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Aflatoxins and Their Impact on Human and Animal Health: An Emerging Problem

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1. Introduction

Aspergillus is a fungus that essentially belongs to grains storage flora. It grows optimally at 25 °C with a minimum necessary water activity of 0.75. It starts to produce secondary metabolites at 10-12 °C, but the most toxic ones are produced at 25°C with a water activity of 0.95 (Hesseltine 1976). Those toxic secondary metabolites named aflatoxins (AF) is a group of mycotoxins produced by a large number of Aspergillus species, basically by three phylogenetically distinct sections. The main producers are *A. flavus*, and *A. parasiticus*, but it has been demonstrated that *A. nomius, A. pseudotamarii, A. parvisclerotigenus,* and *A.bombycis* of section Flavi, *A. ochraceoroseus* and *A. rambellii* from section Ochraceorosei and *Emericella astellata* and *E. venezuelensis* from Nidulatans section also generate aflatoxins (IARC 2002; Frisvad et. al., 2005). All of them contaminate a large fraction of the world's food, including maize, rice, sorghum, barley, rye, wheat, peanut, groundnut, soya, cottonseed, and other derivative products made from these primary feedstuffs in low-income countries (Rizzi et al., 2003; Saleemullah et al., 2006; Strosnider et. al., 2006; Masoero et. al., 2007; Caloni, 2010).
Although aflatoxins have been a problem throughout history, until 1960 they have been recognized as significant contaminants within agriculture, because in this year they were initially isolated and identified as the causative toxins in "Turkey-X-disease" after 100,000 turkeys died in England from an acute necrosis of the liver and hyperplasia of the bile duct after consuming groundnuts infected with Aspergillus flavus (Asao et. al., 1965; D'Mello, 1997; Strosnider et. al., 2006).
Williams et al. estimated in 2004 that 4.5 billion of the world's population is exposed to aflatoxins because they are also everywhere. Some essential factors that affect aflatoxin contamination include the climate of the region, the genotype of the crop planted, the soil type, the minimum and maximum daily temperatures, and the daily net evaporation (Strosnider et. al., 2006). Moreover, aflatoxin contamination is also promoted by stress or damage to the crop due to drought before harvest, the insect activity, a poor timing of harvest, the heavy rains during and after harvest, and an inadequate drying of the crop before storage. Levels of humidity, temperature, and aeration during storage are also important factors that are

intimately related with the actual problems of climate changes and environmental warming around the whole world (Cotty & Jaime-García, 2007; Paterson & Lima, 2010).

There have been identified 18 types of aflatoxins, nevertheless, the naturally occurring and well-known ones are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) (Gimeno, 2004; Saleemullah et. al., 2006; Strosnider et. al., 2006). These names were given due to their blue (B) or green (G) fluorescence properties under ultraviolet light and their migration patterns during chromatography (Wogan & Busby, 1980; Dikeman & Green, 1992). The International Agency for Research on Cancer (IARC, 2000) has classified aflatoxin B1 as a group 1 carcinogen (that means carcinogenic to humans) since 1987, and a group 1 carcinogenic agent since 1993 due to the exposure to hepatitis B virus (Castegnaro & McGregor, 1998). AFB1 is the most prevalent aflatoxin usually found in cases of aflatoxicosis, and is responsible for acute toxicity, chronic toxicity, carcinogenicity, teratogenicity, genotoxicity and immunotoxicity. AFM1 is a metabolic derivate of AFB1, and AFM2 is a metabolic derivate of AFB2; both come from the metabolism of some animals, and are normally found in milk and urine (Gimeno, 2004; Strosnider et. al., 2006).

The B-toxins are characterized by the fusion of a cyclopentenone ring to the lactone ring of the coumarin structure, while G- toxins contain an additional fused lactone ring. Aflatoxin B1 and to a lesser extent AFG1, are responsible for the biological potency of aflatoxin-contaminated feed. These two toxins possess an unsaturated bond at the 8,9 position on the terminal furan ring. Aflatoxin B2 and AFG2 are essentially biologically inactive unless these toxins are first metabolically oxidized to AFB1 and AFG1 in vivo (Verma, 2004). AFM1 and AFM2 are hydroxylated derivatives of AFB1 and AFB2 that may be found in milk, milk products or meat (hence the designation M1). They are formed by the metabolism of B1 and B2 in the body of the animals following absorption of contaminated feeds (Gimeno, 2004; Verma, 2004; Wild & Gong, 2010).

In animals, aflatoxins impair growth and are immunosuppressive. B aflatoxin has been reported to induce liver and kidney tumors in rodents, and there has been found a possible link to increased esophageal cancer. Aflatoxins have been recently considered as an important sanitary problem because it has been demonstrated that human exposure to mycotoxins may result from consumption of plant derived foods that are contaminated with toxins and their metabolites (which are present in animal products such as milk, meat, visceral organs and eggs) or exposure to air and dust containing toxins (Jarvis, 2002). It has been reported that aflatoxins, once ingested (because of their low molecular weight), are rapidly adsorbed in the gastro-intestinal tract through a non-described passive mechanism, and then quickly appear as metabolites in blood after just 15 minutes and in milk as soon as 12 hours post-feeding (Yiannikouris & Jouany, 2002; Moschini et. al., 2006). Aflatoxins are hepatocarcinogenic particularly in conjunction with chronic hepatitis B virus infection, and cause aflatoxicosis in episodic poisoning outbreaks. Recent studies also suggest that the B aflatoxins may cause neural tube defects in populations that consume maize as a staple food (Wild & Gong, 2010).

Due to this important global issue, some organizations and institutions have been purposing a great number of practical primary and secondary prevention strategies, especially for developing countries, in order to reduce the risks given by this public problem, but they could be beneficial if political wills and financial investments are applied to what remains a largely ignored worldwide health matter.

2. Aflatoxins in food products from contaminated grains

In many low-income countries, mycotoxins, and particularly aflatoxins, affect staple foods including cereals (maize, wheat and rice principally) and their derivates; oilseeds (cotton, peanut, rapeseed, coconut, sunflowers and others), cassava, groundnuts and other nuts, and a great variety of foods which are consumed by humans like dry fruits, delicatessen products, spices, wines, legumes, fruits, milk and milk derivates (Gimeno, 2004; Wild & Gong 2010). Maize and groundnuts are major sources of human exposure because of their greater susceptibility to contamination and frequent consumption throughout the world. Table 1 shows some of the most important commodities affected by aflatoxins producer species, according to a review made by Abdin and collaborators in 2010.

Type of aflatoxin	Producer fungal species	Affected commodities
B (B1, B2)	A. flavus, A. parasiticus, A. tamarii, A. pseudotamarii, A. bombycis, A. parvisclerotigenus, A. nomius, A. minisclerotigenes, A. oryzae, A. toxicarius, A. versicolor, A. rambellii, A. arachidicola, A. ochraceoroseus, Emericella astellata, E. venezuelensis.	Cotton seed, peanuts, peanut butter, pea, sorghum, rice, pistachio, maize, oilseed rape, maize flour, sunflower seed, figs, spices, meats, dairy products, fruit juices (apple, guava)
G (G1, G2)	A. parasiticus, A. nomius, A. bombycis, A. pseudotamarii, A. terreus, A. versicolor, A. arachidicola, A. toxicarius, A. minisclerotigenes.	Peanuts, cotton seed, sunflower seed, tree nuts, pistachio, peanut butter, maize flour, pea, cereals, corn, figs, meats, spices, dairy products, fruit juices (apple, guava)

Table 1. Major commodities affected by aflatoxins (Taken from Abdin et. al., 2010.)

Aflatoxins are most prevalent in latitudes between 40° N and 40° S of the equator, but the greatest health risk lies within developing countries in tropical regions, which rely on these commodities as their staple food source (Strosnider et. al., 2006). Even, in some processed typical food like Mexican pozol, there have been found large amounts of aflatoxins, being the AFB2 the more prevalent and abundant toxin, suggesting that AFB2 is more resistant than AFB1 to the alkaline conditions given during hard processes like nixtamalization (Kamimura, 1989; Méndez & Moreno, 2004).

In wealthy grain-producing countries of the world, economic resources exist to ensure that regulations to limit aflatoxin exposure in the food supply are implemented. Furthermore, in markets of grain commodities, the prices of corn and groundnuts are often dictated by aflatoxin content, which contributes to lower levels of exposure in wealthy countries. Thus, a result of these regulations and market forces is that people in economically developing countries are exposed to far higher levels of aflatoxins in the diet (Groopman et. al., 2008).

