bees. In one species of bees, present in the area, there is a tendency for the bees to swarm when they defecated, as a cleansing ritual, which could give the appearance of yellow rain falling. News of such chemical attacks soon stopped and many civilian scientists were convinced that the entire yellow rain incident was a hoax that was carried out by the military to increase funding for defensive chemical and biological weapons. While a plausible alternative was given as to the cause of the yellow rain, the eye witness accounts while questionable, contradicted this theory. To date, the question as to what caused the yellow rain has still not been satisfactorily resolved and may never be (Hsueh et al., 1999).

2.3 Zearalenone

Zearalenone is a widely distributed oestrogenic mycotoxin occurring mainly in Maize, in low concentrations, in the developing countries, Europe, Japan, and North America (Hussein and Brasel, 2001). The concentrations in developing countries can be very high, especially when maize is grown in highland regions, under more temperate conditions. Zearalenone is co-produced with deoxynivalenol by *F. graminearum* and has been implicated with DON, in outbreaks of acute human mycotoxicoses (Prelusky et al., 1989). The exposure to zearalenone-contaminated maize has caused hyperoestrogenism in livestock, especially pigs, characterized by vulvar and mammary swelling and infertility (Bennett et al., 1988). There is limited evidence in experimental animals for the carcinogenicity of zearalenone (Kuiper-Goodman, 1991; Ding et al., 2006).

2.3.1 The fumonisins

The fumonisins are group of recently characterized mycotoxins produced by *F. moniliforme*, a mould that occurs world-wide and is frequently found in maize. Fumonisin B_1 has been reported in maize and maize products from a variety of agroclimatic regions including Brazil, Canada, USA, Austria, Italy, France and South Africa (Ding et al., 2006). The toxins especially occur when maize is grown under warm, dry conditions. Exposure to fumonisin B_1 (FB_1) in maize causes leuko-encephalmalacia (LEM) in horses and pulmonary oedema in pigs (Nair, 1998). LEM has been reported in many countries such as Argentina, Brazil, China, Egypt, South Africa and USA. FB1 is also toxic to the central nervous system, liver, pancreas, kidney and lungs of a number of animal species. The presence of fumonisins in maize has been linked with the occurrence

of human oesophageal cancer in the Transkei, southern Africa and China (Rheeder et al., 1992). There is however, sufficient evidence in experimental animals for the carcinogenicity of cultures of *F. moniliforme* that contain significant amounts of the fumonisins, whereas there is limited evidence, in experimental animals, for the carcinogenicity of fumonisin B_1 (Naiker and Odhav, 2004).

2.4 Ochratoxin A

Ochratoxin A (OA) is caused by the fungi *Aspergillus ochraceous*, *A. parasiticus, A. niger* and *Penicillium verrucosum*, (Kuiper-Goodman, 1991; Blumenthal, 2004). This toxin is produced within the temperature range of 15-37°C, with an optimal production at 25-28°C. The exposure to ochratoxin A occur mainly in wheat and barley growing areas in temperate zones of the northern hemisphere (Abarca et al., 2001). The levels of ochratoxin A reported in these products ranges from trace amounts to 600μg/kg, in Canadian wheat. In the United Kingdom, reported levels have included 5000 and 2700μg/kg in barley and wheat respectively (Anderson & Conning, 1993). It also occurs in maize, rice, peas, beans and cowpeas; developing country origins of ochratoxin A include Brazil, Egypt, Chile, Senegal, Tunisia, Nigeria, India and Indonesia (Wild and Hall, 2000). The ability of OA to transfer from animal feeds to animal products has been demonstrated by the occurrence of this toxin in retail pork products, and the blood of pigs in Europe (Fazekas et al., 2001; Friensen et al., 2008). It has been suggested that pork products are a significant human dietary source of OA which has been found in blood (and milk) from individuals in a variety of European countries, such as France, Italy, Germany, Dewnmark, Sweden, Poland the former Yugoslavia, and Bulgaria (Abarca et al., 2001)). One of the highest reported levels is 100ng/ml OA in blood from the former Yugoslavia (Fuch et al., 1991), while a level of 6.6 ng/ml OA in milk has been reported in Italy (Micco et al., 1991; Huffman et al., 2010).

