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Carcinogens in West Africa with Special Reference to Fungal Metabolites from Fusarium species

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SUMMARY
The presence of fungal toxins in our food systems and tissues has enormous public health implications, this is because mycotoxins are nephrotoxic, teratogenic, immunotoxic and mutagenic. Warm and humid climates, as well as poor conditions of storage and handling of agricultural commodities are favorable to fungal colonization and development, which lead to the accumulation of mycotoxins capable of causing cancer. The fungal metabolites are also capable of causing acute and chronic effects in man and animals ranging from death to disorder of central nervous, cardiovascular, pulmonary systems and intestinal tract. In human, Fumonisin B_1 causes human hepatoma and esophageal cancer, increased susceptibility to diseases, especially in children and childhood pre-five mortality and reduced life expectancy. The complete elimination of carcinogen-contaminated commodities is not easily achievable, hence good agricultural practices, followed by the implementation of good manufacturing practices during the handling, storage, processing, and distribution of agricultural produce for human food and animal feed seek to reduce the level of contamination. Known cancer-causing mycotoxins including aflatoxin B_1, fuminisin B_1, ochratoxin A pose serious economic and health risks.

Introduction
Carcinogen is any substance involved in causing cancer due to its ability to damage the genome or disruption of cellular metabolic processes for example Radon, aflatoxin (Atanda *et al*., 2013). Mycotoxins are chemical substances that contaminate agricultural commodities, either before or after harvest for example aflatoxin, trichothecenes and fumonisins. Toxigenic fungi produce secondary metabolites that cause nutritional losses and represent a significant hazard to the food chain (Magan and Aldred, 2007). Carcinogens cause tumors by interacting with the genetic material (genotoxic). A genotoxic agent has the ability to induce mutations or so-called indicator effects which are mechanistically associated with the formation of mutations (like induction of DNA modifications, DNA repair, or recombination). Mutations are alterations of the genetic material within living cells, which can be transmitted from one cell generation to another (somatic mutations) or to the progeny of affected individuals through germ cells (germinal mutations). Mutations include gene mutations, structural chromosome mutations, and genome mutations (Okaka *et al*., 2013).

Some carcinogens do not affect DNA directly, but lead to cancer in other ways. These may cause cells to divide at a faster than normal rate, which could increase the chances that DNA changes will occur. Cancer a consequence of multiple genetic alterations arising from inherited mutations in germ cells or as a consequence of mutations in somatic cells, resulting in altered growth (CNRS, 2011). Carcinogens do not cause cancer in every case, all the time. Substances labeled as carcinogens may have different levels of cancer-causing potential. Some may cause cancer only after prolonged, high levels of exposure. And for any particular person, the risk of developing cancer depends on many factors, including how they are exposed to a carcinogen, the length and intensity of the exposure, and the person's genetic makeup. Changes in the genetic blueprint may be inherited from our parents, while others may be caused by outside exposures, which are often referred to as environmental factors. Environmental factors can include a wide range of exposures, such as: Lifestyle factors (nutrition, tobacco use, physical activity, etc.), naturally occurring exposures (ultraviolet light, radon gas, infectious agents, etc.), medical treatments (chemotherapy, radiation, immune system-suppressing drugs, etc.), workplace exposures, household exposures, and pollution (Hansen, 2001).

People are exposed to many substances at any given time, including those they encounter at work, school, or home., in the food they eat., and in the air they breathe. It's very unlikely they know exactly what they've been exposed to or that they would be able to remember all of their exposures if asked by a researcher. And there are usually many years (often decades) between exposure to a carcinogen and the development of cancer. Therefore, it can be very hard to definitely link any particular exposure to cancer. By combining data from studies, scientists do their best to make an educated assessment of a substance's cancer-causing ability. When the evidence is conclusive, the substance is labeled as a carcinogen. When the available evidence is compelling but not felt to be conclusive, the substance may be considered to be a probable carcinogen. But in some cases there simply isn't enough information to be certain one way or the other (IARC, 2011).

Classification of Carcinogens

Specific considerations for classification of substances as carcinogens

Classification as a carcinogen is made on the basis of evidence from reliable and acceptable studies and is intended to be used for substances which have an intrinsic property to cause cancer. The evaluations shall be based on all existing data, peer-reviewed published studies and additional acceptable data (CNRS, 2011). Fungal carcinogens as a group cannot be classified according to their mode of action, toxicology or metabolism. These vary according to the different chemical structures, sensitivity of the species to the toxin and also factors such as sex, age, health and diet. Additionally, additive and synergistic effects can occur in the presence of two or more mycotoxins.

Classification of a substance as a carcinogen is a process that involves two interrelated determinations: evaluations of strength of evidence and consideration of all other relevant information to place substances with human cancer potential into hazard categories. Some important factors which may be taken into consideration, when assessing the overall level of concern are:

a) tumour type and background incidence.,
b) multi-site responses.,
c) progression of lesions to malignancy.,
d) reduced tumour latency.,
e) whether responses are in single or both sexes.,
f) whether responses are in a single species or several species.,
g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity.,
h) routes of exposure.,
i) comparison of absorption, distribution, metabolism and excretion between test animals and humans.,
j) the possibility of a confounding effect of excessive toxicity at test doses.,
k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity

Several agencies (national and international) are responsible for determining the cancer-causing potential of different substances.

i) International Agency for Research on Cancer

The International Agency for Research on Cancer (IARC) is part of the World Health Organization (WHO). Its major goal is to identify causes of cancer. The most widely used system for classifying carcinogens comes from the IARC. In the past 30 years, the IARC has evaluated the cancer-causing potential of more than 900 likely candidates, placing them into one of the following groups:

a) Group 1: Carcinogenic to humans (like aflatoxin, asbestos, estrogen, diesel smoke, tobacco)
b) Group 2A: Probably carcinogenic to humans (like chloramphenicol, frying at high temperature, hairdressers, Ochratoxin, Progesterone, metronidazole)
c) Group 2B: Possibly carcinogenic to humans (like fumonisin $B_{1)}$
d) Group 3: Unclassifiable as to carcinogenicity in humans
e) Group 4: Probably not carcinogenic to humans(IARC, 2011)

ii) National Toxicology Program

The National Toxicology Program (NTP) is formed from parts of several different US government agencies, including the National Institutes of Health (NIH), the Centers for Disease Control and Prevention (CDC), and the Food and Drug Administration (FDA). The NTP updates its Report on Carcinogens (RoC) every few years. The Report on Carcinogens identifies 2 groups of agents:

a) "Known to be human carcinogens" (like aflatoxin, asbestos)
b) "Reasonably anticipated to be human carcinogens" (like chloramphenicol)

iii) Environmental Protection Agency

The US Environmental Protection Agency (EPA) maintains the Integrated Risk Information System (IRIS), an electronic database that contains information on human health effects from exposure to certain substances in the environment. The EPA uses a rating system similar to that of IARC when describing the cancer-causing potential of a substance:

a) Group A: Carcinogenic to humans
b) Group B: Likely to be carcinogenic to humans
c) Group C: Suggestive evidence of carcinogenic potential
d) Group D: Inadequate information to assess carcinogenic potential
e) Group E: Not likely to be carcinogenic to humans (EPA, 2005)

iv) Other agencies and groups

Other federal agencies, such as the CDC's National Institute for Occupational Safety and Health (NIOSH), the Food and Drug Administration (FDA), and the National Cancer Institute may comment on whether a substance or exposure may cause cancer and/or what levels of exposure to a particular substance might be considered

acceptable. Some state agencies also keep lists of known or probable carcinogens. For example, the California Environmental Protection Agency (CalEPA) maintains a list of "chemicals known to the state to cause cancer or reproductive toxicity." Much of this list is based on the IARC and NTP lists (US PHS, 2011).

Types of Carcinogens

i) Environmental carcinogens

Several environmental factors often present serious risks for human health. A growing body of evidence links exposure to certain environmental pollutants to cancer. Examples of these include (but are not limited to):

a) Polluted drinking water: Water is essential to sustain life. Poor water quality continues to pose a major threat to human health. Diarrhoeal disease alone is responsible for 1.8 million deaths every year (2004 data). Several microbial, chemical and radiological factors (naturally occurring radionuclides such as radon) contribute to poor water quality.
b) Poor (indoor) air quality: Polluted air is responsible for an estimated 3.1 million deaths worldwide every year. Air pollutants have been linked to a range of health problems such as respiratory infections, cardiovascular diseases, lung cancer and many others.
c) Ionizing radon radiation: Radon, a natural gas that escapes from the ground and surface water, is the most prominent source of environmental radioactivity. While there is usually a rather low outdoor concentration, radon tends to concentrate in houses. Exposure to radon is the second leading cause of lung cancer, after smoking. In the US, radon causes about 21,000 lung cancer deaths every year (out of a total of about 160,000 annual lung cancer deaths) (US EPA, 2003).

ii) Chemical Carcinogens

a) Chemical pollutants (such as asbestos): Asbestos exposure mostly occurs through inhalation of fibres in the working environment, but also through inhalation of air in the vicinity of sources such as factories handling asbestos or indoor air in houses and buildings containing asbestos. In 2004, asbestos-related lung cancer and asbestosis from workplace exposure resulted in 107,000 deaths.
b) Food chemicals (such as dioxins): Human exposure to dioxins and dioxin-like substances has been associated with a range of toxic effects, including immunotoxicity, developmental and neurodevelopmental effects, changes in thyroid and steroid hormones and reproductive function.

iii) Occupational Carcinogens

The most important lung carcinogens in occupational settings are asbestos, radon, arsenic, chromium, silica, beryllium, nickel, cadmium and diesel exhaust. The most important agents for leukaemia are benzene, ionizing radiation and ethylene oxide (Driscoll, *et al.,* 2004). Asbestos is a causal agent of asbestosis, lung cancer and malignant mesothelioma, and silica causes silicosis in addition to lung cancer.

Exposure to Carcinogens

Exposure to these chemicals can be exogenous or endogenous. People are exposed exogenously when these carcinogenic agents are present in food, air or water, while endogenous exposure takes place when the agent is a product of metabolism or pathophysiologic states such as inflammation. Numerous endogenous processes frequently result in cellular DNA damage, including oxidation, deamination, exocyclic adduct formation.

Human and animals are exposed directly through foods intake or indirectly through eating food of animal origin (kidney, liver, milk and eggs) (FDA, 2000). Exposure to carcinogens can be through the respiratory tract, skin and the gastrointestinal tract which can be exogenous (agents are present in things we consume like food, water and air) or endogenous (agents are products of metabolism (catabolic or anabolic processes) or pathophysiologic state and response(s) (such as inflammation). Numerous endogenous processes frequently result in cellular DNA damage, including oxidation, deamination, exocyclic adduct formation, depurination.

Fungal Carcinogens

Fungi are ubiquitous plant pathogens that are major spoilage agents of foods and feedstuffs. The infection of plants by various fungi not only results in reduction in crop yield and quality with significant economic losses but also contamination of grains with poisonous fungal secondary metabolites called mycotoxins. The ingestion of such mycotoxin-contaminated grains by animals and human beings has enormous public health significance, because these toxins are capable of causing diseases in man and animals (Atanda *et al.,* 2013).

Mycotoxins are low molecular weight, toxic compounds produced by certain strains of a variety of filamentous fungi., under appropriate conditions (such as moisture and temperature) and all over the world, they cause enormous economic losses annually to the grain trade and the marketing of foods and feeds ((Turner *et al.,* 2009). Contamination of grains by aflatoxins alone inflicts annual losses of more than $750 million in Africa and is a major economic and health problem for the continent (*Goyal et al*., 2003). Mycotoxins have attracted worldwide attention due to the significant losses associated with their impact on human and animal health and consequent national economic implications (Makun *et al*., 2009). The scientific study of mycotoxin began in 1960, when a large number of turkey poults died in England due to consumption of contaminated ground nut meal imported from Brazil (Blount, 1961*).* A toxigenic fungus identified as *Aspergillus flavus* was isolated from the groundnuts

and the toxigenic principle was named aflatoxin, meaning *A. flavus* toxins. Over 300 mycotoxins have been reported (Jestoi *et al.,* 2004). However, based on extensive analytical studies (IARC, 1993., Bhat and Vashanti, 1999) and detailed study of the distribution of fungi in nature, the five agriculturally important toxins from fungi are aflatoxins, fumonisins, Ochratoxins, Zearalenone and deoxynivalenol.