The presence of aflatoxins in food means a risk for both animals and human beings. This is because not only grains (generally consumed by people), but also whole plants and grasses from which they emerge, could be contaminated by mycotoxins. This is a serious threat for animals, particularly livestock, because the herbaceous food they consume (commonly known as ensilage or forage) contains a large amount of aflatoxins, particularly if field was contaminated. A potentially hazardous feed is ground high-moisture corn, unless it is

treated with adequate preservatives (e.g., propionates); the moisture content promotes the growth of the toxigenic molds and grinding of the kernel destroys the natural barrier to infestation. Hay (unless it contains a large complement of cereal grain infested in the field) is rarely if ever a source of appreciable aflatoxin (however, hay and forage may be sources of other mycotoxins such as ergot alkaloids, sporodesmin, slaframine, etc.). The fungus must gain access to susceptible parts of the plant (e.g., the corn kernel, cotton seed, etc.) before it grows and elaborates aflatoxins. Seasonal peaks in aflatoxin content are seen in key years when drought-damaged plants or insect-damaged crops are rendered more susceptible to fungal invasion. Wet harvest seasons also may contribute to high levels of aflatoxin in certain crops. Aflatoxin sometimes develops in crops stored at levels of moisture content > 15% or properly dried crops stored in leaky bins (Pier, 1992).

Grains for animal feed in the United States are allowed 300 ppb aflatoxin, because this concentration not only provides protection against acute aflatoxicosis but also is low enough to allow most of the grain produced to be traded. In these animal feeding situations, the long-term risk of cancer is not a concern, except for the most susceptible species. Consequently, veterinary research has examined higher levels of exposure but for shorter time periods. This research provides most of the information on the toxicities of aflatoxin at intermediate rates of exposure (100–500 ppb) and is the most potentially relevant information that is appropriate for the human situation in developing countries where no control of aflatoxin is exercised. However, the differences between species in response to aflatoxin introduce a measure of speculation into the extension of farm animal–derived information to the human situation (Williams et. al., 2004).

3. Aflatoxins in food products from contaminated animals

Aflatoxins M1 and M2 (whose names are derived from milk aflatoxins, and then related to meat aflatoxins too), are thermo-resistant hydroxylated metabolites produced by lactating animals consuming aflatoxin contaminated feeds. The ingested AFB1 and AFB2 are metabolized by livestock into AFM1 and AFM2 respectively, with estimated conversion ratio of 1–3% between AFB1 and AFM1 (Barbieri et. al., 1994; Ali et. al., 1999; Herzallah, 2009). The accepted limits of AFB1 and total aflatoxins in foods are 5 and 10 μg/kg, respectively, in more than 75 countries around the world whilst they are 2 and 4 μg/kg in the European Union (López et. al., 2003; Van Egmond & Jonker, 2004).

The most alarming problem through time has been the presence of aflatoxin contaminated milk, because cows and goats (the major producers of drinking milk) are largely affected when eating contaminated forage all around the world (Helferich et. al., 1986; López et. al., 2003). By the way, it is important to consider that AFM1 concentrations in milk vary not only in the cow breed, but also in the concentration of AFB1 in the diet, the amount and duration of consumption of contaminated food and the animal health.

There have been found differences between the amounts of AFM´s produced by different bovine species. In a review, Gimeno (2004) reports that in dairy cows, the relationship between the concentration of AFB1 in the final consumed ration and AFM1 excreted in breast milk could be 300:1; nevertheless this relation is only an approximation because the range is from 34:1 to 1600:1. In Holstein dairy cows consuming final rations with 80, 86, 470, 557, 1493 and 1089 μg of AFB1/Kg (ppb) on dry substance, there were found in milk AFM1 concentrations of 1.5, 0.245, 13.7, 4.7, 12.4 and 20.2 mg/L (ppb) respectively. On the other hand, when diet of Brindle cows was contaminated with 540 ppb of AFB1, 0.92 ppb of AFM1 was produced. In

other cows, the values of contamination in the diet ranged between 64 and 1799 ppb of AFB1 giving some residues in milk between 0.35 and 14.2 ppb of AFM1. With an intake of AFB1 for 2-60 mg / cow / day, AFM1 residues in milk could range between 1 and 50 pp.

It is known that cows can transform AFB1 into AFM1 within 12-24 hours after ingestion of contaminated food. Even at six hours after ingestion, AFM1 residues can appear in milk, and the highest levels are reached after a few days. When the intake of AFB1 is stopped, the AFM1 concentration in the milk decreases to an undetectable level after 72 hours (Gimeno, 2004; Özdemir, 2007).

Many studies have dealt with the transfer of AFB1 in milk as AFM1 when lactating animals ingested contaminated feed continuously, especially in cows. It has been suggested that an increase in AFM1 occurs due to *Staphylococcus aureus* infection and other bacterial infections related with somatic cells diseases (Veldman et. al., 1992; Masoero et. al., 2007). In contrast, little research has been conducted on the transfer of AFM1 into milk as a result of a single assumption of AFB1. From a practical standpoint, the use of highly contaminated feed by dairy farmers is unlikely; however, a single accidental feeding of contaminated feed may happen and can lead to milk AFM1 content above tolerance levels (Mazzete et. al., 2009).

As mentioned before, goats are a clue target of aflatoxins too, so they have been studied as a good model for understanding the generating toxins metabolism (Smith et. al., 1994; Mazzete et. al., 2009). Mazzette and collaborators found that AFB1 ingested by lactating goats is quickly transferred to milk as AFM1. The maximum concentration of AFM1 was reached at 3-6 hours after the single oral administration of pure AFB1. Nevertheless, it showed a negative exponential trend and the toxin was no longer detected after 72 hours from administration. Therefore, an occasional oral assumption of AFB1 can lead to a transient contamination of AFM1 in goat's milk.

Milk has derivates that are consumed principally by humans. Among them we can find cheeses, butter, yogurt, cream and butterfat. The AFM1 distribution in some dairy foods made from contaminated milk is approximately: 40-60% in cheese, 10% in butterfat and <2% in buttermilk. AFM1 is highly soluble in water, so it is incomprehensible why this toxin is deposited in the cheese but not in the milk whey (Yusef & Marth, 1989).

Aflatoxins are not only present in cow, goat and sheep milk and derivates even after pasteurizing processes, there have also been found in other food animal products like turkey and hen eggs. Residues of aflatoxins and their metabolites in foodstuff animal tissues (like beef and sheep meat) may be a source of aflatoxin contamination in human foods (Rodricks & Stoloff, 1977; Herzallah, 2009); nevertheless, milk has been the most studied food, because of its implication in human nutrition at all growing stages.

4. Major human diseases caused by aflatoxins consumption

Populations of developing countries are the most susceptible to aflatoxicosis illness. This is because security blankets in crops at pre-harvest and post-harvest level are not as strict as in developed countries. The same occurs with milk derivates, because developing countries have not accepted and assumed amenities as quick as developed countries. It has been estimated that more than 5 billion people in developing countries worldwide are at risk of chronic exposure to aflatoxins through contaminated foods (Shephard, 2003; Williams et. al., 2004). Because of being an alarming number, aflatoxins have been recently considered as an important public health issue. Adult humans usually have a high tolerance of aflatoxin, and, in the reported acute poisonings, there are usually children who die (Cullen & Newberne, 1994).

The adverse effects of aflatoxins in humans and animals have been categorized in two general forms:

a. Acute aflatoxicosis.

It is produced when moderate to high levels of aflatoxins are consumed. Specific, acute episodes of disease include hemorrhage, acute liver damage which manifests as severe hepatotoxicity with a case fatality rate of approximately 25%, edema, absorption and/or metabolism of nutrients and alteration in digestion. The early symptoms of hepatotoxicity from aflatoxicosis can include anorexia, malaise, and low-grade fever. Acute high-level exposure can progress to potentially lethal hepatitis with vomiting, abdominal pain, jaundice, fulminant hepatic failure and death (Walderhaug, 1992; Cullen & Newberne, 1994; Strosnider et. al., 2006).

b. Chronic aflatoxicosis.

It results from ingestion of low to moderate levels of aflatoxins. The effects are usually subclinical and difficult to recognize. Some of the common symptoms are impaired food conversion and slower rates of growth with or without the production of an overt aflatoxin syndrome (Walderhaug, 1992).