The existing or proposed regulations for OA are in place in about eleven countries, the permitted levels ranging from 1 to 50 μg/kg in foods and from 100 to 1000 μg/kg in feeds. In Denmark, for example, the acceptability of pork products from a specific carcass is determined by analysis of the OA content of the kidney. The pork meat and certain organs can be consumed as food if the OA content of the kidney is no more than 25 and 10 μg/kg respectively (van Egmond, 1989; Wild & Hall 2000).

The WHO/FAO Joint Expert Committee of Food Additives has recommended a provisional tolerable weekly intake of 112ng/kg body weight of OA (WHO, 1991). The ochratoxin A has been linked with the human disease Balkan endemic nephropathy, which is a fatal chronic renal disease, reported in limited regions of the former Yugoslavia, Romania and Bulgaria (Cocker, 1999). OA causes renal toxicity, nephropathy and immunosuppression in several animal species and it is carcinogenic in experimental animals (Abarca et al., 2001; Yoshinari et al., 2007).

3. The metabolism of mycotoxins

Examination of the metabolic fate of aflatoxin B_1 can be used to illustrate the importance of the metabolic process in determining toxicity, and as a means of determining exposure to mycotoxins, by measuring; mycotoxin-macromolecular conjugates, the parent mycotoxin and a biochemical change initiated by the mycotoxin, respectively (Coker 1999).

3.1 Metabolism of aflatoxin

Many animal studies in order to study the metabolic fate of aflatoxins, *in vivo* and *in vitro* studies using animal tissues have been conducted mainly on aflatoxin B_1. Limited studies have also been performed on humans involving the measurement of aflatoxin B_1, and its metabolites, in blood, urine, milk and isolated tissues. Metabolism has been studied in many species and under many different conditions (Dalezios et al., 1973, Yunus et al., 2010).

The metabolic fate of aflatoxins may be considered under the headings of administration, absorption, transformation (activation and detoxification), distribution and excretion.

3.1.1 Administration

Under natural conditions, exposure to the aflatoxins may occur orally (by food ingestion) and by tracheal and bronchial absorption (by the inhalation of contaminated dust particle). In addition to these natural routes, intraperitoneal (ip), intravenous (iv) and dermatitis administration have been used under experimental conditions.

3.1.2 Absorption

Studies using radiolabelled aflatoxin B_1 in rats and monkeys have demonstrated little difference in the distribution and excretion of the toxin after either oral or intraperitoneal administration, therefore implying that absorption after oral exposure is complete (Dalezios et al., 1973; Wilson et al., 2002).

Aflatoxin B_1 can also be absorbed rapidly, by passive diffusion, from the small intestines (especially the duodenum) into the mesenteric venous blood. Given the lipophilic nature of aflatoxin B_1, the composition of the intestinal epithelium is an important criterion. Although the liver is regarded as the main site of aflatoxin transformation, gastrointestinal metabolism will reduce the exposure of the liver to aflatoxin B_1 and, in terms of hepatic toxicity, is an important means of detoxification (Hsieh & Wong, 1994; Avantaggiato et al., 2004).

3.1.3 Transformation

The transformation of aflatoxin B_1 results in both the activation and the detoxification of the toxin and may be considered as occurring in two phases (Ben-Ami et al., 2010). Firstly, the transformation of the toxin to a selection of metabolites and, secondly, the conversion of some of these metabolites to either water soluble conjugates or macromolecular adducts (Huffman and Gerber, 2010). The transformation process will be modulated by numerous factors including the genetic make-up of the species, nutritional and health status, and exposure to metabolic modifiers in foodstuffs (Meggs, 2009)

The major metabolites of aflatoxin B_1 includes aflatoxin B_1 -8,9-epoxide, -8,9-dihydro-8,9-diol;the aflatoxins-B2a, -P_1 , M_1 -Q_1; aflatoxicol, aflatoxicol H_1 and aflatoxicol M_1 (Essigmann et al., 1982; Klich, 2009)(Figure 7). However, not all metabolites have been identified in all species.