Table 1: Carcinogens, producer fungi and principal toxic effects

Carcinogens	Class of Carcinogen	Sources	Producer fungi	Toxic effects
Aflatoxins	Class 1	Kulikuli, yam chips, rice	*Aspergillus flavus, A. oryzae, A. parasiticus A. nominus*	Potently carcinogenic, mutagenic and teratogenic
Fumonisins B1	Class 2B	Maize, maize products	*Fusarium moniliforme, F. poae, F. verticillioides* F. *proliferatum F. anthophilum, F. globosum, F. nygamai* , F. *dlamini, F. napiforme, F. pseudonygamai, F. andiyazi, F. polyphialidicum*	carcinogenic, pulmonary oedema in pigs, leucoencephalomalacia in horses, hepatotoxic and carcinogenic to rats
Ochratoxins A	Class 2A	cereals, dried fruits, cocoa, wine, poultry eggs and milk,pork	*A, ochraceus, A. flavus, Penicillium viridicatum, Penicillium verrucosum, A. niger, A. turbingensis*	Hepatotoxic, nephrotoxic,, teratogenic. Possible human carcinogen, immunotoxic and neurotoxic
Sterigmatocystin	Class 2B		*A. versicolor, A. nidulans, A. rugulosus*	Carcinogenic

Source: Coronel *et al*., 2010

Table 2: General Information on Fungal Carcinogens

	Aflatoxin B_1	Ochratoxin A	Fumonixin B_1
Molecular formula	$C_{17}H_{12}O_6$	$C_{20}H_{18}ClNO_6$	$C_{34}H_{59}NO_{15}$
Appearance	Off-white powder, blue fluorescence	Colourless crystalline compound	white to off-white powder
Other name	6-Methoxydifuro-coumarone	(-)-*N*- [(5-Chloro- 8-hydroxy- 3-methyl- 1-oxo- 7-isochromanyl) carbonyl]- 3-phenylalanine	Macrofusine
Melting pt	Not known	169 °C., 336 °F., 442 K	Not known (has not been crystallized)
Molar Mass	312.27 g mol^{-1}	403.82 daltons	721
Exposure	oral route, inhalation, dermal	Oral route, skin permeation	Oral route
Biomarker	aflatoxin/albumin adducts in blood serum	ochratoxin protein adduct in serum	free sphingoid bases in the serum and urine

Aflatoxin B_1

Introduction

Aflatoxins are a naturally occurring carcinogenic by-product of common fungi on grains and other crops, particularly maize and groundnuts. They are a kind of mycotoxin, a highly toxic product of fungi *Aspergillus flavus* and *A. parasiticus* that occurs on almost all agricultural commodities worldwide (Strosnider *et al*., 2006). Aflatoxin is not always obvious, and even grains that appear normal could actually be infested with high levels of the toxin-producing fungus, which thrives under poor storage conditions. The occurrence of aflatoxins is largely dependent on "geographic location, agricultural and agronomic practices, and pre- and post-harvest handling. If crop drying is delayed or storage is not properly handled, the effects can be greater, with insect and rodent infestations facilitating the invasion of fungal-producing aflatoxins and contaminating stored products." Aflatoxin poisoning is reported from all parts of world in almost all domestic and non domestic animals like cattle, horses, rabbits, and other non human primates. Aflatoxicoses is also reported in humans in many parts of the world.

Aflatoxins are acutely toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic compounds. The main target organ for toxicity and carcinogenicity is the liver. The evaluation of epidemiological and laboratory results carried out in 1987 by the International Agency for Research on Cancer (IARC) found that there is sufficient evidence in humans for the carcinogenicity of naturally occurring mixtures of aflatoxins, which are therefore classified as Group 1 carcinogens, except for aflatoxin M1, which is possibly carcinogenic to humans (Group 2B) (Makun *et al*., 2012).

Aflatoxins are divided into six major toxins according to their fluorescent properties under ultraviolet light (ca. 365 nm) and their chromatographic mobility. *A. flavus*, produces only aflatoxins B, and *A. parasiticus*, produces aflatoxins B and G. Aflatoxins M1 and M2 are oxidative metabolic products of aflatoxins B_1 and B_2 produced by animals following ingestion, and so appear in milk (both animal and human), urine and faeces. Aflatoxicol is a reductive metabolite of aflatoxin B1. Aflatoxins B_1 and B_2 produce a blue fluorescence while G_1 and G_2 a green one. There are also two metabolic products aflatoxin M_1 and M_2 which occur in the milk of lactating mammals which have consumed aflatoxin contaminated feed. Aflatoxin B_1 is the most toxic and the most prevalent among this family.

Recorded Cases of Aflatoxins in West Africa

According to the International Food Policy Research Institute (IFPRI) aflatoxins contaminate one-quarter of the global food supply and over half the world's population., 4.5 billion people are exposed to high, unmonitored levels, primarily in developing countries. In sub-Saharan African alone, an estimated 26,000 people die annually of liver cancer associated with aflatoxin exposure (El-Serag *et al*., 2007). Hepatocellular carcinoma (HCC) cases (> 80%) occur in sub-Saharan Africa or Eastern Asia. HCC accounts for 70%-90% of primary liver cancers, making it the third leading cause of cancer-related deaths world-wide (Venook *et al*., 2010., Yu and Yua, 2004).

In parts of West Africa, e.g., The Gambia and Guinea Conakry, aflatoxin exposure has been linked to the consumption of groundnuts with a seasonality occurring in exposure levels (Turner et al., 2000). In Benin and Togo, maize is consumed and stored across all agroecological zones and, depending on agroecology, crop management, and length of storage, aflatoxin contamination levels averaging over 100 ppb in up to 50 percent of grain stores sampled have been recorded (Hell *et al*., 2000). Another factor in risk of food contamination with aflatoxin is the inherent toxicity of the resident *A. flavus* strains in the different agroecological zones. The *A. flavus* L (large sclerotia) strain, which produces either no alfatoxin or only aflatoxin B1, is found predominantly in moist zones., while the highly toxigenic African S (small sclerotia) strain , an abundant producer of aflatoxins B_1, B_2, G_1, and G_2, is prevalent in dry zones (Cardwell and Cotty, 2000).

Rural populations in Benin and Togo rely on both groundnuts and maize as dietary staples and both crops are stored up to one year in most households. Maize is the principle weaning food in these countries used by 98 percent of households surveyed. Thus, quality degradation of maize during storage may have a direct effect on weaning children.

The economic and country assessment conducted in 2012 by Abt Associates in collaboration with representatives of the Mycotoxicology Society of Nigeria (MYCOTOXSON) and Nigeria's National Agency for Food and Drug Administration and Control (NAFDAC) concluded that the largest impact of aflatoxins in Nigeria is on health, especially human. The assessment found little awareness about aflatoxins among farmers, rural traders, and consumers. Despite aflatoxin standards, unpackaged food and found bound for domestic consumption are not regulated. This means that aflatoxin-contaminated grain can easily enter the Nigerian consumption stream.

Among staple cereals in the Nigerian diet, maize has the highest levels of aflatoxin contamination (Bandyopadhyay *et al.,* 2007). There is also evidence of high levels of contamination in Nigerian groundnuts. Over the past 5 years, there were 12 published studies assessing aflatoxin prevalence in Nigeria (7 assessing aflatoxin prevalence in maize and 5 assessing prevalence in groundnuts). The evidence does suggest that aflatoxin contamination in Nigeria warrants attention.

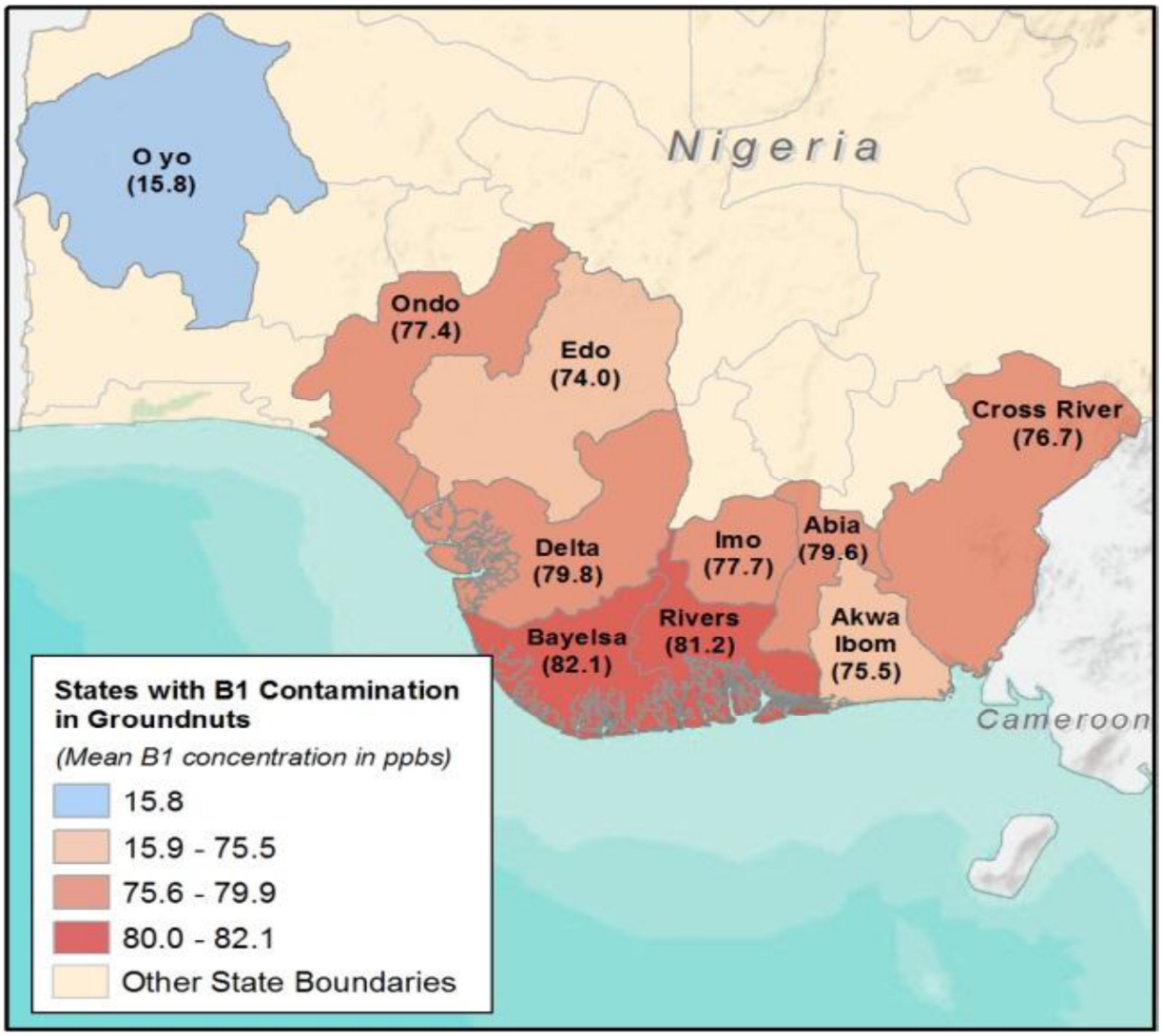

Figure 1: Aflatoxin B1 Contamination in Maize in 2010 (Source: Abt Associates, 201)

