BACKGROUND

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world with 80% of cases occurring in developing countries. The incidence of HCC is increasing, ranging between 3% and 9% annually depending on the geographical location, and variability in the incidence rate correspond closely to the prevalence and pattern of the primary etiologic factors. According to recent reports, the incidence of HCC has increased sharply in the last 5–10 years with an especially high incidence in Egypt. This has been related to several biological factors e.g. hepatitis B and C virus infections. While HBV and HCV may account for the majority of HCC in Egypt, there is another suggestive etiologic role in hepatocellular carcinoma including environmental factors (e.g. aflatoxins, AF), other factors such as cigarette smoking, occupational exposure to chemicals such as pesticides, and endemic infections in the community, such as schistosomiasis, may have additional roles in the etiology or progression of the disease.[1,2]

Location and environmental condition in Egypt make it prone to aflatoxins, Egypt is located in North Africa and Southwest Asia. The majority of the country is located in northeastern Africa, but its Sinai Peninsula extends out into Southwest Asia, connecting the two continents. Egypt has water boundaries along the Mediterranean Sea and the Red Sea. The country of Egypt is in the Africa continent and the latitude and longitude for the country are 28.8013° N, 31.1711° E a region where aflatoxins spread. The exposure to aflatoxins and development of HCC in Egypt was less clear in the past, but recent food surveys indicated that both local and imported samples were positive for aflatoxin B_1 (AFB_1). The level of AFB_1 was dependent on the area of collection and the season of the year.[2]

Studies suggest that aflatoxins are expected to spread and become more problematic with future climate conditions. There are many climatic reasons for an increase in aflatoxins, including an increase in temperature , humidity and moisture.[3]

Aflatoxins are a group of mycotoxins produced in tropical and sub-tropical regions.[4]. Aflatoxins are hepatic and carcinogenic secondary metabolities of moulds that produce mainly from *Aspergillus flavus* and *Aspergillus paraciticus*. Aflatoxins contaminate a variety of agricultural commodities in countries with hot and humid climates.[2] Host crops are particularly susceptible to infection by *Aspergillus* following prolonged exposure to a high-humidity environment, or damage from stressful conditions such as drought, a condition that lowers the barrier to entry. Cereals are a very important part of human diets particularly in developing country as Egypt. Cereals such as rice (*Oryza sativa L.)*, wheat (*Triticum aestivum L.*) and maize (*Zea mays L.*) are members of the grass family *(Poaceae or Gramineae)* and they are particularly important to humans because of their role as staple food. The grass family offers many important economic and ecological features including food, forage, fiber (paper and rope), renewable energy and oil.[5]

The three major species, wheat, maize and rice, account for a large proportion of the calories and protein in human diets. The importance of cereals in the food chain is also attributable to the extensive use of cereals in the diets of animals. The major constituents of cereals are the carbohydrates and proteins. Other grain components such as lipids and

vitamins may be of great significance in human nutrition because of the large contribution of cereals to the diet. Cereals are prone to a range of diseases caused by micro-organisms, predominantly fungi, which can attack the roots, stems, foliage and/or the ear, causing substantial losses of yield and frequently having a detrimental effect on grain quality. The presence of disease in an otherwise healthy crop is first recognized in the field by the appearance of well defined symptoms resulting from earlier activity on the part of the pathogen. If the disease is allowed to progress then the pathogen itself becomes more obvious, but often more difficult to control at this stage of its development. Grain quality is largely determined during the growing season. Once the grain has been harvested it is difficult to improve its quality, although quality can easily be destroyed by conditions during harvest and subsequent drying and storage. Indeed grain quality can start to deteriorate in the field prior to harvest. Rainfall prior and during the harvest period can encourage ear diseases and premature sprouting.[6]

Occurrence of mycotoxin in cereal foodstuff is unavoidable.[7] The attendant challenges to crop production, yield and quality loss is about 25% annually.[8] The mycotoxin contamination of cereal commodities is a much older problem than its detection and characterization. Molded grains often caused different animal and human health problems long before the causal agents were known. The first mycotoxin detected is the aflatoxin B_1 in the 1960s and since then hundreds of toxins have been described.[9]

Aflatoxin B_1 is the most potent hepatocarcinogen known in animals and it is classified by the International Agency of Research on Cancer (IARC) as Group I carcinogen,[10] meaning that it is a proven cancer-inducing agent. It also occurs in the environment contaminating a lot of different food and feed commodities.