There have been memorable clinic cases that had woken up the interest of scientists on investigating deeply the mode of action of aflatoxins in humans and take matters into. One of the most peculiar cases occurred in 1976, with a 68 old british chemical engineer who worked for three months on a method of sterilizing Brazilian peanutmeal which was contaminated by *Aspergillus flavus*. Three months after finishing this work he became ill with high fever and began to expectorate thick, white sputum. X-ray examination showed cavitation in the left lower lobe of the lung. At first the process was considered to be due to tuberculosis, and later to mycotic disease. After two months further lesions developed in both lungs. The condition of the patient became worse and he died 11 months after the onset of his illness. Necropsy showed enlarged, heavy lungs diffusely infiltrated with firm yellow-white or reddish lesions, mitotic figures were rare, the picture was of pulmonary adenomatosis, no metastases or tumors in other organs were found and bacteriological examination was negative. A sample of lung tissue was taken for chemical investigation. Thin-layer chromatography of the extract showed a blue fluorescent spot in 365 nm UV light similar to that of a commercial sample of aflatoxin B1, the same color change as standard B1 when treated with 50% H_2SO_4, and an RF value identical to that of the commercial aflatoxin sample B1. A colleague of this patient who had been doing the same work died three years before of pulmonary adenomatosis, but no chemical investigations were done in his case. The conclusion was that his illness was caused by the inhalation of A. flavus spores that, together with another factors lead to chronic damage to the lung, determining that aflatoxicosis is an opportunistic disease. Also, there is evidence that air-borne particles of dust contaminated by aflatoxins contribute to the development of pulmonary cancer (Dvorackova, 1976).

Aflatoxicosis is not only caused by inhalation, but also, as mentioned before, is caused by aflatoxin ingestion. In places like Brazil and Abu Dhabi, there have been found lots of cases in which infants were exposed to aflatoxin M1 from mother's breast milk. Aflatoxins have also been found in infant formula (Aksit et. al., 1997; Saad et. al.,1995; Navas et. al., 2005). There are lots of earlier studies reporting the presence of aflatoxins and derivatives in human urine, blood, and human cord blood that apparently can enter the developing fetus in humans and animals (Denning et. al., 1990).

The 80's and 90's were globally fatal decades. In India, at least 400 people were affected by eating infected corn, and 104 of them died. In Kenya, 12 people were also killed by high consumption of aflatoxins (Mehan & Mc Donald, 1991). In Southeast Asia, 19 patients after eating rice and pasta became jaundiced and sick within hours; 17 of them presented symptoms of hepatitis, and in total, 14 died because of liver failure and 7 because of renal failure. In biopsies, there were found high concentrations of aflatoxin in liver, lungs and other organs (Hendrickse, 1999).

It has been well documented that chronic aflatoxin exposure causes Hepatocellular Carcinoma (HCC), generally in association with hepatitis B virus (HBV) or other risk factors. That's why the International Agency for Research on Cancer (IARC) recognized aflatoxins as carcinogenic in 1976 (Chen et. al., 2001; Henry et. al., 2002; Omer et. al., 2004; Qian et. al., 1994; Wang et. al., 1996). HCC is the sixth most prevalent cancer worldwide. Developing countries have a higher incidence rate, with approximately 82% of the 600,000 new cases each year occurring in developing countries (Parkin et. al., 2005).

Unsafe sex associated with aflatoxicosis has been identified as a risk factor largely because of the HIV epidemic. Whereas the risk is behavioral, the disease is viral, and the progress of the epidemic is determined by disease transmission, rate of disease progress, and opportunistic infections. The disease of HIV is complicated, and the ways in which the virus interacts with another immunocompromising agent is also likely to be complicated. The animal data on immune suppression and nutritional interference has shown aflatoxicosis symptoms to be similar to HIV infection symptoms, differing mainly in that the removal of aflatoxin from the diet reverses the symptoms. The animal data on immune suppression suggest that the parameters of the epidemiologic model are likely to be modulated by aflatoxin at some level of exposure, either directly or indirectly through the known toxicities of aflatoxin. Nutrition is also a general area in which aflatoxin exposure can be expected to modulate HIV (Williams et. al., 2004).

5. Major animal diseases caused by aflatoxins consumption

Effects of aflatoxin consumption are similar in all animals; however, the susceptibility varies by species, age, and individual variation. Symptoms of acute aflatoxicosis consist of depression, anorexia, weight loss, disease, gastrointestinal bleeding, pulmonary edema and liver damage. Signs of acute hepatic injury are seen as coagulopathy, increased capillary fragility, hemorrhage, and prolonged clotting times. Blood pigments may appear in the urine and mucous membranes are icteric. The liver shows gross changes caused by centralobular congestion and hemorrhage and fatty changes of surviving hepatocytes. Death of the animal may occur within hours or a few days. Symptoms of prolonged exposure to moderate to aflatoxins may be reflected in a decline in feed consumption and production (growth and production of eggs and milk). It can also affect the quality of milk and milk products, and represent a risk for the presence of AFM1 as derived from AFB1 consumed by lactating females. In chronic aflatoxin poisoning, most of the effects are still referable to hepatic injury, but on a milder scale. The most sensitive clinical sign of chronic aflatoxicosis is reduced rate of growth of young animals. Other signs include prolonged clotting time, increases in serum glutamic oxalacetic trans-aminase, ornithine carbamyl transferase, and cholic acid levels. Hepatic pathology includes a yellow to brassy color, enlarged gall bladder, dilute bile, histologic signs of fatty changes in the hepatocytes, and bile duct proliferation. Frequently the signs of chronic aflatoxins are so protean that the condition

goes undiagnosed for long periods. Chronic aflatoxin poisoning, however, is the manner in which animals are most frequently affected and the economic consequences are often considerable (Pier, 1992; Denli & Pérez, 2006).

AFB1 is absorbed via the gastrointestinal tract into the portal blood system and is carried to the liver where it is metabolized. A portion of aflatoxin is activated and set in hepatic tissues. Some water-soluble conjugated metabolites of AFB1 are excreted into the bile and go to the stool. Other water-soluble conjugated metabolites, AFB1 degradation products and non conjugated metabolites are excreted into the blood circulatory system and distributed systemically. Eventually, these residues are referred to milk, eggs, muscle and edible tissues (Dennis & Hsieh, 1981). AFM1 is one of those metabolic derivatives that taint milk. Other metabolites are formed from AFB1, including aflatoxicol (18 times less toxic than AFB1) and aflatoxin B2a (not toxic). The animal organism usually produces those metabolic products as an autodetoxification system (Gimeno, 2004).

AFB1 mainly affects birds, pigs and other monogastric animals. Ruminants are less vulnerable to aflatoxin ingestion. In monogastric animals, clinical symptoms may occur after consumption of feed contaminated with concentrations above 50 ppb while the symptoms in cattle occurs at concentrations above 1.5 to 2.23 mg/kg. Depending on the presence of other concurrent factors, small amounts of AFB1 (greater than 20 ppb) can cause toxic effects. In these conditions, an aflatoxin level above 100 ppb may be also toxic in ruminants (Denli & Pérez, 2006).

Experimental animal evidence suggests that chronic exposure to aflatoxins may lead to impaired immunity and reduced uptake of nutrients from the diet too (Hall & Wild, 1994; Miller & Wilson, 1994). Furthermore, diseases caused by aflatoxins can cause subclinical losses in production, and increase the risk and incidence of other diseases (Denli & Pérez, 2006).

Below, we describe some of the most important diseases that some animal species develop when they eat aflatoxin contaminated food or, in some cases, inhale the fungal spores from the air. Those data are summarized in Table 2.

5.1 Horses

There have been reported some cases of aflatoxicosis on horses since 1976. The reported symptoms included anorexia, icterus and rapid weight loss immediately prior to death. On post mortem examination, the liver was described as being black, of firm consistency and enlarged. Histopathological examination revealed marked centrilobular hepatic necrosis and necrotic areas were engorged with erythrocytes. Kupffer cells were prominent and many contained phagocytosed haemosiderin, which was the likely cause of the black coloured liver. Bile-duct hyperplasia, congestion of renal vessels and adrenal cortex were found. Samples of the feed revealed AFB1 levels of 58.4 μg/kg, which exceeded the limit recommended by the FDA (20 μg/kg) (Greene & Oehme, 1976). Other reports mention that AFB1 content in horse feeds was also within tolerable limits (10 μg/kg), with an average AFB1 concentration of 1.98 ± 0.71 μg/ kg (Greene & Oehme, 1976; Basalan et. al., 2004; Caloni & Cortinoivis, 2010).

It is thought that a possible link between chronic obstructive pulmonary disease (COPD) and inhaled mycotoxins exist. A. fumigatus and Mycropolyspora faeni are potential causes of COPD in horses, which is characterized by asthma-like symptoms, such as chronic cough, nasal discharge, expiratory dyspnoea and reduced exercise tolerance. The olfactory and

respiratory mucosa of horses may be exposed to mycotoxins and other xenobiotics via inhalation of contaminated feed-dust particles (for a complete review see Caloni & Cortinovis, 2010).