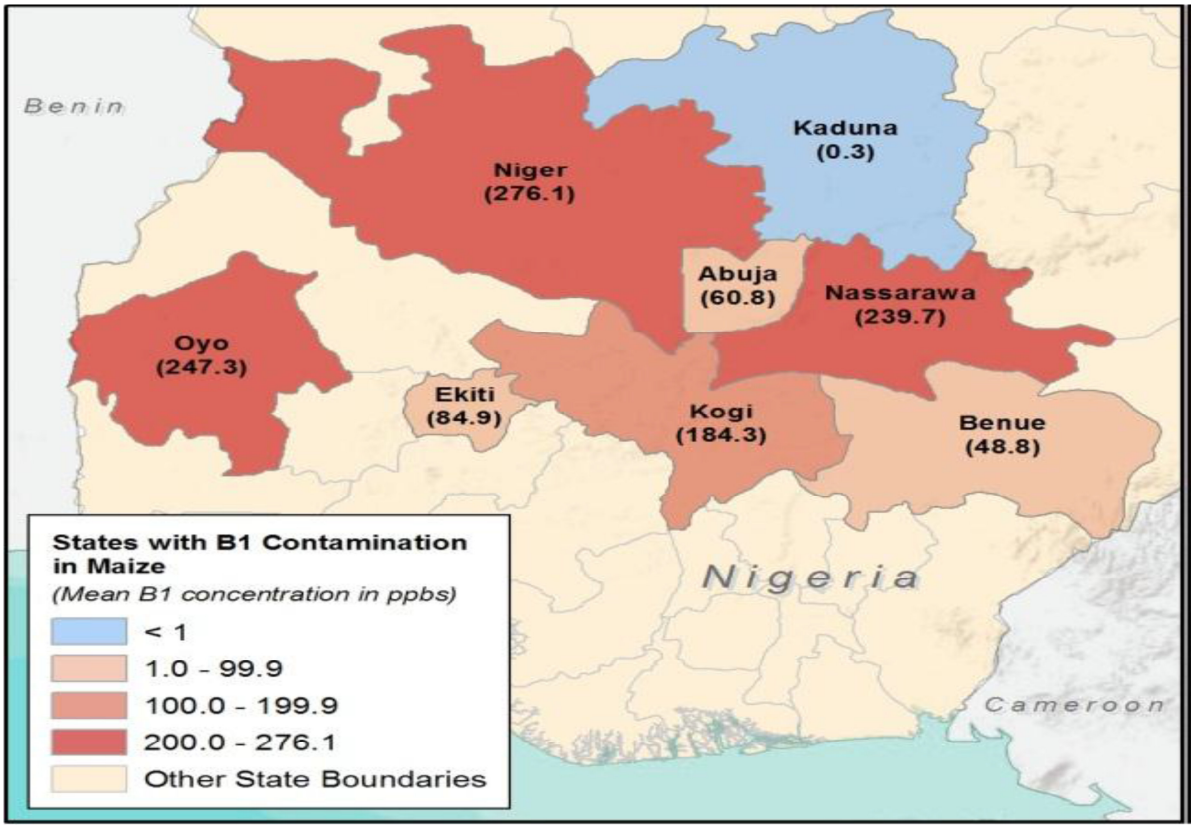

Figure 2: Aflatoxin B1 Contamination in Groundnuts in 2010 (Source: Abt Associate, 2013)

Incidence of aflatoxins in Nigeria

- 1965: Aflatoxin outbreak in Northern Nigeria
- 1966: palmotoxin Bo and Go isolated from palm wine contaminated by *Aspergillus flavus* (Bassir and Adekunle, 1968)
- 1982: mycotoxicosis of pecking ducklings and horses
- 1988: pupils in Ibadan died after eating groundnut cake ("kulikuli") (Akano and Atanda, 1990)
- 2010: Nigeria reported the highest estimated number of hepatocellular carcinoma (HCC-liver cancer) in the world attributable to aflatoxins in 2008-2009 (Liu and Wu, 2010).
- 2013: 7,761 out of 10,130 estimated liver cancer cases per year caused by aflatoxin contamination of maize and groundnuts in Nigeria (Abt Associates, 2013)

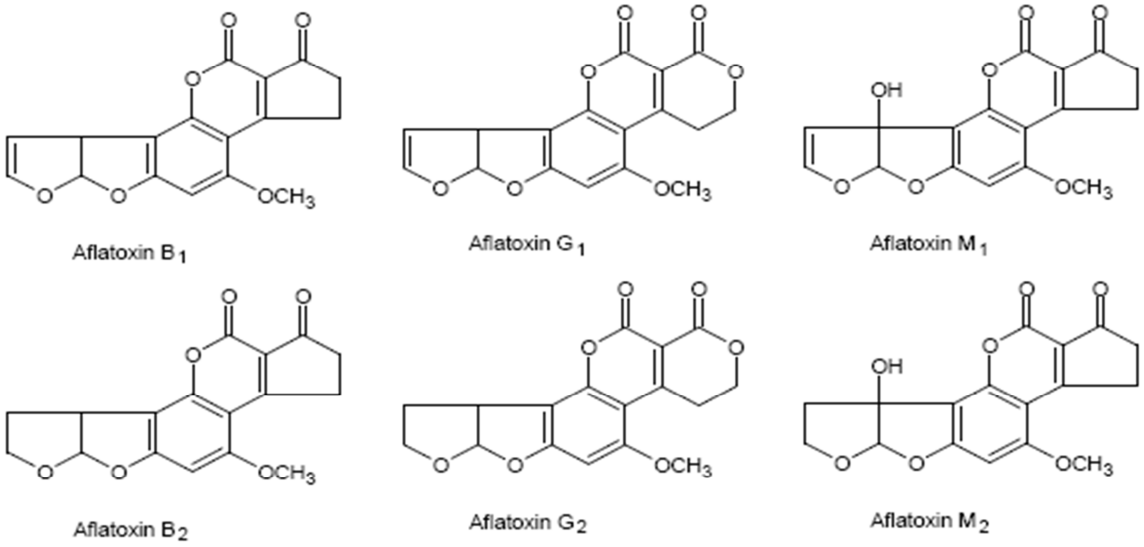

Figure 3: Chemical structure of the different aflatoxins (Source: William *et al.*, 2004)

Exposure to Aflatoxin

As a result of aflatoxins' common occurrence in feedstuffs, feeds and milk products, these mycotoxins pose a serious threat to humans and animal species. Although the oral route is the main contamination means, inhalation may also occur as a result of people or animals being exposed to the grains' dust. After respiratory exposure, AfB_1 may appear in the blood more quickly than after oral exposure. Nevertheless, after 4 hours the plasmatic concentration does not differ between the two routes of contamination. Following ingestion, aflatoxin B_1 is efficiently absorbed in the intestinal tract, of which the duodenum appears to be the major site of absorption. Due to the particle's low molecular weight, the main mechanism of absorption of mycotoxin, as suggested by several authors, is passive diffusion, in which no efflux pumps or transporters are involved (William *et al*., 2004)

Absorption into the organism

Metabolism

The main metabolizing organ for aflatoxin is the liver, but this can also occur directly at the site of absorption, in the blood or in several extra-hepatic organs. The metabolism of AfB_1 can be divided into three phases: 1) Bioactivation., 2) Conjugation., 3) Deconjugation.

Bioactivation

In this phase aflatoxins exert their toxic effects. At this first stage, aflatoxin B_1 is oxidized into several hydroxylated metabolites. The metabolic pathways for AfB_1 include o-demethylation to AfP_1, reduction to aflatoxicol and hydroxylation to AfB_1-8,9-epoxide (acutely toxic, mutagenic, and carcinogenic), AfM_1 (acutely toxic), $AfFQ_1$, or AfB_2 (both relatively non-toxic).

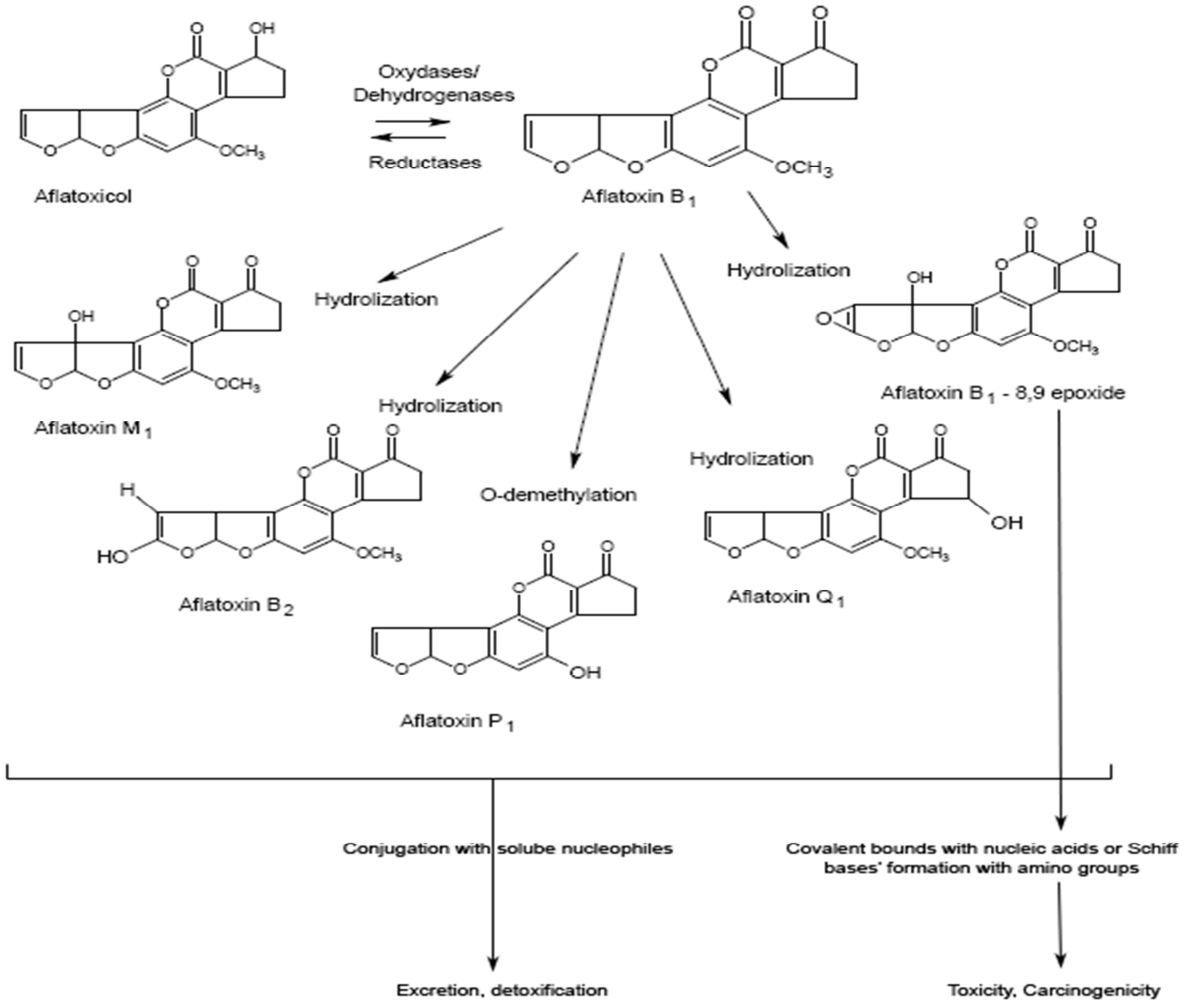

Figure 4: Aflatoxin B_1 pathways (Source: Yiannikouris and Jouany, 2002)

Aflatoxin B_1– 8,9 epoxide is highly unstable, thus several reactions may occur, depending on the second molecule present: Biological nucleophils (such as nucleic acids) – stable links to RNA and DNA are formed., inducing point mutations and DNA strand breaks. These reactions and the formation of AfB_1-DNA adducts are highly correlated with the carcinogenic effect of AfB_1 in both animal and human cancer cases. Water – in the presence of water molecules, Aflatoxin B_1– 8,9 epoxide will be hydrolyzed into AfB_1– 8,9-

dihydrodiol and become available to be linked with serum proteins, such as lysine and albumin. This mechanism may explain the toxic effects of aflatoxin (Yiannikouris and Jouany, 2002).