3.1.4 Activation

In the liver, aflatoxin B_1 may interact with both DNA and proteinto elicit the carcinogenic and acutely toxic effects of aflatoxin, respectively. Initially, aflatoxin B_1 is converted, by cytochrome P450, to the highly reactive aflatoxin B_1 -8,9-epoxide which in turn may be
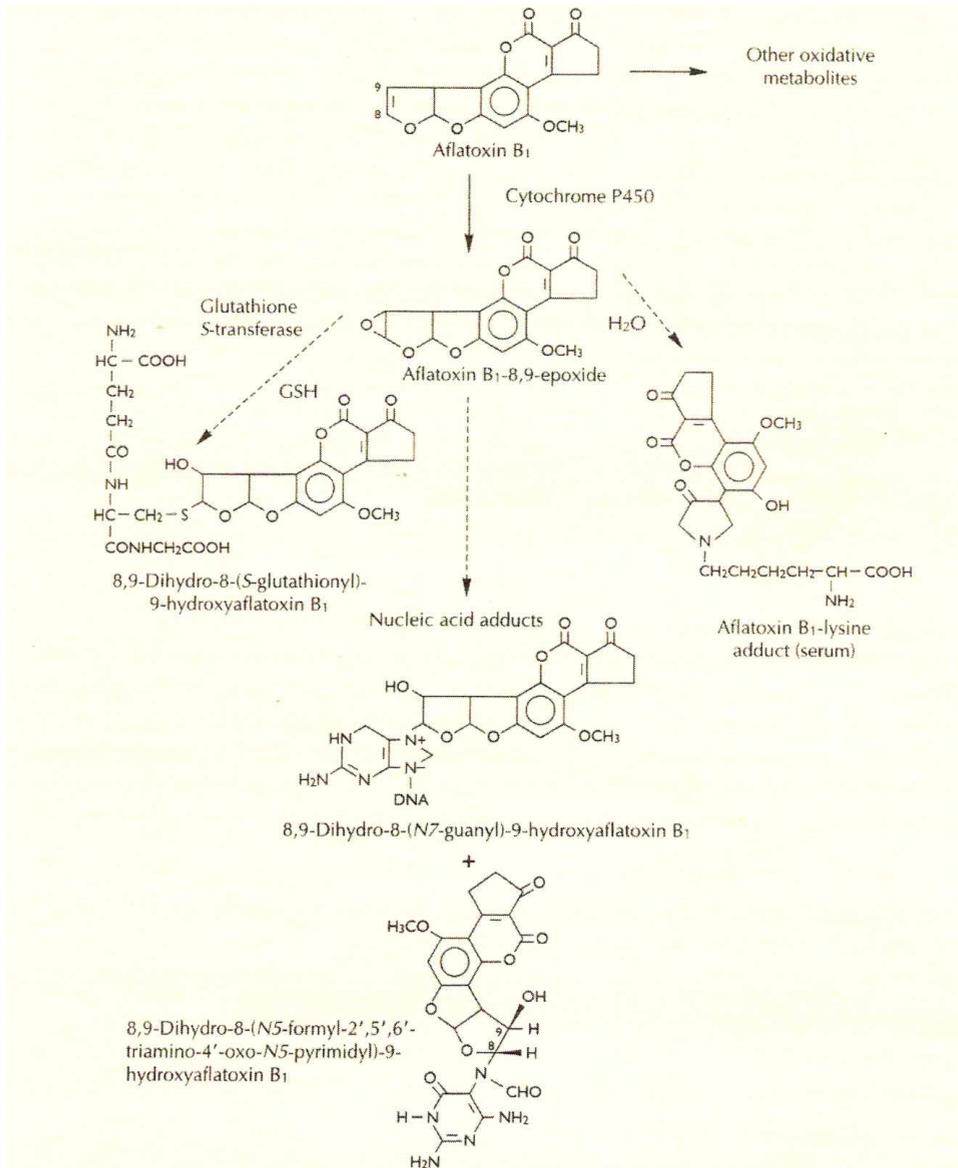
converted to aflatoxin B_1-dihydrodiol (Figure 8) (Baertschi et al., 1989; Coker, 1999; Kremer et al., 2007)