The existing information on aflatoxicosis in horses is inconclusive, although a total dietary concentration of 500–1000 μg/kg has been shown to induce clinical changes and liver damage, depending on the duration of exposure (Meerdink, 2002). Horses suffering from aflatoxicosis exhibit non-specific clinical signs, such as inappetence, depression, fever, tremor, ataxia and cough. Necropsy findings include, as intoxication by feeding, yellow–brown liver with centrilobular necrosis, icterus, hemorrhage, tracheal exudates and brown urine (Caloni & Cortinoivis, 2010).

5.2 Chickens

Broiler-type chickens are considered to be more resistant to aflatoxin toxicity than are other poultry species (Arafa et. al., 1981). In the poultry industry, AFB1 is called "the silent murderer" because its chronic consumption at levels below 20 ppb does not induce evident clinical symptoms; however, it reduces the absorption of food and causes immunosuppression. The final result is a low productivity, because birds show a low growth and low stance. Additionally, due to induced immunosuppression, birds are much more susceptible to opportunistic infectious agents and respond poorly to vaccination programs (AgroBioTek, 2009).

Some studies conducted on the 1960's decade, showed that aflatoxins ingestions caused periportal fatty infiltrations, increase in connective tissue and hemorrhages in most of sick chickens (Newberne & Butler, 1969). Later, in 1984, Chen and collaborators made an study un which they fed some broiler chicken with aflatoxins contaminated food, and 3 hours after the withdrawal of the contaminated feed, measurable amounts of AFB1 and AFB2 were found in all of the tissues of the birds that had been fed the aflatoxin-contaminated ration. The highest levels were found in the gizzards, followed by the livers in second place, and kidney contained the third highest levels. The capacity of the liver and kidneys to concentrate aflatoxins is probably associated with their important role in the metabolism and elimination of xenobiotics. After four days on an aflatoxin-free diet, there were no detectable levels of aflatoxins in any of the tissues. This suggests that four days on an aflatoxin-free diet before slaughter is adequate to remove detectable levels of free aflatoxins and their metabolites from the tissues of chickens that had previously been fed a highly contaminated diet. These aflatoxin residues are rapidly cleared from the tissues alter removal of the contaminated food (Chen et. al., 1984).

It has been demonstrated that broiler chickens fed with a diet rich in aflatoxins record significantly lower performances, growth and survival rate than controls. That's why lowered growth rate and increase of mortality have been associated with contaminated feeding broiler diets (Oguz & Kutoglu, 2000). In 2010, Okiki and collaborators confirmed this, because they found that chickens fed with aflatoxin decreased their growth rate and showed a weight loss of up to 400g when compared with controls after having been fed for 56 days with contaminated food.

5.3 Pigs

In pigs, aflatoxin also induces a low growth rate and increases the expression of opportunistic infections to cause immunosuppression (Gimeno, 2004). Since old times, major

lesions have been identified in liver, which turns swollen, congested, and friable; liver surface shows occasional petechiae and animals surviving beyond 24 hours often had ascites and hydrothorax. The gall bladder seems edematous and the mucosa turns petechiated and ecchymotic (Newberne & Butler, 1969).
In some studies with guinea pigs, it was found that aflatoxins cause acute gastrointestinal effects too (Luzi et. al., 2002). In a study conducted in 1982, swine with a rich-aflatoxins diet presented a peracute toxicity that caused collapse and deaths within several hours while acute toxicity caused deaths within 12 hours; subacute toxicity deaths occurred after 3 weeks on a toxic ration. Anorexia and ill thrift affecting only growing animals were seen with chronic toxicity. Extensive centrilobular liver necrosis and haemorrhage occurred with peracute toxicity and in cases of acute poisoning there was hepatic centrilobular cellular infiltration, hepatocyte swelling and bile stasis. With subacute toxicity hepatocyte vacuolation together with bile stasis and bile ductule hyperplasia was seen (Ketterer et. al., 1982).
Although aflatoxicosis in pigs is a big health problem, it has not been considered as important as fumonisin toxicosis, which nowadays is the biggest swine threat in this specie (Mallman & Dilkin, 2007; D'Mello et. al., 1999; Placinta et. al., 1999; Straw et. al., 1999).

5.4 Cattle

The first case of poisoning in cattle by groundnut was reported in 1961. Calves (3-9months of age), had eaten for at least six weeks a compounded aflatoxin contaminated groundnut. Livers of animals exhibited areas of fibrosis with biliary proliferation and venocclusive disease. In other reported cases, it was found an increase in connective tissue too, and degeneration of centrilobular hepatic cells was described. Icterus, weight loss and dead were reported (For a review look for Newberne & Butler, 1969).
Milk from cattle is mainly affected because of the infection mechanism it suffers. Pathological, hematological and plasma enzymatic studies were made on milk cattle affected by chronic aflatoxicosis caused by the prolonged feeding of concentrate feed mixtures containing contaminated groundnut cake having aflatoxin B1 (110 µg/kg groundnut cake at the time of sampling), B2, G1 and G2. Clinical and necropsy observations on liver included proliferation of connective tissue along portal triads leaving small group of hepatocytes intact. Liver function tests showed liver damage in three of the four affected animals studied (Vaid et. al., 1981)
In dairy cattle, aflatoxin B1 in contaminated food consumed is metabolized and processed in approximately 5% of aflatoxin M1, which is secreted in milk. Although the transformation from B1 to aflatoxin M1 turns it about 1,000 times less toxic, M1 levels in milk are regulated to 0.5 ppb, because the milk is consumed primarily by children and is at the stage of development when immune system is more susceptible to the suppressive effects of aflatoxin. Therefore, milk with aflatoxin M1 levels above 0.5 ppb is not fit for human consumption (Gimeno, 2004).
Goats, because of being one of the major fonts of milk production as in cows, are very susceptible to present liver damages and milk contaminations.

5.5 Other species

Species described above have been the most studied ones because they are the basis of human feeding, and furthermore, they are the ones who imply the most economic gain worldwide. Nevertheless, there are other specific susceptible species to be affected by aflatoxins like turkeys (Richard et. al., 1986; Mckenzie et. al., 1998, Klein et. al., 2002) ducks

(Ostrowski-Meissner,, 1983; Cova et. al., 1990; Bintvihok, 2001), sheep, rats, mouse, frogs, dogs, cats, rabbits and monkeys to name a few (Newberne & Butler, 1969)

SPECIE	DISEASE	SYNTOMPHS	REFERENCES
Horses	When eating: Liver damage, centrilobular hepatic necrosis phagocytosed haemosiderin in Kupffer cells, bile-duct hyperplasia, congestion of renal vessels and adrenal cortex. When inhaling: Chronic obstructive pulmonary disease (COPD), yellow–brown liver with centrilobular necrosis, icterus hemorrhage, tracheal exudates and brown urine.	When eating: Anorexia, icterus, rapid weight loss and dead. When inhaling: Chronic cough, nasal discharge, expiratory dyspnoea reduced exercise tolerance, inappetence, depression, fever, tremor, ataxia, cough and dead.	Greene & Oehme, 1976; Meerdink, 2002; Basalan et al., 2004; Caloni & Cortinovis, 2010.
Chickens	Immunosuppression, liver and kidney damage, periportal fatty infiltrations, increase in connective tissue, hemorrhages, susceptibility to opportunistic infectious agents and poor response to vaccination programs.	Low productivity, low growth, low weight, low stance, but no evident clinical symptoms and death.	Newberne & Butler, 1969; Arafa et al. 1981; Chen et al., 1984; Oguz & Kutoglu, 2000; Okiki et al. 2010.
Swine	Immunosuppression, expression of opportunistic infections, liver swollen, liver congestion, hydrothorax, edematous gall bladder, petechiated and ecchymotic mucosa, extensive centrilobular liver necrosis, haemorrhage, hepatic centrilobular cellular infiltration, hepatocyte swelling, bile stasis, hepatocyte vacuolation and bile ductule hyperplasia.	Low growth rate, gastrointestinal problems, anoroexia, ill thrift and dead.	Newberne and & Butler, 1969; Ketterer et al, 1982; Luzi et al, 2002; Gimeno, 2004.
Cattle	Fibrosis with biliary proliferation in livers, venocclusive disease increase in connective tissue, degeneration of centrilobular hepatic cells, proliferation of connective tissue, generalized liver damage and immunosupression.	Icterus, rapid weight loss and dead.	Newberne and & Butler, 1969 Vaid et al. 1981) (Gimeno 2004.
Other Animals	Pulmonary edema, generalized liver damage, coagulopathy, capillary fragility, hemorrhage, prolonged clotting times, urine pigmentation, icterus and hepatic injury.	Depression, anorexia, weight loss, bleeding, decline in feed consumption and production, gastrointestinal damage and death.	Newberne & Butler, 1969; Ostrowski-Meissner, 1983; Richard et al, 1986; Cova et al. 1990; Mckenzie et al, 1998; Klein et al, 2002; Bintvihok 2001.