Conjugation

Phase I metabolites may undergo phase II biotransformation involving the enzymes glutathione S-transferase (GST), ß-glucuronidase, and/or sulfate transferase which produce conjugates of AfB_1-glutathione, AfB_1-glucuronide, and AFB_1-sulfate, respectively. The major conjugate of AfB_1-epoxide identified is the AfB_1-glutathione conjugate. This conjugation is the principal detoxification pathway of activated AfB_1 in many mammals which is essential in the reduction and prevention of AfB_1 induced carcinogenicity. The resulting conjugates are readily excreted via the bile into the intestinal tract. It has been accepted that cytosolic GST activity is inversely correlated to the susceptibility of several animal species to AfB_1 carcinogenicity (Yiannikouris and Jouany, 2002).

Deconjugation

This phase can occur in the intestinal tract as a result of bacterial activity. Deconjugation is part of the metabolic role of the large intestine flora, which results in reabsorption and an enterohepatic circulation is established.

Excretion and residues in animal products

The excretion of AfB_1 and its metabolites is mainly made through bile liquid and urine. In lactating animals, AfM_1 and other metabolites are excreted in the milk. Many studies exist showing the carry-over of aflatoxin into animal products such as eggs, milk and milk products.

Toxicity

In 2004, an outbreak of aflatoxicosis in Kenya resulted in 125 deaths from the 317 cases of poisoning. The cause for this 39% fatality rate was contaminated maize with aflatoxin levels up to 8mg/kg. Besides carcinogenic effects, immunomodulatory effects are also observed in the humans along with infectious disease and growth problems in children.

In animals, the effects of aflatoxins are variable depending on sex, age, species and even animal breed. The main target organ for aflatoxins is the liver. Nevertheless, due to the toxins' interference and reactions with nucleic acids, RNA and DNA, proteins and enzymes, their effects on domestic animals are not only hepatotoxic and expressed by toxic hepatitis and jaundice but involve a broad range of organs, tissues and systems (Yu, 1995).

Human Exposure to Aflatoxins

In the last few years, new technologies have been developed that more accurately monitor individual exposures to aflatoxins. Particular attention has been paid to the analysis of aflatoxin DNA adducts and albumin adducts as surrogates for genotoxicity in people. Autrup *et al.*(1983) pioneered the use of synchronous fluorescence spectroscopy for the measurement of aflatoxin DNA adducts in urine. Urine samples collected after exposure to alfatoxins were found to contain 2,3-dihydroxy-2-(N7-guanyl)-3-hydroxyaflatoxin B1, trivially known as AFB-Gual . Wild *et al*. (1986) used highly sensitive immunoassays to quantitate aflatoxins in human body fluids. An enzyme linked immunosorbent assay (ELISA) was used to quantitate aflatoxin B1 over the range of 0.01 ng /ml to10 ng/ml, and was validated in human urine samples. Using this method, aflatoxin-DNA adduct excretion into urine was found to be positively correlated with dietary intake, and the major aflatoxin B1-DNA adduct excreted in urine was shown to be an appropriate dosimeter for monitoring aflatoxin dietary exposure.

Fumonisin B_1

Introduction

Fumonisins are ubiquitous contaminants of corn and other grain products produced by Fusarium verticillioides (synonymous with Fusarium moniliforme) and several other Fusarium species (Voss et al., 2001). There are at least 28 known analogues of fumonisins, of which fumonisin B1 (FB1) is the most plentiful. Different subtypes of fumonisin have been known, but only fumonisin B1 (FB1), FB2 and FB3 are naturally found in contaminated foods (Marasas, 1996). FB1, the most toxic form, is one of the secondary metabolites that commonly contaminate many kinds of cereals including rice, corn and wheat (Stockmann and Savolainen, 2008). Its occurrence in corn varies seasonally and geographically. Levels of FB1 in corn can range from undetectable (less than a few parts per billion) to as high as 150 parts per million (Shephard et al., 1996).

Previous investigation has shown that stored maize was readily attacked by various fungi and *Fusarium* was one of the most important fungal genera isolated in the maize samples (Ameh *et al.*, 2008). Furthermore, Ameh et al. (2008) reported the presence of various species of *Fusarium* in twenty samples corresponding to 37.73% infection rate, while the moisture content values of the grains ranged from 5.3 to 13% which is well below the minimum range of 18 to 20% required for the growth of *Fusarium verticilloides* (Munkvold and Desjardins, 1997). *Fusarium* species such as *Fusarium proliferatum* and *F. verticilloides* have been reported to be prolific producers of toxins (Marasas, 2001) which are associated with various health conditions in humans and animals.

As a result of the widespread problem of mycotoxin contamination of maize, the Joint Food and

Agricultural Organisation and World Health Organisation (FAO/WHO) Expert Committee on Food Additives (JECFA) allocated a Provisional Maximum Tolerable Daily Intake for humans of 2 µg/g for fumonisins (WHO, 2002).

Fumonisins in West Africa

Fumonisins are prevalent on crops in Sierra Leone and Ghana (Gelderblom *et al.,* 1991). Villages in Burkina Faso experienced fumonisin contamination on maize with levels as high as 29,000ppb (Shephard *et al.,* 1990). The authors confirmed that all the 72 samples from some local markets tested positive for fumonisin. Given the ubiquity of the toxin on maize, the authors raised alarm on chronic exposure to the human population and the attendant consequences. Ghana, Nigeria, Senegal, Togo and Burkina Faso all have a record of contamination due to aflatoxin on sorghum, maize, cotton seeds, ground nut and its products, shelled mellon, yam and cassava chips all at varying levels, most time exceeding the EU and FDA standards (Ross *et al.,* 1990). The aflatoxin contamination of groundnuts in Burkina Faso has always been a source of concern (Ross *et al.,* 1991) compared to fumonisins.

In Nigeria the fumonisin contamination of maize grains is above the limit set for human consumption (Afolabi *et al*, 2008). The prevalence of toxigenic *Aspergillus* species on maize kernels from 3 agroecological zones in Northern part of Nigeria is already established by Atehnkeng *et al* (2008) although the Nigeria Mycotoxin Awareness and Study Network has put in place structures that will come up with a mycotoxin map of the country. This is expected to enhance further studies and management. Some *Fusarium* toxins had also been implicated as contaminants of major crops (Afolabi *et al*., 2006).

History of fuminisins

- **1970:** Fumonisin was characterized in South Africa (EHP, 2001)
- **1984:** *F. verticillioides* MRC 826 caused primary hepatocellular carcinoma and cholangiocarcinoma
- **1988**, The structures of the fumonisins elucidated
- **1988:** equine leukoencephalomalacia (ELEM) caused by Fumonisin B1
- **1989 -1991:** Large-scale outbreaks of ELEM in United States.

2000 till now: work still going on Millennium

Description and Chemistry

Fumonisin B1 (FB1) belongs to the toxins fumonisins which are produced by *Fusarium verticilloides* and *F. proliferatum*, fungi that commonly contaminate maize. It has been also claimed that *F. napiforme, F. anthophilum, F. dlamini* and *F. nygamai* are able to produce FB1 (ECH, 2000., NTP, 1999). FB1 has been found as natural contaminant in maize and maize based food from many parts of the world, e.g. the US, Canada, South Africa, Nepal, Australia, Thailand, The Philippines, Indonesia, Mexico, France, Italy, Poland, and Spain (ECH, 2000).

Chemistry

FB1: 1,2,3-propanetricarboxylic acid, 1,1'-[(12-amino-4,9,11-trihydroxy-2- methyltridecyl)-2-(1-methylpentyl)-1,2-ethanediyl] ester, C34H59NO15. MW: 721.838, CAS no.: 79748-81-5. FB1 is stable during most types of processing. Dry milling of maize results in the distribution of FB1 into the bran, germ and flour. However, the concentration of FB1 is reduced during the manufacture of cornstarch by wet milling, since FB1 is water-soluble. A number of factors make it difficult to extract FB 1 from processed food (Norred, 1998). Nixtamalisation (calcium hydroxide processing) and ammoniation lead to hydrolysed FB1 (AP1or HFB1) and aminopentol, respectively. These treatments reduce the fumonisin content while increasing the concentration of hydrolysed fumonisins without eliminating the toxic product (Flynn *et al.,* 1997).

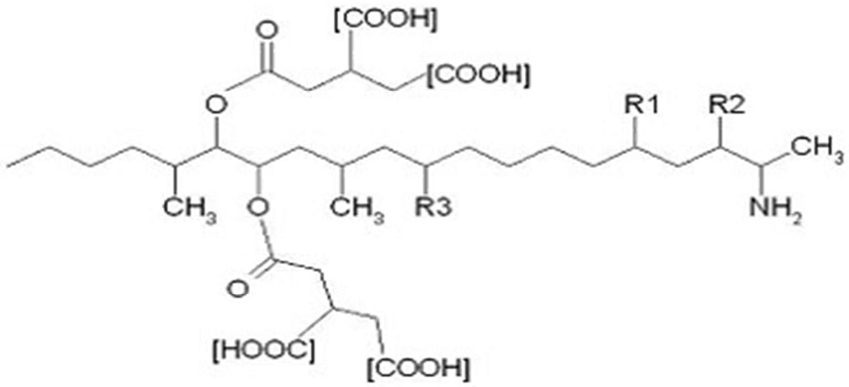

Figure 5: Chemical Structure of fumonisin B_1

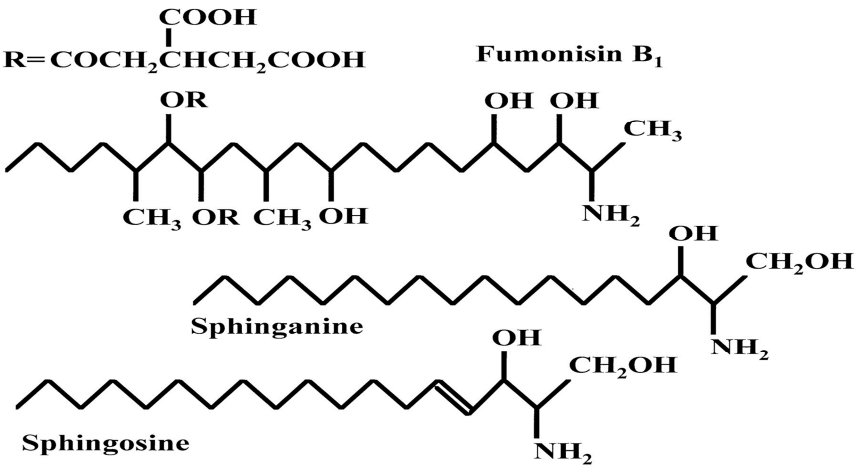

Figure 6: Similarity in chemical structures of sphingosine, sphinganine, and FB1 (Source: Dragan *et al,* 2001).

Exposure data on fumonisin B1

Within the EU, there is no systematic collection of exposure data available yet. However, real exposure assessments have rarely been reported.

Table 3: Recommended maximum levels of fumonisins in human food products and animals feeds

Product	**Recommended maximum level (ppm FB1 + FB2 + FB3)**	
Human Food Products		
Degermed dry milled corn products	2	
Whole or partially degermed dry milled corn products	4	
Dry milled corn bran	4	
Cleaned corn intended for masa	3	
Cleaned corn intended for popcorn	3	
Animal Feeds	**Corn**	**Total diet**
Equids (horses) and rabbits	5	1
Catfish	20	10
Swine		10
Ruminants	60	30
Poultry	100	50
Ruminant, mink, and poultry breeding stock	30	15
All other livestock species and pets	10	5

Source: US Food and Drug Administration, June 6, 2000.

Cell Death and Carcinogenesis

Cell death a fundamental biological process have two major categories distinguished., apoptotic and oncotic necrosis. Apoptotic necrosis is morphologically identified by cytoplasmic shrinkage and karyorrhexis, while oncotic necrosis is identified by cytoplasmic swelling and karyolysis. Either cell death process can occur in individual cells or in multiple cells in clusters. Apoptosis has frequently been referred to as programmed cell death. Thus, an agent can increase the risk of carcinogenesis either by damaging DNA so that there are more

mistakes each time DNA replicates, by increasing the number of DNA replications, or by a combination of both processes (Cohen and Ellwein, 1991). If the agent, or a metabolite, forms a mutagenic adduct and causes DNA damage, it is commonly referred to as genotoxic (DNA reactive). Agents that increase carcinogenesis by increasing the number of DNA replications have been referred to by a variety of names, including non-genotoxic carcinogens (Dragan *et al,* 2001).