The importance given to AF is because of its discovery after a condition observed in Britain called Turkey X disease, where it was associated with deaths of tens of thousands of turkey poults and many of the symptoms of the disease fit those of cyclopiazonic acid (CPA) and after analysis of the groundnut that used to produce feed for these birds it was found that it contains (CPA) in addition to AFB_1.[11]

The mycotoxins most commonly associated with cereal grains are aflatoxins, ochratoxin A, fumonisins, deoxynivalenol and zearalenone. The mycotoxins that commonly occur in cereal grains and other products are not completely destroyed during food processing operations and can contaminate finished processed foods. Very high temperatures are needed to bring about much of a reduction in mycotoxin concentrations.[12] As mycotoxins are usually classified as stable compounds, the different processes which are conducted on the grains before they are consumed, such as cooking at normal temperature ranges (less than 150^{o}C), do not decrease toxicity levels in the majority of cases.[13]

Practical decontamination procedures must: inactivate, destroy, or remove the toxin, not produce or leave toxic residues in the food/feed, retain the nutritive value of the food/feed, not alter the acceptability or the technological properties of the product, and, if possible, destroy fungal spores.[14]

The stability of aflatoxins with respect to different physical and chemical agents is well known. Possible interventions include biological control, chemical control, decontamination, breeding for resistance as well as surveillance and awareness creation.[15]

Natural methods of aflatoxins removal are cost effective.[16] Chemical methods led to a number of environmental and health problems because they are themselves carcinogenic, teratogenic, and highly toxic with long degradation periods.[17] Using of heat for the mycotoxins destruction is ineffective because the temperatures reached during the detoxification process affect vitamins and food proteins. Auoclaving at 120^{o} for up to 1h does not destroy this mycotoxin and even after sterilization in an acid or alkaline medium, slight mutagenic activity is still detectable in vitro. The stability of this mycotoxin is, however limited in highly polar solvents. UV light are also able to stop biological activity but these sources of radiation have a low penetration capacity in solid and liquids.[18] Therefore, this study evaluates the action of gamma rays on aflatoxin B_1 stability.

Food irradiation is a mechanized process of exposing food stuff to carefully controlled amount of energy in the form of high-speed rays. The choice of the mode of irradiation operation depends on the type of products, quantity of products, size, shape, density and the required dose preventing microbial contamination, extending shelf-life under recommended conditions of storage; and overcoming quarantine barriers in international trade. It is a fast treatment and its efficacy is not temperature dependent. It is a safer and better alternative to chemical fumigation.[19]

Unlike methyl bromide, irradiation is not an ozone depleting substance. It does not leave toxic residues on treated products. It meets quarantine requirements reported in IAEA series. Irradiation technology is easy to apply, clean, and environment-friendly. It is a direct, simple and efficient on-line process. Gamma irradiation has been implicated in eliminating micro-organisms in oil-rich and starch rich foods.

Gamma radiation is an emerging new technique in a number of countries where consumption of irradiated food (Appendix 1), whether needing processing or at the ready-to-eat stage, is of major concern. Interest in irradiation due to high food infestation losses and spoilage is increasing concern due to food borne illness as well as growing international trade in food commodities that must meet strict quarantine quality standard.[19]

REVIEW OF LITERATURE

Aspergillus (Appendix 2) is a fungus that essentially belongs to grains storage flora. The optimium temperature for fungal growth is at 25 °C with a minimum water activity of 0.75. It starts to produce secondary metabolites at 10-12 ºC, but the most toxic ones are produced at 25°C with a water activity of 0.95.[20] 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. pseudo tamarii*, *A. parvisclerotigenus*, and *A. bombycis* of section *Flavi*, *A. ochreceoroseus* and *A. rambellii* from section *Ochraceorosei* and *Emericella astellata* and *E. venezuelensis* from *Nidulatans* section also generate aflatoxins.[21] 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 developing countries.[22]

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.[23] 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.[4]

1. Historical background.

It is difficult to discuss the discovery of the aflatoxins. It began as a veterinary problem. Moulds of many types have been recognized as spoilage agents of different foods and animal feeds for a long time, the growth of mould was associated with changes in texture, colour, or flavour and when mould appeared in certain foods, such as bread, the mouldy portion was often removed and the rest was considered satisfactory. Due to the recognition that penicillin and other antibiotics are effective in treating infections, many people understood that fungal metabolites and fungi were salutary or benign. Before this decade, studies of fungal contamination centered on economic aspects of spoilage rather than on the health hazards. Many reports suggesting that some illnesses, or even deaths, of certain farm animals were associated with ingestion of mouldy feed, but mostly the toxicoses were not wide spread and not of major economic significance. Forgacs and Carll (1962)[24] referred to mycotoxicoses as the neglected diseases and they wrote "Scientists tend to approach the causes of animal diseases through a process of elimination: if the causal agent is not found to be bacterial, viral or nutritional, it is concluded to be chemical in nature". The possibility that the source of such toxic chemicals may be fungal was usually ignored, This situation changed dramatically with the development of a new disease in England in 1960 which was termed the 'Turkey-X disease' after 100,000 turkeys died in England from liver acute necrosis and bile duct hyperplasia after consuming groundnuts infected with *Aspergillus flavus*, these dramatic reports of thousands of mortalities focused attention upon the practical problem, and stimulated the investigation of scientific community not only of veterinarians but also of biologists, chemists,

microbiologists, and other scientists of other disciplines. Focus on Turkey-X disease provided a fascinating illustration to the solution of an important problem.[11,23]