Table 2. Major diseases caused by aflatoxicosis in some animal species.

6. Aflatoxins metabolism and mutagenesis

Most of the research for understanding the metabolism and mutagenesis of aflatoxins inside the consumer organism have been done using different animals as models. Those

investigations have let us know that aflatoxin B1 may not itself be a toxic molecule but is metabolized in the animal body in a complex network of reactions and it is the result of this metabolism which determines both the acute and chronic toxicity (Moss, 2002).

When AFB1 is ingested, once inside the body, it is absorbed by the intestine and carried to the liver. There, AFB1 is activated and metabolized by cytochromes p450 (CYP) of hepatocytes to AFB1-8,9-exo-epoxide and AFB1-8,9-endo-epoxide. CYP3A4, 3A5, 3A7 and 1A2 are the enzymes involved in aflatoxin metabolism. Aflatoxin undergoes enzymatic conversion by the microsomal mixed function oxidase (MFO) primarily present in the liver, but probably also present in the lungs, kidneys and elsewhere. The overall contribution of these enzymes to AFB1 metabolism in vivo will depend on affinity and expression; CYP3A4 appears to be the most important, with the relative contribution of CYP3A5 varying by individual. Polymorphisms identified in the CYP3A5 promoter region have been associated with different levels of aflatoxin biomarkers, suggesting that this interindividual variation could influence susceptibility to aflatoxin. Given the fact that aflatoxin is known to cross the placenta, it is also of interest that CYP3A7, a major CYP in human fetal liver, has the capacity to activate AFB1 to 8,9-epoxide (Hendrickse, 1991; Moss, 2002; Wild & Turner, 2002; Kamdem et. al., 2006).

AFB1-8,9-exo-epoxide is highly unstable when joining to the nitrogen of guanine, which binds to DNA to form the predominant 8,9-dihydro-8-(N7-guanyl)-9-hydroxy-AFB1 (AFB1–N7-Gua) adduct. AFB1–N7-Gua adduct confers the mutagenic properties of the compound. This may be the most important product from the carcinogenic point of view. The binding of the exo-epoxide to guanine reflects the geometry of intercalation between base pairs in the DNA helix; 5′ intercalation appears to facilitate adduct formation by positioning the epoxide for in-line nucleophilic reaction with the N7 guanine. The epoxide ring is positioned above the plane and in trans to the 5a and 9a protons in the endo-epoxide, hindering reaction, but in the exo-epoxide, the epoxide ring is positioned below the plane and in cis to the 5a and 9a protons, assisting reaction (Wild & Turner, 2002; Verma, 2004).

The 8,9-epoxide is not only known to react with DNA, but also to do so at the guanine residues of specific sites, one of these being the third base position of codon 249 of the p53 gene. A considerable insight into the nature of this reaction is provided by the chemical synthesis of aflatoxin B1 epoxide and its use in reactions with model oligodeoxynucleotides. Indeed, there is evidence that a dose-dependent relationship between dietary aflatoxin B1 intake and codon 249ser p53 mutations was observed in hepatocellular carcinoma from Asia, Africa and North America (Moss, 2002).

Cytochrome p450 3A4 can activate and detoxicate AFB1. Only the 8,9-exoepoxide appears to be mutagenic and others are detoxification products. The putative AFB1 epoxide is generally accepted as the active electrophilic form of AFB1 that may attack nucleophilic nitrogen, oxygen and sulphur heteroatoms in cellular constituents (Verma, 2004).

Both humans and animals possess enzymes systems, which are capable of reducing the damage to DNA and other cellular constituents caused by the 8,9-epoxide. For example, glutathione-S-transferase mediates the reaction (termed conjugation) of the 8,9-epoxide to the endogenous compound glutathione. This essentially neutralizes its toxic potential. The exo and endo-epoxides can also undergo rapid non-enzymatic hydrolysis to AFB1-8,9-dihydrodiol that in turn is subject to slow, base-catalysed ring opening to a dialdehyde phenolate ion. The dihydrodiol can react with the e-amino group of lysine in serum albumin resulting in aflatoxin–albumin adducts, used as biomarkers. A further metabolic step involves aflatoxin aldehyde reductase catalysing the nicotinamide adenine dinucleotide

phosphate (NADPH) dependent reduction of the dialdehydic phenolate ion to a dialcohol. Animal species such as the mouse that are resistant to aflatoxin carcinogenesis have 3-5 times more glutathione-S-transferase activity than susceptible species such as the rat. Humans have less glutathione-S-transferase activity or 8,9-epoxide conjugation than rats or mice suggesting that humans are less capable of detoxifying this important metabolite (Guengerich, 1996; Verma, 2004; Johnson et. al., 2008).

In addition to errors in DNA transcription due to its binding to AFB1 exo-8-9- epoxide, it can be configured a similar adduct when binding to albumin or lysine; that's why this two compounds are used at clinical level to determine the consumption of AFB1. AFM1 also been detected in urine, indicating that this toxin is also capable of reacting with DNA and form adducts (Unusan, 2006). In circulation, aflatoxin binds with plasma proteins (especially albumin) to form an aflatoxin-albumin adduct. The protein adduct by binding with 8,9-epoxy aflatoxin, initially forms dihydrodiol with sequential oxidation to dialdehyde and condensation with the S-amino group of lysine. This protein adduct is a completely modified aflatoxin structure retaining only the coumarin and cyclopentenone rings of the parent compound. These adduct represent the cumulative dose of aflatoxin intake over previous weeks. The average half-life of albumin in people is about 20 days. Therefore, an accumulated dose of aflatoxin will be present in albumin long after the dietary exposure has ceased. This is a property not found for DNA adduct because the half-life of DNA adduct is about 12 hour and then rapidly excreted in urine (Verma, 2004).

In a next phase, the challenge is to stabilize and inactivate the epoxide, hydrolyzing and conjugating it with glutathione to form AFB1-Glutathione (AFB-SG) that will be excreted in urine. In this metabolic stage are also originated three major hydroxylated metabolites: AFQ1, AFP1 and AFM1, which begin to distribute systemically and can be found in milk, eggs and tissues from intoxicated animals. Another important derivative from AFB1 metabolism is aflatoxicol, which extends the presence of AFB1 in the organism; it comes from reducing AFB1, and it can be reoxidized back to AFB1 by NADHP (Arangurén & Argüelles, 2009).

In an extensive review made by Verma in 2004, he mentions that aflatoxin concentration recorded in the serum of human beings varies with the amount and duration of aflatoxin-ingested and the physiological state of the body. Unmetabolized (B1, B2, G1 and G2) and metabolized forms (aflatoxicol, M1 and M2) of aflatoxins are excreted in the urine, stool, milk and saliva. Aflatoxin excreted/secreted through saliva might be getting absorbed in gastrointestinal tract and passing again to the blood stream. This explains a sort of recycling of aflatoxin in the body. Aflatoxin (0.35-3.5 μg/ml) exposure to hepatocytes in vitro caused pronounced swelling, polymorphic condition, bleb formation and cell lysis. Aflatoxin B1 is reported to induce cytotoxicity and transformation in culture cells. The earliest effect of aflatoxin is to reduce protein biosynthesis by forming adducts with DNA, RNA and protein, to inhibit RNA synthesis and DNA dependent RNA polymerase activity and to cause degranulation of the endoplasmic reticulum. Some of this information can be well understood in Figure 1.

In summary, once the toxin has entered the liver cell, the agency causing tissue injury in particular animal species is dictated by the rate and pattern of aflatoxin metabolism. When it is metabolized slowly, the untransformed toxin activates the molecular species that cause chronic liver damage as the most probable result. When it is metabolized rapidly, metabolites are the ones involved in diseases. Acute liver damage may be caused by the intracellular formation of aflatoxin hemiacetal in many species (Patterson, 1973).