Mechanism of action

Fumonisins toxicity are based on a structural similarity to the sphingoid bases, sphingosine and sphinganine (see figure 6). They are inhibitors of sphinganine (sphingosine) N-acyltransferase (ceramide synthase), a key enzyme in the lipid metabolism, resulting in a disruption of this pathway. This enzyme catalyzes the acylation of sphinganine in the biosynthesis of sphingolipids and also deacylation of dietary sphingosin and the sphingosine that is released by the degradation of complex sphingolipids (ceramid, sphingomyelin and glycosphingolipide) (Soriano *et al*., 2005).

Sphingolipids are important for the membrane and lipoprotein structure and also for cell regulations and communication (second messenger for growth factors). Sphingosine is the backbone of sphingolipids. As a consequence of this disruption many bioactive intermediates are elevated, others reduced. The main points are: rapid increase of sphinganine (sometimes sphingosine), b) Increase of sphinganine degradation products like sphinganine 1-phosphate., c) decrease of complex sphingolipids

Free sphingoid bases are toxic to most cells by affecting cell proliferation and inducing apoptosis or necrotic cell death or increasing in kidney and serum sphinganine 1-phosphate. The accumulation of sphinganine is associated with hepato- and nephrotoxic effects. Complex sphingolipids are important for cell growth regulation and also cell-cell interactions. Fumonisin B_1 impairs the barrier function of endothelial cells in vitro. These adverse effects on endothelial cells could indirectly contribute to the neurotoxicity and pulmonary edema caused by fumonisins. Sphingosine 1-phosphate activates the endoplasmic reticulum calcium release and also acts as a ligand for extracellular receptors in the vasculature (S1P receptors).

The accumulation of free sphingoid bases in the serum and urine is a useful biomarker for the exposure of fumonisins (Dragan *et al,* 2001)..

Exposure and absorption into the organism

Several studies have indicated that fumonisins are poorly absorbed from the gastrointestinal tract and rapidly cleared from the blood. Fumonisins only accumulate insignificantly in the tissues. The most important target organs are the liver and the kidneys depending on species and dosage. It causes apoptosis followed by mitosis in the affected tissues.

Excretion and residues in animal products

There is no evidence for the carryover of fumonisins to eggs. Additionally the exposure of the toxin through milk does not pose a production or health concern to consumers or animals because there is only a minimal carryover.

Toxicity

In humans, fumonisins have been found to cause oesophageal cancer in certain regions (South Africa, China, and Italy) after ingestion of contaminated grains. According to IARC (International Agency for Research on Cancer) fumonisins are classified as possible human carcinogens.

In the case of animals, horses are the most sensitive species to fumonisin toxicity. The mycotoxin causes a disease syndrome which is called equine leukoencephalomalacia (ELEM) and affects the central nervous system. Several studies have indicated that fumonisins can cause porcine pulmonary edema (PPE). The first sign of fumonisin contamination is often a decreased feed intake. Within 4 – 7 days of being fed contaminated feed pigs show respiratory disorders followed by death due to acute pulmonary edema. In general, the most affected organs are the liver and kidneys but fumonisins also cause a broad range of effects on other systems (IPCS, 2000; Bucci and Howard, 1996).

Biochemical mode of action

Studies have shown that fumonisins are competitive inhibitors of de novo sphingolipid biosynthesis and metabolism. umonisins are structurally similar to sphingoid bases such as sphingosine, which is a component of the sphingolipid molecule, and are able to inhibit sphingosine-sphinganin-transferase and ceramide synthase. In mammals sphingolipid biosynthesis can occur in all kinds of tissue. The biosynthesis consists of a cascade of reactions that are regulated and catalysed by several enzymes. Briefly the biosynthesis starts with serine and palmitoyl-CoA forming 3-ketosphinganine, then sphinganine which is converted to sphingosine, which in turn can be converted to glycosphingolipids such as ceramide, which can be converted further into sphingomyelin or other complex glycosphingolipids. The first classes of sphingolipids were named for the tissues from which they were isolated (e.g. sphingomyelin, cerebrosides). However, sphingolipids can be found in all eukaryotic cells, where they primarily occur in cell membranes and related intracellular membranes, such as Golgi and lysosomal membranes. In addition to biosynthesis, metabolism of sphingolipids also occurs. In the intestines sphingomyelin and other complex glycosphingolipids are digested and the gut cells absorb ceramide and sphingosine. The different intermediates of sphingolipids have various effects on cellular processes (Dragan *et al,*

2001). .

Sphingosines play a role in the regulation of cell growth, cell differentiation, cell morphology, apoptosis and endothelial cell permeability. The glycosphingolipid ceramide plays a role in the regulation and differentiation of cells, apoptosis and protein secretion, induction of cellular senescence and other processes. The ultimate effects are dependent of concentration and cell type. Another feature of the sphingoid bases (sphinganines, sphingosines) is the inhibition of cell transformation (Merrill *et al.,* 1997) Important for the involvement of sphingolipids is that inhibition of the biosynthesis is already seen a few hours after exposure to FB1 (Riley *et al.,* 1996).

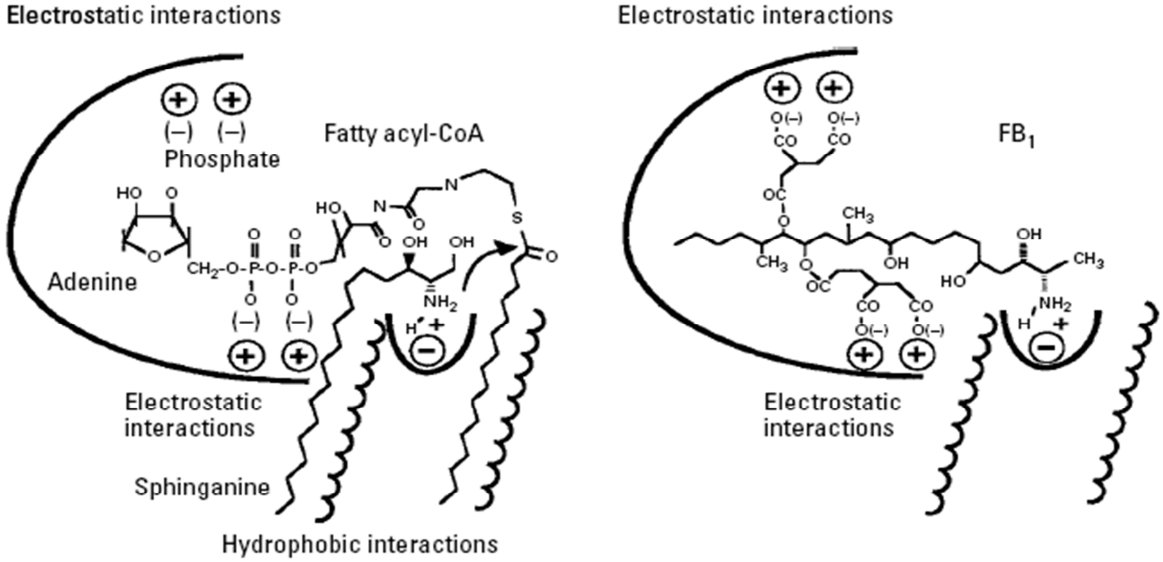

Figure 7: Proposed mechanism of action of ceramide synthase inhibition by FB_1., FB_1 mimics regions of the sphingoid base and the fatty acyl-CoA substrates. (Merrill *et al*., 2001)

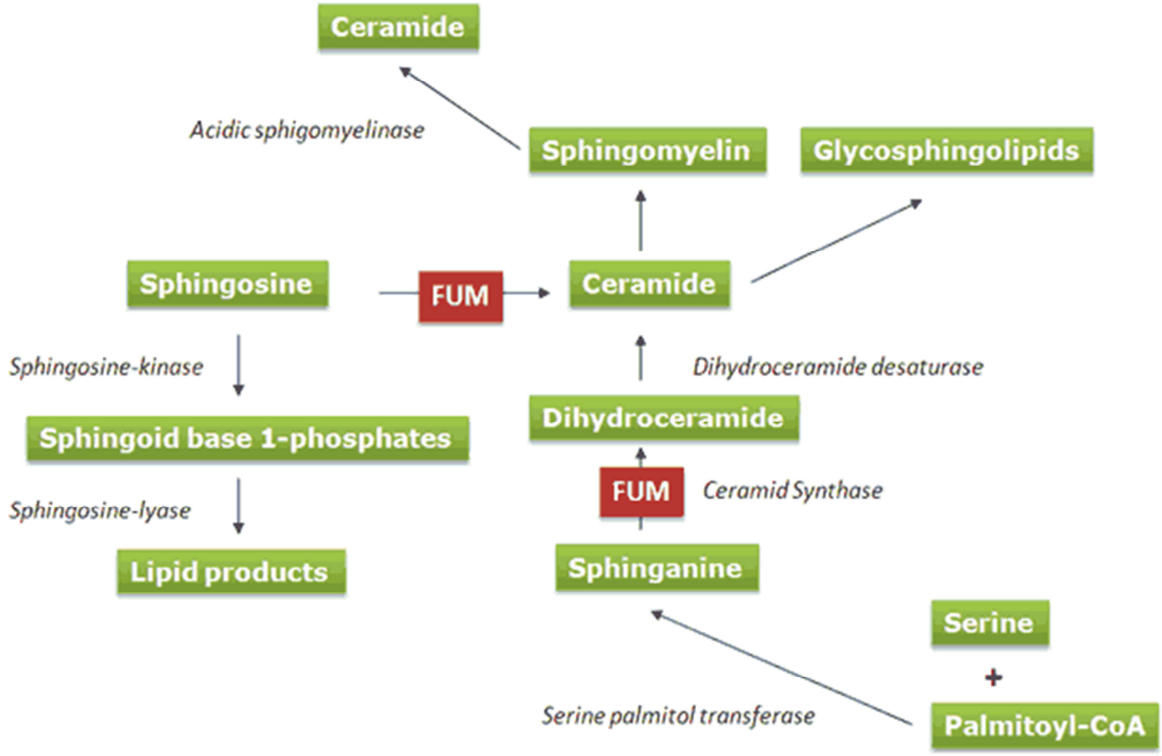

Figure 8: Sphingolipid metabolism showing the inhibition of ceramide synthase (x) by fumonisins and the changed concentrations of other compounds caused by this inhibition. (Voss *et al*., 2007)

Toxicokinetics

Absorption

There are no studies of fumonisin absorption following inhalation and/or dermal exposure. FB_1 is taken orally via food. Overall FB_1 is poorly absorbed, less than 6% (EC, 2000) Absorption of orally administered fumonisin B_1 (10 mg/kg body weight) to rats is low (3.5% of dose) but rapid (Tmax = 1.02h). FB_1 does not significantly permeate through the human skin and hence has no significant systemic health risk after dermal exposure (Boonen *et al.,* 2012., Alizadeh, *et al.,* 2012).

Distribution

After absorption, some appears to be retained in liver and kidneys. For rats that were fed diets containing fumonisins for several weeks, the concentrations of the fumonisins in the kidneys were approximately 10-fold higher than in the liver (Riley and Voss, 2006). Plasma distribution of the absorbed dose conformed to a two-compartment open model and the tissue (liver, kidney) concentration time results were consistent with a one-compartment open model. Other studies have also shown that there is no distribution of FB1 to the maternal milk (Scott et al., 1994). Voss *et al.* (1996a) injected pregnant rats on gestation day 15 with [14C]-FB1. After 1 hr, which allowed for approximately 98% of the dose to be cleared from the maternal blood, negligible amounts of radioactivity were found in the foetuses. Other studies confirmed that FB1 did not cross the placenta in rats, mice and rabbits (Laborde *et al.,* 1997). Studies on other species such as the monkey with different type of placenta (similar to the human) were not available. Also an increased Sa/So ratio indicative of exposure to FB1 was not observed (Voss *et al.,* 1996b). Becker *et al*. (1995) studied the effects of feeding FB1 in lactating sow and their suckling pigs. When sows ingested sub-lethal concentrations of FB1 (100 mg/kg feed) for 17 days, no detectable amounts of FB1 in the sow milk were found.