It is reported that no known microorganism had been isolated and no biological transmission, so the possibility was remained that the birds were being 'poisoned' and all attempts associated the disease with the presence of organic or inorganic poisonous plant material.[11]

During this time a test was developed using young ducklings. These were found to be suitable to evaluate the toxicity associated with death or the appearance of characteristic liver lesions. About this time reports were received from Kenya and Uganda of severe losses of ducklings and examination revealed the presence of characteristic histological lesions in the liver. These ducklings had been fed peanut meal processed locally from peanuts grown in Uganda and Tanganyika. This was the first indication that it was not only Brazilian groundnut meal that could cause the disease. A test was performed to monitor the extraction and concentration of the toxin through classical procedures. Toxic extracts emitted a characteristic bright blue fluorescence when illuminated under ultraviolet light. Furthermore the amount of fluorescent material, as estimated visually generally afforded a guide to the toxicity of the sample. Thus was provided for the first time the basis for routine chemical assay of the toxin. Using these tests, it was soon found that feedstuffs from many countries were sometimes contaminated and that the toxin was not confined to ground nuts.[25]

Reports are made during 1960 suggested that the nature of the toxin might be of fungal in origin. Sargeant and his co-workers (1961)[26] in their epoch made a report to explain the isolation of the toxin-producing fungus, identified by J. J. Elphick as *Aspergillus flavus* Link ex Fries, from toxic kernels obtained from Uganda, Later the toxin was given the name 'Aflatoxin' in view of its origin.

In December16, 1961 appeared the first report made by Lancaster and his colleagues (1961)[27] which explained that; after six months feeding of 20% (toxic) Brazilian groundnut meal in a purified diet, nine out of eleven rats developed multiple liver tumours, and two of these had lung metastases. This finding indicates that this diet is carcinogenic. This explains the problem and the results have been amply confirmed.

Researchers studied lots of ways to fight against this threat; but after more than a half century, aflatoxins are still a big problem that is difficult to deal with, because humans are not able to manipulate essential factors that affect aflatoxin contamination like the region weather, the crop genotype, the soil type, the minimum and maximum daily temperatures and the daily net evaporation.[23]

2. Significance of Mycotoxin Contamination.

2.1 Mycotoxins and Aflatoxins:

Mycotoxins are commonly found in foods and feeds all over the world. It has been estimated that a quarter of the world's crops are contaminated to some extent with mycotoxins.[28] Mycotoxins considered as the most important noninfectious, chronic dietary risk factor, higher than synthetic contaminants, plant toxins, food additives, or pesticide residues.[29] Mycotoxins are low molecular weight secondary metabolites

produced by filamentous fungi that display various degrees of toxicity to vertebrates, invertebrates, plants and microorganisms.[30]

2.2 Types of mycotxins:

Table 2.1 Types of fungi producing mycotoxins and effects on health.[9]

Toxins	Producing fungi	Toxicities
Aflatoxins	*A. flavus, A. parasiticus*	Hepatocellular carcinoma and fatty liver
Citreoviridin	*Penicillium virdicatum*	Cardiac beri-beri
Citrinin	*Penicillium vindicatum Penicillium citrinum*	Nephrotoxin
Cyclocholrotine	*Penicillium islandicum*	Hepatotoxin
Cytochalasin E	*A. clavatus*	Cytotoxicity
Maltoryzine	*A. oryzae*	-
Ochratoxins	*A. ochraceus*	Hepatotoxin
Rubratoxin	*Penicillium rubrum*	Liver hemorrhage and fatty infiltration
Tremorgens	*Penicillium and Aspergilllus*	Hepatocarcinogen
Trichothecenes	*Fusarium graminearum*	Cytotoxicity
Vomitoxin (Deoxynivalenol)	*Fusarium graminearum*	Vomiting
Zearalenone	*Fusarium*	Hyper-estrogenic effect Teratogenic effect
Patulin	*Penicillium-expansum* *Penicillium patulum*	Brain and lung hemorrhage Carcinogenicity
PR toxin	*Penicillium requeforti*	-
Rugulsion	*Penicillium islandicium*	Nephrosis and liver damage
Steirgmatocystin	*A. flavus and A. versicolor*	Hepatocellular carcinoma