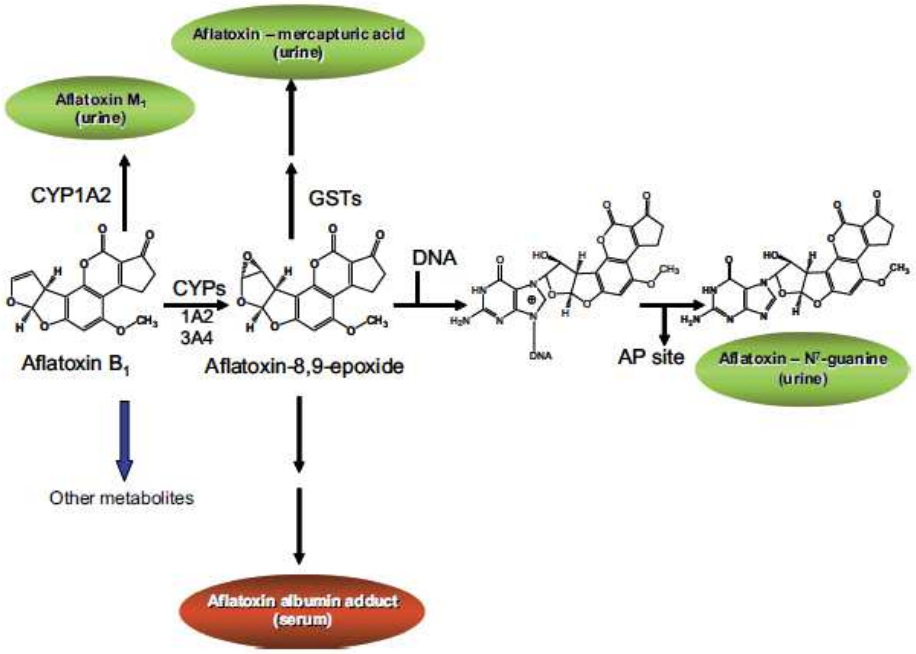

Fig. 1. Biomarkers of aflatoxin exposure in an internal dose and a biologically effective dose. Biomarkers of exposure include aflatoxin M1, the internal dose includes the aflatoxin-mercapturic acid and aflatoxin- albumin adduct, and the biologically effective dose is reflected by the excretion of the aflatoxin-N7-gua- nine adduct formed by depurination leading to an apurinic (AP) site in DNA (Taken from Groopman et. al., 2008).

7. Biomarkers and immunoassays

Since the chemical structures of the major aflatoxin-DNA and aflatoxin-protein adducts were identified, an extensive research has been conducted to validate these structures for biomarker applications. Groopman and collaborators, in their review published in 2008, mention that early experimental studies around 1980 demonstrated that the major aflatoxin-nucleic acid adduct, AFB1- N7-Gua, was excreted exclusively in the urine of exposed rats. The serum aflatoxin-albumin adduct was also examined as a biomarker of exposure because the longer half-life of albumin would be expected to integrate exposures over longer time periods, i.e., months instead of days. Studies in experimental models found that the formation of aflatoxin-DNA adducts in liver, urinary excretion of aflatoxin-nucleic acid adduct and formation of the serum albumin adduct were highly correlated events. These investigations provided the rationale for exploring the application of these biomarkers in human studies. An immunoaffinity clean-up/HPLC procedure was developed for aflatoxin metabolites in urine samples. With this approach, initial validation studies investigated the dose-dependent excretion of urinary aflatoxin biomarkers in rats after a single aflatoxin B1 (AFB1) exposure. Investigators found a linear relationship between AFB1 dose and excretion of the AFB1-N7-Gua adduct in urine over the initial 24 hours period. Subsequent studies in

rodents that assessed the formation of aflatoxin macromolecular adducts after chronic administration also supported the use of DNA and protein adducts as molecular measures of exposure. For example, in rats treated with relatively low doses of AFB1 (3.5 μg) twice daily for 24 days there was an accumulation of aflatoxin binding to peripheral blood albumin followed by steady-state levels, which illustrated the potential for this biomarker (aflatoxin-albumin adduct) to integrate exposure over time. Many different analytical methods are now available for quantitation of chemical adducts in biological samples, each with unique specificity and sensitivity (Santella, 1999; Poirier, 2004; Wogan et. al., 2004; Scholl et. al., 2006).

Initial studies of aflatoxin biomarkers in human populations began in the Philippines, where investigators demonstrated that an oxidative metabolite of aflatoxin, AFM1, could be measured in urine as an internal dose marker. Subsequent works conducted in China and Gambia (areas with high incidences of HCC) determined that the levels of urinary aflatoxin biomarkers followed a dose-dependent relationship with aflatoxin intake. However, as in the earlier experimental studies, this relationship was dependent on the specific urinary marker under study; for example, AFB1-N7-Gua and AFM1 showed strong correlations with intake, whereas urinary AFP1, a different oxidative metabolite, showed no such link. In other studies, levels of aflatoxin-albumin adducts were measured and there was observed a highly significant association between intake of aflatoxin and level of adduct. This kind of studies, to measure dietary aflatoxin intake and biomarkers at the individual level, is crucial to validate a biomarker for exposure assessment and is often over-looked in molecular epidemiology. In Gambia, there was observed that urinary aflatoxin metabolites reflected day-to-day variations in aflatoxin intake, whereas the aflatoxin-albumin adducts integrated exposure over the week-long study. Data from these initial cross-sectional biomarker studies demonstrated short-term dose-response relationships for a number of the aflatoxin metabolites, including the major nucleic acid adduct, serum aflatoxin-albumin adduct, and AFM1. This supported the validity of these exposure biomarkers for use in epidemiological studies, including investigations of intervention strategies and studies of the mechanisms underlying susceptibility (Groopman et. al., 2008).

Thin layer chromatography (TLC), High Performance Liquid Chromatography (HPLC), minicolumns, immunoassays such as Enzyme-Linked Immunosorbent Assay (ELISA) and Immunoaffinity Columns (IAC) are employed in testing biological samples like blood, serum, plasma, urine, stool, breastmilk and other body exudates. Taking cost, speed of analysis, availability of personnel and facilities as well as the characteristics of the tests (sensitivity, specificity and reproducibility), TLC, HPLC, ELISA and other immunoassays have been identified as the preferred methods for aflatoxins detection. Since mycotoxins were added to the list of materials covered by international conventions relating to bio-terrorism, maintaining standards has become a major issue (WHO, 2005).

Nevertheless, information regarding the interpretation and application of AFB1 adducts and urine immunoassay is also limited. Aflatoxin metabolites or adducts in urine and serum indicate exposure, but do not necessarily equate to adverse health effects. Some studies have examined the correlation of aflatoxin intakes to biomarker levels (Reviewed by Strosinder et. al., 2006). Aflatoxin B1 adducts and urine immunoassay for epidemiologic studies, biomarkers in serum and urine provide a better estimate of aflatoxin exposure than food analysis. Aflatoxin metabolites in urine reflect recent exposure (i.e. 2-3 days) whereas the measurement of aflatoxin albumin adducts in blood reflects exposure over a longer period (i.e. 2-3 months); these analysis are labor-intensive and expensive (Groopman et. al., 1994; FAO, 2005).

More research is needed to further elucidate the correlation between aflatoxin levels in biologic specimens and adverse health effects. Research must also clarify the relationship between aflatoxin levels in biologic specimens and levels in food.

8. Permissible worldwide aflatoxin levels

Aflatoxins are considered unavoidable contaminants of food and feed, even where good manufacturing practices have been followed. The FDA has established specific guidelines on acceptable levels of aflatoxins in human food and animal feed by establishing action levels that allow for the removal of violative lots from commerce. However, it is very difficult to accurately estimate aflatoxins concentration in a large quantity of material because of the variability associated with testing procedures; hence, the true aflatoxin concentration in a lot cannot be determined with 100% certainty. Table 3 summarizes some FDA action levels for aflatoxins (Cornell University, 2009).

Aflatoxins are regulated quite differently than food additives and other chemical substances included in food. In developed countries, human populations are protected because regular surveillance keeps contaminated foods out of the food supply. Unfortunately, in countries where populations are facing starvation, or where regulations are either nonexistent or unenforced, routine ingestion of aflatoxin is very common (FAO, 1997).

However, not only the FDA in USA, but also some European countries have been establishing special committees and commissions to create and recommend guidelines, test standardized assay protocols, and maintain up-to-date information on regulatory statutes of aflatoxins and other mycotoxins. Those guidelines are developed from epidemiological data and extrapolations from animal models, taking into account the inherent uncertainties associated with both types of analysis. Estimates of "safe doses" are usually stated as a "tolerable daily intake". For example, in the United States, the Food and Drug Administration guideline is 20 ppb total aflatoxin in food destined for human consumption and 100 ppb is the limit for breeding cattle and mature poultry (FDA, 1998; Bennett et. al., 2007).