Excretion

FB1 is rapidly eliminated by biliary excretion in several animal species including laying hen, swine, cow, rat, mouse and non-human primates. There are no human data available. Enterohepatic recycling is clearly important in some animal species. Small amounts (less than 1 %) are excreted in urine. A small but persistent (and biologically active) pool of [14C]-labelled FB1 or its metabolites appears to be retained in liver and kidneys. Elimination half-life in rats is 3.15h for plasma, 4.07h for liver, and 7.07h for kidney. However, FB_1 is rapidly excreted mostly in its original form. Small amounts are excreted in urine., the most are excreted in feaces (EC, 2000).

Metabolism

Only very sparse information concerning metabolism is available. No metabolites were detected in rat studies independent of the route of administration, whereas analysis of non-human primate's faeces extract revealed a metabolite of FB1 (Eriksen and Aleander, 1998). This metabolism most likely occurred in the gut since partially hydrolysed and fully hydrolysed FB1 were recovered in faeces but not in bile of Vervet monkey (ECH, 2000). In pigs it was suggested that similar metabolism occurred, but this was not demonstrated (Prelusky *et al*., 1994).

Toxicodynamics

As a result of their similarity, fumonisins are able to inhibit sphingosine-sphinganin-transferases and ceramide synthases and are therefore competitive inhibitors of sphingolipid biosynthesis and metabolism.

Chapter Four

Ochratoxin A

Introduction

Ochratoxins are a small group of chemically related toxic fungal metabolites (mycotoxins) produced by *Aspergillus* and *Penicillium* growing on a wide range of food commodities. In tropical and sub-tropical regions, OTA as it is commonly called is produced mainly by *Aspergillus ochraceus*. But in temperate climates, the main producer is *Penicillium* verrucosum and *P nordicum* (Frisvad *et al*., 2004). OTA is carcinogenic in animals and is classified as a class 2B, possible human carcinogen by the International Agency for Research on Cancer [Reddy and Bhoola, 2010]. The National Toxicology Program (NTP) has designated OTA as "reasonably anticipated to be a human carcinogen" based on sufficient evidence of carcinogenicity in experimental animals (Clark and Snedeker, 2006).

Ochratoxin A was first isolated in 1965 from cultures of *Aspergillus ochraceus* (van der Merwe *et al*., 1965) now known as *A. alutaceous*. OTA production is favored by relatively high temperatures (13^{o}C to 37^{o}C), but *P. verrucosum* grows and produces the toxin at temperatures as low as 0^{o}C. *A. ochraceus* is able to produce OTA at water activities down to 0.80, while the lower limit for significant toxin production by *P. verrucosum* is thought to be about 0.86. Both are considered to be storage fungi. The only other ochratoxin apart from OTA found in food is ochratoxin B, which is rare and much less toxic. Other structurally related ochratoxins include ochratoxin C, α and β (Searcy *et al*., 1969).

OTA has been found in a very wide range of raw and processed food commodities all over the world. It was first reported in cereals, but has since been found in other products, including coffee, dried fruits, wine,

beer, cocoa, nuts, beans, peas, bread and rice. It has also been detected in meat, especially pork and poultry, following transfer from contaminated feed. OTA levels in different food products vary, but are generally low in properly stored commodities (mean value less than 1 µg/kg for cereals from temperate region

Occurrence of Ochratoxin A

Recent studies on rice from Nigeria have shown the presence of OTA in 66.7% (Adejuyo and William, 2008) and 98% (Makum *et al*., 2010) of rice samples from Lagos markets and Niger State respectively. There was also a wide gap in the levels detected in the two studies. Adejuyo and colleagues detected low levels of OTA (0.01-2.18 ng g-1), whereas Makun and colleagues detected levels as high as 134 - 341ug/kg. Cola nuts (*Cola nitida*) from Nigeria have been shown to contain OTA. Levels as high as 65.3ug/kg and 19.1ug/kg have been recorded in white and red cola nuts respectively (Dongo *et al.,* 2007).

There are no reports in the literature of acute ochratoxicosis in humans. Ochratoxicosis appears to be a well established disease entity in poultry and to a lesser extent in swine (Schaeffer and Hamilton, 1986). Immune suppressive nature (Dwivedi and Burns, 1984) Anemia , nephropathy, teratogenicity and carcinogenicity

Chemical and physical properties

OTA, $C_{20}H_{18}ClNO_6$ (molecular weight: 403.82 daltons), is a phenylalanyl derivative of a substituted isocoumarin. It is listed in Chemical Abstracts' index as L-phenylalanine N-[5-chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1H2-benzopyran-7-yl]carbonyl-(R)-(C.A. No. 303-47-9). The chlorine containing metabolite, designated Ochratoxin A is the major component of extracts from *A. ochraceus* (van der Merwe *et al.,* 1965).

OTA is structurally similar to the amino acid phenylalanine (Phe). For this reason, it has an inhibitory effect on a number of enzymes that use Phe as a substrate, in particular, Phe-tRNA synthetase, which can result in inhibition of protein synthesis. For the same reason, OTA may also stimulate lipid peroxidation. The Ochratoxins, A, B and C occur naturally and are essentially phenylalanine derivatives of an isocoumarin nucleus (Searcy *et al.,* 1993).

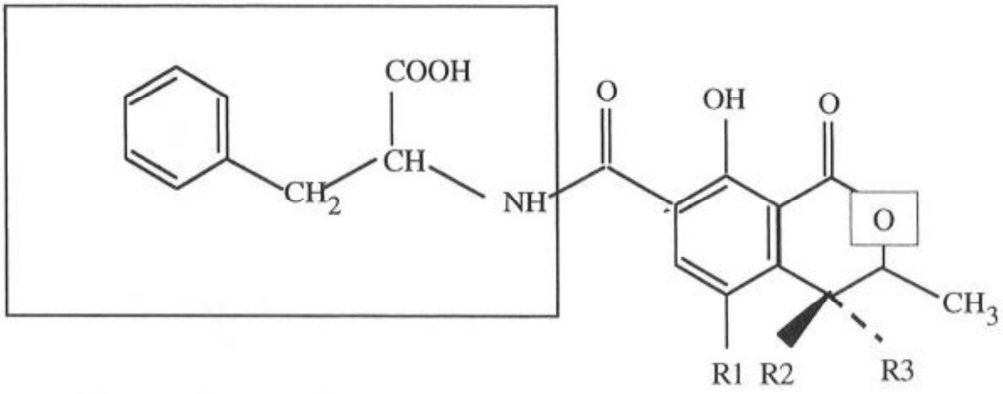

Figure 9: Structure of Ochratoxin

OTA is a colorless crystalline compound, soluble in organic solvents and in alkaline water. It crystallizes from benzene to give a product melting at 90 ºC containing one molecule of benzene. This can be removed under vacuum at 120 ºC to give a substance melting at 168 ºC. It crystallizes in a pure form from xylene. OTA is optically active and exhibits blue fluorescence under UV light, but the ultraviolet spectrum varies with pH and with the solvent polarity. Fluorescence emission is maximum at 467 nm in 96 % ethanol and 428 nm in absolute ethanol (Scott, 1994).

Inhalational and Dermal Exposure to Ochratoxin A

OTA has been identified in studies of water-damaged buildings from air (Wang *et al.,* 2008), dust (Richard *et al.,* 2008), wallpaper (Polizzi *et al*., 2009), and agricultural dust and conidia (Skaug *et al.,* 2001). Hooper et al. have reported elevated concentrations of OTA in the urine of individuals exposed to water-damaged buildings versus unexposed controls (Hooper *et al*., 2009). The concentrations of mycotoxins for controls not exposed to water damaged buildings were below the detection limit which is 2.0 ppb for ochratoxin. In a study by Skaug et al., dust and aerosol samples were collected from three Norwegian cowsheds (Skaug *et al.,* 2001). OTA was detected in 6 out of 14 samples with concentrations ranging from 0.2 to 70 µg/kg (ppb) (Skaug *et al.,* 2001). Collected conidia also contained OTA. The authors concluded that airborne dust and conidia can be sources of OTA and that peak exposures and absorptions from this route can be considerable, especially given the efficiency with which OTA is absorbed through the lung (Skaug *et al*., 2001).

Ochratoxin A can permeate through the human skin (Boonen *et al.,* 2012). Although no significant health risk is expected after dermal contact in agricultural or residential environments, skin exposure to ochratoxin A should nevertheless be limited.

Stability of OTA

OTA is a very stable mycotoxin in different solvents. It can be stored in ethanol for at least one year under refrigeration and protected from light, as photolysis may occur on exposure to fluorescent light (Neeley and West, 1972). It has been reported that OTA solutions in methanol stored at -20 ºC are stable over a period of some years (Valenta, 1998). OTA is a relatively heat-stable molecule and survives most cooking processes to some extent, although the reduction in concentration during heating depends on factors such as temperature, pH and other components in the product. For example, heating wet wheat at 100°C for 2.3 hours gave a 50% reduction in OTA concentration, but in dry wheat, the same reduction took 12 hours.

Processes such as coffee roasting and baking of cereal products and biscuits can produce significant losses in OTA levels, but processes like pasta manufacture produce little reduction. OTA also survives brewing and winemaking and can be found in a variety of processed consumer food products. OTA is destroyed by acid and alkaline hydrolysis and by the action of some oxidizing agents.

Mechanism of action and metabolism

In the blood, OTA binds to albumin and the bound fraction constitutes a mobile reserve of OTA (Benford *et al.,* 2001). The relative contribution of each excretory route is influenced by the degree of serum macromolecular binding as well as differences in the enterohepatic recirculation of OTA (Hagelberg *et al.,* 1989). Ochratoxins primarily affect the enzymes involved in phenylalanine metabolism. They inhibit the enzyme involved in the synthesis of the phenylalanine-tRNA complex. Ochratoxins might also interact with other enzymes that use phenylalanine as a substrate. For example the phenylalanine hydroxylase which catalyzes the irreversible hydroxylation of phenylalanine to tyrosine.

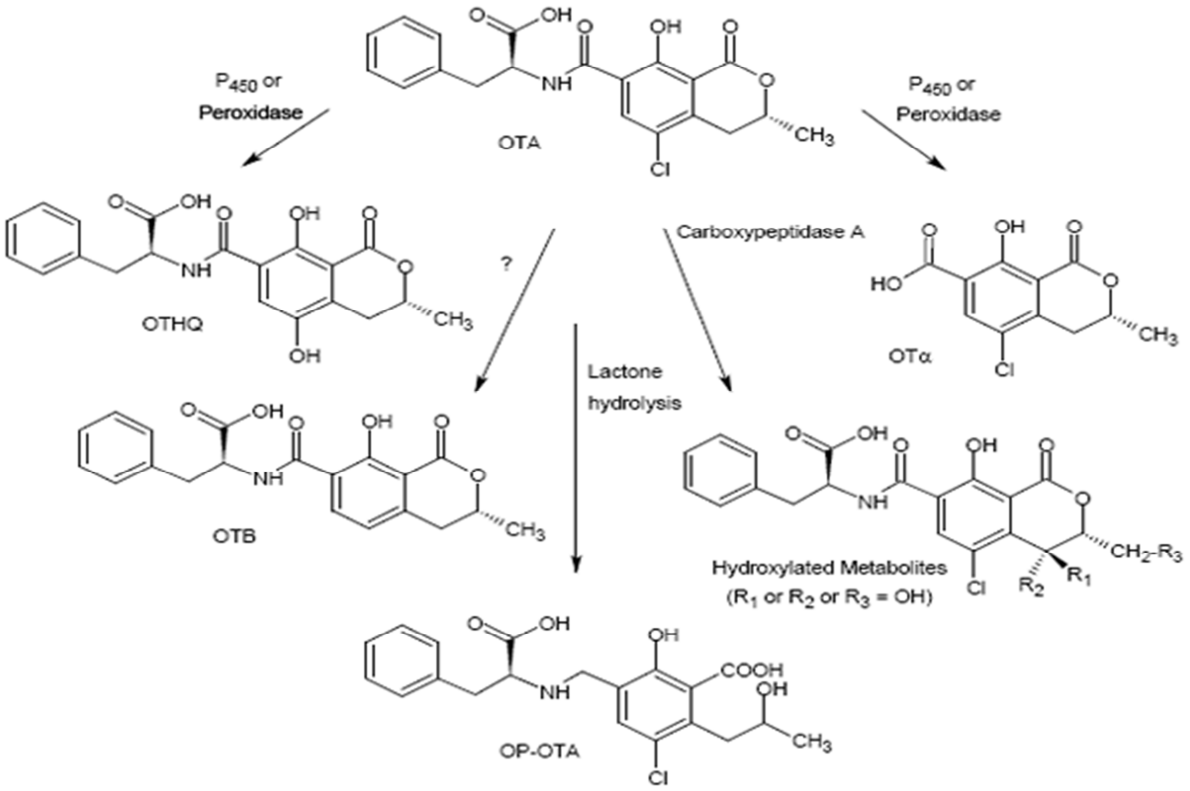

Figure 10: Metabolism of OTA (Source: Pfohl-Leszkowicz *et al.*, 2007).