Fig. 7. The biotransformation of aflatoxin B_1 (Baertschi et al., 1998)

Aflatoxin B_1 is converted to at least seven metabolites, including a proposed unstable metabolite, the aflatoxin B_1-8,9-epoxide, which is the so called ultimate carcinogenic form (Hsieh and Wong, 1994; Magan et al., 2010). Aflatoxin M_1 occurs in milk of cows fed on aflatoxin B_1-containing feeds. This metabolite is found in the liver, kidneys and urine of sheep and in the livers of rats treated with aflatoxin B_1 (Appleton et al., 1982; Micco et al., 1991). The carcinogenicity of aflatoxin B_1 arises from interaction with guanine moiety of DNA, to produce the aflatoxin-N[7]-guanine adduct (Baerstchi et al., 1989), whereas the acute toxicity of aflatoxin B_1 is believed to stem from interaction between the dihydrodiol and protein amino groups to produce Schiff base adduct (Autrup et al., 1987).

Fig. 8. Biotransformation process of aflatoxin B_1 (Parker et al., 1998)

The major metabolites of aflatoxin B_1 includes B_1-8, 9-dihydro-8-9-diol; the aflatoxins –B_2a,-P_1, M_1, -Q_1; aflatoxicol, aflatoxicol H_1 and aflatoxicol M_1 (Essigmann et al., 1982; Smith et al., 2007). However, not all metabolites have been identified in all species. Aflatoxicol is a major aflatoxin B_1 in rat plasma (Wong and Hsieh, 1978; Pestka & Bondy 1990). It is reported as having equivalent carcinogenic potency as aflatoxin B_1 (Schoenhard et al., 1981; Pasquali et al 2010), and about 70% the mutagenicity (Coulombe et al., 1982Porbst et al., 2007). Since

aflatoxicol can be readily converted back to aflatoxin B_1, it has been proposed that aflatoxicol may act as a reservoir for aflatoxin B_1, *in vivo*, thereby prolonging the lifetime of the toxin in the body (Wong and Hsieh, 1978).

Total conversion of aflatoxin B_1 to M_1 in cow's milk is estimated to be about 1%. In comparing carcinogenic activity in rats, aflatoxin M_1 is less than one-tenth as active as aflatoxin B_1. However, the acute toxicities of these substances are almost similar. The aflatoxin metabolites M_1 and P_1 can also form DNA adduct (Essigmann et al., 1983). Similarly, there is evidence that aflatoxin G_1 can bind to DNA (Garner et al., 1979; O'brian et al., 2007).

3.2 Detoxification

Many methods have been used in an effort to detoxify contaminated feeds. Physical separation of obviously contaminated materials has proven successful in controlling aflatoxin contamination in peanuts. *Aspergillus flavus* and several other fungi emit a bright yellow-green fluorescence under ultraviolet light (Takayuki and Bjeldanes, 1993; Wilson et al., 2002). This telltale signal of fungal contamination has been useful in the physical separation of contaminated peanuts and corn as well as a few other crop samples.

Heat treatment of contaminated crops has also been used to detoxify food or feed material. Generally, under dry conditions the aflatoxins are quite heat stable. Normal roasting conditions can reduce the aflatoxin B_1 content in peanuts by 80% after an hour. Heating under conditions similar to the moist conditions used for autoclaving is much more effective in reducing aflatoxin content than dry heating (Park et al., 1988; Pitt & Miscamble, 1995).

Several chemicals such as hydrogen peroxide, ozone, and chlorine have been used to destroy aflatoxins. These substances react readily with aflatoxins in food as well as with many desired substances, including vitamins. A more useful method of chemical detoxification of contaminated feed is treatment with ammonia.

3.2.1 Ammonia detoxification

Ammonia was first used for the detoxification of aflatoxin-contaminated cottonseed meal, in the USA in the late 1960s (Park et al., 1988). The use of ammonia to detoxify corn meal and cotton meal increases the nutritional value of the feed.