Commodity	Level (ng/g)
All products, except milk, designated for humans	20
Milk	0.5
Corn for immature animals and dairy cattle	20
Corn for breeding beef cattle, swine and mature poultry	100
Corn for finishing swine	200
Corn for finishing beef cattle	300
Cottonseed meal (as feed ingredient)	300
All feedstuff other than corn	20

Table 3. FDA action levels for aflatoxins (Taken from Cornell University,2009)

According to Tedesco et al, (2008), few countries regulate AFB1 in feedstuffs for dairy cattle. Limiting AFB1 in animal feeds is the most effective means of controlling aflatoxin M1 in milk. A limit of 5 µg AFB1/kg feed for dairy cow and a limit of 20 µg AFB1/kg in feed for cattle, sheep, goats, swine and poultry are applied in the European Union countries. This

limit is applied by countries in the European Free Trade Association (EFTA), in many of the candidate EU countries and sporadically outside Europe. A limit of 20 μg AFB1/kg feed for dairy animals and a limit of 100 μg AFB1/kg intended for breeding beef cattle, breeding swine, or mature poultry is applied in the United States, Africa and Latin America. Regulations for AFM1 existed in 60 countries at the end of 2003, a more than threefold increase as compared to 1995 (FAO, 2005). EU, EFTA, candidate EU countries and some other countries in Africa, Asia and Latin America, apply a maximum level of 0.05 μg AFM1/kg in milk and a maximum level of 0.025 μg AFM1/kg in infant formula. A limit of 0.5 μg AFM1/kg in milk is applied in the United States, several Asian, European countries and in Latin America, where it is also established as a harmonized MERCOSUR (a trading block consisting of Argentina, Brazil, Paraguay and Uruguay) limit.

The Codex Committed on Food Additives and Contaminants (CCFAC) contain the result of discussions envisaged for the maximum level of AFM1 contamination. Given the public health concerns, the EU continues to maintain the maximum level of 0.05 ppb in milk AFM1 and 0.025 ppb in dairy foods for infants (CCFAC, 1999, 2000, 2001). This contradicts the regulations in America, where 0.5 ppb is an aflatoxin permissible level.

According to aflatoxins levels in human and animal health, Gimeno reviewed in 2004 that, after studies presented by the World Health Organization in 2005, it is known that the risk of liver cancer is almost null if concentrations of 0.05 ppb to 0.5 ppb are present; but exposure to any level of genotoxic carcinogens as AFM1, may pose a health risk to consumers, especially for children, so, the exposure level should be zero for a zero risk to liver cancer that may be caused by aflatoxins in general. Countries which defended an AFM1 maximum level of 0.5 ppb argue that those concentrations they could cause adverse economic consequences due to the difficulty of milk exports to countries that accept only a maximum level of 0.05 ppb. Delegates from some other countries argue that the level of 0.05 ppb is difficult to achieve in most regions of the world, so, a level of 0.5 ppb is enough to promote public health protection.

The Codex Alimentarius Comitee has reported some recommendations to institutions and consumers in general if AFB1 is detected. Some of the most important ones are:

1. In all cases, be sure that the level of aflatoxin B1 in the finished feed is suitable for its intended purpose (i.e., according to the maturity and animal species which are going to be fed) and if it conforms to codes and guidelines or qualified veterinary advices.
2. Consider the restriction of contaminated feed with aflatoxin B1 to a percentage of daily rations, so that the daily intake of AFB1 does not lead the presence of significant residues of AFM1 in milk.
3. If the feed restriction cannot be put into effect, the use of contaminated feed could be diverted to non-dairy animals.

Nevertheless, it is important to unify regulations of permissible aflatoxin levels in order to homogenize the consumption laws and amenities worldwide, in order to avoid risks and health problems derived from importing and exporting contaminated food.

9. Treatment and prevention of diseases caused by aflatoxins

As it has been mentioned before, most aflatoxicosis results from eating contaminated foods. Unfortunately, except for supportive therapy (e.g., diet and hydration) there are almost no treatments for aflatoxin exposure. However, there have been described few and specific methods for veterinary management of mycotoxicosis; for example, there is evidence that

some strains of Lactobacillus effectively bind dietary mycotoxins. Similarly, clay-based enterosorbents have been used to bind aflatoxins in the gastrointestinal tract. It has been demonstrated that selenium supplementation modifies the negative effects of aflatoxin B1 in Japanese quail, while butylated hydroxytoluene gives some protection in turkeys. Oltipraz, a drug originally used to treat schistosomiasis, has been tested in human populations in China with some apparent success (Bennett et al, 2007). In Figure 2 we reproduce an overview for preventing acute aflatoxicosis in countries in development purposed in 2006.

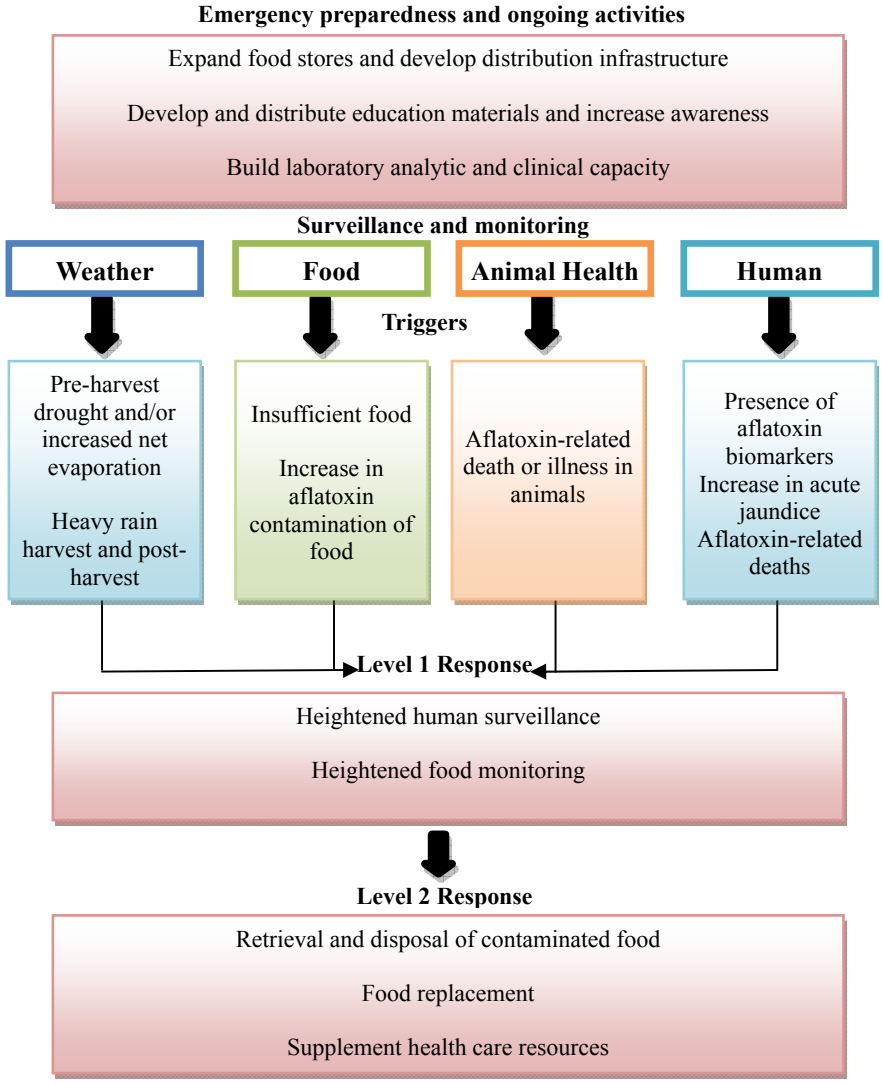

Fig. 2. Overview of preparedness, surveillance, and response activities for preventing acute aflatoxicosis in countries in development (Strosnider et. al.,2006).

Methods for controlling aflatoxin exposure are largely prophylactic. In a primary prevention trial, the goal is to reduce exposure to aflatoxins in the diet. A range of interventions includes planting pest-resistant varieties of staple crops, attempting to lower mold growth in harvested crops, improving storage methods following harvest, and using trapping agents that block the uptake of unavoidably ingested aflatoxins. In secondary prevention trials, one goal is to modulate the metabolism of ingested aflatoxin to enhance detoxification processes, thereby reducing internal dose and subsequent risk (Groopman, 2008). The aflatoxin problem sits at the interface of agriculture, health and economics, whose detailed explanation is presented below:

9.1 Agricultural sector

It consists principally in a good agricultural practice, including an appropriate drying of crops after harvest and avoidance of moisture during storage.