In addition, it alters the mitochondrial membrane transportation system and inhibits ATP production and enhanced membrane lipid peroxidation and superoxide and hydrogen peroxide radical formation. Inhibition of microflora in the lower GI tract of rats by neomycin results in decreased hydrolysis of OTA to ochratoxin alpha resulting in elevated levels of OTA (Madhyastha *et al.,* 1992). In addition, administration of radiolabeled OTA to rats indicated that effective metabolism of OTA was lacking in most tissues other than the intestines (Galtier *et al.,* 1979).

Absorption

Between 40 and 66% of ochratoxin is absorbed from the gastrointestinal tract depending on the different species. The small intestine has been shown to be the major site of absorption and maximal absorption occurs in the jejunum. Ochratoxins bind rapidly to serum albumin and are distributed in the blood mainly in bound form. Generally, the toxin has a long biological half life due to its high rate of binding to serum protein but there are

differences between species. Therefore the ochratoxin protein adduct in serum can be used as a biomarker for ochratoxins exposure. Intestinal microflora contributes significantly to the metabolism of OTA via hydrolyzation to the less toxic ochratoxin alpha in rats (Galtier, 1978).

Excretion

It primarily accumulates in the kidneys followed by the liver, muscle and fat. Due to this accumulation there has been a concern over the potential carryover into meat. Elimination of OTA in urine and feces is felt to be relatively slow and has been shown to vary by species and gender as well as specific genotype that may affect the biotransformation of OTA (Zepnik *et al.,* 2011). The importance of digestion in the detoxication of OTA is also supported by the observation that OTA does not readily accumulate in ruminants due to rapid detoxification in the extensive ruminant stomach (WHO, 1990). The toxin is excreted primarily in the urine, and to a lesser degree in the faeces, as ochratoxin α or OTA, in bile and also in milk.

Carcinogenesis

Oral administration of OTA produced renal tumours in rats and mice (Boorman, 1989). Moreover, in mice OTA give rise to liver tumours in both sexes (Kuiper-Goodman and Scott, 1989). Nephrotoxic effects have also been demonstrated in other mammalian species. In the early 1970s, observers in Denmark noted a high incidence of nephritis in pigs (Krogh, 1972), a disease known nowadays as Danish porcine nephropaty, which was associated with the use of mouldy rye, and particularly, with the presence of OTA in feed samples. Given that OTA is a kidney toxin in all mammals tested, it would appear prudent to assume it is also a kidney toxin in humans. Particularly, kidney failure rates in rural Scandinavian populations were proved high, and a possible cause was the ingestion of those pig tissues containing excessive levels of OTA (Krogh *et al*., 1976., 1977).

Observational studies have associated OTA with two human disease states:

- Balkan endemic nephropathy (BEN).
- Urothelial tumours (UT).

Apoptosis

OTA also induces apoptosis (programmed cell death) in a variety of cell types *in vivo* and *in vitro* (Seegers *et al*., 1994). The apoptosis is also mediated through cellular processes involved in the degradation of DNA.

Half-life

Ochratoxin A has a half-life of about 35 days in humans and since OTA binds readily to serum albumin, the blood concentrations are considered to represent a convenient biomarker of exposure (Perry *et al.,* 2003).

This bond with serum albumin result in the generation of a mobile reservoir of ochratoxin, which is slowly released and hence rendered bioavailable over extended periods of time and furthermore, retard the elimination of OTA from the body (O'Brien and Dietrich, 2005).

The half-life of experimentally orally ingested OTA is shorter than intravenously injected OTA, as part of the toxin is subjected to a hepatic first-pass elimination and is removed by the bile before it can enter the systemic blood circulation. Following intravenous administration OTA is eliminated with a half-life from body in rats in 3 days, in 3-5 days in pigs (Galtier *et al*., 1981) and in vervet monkeys in 19-21 days (Hagelberg *et al*., 1989., Stander *et al*., 2001). Studer-Rohr (1995) showed human serum half-life of OTA to be 35 days after oral ingestion. Assuming that it takes eight-times the half-life to reach a zero value, a detectable serum level would still be found in humans 280 days after a single uptake

Chapter Five

Implications

Effects of fumonisin B_1

In human

There are no reports on the acute effects of fumonisins on humans, although instances of very high fumonisin concentrations have been reported from home grown maize in South Africa and China (118 –155 mg/kg food).

i Neural tube defects

Neural Tube Defect (NTD) are defects of the brain and spinal cord in the embryo resulting from failure of the neural tube to close. Epidemiological studies and clinical trials have pointed out folate deficiency as a major risk factor for NTD (Blom et al; 2006). FB_1 disrupts sphingolipid metabolism and therefore this could affect folate uptake and cause NTD (Marasas, 1996). In 1990 and 1991 a sudden outbreak of neural tube defects occurred along the Texas-Mexico border. It is believed that this outbreak might have been due to high levels of FB_1 that were observed in corn during previous years (Missmer et al; 2011). Also regions in China and South Africa with high corn consumption show a high prevalence of NTD (Marasas, 1996).

ii Esophageal cancer

It is thought that there is a relationship between the occurrence of *F.verticillioides* and human esophageal cancer. A low socioeconomic status and a less variated diet that mainly exists of corn and wheat, is associated with the appearance of esophageal cancer (Marasas, 1996). Studies showed that a higher level of concentrations of FB_1, FB_2 and *F. verticillioides* are present in corn growing in regions with a high percentage of esophageal cancer.

This is in contrast with regions with low levels of *F.verticillioides*, FB_1 and FB_2 in corn (Wild and Gong, 2009).

iii Acute mycotoxicosis

Acute mycotoxicosis is food poisoning by food products contaminated by fungi. In 1995 an outbreak of disease characterized by diarrhea and abdominal pain occurred in 27 villages in India. This was the result of consumption of moldy sorghum and corn due to rain damage. This outbreak was studied and the mycotoxicosis was connected to consumption of unleavened bread. Corn and sorghum samples were collected from the households and examined. The corn and sorghum were contaminated by *Fusarium* and *Aspergillus* and contained high levels of FB_1 compared with samples of unaffected households (Marasa, 1996).

Toxic effects in animals

Much research has been done on toxic effects of FB_1 in animals. In vivo studies indicated that liver and kidneys are the main target organs (Marasas, 1996). In pigs and rats there is a wide distribution of FB_1 and small amounts have been found to accumulate only in liver and kidneys. In vervet monkeys, some FB_1 is partially hydrolyzed in the gut (Marasas, 1996).

i Porcine pulmonary edema

Porcine pulmonary edema due to FB_1 is intensively studied after the first report in 1981 of swine with pulmonary edema after exposure to corn contaminated with *F. verticillioides*. Alterations in sphingolipid biosynthesis are reported, especially in lung, heart, kindey and liver tissue. Lethal pulmonary edema was developed within 4–7 days after exposure to feed with concentrations of FB_1 >16 mg/kg body weight (>92 ppm). Doses of 10ppm developed a milder form of pulmonary edema (Marasas, 1996).

ii Equine leukoencephalomalacia

Leukoenchephalomalacia (ELEM) is a neurotoxic disease of horses. Outbreaks of this disease in the 20th century resulted in a number of studies. FB_1 induces cardiovascular function, because of the elevated sphinganine/sphingosine ratio.

Effects of aflatoxin B_1

Evidence of acute aflatoxicosis in humans has been reported from many parts of the world, namely the Third World Countries, like Taiwan, Uganda, India, and many others. The syndrome is characterized by vomiting, abdominal pain, pulmonary edema, convulsions, coma, and death with cerebral edema and fatty involvement of the liver, kidneys, and heart. There is also some evidence to suggest that aflatoxin exposure can affect child stunting and lead to greater susceptibility or exacerbation of symptoms associated with human immunodeficiency virus (HIV), tuberculosis, and malaria (Moss, 2008).

Conditions increasing the likelihood of acute aflatoxicosis in humans include limited availability of food, environmental conditions that favor fungal development in crops and commodities, and lack of regulatory systems for aflatoxin monitoring and control.

Effects of ochratoxin A

The evidence in experimental animals is sufficient to indicate carcinogenicity of ochratoxin A. It was tested for carcinogenicity by oral administration in mice and rats. It slightly increased the incidence of hepatocellular carcinomas in mice of each sex and produced renal adenomas and carcinomas in male mice and in rats (carcinomas in 46% of males and 5% of females). In humans, very little histology data are available; however, the incidence of transitional cell (urothelial) urinary cancers seems abnormally high in Balkan endemic nephropathy patients, especially for the upper urinary tract (Castegnaro *et al*., 2006).

Ochratoxin A has a strong affinity for the brain, especially the cerebellum (Purkinje cells), ventral mesencephalon, and hippocampal structures. The affinity for the hippocampus could be relevant to the pathogenesis of Alzheimer's disease, and subchronic administration to rodents induces hippocampal neurodegeneration. Ochratoxin causes acute depletion of striatal dopamine, which constitutes the bed of Parkinson's disease, but it did not cause cell death in any of brain regions examined. Teams from Zheijiang Univ. and Kiel Univ. hold that ochratoxin may contribute to Alzheimer's and to Parkinson's diseases. Nonetheless, their study was performed *in vitro* and may not extrapolate to humans. The developing brain is very susceptible to ochratoxin, hence the need for caution during pregnancy (Pfohl-Leszkowicz and Manderville, 2007).

Economic Implications

Mycotoxins contamination can affect the entire supply chain for susceptible crops.

Control measures (or their absence) taken along the supply chain can directly affect the availability of mycotoxin-free crops to households for both their own consumption and for sale to the consuming public. The sum total of action and inaction can impact all four pillars of food security: availability of food, access to food (by affecting incomes), utilization of food (by affecting what households consume), and stability (in terms of continuity of safe food supply as well as associated price determination) (FAO, 1997).

Mycotoxins contamination impacts three sectors: agriculture, trade, and health.

If crops with very high levels of aflatoxin contamination are consumed by humans, poisoning (i.e., aflatoxicosis) and even death can occur. Chronic exposure to low levels of contamination in crops consumed regularly increases liver cancer risk and can suppress the immune system, particularly for populations that test positive for

the hepatitis B virus (HBV). Aflatoxins can also enter the human diet through livestock products if the livestock are given contaminated feed. Children can be affected through breast milk or direct consumption of weaning foods.

Aflatoxin contamination can also lead to rejection of specific export shipments and increased inspection and sampling rates. If plant quarantine authorities perceive the contamination as chronic, they can curtail the right of countries to export susceptible products. These effects on trade result in lost revenues. Economic losses to producers and traders can also occur in the domestic market if either consumer awareness about the problem rises, leaders in marketing channels begin to pay more attention or regulations is tightened or more strictly enforced. Thus, aflatoxin contamination can adversely affect both individual livelihoods and agricultural sector output (Mutegi *et al*., 2009).