The detoxified feed supports the growth of trout, cows, and other animals without apparent ill effects. An ammoniation process developed in Arizona involves placing a mixture of aqueous ammonia and cottonseed in large plastic bags used for silage (Cocker, 1999; Wu & Munkvold, 2008). The bags are sealed and allowed to stand in the sun for several weeks. The process has been shown to be effective in reducing the levels of aflatoxin in highly contaminated cottonseed (800ppb) to less than the 100 ppb action levels set by the FDA (Pepplinski et al., 1983).

Detoxication process involves numerous oxidising agents, aldehydes,acids and bases (inorganic and organic) that have been reported as potential chemical detoxification agents (Wild and Hall, 2000).

3.2.2 Chemistry of ammoniazation

The nature of the reaction products produced by the ammoniation of aflatoxin is still poorly defined. Most studies have focused on the reaction products of aflatoxin B_1 produced under a variety of conditions including the treatment in vitro, of pure toxin or of pure toxin on an inert carrier. The ammoniazation process of aflatoxin B_1 is illustrated in figure 8. Ammoniation, *in*

vitro, of pure aflatoxin B$_1$ has afforded four compounds of molecular weights (MW) 286, 256, 236 and 206, together with many unidentified compounds of MW less than 200. The compound of MW 286 has been characterized as the decarboxylated derivative (aflatoxin D1) of aflatoxin B1, whereas the compound of MW 206 lacks the cyclopentenone ring of aflatoxin D1. The loss of the methoxy group from aflatoxin D$_1$ affords the compound of MW 256. The reaction product of molecular weight 236 is still to be identified
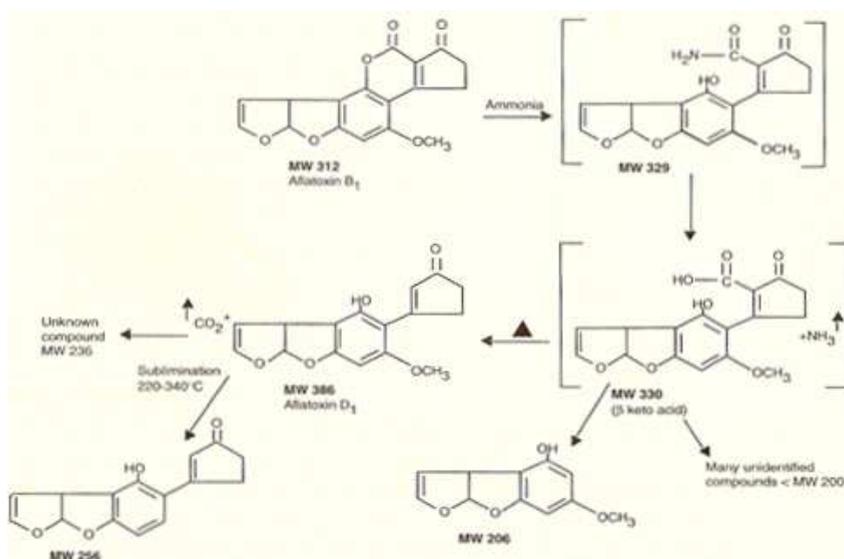
Fig. 9. Ammoniazation process of aflatoxin B1 (Parker et al., 1998)

3.2.3 Ammoniation and feed toxicity

The interaction of ammonia with both aflatoxin and nutritional components of feedstuffs has been. The resultant composition of these reaction products will determine the effect of ammoniation on both the nutritional and toxicological properties of treated commodity. These properties in turn, will determine the productivity of animals fed ammoniated feeds, together with the acceptability of animal products (milk, meat and eggs) used as human food.

3.2.4 Toxicity of ammonization reaction products

The toxicity of the reaction product, aflatoxin D$_1$, has been compared to that of the aflatoxin B$_1$ using a) the Ames test (Salmonella mutagenicity), b) the DNA covalent binding index CBI and c) the chick embryo bioassay as indicators of toxicity. Aflatoxin B$_1$ was reported (Lee et al., 1981; Yunus et al., 2010), as representing a 450-fold decrease in mutagenic potential, a 300-fold decrease, at least, in the DNA CBI (46), and (c) a 20-fold decrease, in toxicity to check the embryo (Lee et al., 1981; Kisoh et al., 2004). The reaction product MW 206, was over 600 times less mutagenic than aflatoxin B$_1$ (Hawarth et al., 1989; Faisal et al., 2008).