Pre-harvest interventions

Although the initial focus of research was on the prevention of contamination in storage, it was established in about 1970 that fungal contamination could start in the field before harvest. For peanuts, environmental conditions such as drought during the grain growth stage, insect damage in the field, variety and soil characteristics have proven to be determining factors in pre-harvest contamination. These conditions are now sufficiently well understood for computer simulation models to describe the risk of contamination of major crops (Williams et. al., 2004). Pre-harvest would be the most effective point of control because this is the point at which the crop is first infected by the toxin-producing fungus (Wild & Turner, 2002)

According to extensive reviews (Cotty & Bhatnagar, 1994; Williams et. al., 2004; Strosnider et. al., 2006; Bennett et. al., 2007; Wild & Gong, 2010), the presence and growth of Aspergillus on pre-harvested crops can be reduced through agricultural practices such as proper irrigation and pest management. Pre-harvest interventions include choosing crops with resistance to abiotic stresses (like drought, temperature and moisture content) and reducing crop stresses in general, developing host resistance through plant breeding, and choosing varieties that are genetically more resistant to fungal growth and aflatoxins production, diseases and pests. However, these processes may not be economically feasible in many high risk regions. The use of staple crops resistant to fungal colonization or genetically modified crops that inhibit fungal invasions (transgenic crops), joined to the elimination of inoculum sources (such as infected debris from the previous harvest) may prevent infection of the crop. Years before, the use of fungicides, pesticides and insecticides were a good way for controlling infections, but nowadays, the use of biocontrol agents is the most appropriated in order to avoid consumers chemical intoxications. For example, biopesticides consisting of a nonaflatoxigenic strain of Aspergillus may competitively exclude toxic strains from infecting crops, but the allergenic and human health aspects of the atoxigenic strain need still to be evaluated.

Post-harvest interventions

Post-harvest interventions can be practiced at three stages: drying level, storage level and in food preparation; nevertheless, the last mentioned is not practiced as commonly as the first ones, which are properly physicochemical methods practiced by grain producers.

Before storage, properly drying crops can prevent the development of aflatoxins. Sorting and disposing of visibly moldy or damaged kernels before storage is an effective method for

reducing, but not eliminating, the development of aflatoxins (Fandohan et. al., 2005; Turner et. al., 2005). Moisture, insect and rodent control during storage can prevent damage to the crop, which would promote aflatoxin development.
Aflatoxins often accumulate during food storage and therefore post-harvest control at the subsistence farm aims to minimize fungal growth and aflatoxin production. The growth of Aspergillus is influenced most critically by temperature, moisture content and storage time. Studies conducted in Guinea, revealed a high HCC incidence and aflatoxin exposure mainly attributable to contamination of groundnuts following storage. A primary prevention study is underway where the intervention incorporates a package of post-harvest procedures, including improved sun drying prior to storage, drying on cloth rather than directly on the earth, removal of visibly mouldy nuts by hand sorting, storage in jute sacks rather than plastic, use of wooden pallets for storage to avoid contact with the earth and to improve ventilation and, finally, use of insecticides to control insect damage and spread of fungal spores. The outcomes of the study are being determined by measuring both food levels of the toxin and, more importantly, blood AF–albumin biomarker levels at three time points post-harvest. Primary intervention strategies to reduce mycotoxin exposures at the post-harvest level may have a significant impact in high exposure populations, but are unlikely to eliminate exposure. In addition, these approaches cannot be targeted specifically to high risk individuals. Therefore, intervention strategies also encompass chemoprevention, using compounds that interfere with the absorption or metabolism of aflatoxins once ingested (Reviewed by Wild & Turner, 2002). From here derives the health sector.

9.2 Health sector

It refers basically to those kinds of food we can eat and how hygienically does food is prepared.
Chemoprotection is one of the major used post-harvest techniques, and consists in the use of chemicals (e.g. oltipraz [4-methyl-5-(2- pyrazinyl)-1,2-dithiole-3-thione], chloro- phylin) or dietary intervention (e.g., eating broccoli sprouts, drinking green tea) to alter the susceptibility of humans to carcinogens, and has been considered as a strategy to reduce the risk of HCC in populations with high exposures to aflatoxins (Strosnider, 2006). The dietary intervention is maybe the easiest way to prevent cancer disease; however, for many communities in developing countries a change in diet is simply not feasible because they do not have the culture of eating a balanced diet, joined to a great skepticism about eating organic food, and moreover, that money isn´t enough to buy non-staple food.
Finally, is important to consider that simple food preparation methods such as sorting, washing, crushing, and grain dehulling, may reduce aflatoxin levels (Fandohan et. al., 2005; Park, 2002). In the case of maize, the fight against the fungal species has focused mainly through processes such as nixtamalization in which product aflatoxins are eliminated (Méndez & Moreno, 2009), or by the addition of low concentrations of Sodium Hydroxide which achieves the elimination of a large amount of aflatoxins (Carrillo 2003). Aflatoxin may be prevented by packing the dried products in polyethylene or propylene bags (Siriacha, et. al., 1990).
Most efforts to address the mycotoxin problem involve analytic detection, government regulation, and diversion of mycotoxin-contaminated commodities from the food supply. Basic research on the biosynthesis and molecular biology of aflatoxins has been a priority because a full understanding of the fundamental biological processes may yield new control strategies for the abolition of aflatoxin contamination of food crops.

9.3 Economical sector

This is maybe the post complicated treatment and preventive sector, because it includes the government security blankets to face this global problem. Because of the global threat that aflatoxicosis represents, the World Health Organization has started to respond and highlight the need for action (Strosnider et. al., 2006). However, aflatoxins and mycotoxins in general have not been widely prioritized from a public health perspective in low-income countries. This is because knowledge of mycotoxins and the full range and scale of their adverse health effects is incomplete and the known risks are poorly communicated to governments in regions where the contamination is greatest (Wild & Gong, 2010). Matters that have to be considered by government to avoid diseases from aflatoxicosis are: an opportune and non-expensive analytic detection, unifying worldwide government regulations, deviation of aflatoxin-contaminated commodities from the food supply, improving research on the biosynthesis and molecular biology of aflatoxins, and designing new control strategies for the abolition of aflatoxin contamination of food crops, inter alia.

10. Conclusions

Aflatoxins are not only a big problem at crop production level, but also it has become a global health issue because of the consequences that the consumption of this toxin generates in animals and human beings. Diverse worldwide established groups have the challenge of identifying public health strategies, which complement the agricultural ones in order to reduce aflatoxin exposure, especially in developing countries. Although there have been documented extensive researches about how to prevent and control aflatoxicosis, populations of developing countries know just a little about aflatoxin exposure and the resulting health effects.

It is known that acute aflatoxicosis is preventable, and chronic exposure can be reduced, even without a complete understanding of the public health problem caused by aflatoxins. Efforts to reduce aflatoxin exposure require the commitment of sufficient resources and the collaboration between the agriculture and public health communities as well as local, regional, national, and international governments.

Because of the recent investigations conducted in this area, it is important to take actions to prevent damage and diseases; that´s why, at first, governments supported by scientific research groups should report publicly the risks that aflatoxins consumption means by quantifying the human health impacts and the burden of disease due to the toxin exposure; then, they should compile inventory and worldwide statistics in order to evaluate the efficacy of the current intervention strategies. It is also important to increase disease surveillance, food monitoring, laboratory detection of mycotoxins and public health response capacity of affected regions. Public health services should offer immediate attention to aflatoxicosis diagnoses and opportunistic diseases caused by them in order to reduce mortality rates in humans and animals. Finally, it is important to develop response protocols to be used in an event of an outbreak of acute aflatoxicosis, which could become in an epidemic stage.

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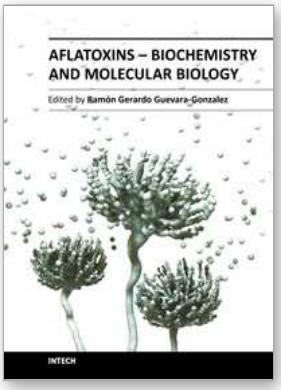

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Aflatoxins â€“ Biochemistry and Molecular Biology is a book that has been thought to present the most significant advances in these disciplines focused on the knowledge of such toxins. All authors, who supported the excellent work showed in every chapter of this book, are placed at the frontier of knowledge on this subject, thus, this book will be obligated reference to issue upon its publication. Finally, this book has been published in an attempt to present a written forum for researchers and teachers interested in the subject, having a current picture in this field of research about these interesting and intriguing toxins.

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