Mycotoxins disproportionately impact women and the poor

Food-insecure households are more likely to consume contaminated food rather than sell it at lower prices or discard it. The poor may also not be able to adopt costly control strategies. Even a well-intentioned awareness campaign can reduce prices for mycotoxin-contaminated food, resulting in direct market losses for the poor and more severe health impacts because of farmers' own consumption of low-price-yielding, contaminated grain. Women are also less likely to have access to information and resources for mycotoxin control and mitigation. Lack of decision making power with women may inhibit adoption of mitigation strategies even if information and resources are not a constraint.

Implications for Agriculture and Food Security

Mycotoxin contamination can reduce the volume, value, and availability of safe, locally produced, or imported food. However, because contamination often does not cause visible damage to the crop, the perceived impact of mycotoxin contamination on agriculture and food security has so far been negligible. The market does not differentiate between mycotoxin-free and mycotoxin-contaminated food; therefore farmers do not incur any costs for mitigating mycotoxin. This in turn results in increased risk that mycotoxin-contaminated grains leave the farmers' fields and enter the food and feed supply (Abt Associates, 2013).

The lack of awareness among growers and buyers, combined with the often unobservable effects of mycotoxin, make it very difficult to incentivize and inform farmers of the risks associated with mycotoxins. Farmers are not aware of mycotoxins, or of measures to control mycotoxins in the field, which begin with good agricultural practices (GAP). There is no set ―agenda‖ for agricultural extension messaging about GAP and food safety that includes aflatoxins fumonisins and others. Farmers rarely incur losses for mycotoxin-contaminated grain (or realize premiums for mycotoxin-free grain) due to lack of trader and consumer awareness

Implications for Domestic and International Trade

The exact figures for world economic losses resulting from mycotoxin - contamination may never be available. Apart from the obvious losses of food and feed, there are losses caused by lower productivity; losses of valuable foreign exchange earnings; costs incurred by inspection, sampling and analysis before and after shipments; losses attributable to compensation paid in case of claims; farmer subsidies to cover production losses; research, training and extension programme costs; costs of detoxification; etc. When combined, these costs may be staggering.

Nigeria's NAFDAC enforces commodity standards and does laboratory testing to detect aflatoxins. However, this is only done for packaged foods and foods bound for the formal export market. The vast majority of foods consumed by the Nigerian population are not regulated for aflatoxins. Field research conducted by Abt Associates in the Niger, Kogi, and Ondo states found no evidence of testing for aflatoxins in the domestic maize and groundnut markets in Nigeria. Consequently, aflatoxin-contaminated grain can enter the domestic markets and the informal international markets (e.g., Chad and Niger for maize) due to low awareness about aflatoxins and their health impact among consumers and sellers (Abt Associates, 2013).

There are no regulations governing aflatoxins in animal feed. However, some medium- and large-scale commercial growers of maize and groundnuts, as well as animal and fish feed markets, are vigilant about aflatoxin levels in the feeds due to observable effects of aflatoxins on animals (e.g., negative impacts on productivity and reproduction in poultry, even sudden death). Some of the large-scale livestock growers

Control and Prevention of Mycotoxins

Due to their risks to human and animal health, guidelines for fumonisin content in maize products intended for food and animal feed have been implemented in a number of countries (Van et al; 2007). In South Africa, legislation on tolerable levels for fumonisins generally does not exist or is not enforced (Shephard, 2008; Wagacha and Muthomi, 2008). Therefore, a substantial portion of the maize crop in many parts of southern Africa could be affected when environmental condition does not favor fumonisin accumulation in grain. Thus, there is a need to control ear rots of maize to obtain good quality grain and to reduce the potential health effects associated with consumption of fumonisin contaminated grain.

The most effective approach to controlling *F. verticillioides* ear rots and minimizing the risk of fumonisin accumulation in grain is the development of host resistance. Host resistance would provide producers

with environmentally sound and economical control of Fusarium ear rot and fumonisins. Genetic variation for resistance to Fusarium ear rot has been investigated for a number of decades (Butron et al; 2006). Heritable resistance has been identified in maize (Afolabi *et al*; 2007) but no highly resistant genotypes suited to the production regions in South Africa, where maize is the preferred staple food, are known (Aquino et al, 2001).The potential problems associated with Fusarium ear rot and fumonisin contamination of maize could be averted by proactively screening maize cultivars for resistance to *F. verticillioides* and fumonisin. In addition, sources of resistance should be identified in maize germplasm, preferably in locally adapted inbred lines, for use in breeding programs. Existing cultivars in South Africa are not known to have sufficient resistance to Fusarium ear rot or fumonisin contamination (Rheeder *et al*., 1990), and limited information is available for inbred lines used in South Africa.

Agronomic approaches such as avoiding water stress, minimizing insect infestation and reducing inoculum potential have been suggested and are effective when the farmers can implement such practices. Following good agricultural practices during both pre-harvest and post-harvest conditions would, minimize the problem of contamination by mycotoxins such as aflatoxins, ochratoxin and trichothecene mycotoxins. These include appropriate drying techniques, maintaining proper storage facilities and taking care not to expose the grains or oilseeds to moisture during transport and marketing. The method of segregating contaminated, mouldy, shrivelled or insect-infested seeds from sound kernels has been particularly useful in minimizing aflatoxin contamination in peanuts.

Mycotoxin control measures have been implemented for agricultural commodities entering international trade or located in countries with centralized or large-scale buying and distribution systems. However, in developing countries, where local food consumption or subsistence agriculture is practiced by as much as 70 percent of the population, such measures would be difficult to implement. Because of the stringent mycotoxin control measures being maintained by those countries importing food grains and oilseeds, and because of the need for exporting countries to earn foreign exchange, the best of the commodities are often sold abroad while the substandard or contaminated commodities are retained for domestic use. Such a situation exacerbates the risk to human and animal health

Detoxification

The inhibition of ceramide synthase by FB_1 is thought to be reversible, since the binding is formed by noncovalent interactions. Factors that will probably induce this reversibility are reduction of cellular FB_1-concentration and increasing of cellular concentrations of the substrates for ceramide synthase (Merrill et al; 2001). Also, the rate of removal of the accumulated sphinganine and sphingosine will affect the detoxification. The information on metabolism and biotransformation of FB_1 is very sparse. However, metabolism most likely occurs in the gut since partially hydrolysed and fully hydrolysed FB_1 were recovered in faeces but not in bile of Vervet monkeys (EC, 2000). Bioavailability of FB_1 can be reduced by treating fumonisin-contaminated corn with glucomannans extracted from the cell wall of the yeast Saccharomyces cerevisiae. These polysaccharides are able to bind certain mycotoxins and have a 67% binding capacity for fumonisins (Yiannikouris and Jouany, 2002).

Detoxification of fumonisins in foods and animal feeds has been attempted in the past. For instance, Senegal has been operating commercial facilities to detoxify peanut cake contaminated with aflatoxins by the ammonia process. Any detoxification procedure must be tested for safety and efficacy and invariably results in increased handling and costs. In addition, the detoxified product has been considered suitable only for animal feed purposes and not for human consumption. Several countries have introduced legislation concerning mycotoxins. Most of this legislation pertains to aflatoxins, ergot alkaloids, deoxynivalenol and ochratoxins. Although various legislative measures have yet to be harmonized among countries, the Codex Alimentarius Commission is making efforts to establish international guideline levels for mycotoxins, and aflatoxins in particular.

Chemical carcinogenesis and chemoprevention

Cancer chemoprevention also known as anti-carcinogenesis is the process of exposure of an animal to a substance that will reduce the incidence of cancer that would otherwise develop. In chemoprevention, it is desirable to be able to identify and test compounds or agents effective against a specific carcinogenic mechanism. While this field is gaining an increasing and sustained merited attention in the developed countries, it has received very little attention in the rapidly industrializing developing countries where the incidence of cancers appears to parallel the pace of industrialization (Anetor et al; 2008).

Over 67% of cancers are now attributed to environmental factors of which chemicals occupy a predominant proportion (Lichtenstein et al., 2000). Majority of chemical carcinogens

Challenges

In developing countries there is little doubt that high levels of exposure of people to food-borne mycotoxins is a serious threat to public health. It is a developmental issue, which embraces childhood survival, demographics, immune system function, the economic and human resource drain due to cancers, as well as food security where

livestock feeds are contaminated.

Research is needed on inexpensive and appropriate sampling and testing protocols. Research on identification and application of appropriate technologies for obtaining low grain moisture at harvest and maintaining low grain moisture during storage are needed. Research is needed on traditional food preparation technologies, such as fermentations and nixtimalization, or chelating additives such as clays or yeasts that may lower mycotoxins in prepared foods. Research must continue to develop crop plant cultivars that are resistant (or at least not susceptible) in the field to infection by mycotoxin-producing fungi. Breeding for high yield alone is not enough.

Control and Prevention Steps are summarized below

Intervention Strategies:

1st Strategy: STOPPING THE INFECTION PROCESS

i) Breeding for resistance

screening maize cultivars for resistance to *F. verticillioides aflatoxin, ochratoxin* and fumonisin producers

ii) Biological control using

a) afla-guard and AF36 in USA, aflasafe
b) use of an endophytic bacterium - *Bacillus mojavensis* (Bacon and Hinton, 2000).
c) iodine-based product called Plantpro 45. as a biocompatible control of the fungus. (Yates *et al*., 2000)
d) use of non-producing strains of *F. verticillioides* aiming to minimise fumonisin levels in maize (Plattner et al., 2000).

2nd Strategy: CONTROL OF ENVIRONMENTAL FACTORS

3rd Stategy: PRE HARVEST CROP MANAGEMENT PRACTICES

- a) fertilizer use
- b) fungicide application

4th Strategy: POST HARVEST CROP MANAGEMENT PRACTICES

a) early harvesting,
b) proper drying,
c) sanitation,
d) proper storage
e) insect management
f) surveillance and awareness creation.
g) Fumigation:

- ethylene oxide and methyl formate reduce the incidence of fungi in groundnuts and melon seeds
- sodium chloride, propionic acid, acetic acid inhibits aflatoxin B1 production in *A. flavus*

Challenges and Current needs

- Need for efficient, cost-effective sampling and analytical methods
- Children and old people with low immunity most affected
- HIV and Hepatitis patients predisposed
- Too costly to do analysis

Recommendations

- Research on fungal carcinogens must continue to have a high priority.
- Eat plant-based foods, fibers, lean meat (fish, chicken, turkey), antioxidants, muchroom, green tea, cabbage and fresh garlic
- Know what carcinogens you work with
- Get screened
- Be conscious of chemicals around your home and use them properly.

Conclusion

This brief was designed to share the results in Nigeria of a country and economic assessment based on a new methodology for assessing the situation, outlook, and needs of any developing country. Its purpose was to establish the evidentiary basis for policy and institutional reform, then stimulate regulatory improvement and concerted action by both public and private stakeholder groups, and ultimately to foster behavioral change by actors within value/supply chains, as well as consumers.

While solutions for aflatoxin control are readily available at all stages of food production, resources are scarce in comparison to the many development challenges that Nigeria faces. Interventions must be prioritized based on country-led perceptions of risk to vulnerable populations, reward in terms of prevention or mitigation, capacity to pay, and degree of political and institutional support.

Mitigation strategies should be multi-sectoral in nature, supported by relevant public and private sector

institutions and respected professionals that represent plant and animal agriculture, human and animal health, and trade. Ideally, their actions should be coordinated through an entity that can meld and reconcile competing interests, champion the cause, and provide continuity of attention over time. The recent stakeholder workshop held in Abuja, which concluded the country and economic assessment effort undertaken by Abt Associates Inc., gave participants the opportunity to review the country assessment findings and discuss recommendations for the country.

Definition of Terms

- Carcinogens: A carcinogen is any substance involved in causing cancer due to its ability to damage the genome or disruption of cellular metabolic processes eg Radon, aflatoxin
- Mutagens: agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms. most mutagens are also carcinogens
- Cancer: a consequence of multiple genetic alterations arising from inherited mutations in germ cells or as a consequence of mutations in somatic cells, resulting in altered growth
- Mycotoxin: chemical substances that contaminate agricultural commodities, either before or after harvest. E.g trichothecenes and fumonisins.

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