3.3 Distribution

After absorption from the intestine, aflatoxin B$_1$ rapidly enters the liver through the hepatic portal vein. The toxin is heavily concentrated in the liver after orla, ip and iv

administration;much less aflatoxin accumulated in the kidney. The ip administration of radiolabelled aflatoxin B1 to rats resulted in the presence of approximately 17% of the label in the liver within 30 minutes. The kidneys and the eviscerated carcass contained about 5 and 27% respectively; traces (< 0.5%) of labelled material were present in the adrenal glands, brain, heart, pancreas, spleen, thymus and testes (Wogan et al, 1967). The radioactivity reduces rapidly, only 10% remaining in the liver after 2 hours.

3.4 Excretion

The excretion of aflatoxin B_1 occurs mainly through biliary pathway and, to a lesser extent, by the urinary pathway, and by excretion into milk of lactating animals.

3.4.1 Biliary excretion

Studies show that when radiolabelled aflatoxin B_1 was feed to rats, the reported plasma half-life for radioactivity was 91.8 hours. Twenty-three days after dosing, 70% of the radioactivity had been excreted; 55% was present in the faeces compared to 15% in the urine (Coulombe and Sharma, 1985; Herwaarden et al., 2006).

3.4.2 Urinary excretion

Urinary excretion shows that approximately 15% of radiolabelled aflatoxin B_1 was excreted in rats' urine 20-24 hours after ip administration. The major metabolites were the aflatoxin M1 (45% radioactivity) and P1 (<10%), and aflatoxin B_1 -N7-guanine (16%). The later is the major degradation product of hepatic B1-DNA adducts (Groopman, 1994).Eighty percent of the excreted B_1 -guanine occurred in the urine during the 48-hour period after dosing (Essigmann et al,1982); a dose-dependent correlation between B_1 and B_1 -guanine has been observed in male rats (Bennett et al., 1981; Baerschi et al., 1989).

3.4.3 Excretion through cow milk

Aflatoxin B1 in dairy feed can be metabolised and transferred to cow's milk in the form of aflatoxin M_1. The percentage carry-over rate typically lies within the range of 1-5% depending upon, for example, the level of aflatoxin within the feed and the productivity of the cow. Generally, the carry-over rate increases as the feed contamination level decreases and as the productivity increases. However, the carry over rate varies significantly from cow to cow and on an individual cow basis.

3.4.4 Excretion through human milk

The aflatoxins have also been reported in human breast milk. In Africa (Sudan, Ghana, Kenya and Nigeria), for example, the aflatoxin M_1, B_1, B_2, G_1 and G_2 have all been found in breast milk (Maxwell et al 1989). Aflatoxin M_1 was the major metabolite, occurring at concentrations ranging from 20 to 1800ng/L, in Ghana.

4. Biomonitoring of mycotoxins

4.1 Biomonitoring of aflatoxins

The introduction of methods for biomonitoring individual members of the population is a major development which will make a significant contribution towards confirming the perceived linkage between mycotoxin exposure and human diseases. Biomarkers for the

aflatoxin, in humans, will now be discussed in terms of their role as markers of internal dose, biologically affective dose, early biological effect and susceptibility.

4.2 Markers of internal dose
4.2.1 Markers in urine

Aflatoxin M1 is a predominant metabolite in human urine. The presence of aflatoxin M_1 in urine may be detected by TLC, HPLC and ELIZA methods. Immunoaffinity columns have also been used to clean-up the samples prior to quantification.

Zhu et al. (1987) have compared dietary exposure to the aflatoxins with the urinary excretion of aflatoxin M_1, over a 3-day period, by analysing 252 urine samples in Guangxi Region of China. Between 1.2 and 2.2% of the dietary aflatoxin B_1 appeared in the urine as aflatoxin M_1, with a good correlation between the ingestion and excreted toxins.

4.2.2 Markers in milk

The occurrence of aflatoxin M1 in human breast milk is both an indicator of exposure of individual mothers to aflatoxin in food, and of the exposure of their infants to this toxin. However, no studies have reported a good correlation between levels of ingested aflatoxin B_1 and levels of aflatoxin M_1 in human breast milk. The occurrence of aflatoxin M_1 in human breast milk has been studied in Zimbabwe and France (Wild et al., 1987). In Zimbabwe , 11% of 54 samples of milk contained up to 0.05ug/L) aflatoxin M_1, whereas none of the 42 samples collected in France was contaminated.

4.2.3 Markers in blood

Unmetabolised aflatoxin B_1 in human blood serum has been used as an indicator of recent exposure to aflatoxins in food. Aflatoxin B1 has been detected (Tsuboi et al., 1984; Denning et al., 1988), for examples, in serum samples collected in Japan, Nigeria and Sudan. The detection methods used were ELIZA and HPLC; up to 3ug/kg B_1 were detected.

The aflatoxin M_1, B_1, B_2, G_1 and G_2 have been detected in cord sera from Ghana (34% of 188 samples); and aflatoxin M_1, M_2 and in B_2 cord sera collected in Nigeria (12% of 78 samples) (Lamplugh et al., 1988).

4.2.4 Markers of biologically effective dose

Two biomarkers of biologically effective dose have been developed. The first marker is a urinary aflatoxin B_1 -DNA adduct whereas the second is an adduct between aflatoxin B_1 and serum protein.

4.2.5 Aflatoxin B1-DNA adduct in urine

Studies in rats have examined the urinary excretion kinetics of specific metabolites after a single exposure to aflatoxin B1-N7-guanine (the primary B1-DNA adduct (Figure 8) accounted for 7.5% of the total detectable aflatoxins, whereas the aflatoxins P1, Q1, M1 and B_1 accounted for 31.5, 3,0, 2.2 and 0.3% of total aflatoxins, respectively (Groopman et al., 1992). Over the 24 hours after exposure, an excellent correlation existed between the oral dose of aflatoxin B_1 and the urinary aflatoxin-N7-guanine adduct. The other metabolites showed no such relationship.

The use of the aflatoxin-albumin adduct as a marker of the biologically effective does offers two advantages over the measurement of the aflatoxin-DNA adduct. Firstly, whereas the

aflatoxin-DNA adduct reflects exposure to aflatoxin on the previous day, the level of aflatoxin-albumin adduct is a measure of chronic exposure to aflatoxin, over the previous 2-3 months (Hall and Wild, 1994). Secondly, the collection of fingerprick samples of peripheral blood is a far more convenient operation than the collected urine.

4.2.6 Aflatoxin-albumin adduct in blood serum

The aflatoxin-albumin adduct has been measured in children and adults from a variety of African, and other countries. In Africa, between 12 and 100% of the samples contained the adduct, whereas samples from Thailand were contaminated at lower levels and incidence. An estimate of the average daily intake of aflatoxin B_1 was performed by determining the aflatoxin-albumin adduct in blood samples collected from population of 100 persons attending health screening at the Bangladesh Institute of Research and Rehabillitation in Diabetes, Endocrine and Metabolic Disorders, BIRDEM) and United Kingdom. A comprehensive studies of the major foods showed that the main staples such as rice, pulses and wheat, were relatively free of mycotoxin contamination, whereas maize and groundnuts were significantly contaminated. Over 60% of the groundnut samples contained aflatoxin, with some samples containing levels of toxin which were 40 times greater than the maximal level permitted in the European Union, EU. About 70% of maize were contaminated, and 17% of these contained more than one mycotoxin; one sample contained five different mycotoxins. Since the use of maize, both as an animal feed and as human food, is being actively encourage in Bangladesh, it is essential that every effort is made to alleviate the occurrence of mycotoxin in this commodity.

4.3 Markers of early biological effect
4.3.1 Measures of mutation spectra

Studies in the field of molecular biology have led to a better understanding of the generic alterations which occur during the progression from initiation to tumour formation, and to the development of sensitivity tests for the diagnosis of tumours.

The p53 tumour suppression gene is mutated in more than 50% of all human tumours (Hollstein et al., 1991). The number and type of mutations in this gene (the mutation spectrum) are not equally distributed, but occur in specific hot-spots which vary with the etiology of tumour formation.

In vitro studies using the human p53 gene have shown that codon 249 is the preferential site for the formation of aflatoxin-N7-guanine adducts (Pusieux et al., 1991). Exposure of cultured human liver cells to aflatoxin B_1 has produced codon 249 mutations. Although the link between aflatoxin exposure and specific p53 gene mutations in human populations has still to be confirmed, the gene mutation spectrum has considerable potential as a marker for exposure to, and damage from, the aflatoxins.

4.4 Markers of susceptibility
4.4.1 Measures of genetic variation in metabolism

Susceptibility to a particular agent will be influenced by the ability of individuals to absorb, distribute and metabolize the agent, and the nature of the metabolic process. The ability of individuals to repair damage inflicted by the agent will also contribute to the level of susceptibility. Studies with human liver microsomes have shown that the cytochrome P450s involved in the activation (epoxidation) of aflatoxin B_1 vary with the level of exposure. The activation of high levels of aflatoxin B_1, for example, is performed by cytochrome P450 3A4

(CYP3A4), with the simultaneous production of aflatoxin Q_1. Conversely, low levels of aflatoxin B_1 are activated by cytochrome CYP 1A2, with the simultaneous formation of aflatoxin M_1.

5. Conclusion

The problem posed by mycotoxin contamination of foodstuff especially in warm humid tropical environment has call for great interest in research in this area. For a better control and alleviation of mycotoxin problems in food it is important that importing nation adapts policies that ensures that the permitted levels of mycotoxin is maintained to an acceptable level of consumer protection. Efforts are in progress to implement these techniques in areas of high aflatoxin contamination in the hope of reducing the incidence of liver cancer. Developing nation needs to work on prevention strategies from mycotoxin through a partnership scheme. This can be achieved through a better knowledge of the role of mycotoxins in the epidemiology of human disease; a better understanding of the metabolism and toxicity of mycotoxins in animals and humans. There is also the need to understand the aetiology of mould and mycotoxin production in the field and the development of detoxification procedures which afford a safe product. In order to foster and intensify research in mycotoxin there is the need for continuous studies in developing a rapid simple, cost effective mycotoxin analysis method which can be used in sub-saharan and developing countries. There is also the need to put in place simple in vitro test for both acute and chronic toxicity and biomarkers for the detection of the exposure of individuals to mycotoxins and for the detection of immunotoxicity.

Continuous analyses of the combined epidemiological data from such studies indicate that high-level intake of aflatoxin in combination with such other diseases as hepatitis is associated with an increased rate of liver cancer. Despite these uncertainties about the role of aflatoxins in human cancer, efforts to minimize human exposures continue. There are well-established methods for harvesting, drying, and storing crops that are effective in the control of fungal contamination and aflatoxin production.

6. Acknowledgement

7. References

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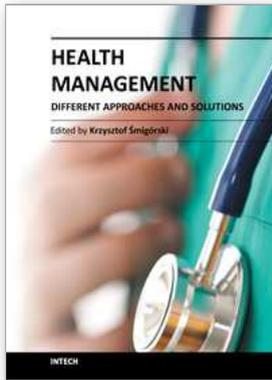
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Edited by Dr. Krzysztof Smigorski

The development in our understanding of health management ensures unprecedented possibilities in terms of explaining the causes of diseases and effective treatment. However, increased capabilities create new issues. Both, researchers and clinicians, as well as managers of healthcare units face new challenges: increasing validity and reliability of clinical trials, effectively distributing medical products, managing hospitals and clinics flexibly, and managing treatment processes efficiently. The aim of this book is to present issues relating to health management in a way that would be satisfying for academicians and practitioners. It is designed to be a forum for the experts in the thematic area to exchange viewpoints, and to present health management's state-of-art as a scientific and professional domain.

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