2.3 Properties of aflatoxins:

2.3.1 Physical properties of aflatoxins:

Aflatoxins are produced both under moderate and under subtropic and tropic climatic conditions. It is a physically and physico-chemically stable molecule, e.g. temperatures more than 250 °C are necessary for effective destruction.[31] When exposed to ultraviolet light aflatoxins are viewed as a complex array of fluorescent compounds they emit fluorescence (B = blue and G =yellow-green) and are named accordingly (B_1, B_2, G_1, and G_2).[32]

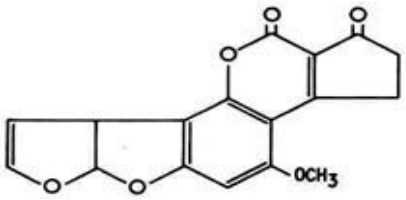
Aflatoxin G_1, B_2 and G_2 are present in small relative amounts, whereas B_1 is usually present in largest yield. The molecular formula of aflatoxin B_1 was established as $C_{17}H_{12}0_6$, and of aflatoxin G_1 as $C_{17}H_{12}0_7$; aflatoxins B_2 and G_2 were found to be the dihydro derivatives of the parent compounds, $C_{17}H_{14}0_6$ and $C_{17}H_{14}0_7$. Some physical properties of the compounds are summarized in Table 2.2. Structures based largely on interpretation of spectral data were proposed for aflatoxins B_1 and G_1 in 1963 and for B_2 and G_2 shortly thereafter. These are shown in (Figure 2.1). The proposed structure of G_1 has been supported by X-ray crystallography.

These closely related compounds are highly substituted coumarins, and the presence of the furocoumarin configuration places them among a large group of naturally occurring compounds with many pharmacological activities. The spectral characteristics of the aflatoxins have been determined by several investigators the ultraviolet absorption spectra are very similar, each showing maxima at 223, 265, and 363 nm. The molar extinction coefficients at the latter two peaks, however, demonstrate that B_1 and G_2 absorb more intensely than G_1 and B_2. Because of the close similarities in structural configuration, the infrared absorption spectra of the four compounds are also very similar.

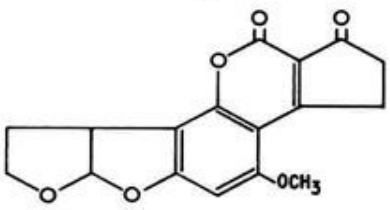
Table 2.2 Molecular formulas, Molecular weights and Melting points of some aflatoxins.[33]

Aflatoxin	Molecular formula	Molecular weight	Melting point
B_1	$C_{17}H_{12}O_6$	312	268-269
B_2	$C_{17}H_{14}O_6$	314	286-289
G_1	$C_{17}H_{12}O_7$	328	244-246
G_2	$C_{17}H_{14}O_7$	330	237-240

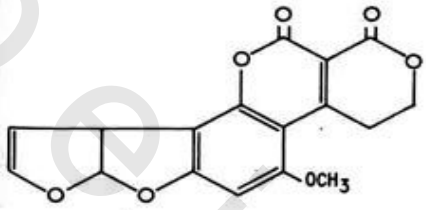
The fluorescence emission maximum for B_1 and B_2 has been reported to be 425 nm and that for G_1 and G_2 is 450 nm. The intensity of light emission, however, varies greatly among the four compounds, a property of significance in the estimation of concentrations of the compounds by fluorescence techniques.



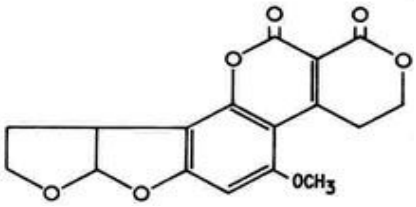
Aflatoxin B_1



Aflatoxin B_2



Aflatoxin G_1



Aflatoxin G_2

Figure 2.1: Chemical structures of some aflatoxins.

2.3.2 Chemical Properties:

The chemical reactivity and behavior of the aflatoxins has received relatively little systematic study beyond work associated with structure elucidation. However, it has been shown that catalytic hydrogenation of aflatoxin B_1 to completion results in the uptake of 3 moles of hydrogen with the production of the tetrahydrodeoxy derivative. Interruption of the hydrogenation procedure after the uptake of 1 mole of hydrogen results in the production of aflatoxin B_2 in quantitative yield.

Aflatoxin B_1 has also been reported to react additively with a hydroxyl group under the catalytic influence of a strong acid. Treatment with formic acid-thionyl chloride, acetic acid-thionyl chloride or trifluoroacetic acid results in addition products of greatly altered chromatographic properties, but relatively unchanged fluorescence characteristics. Ozonolysis results in fragmentation of aflatoxin B_1, and the products of this reaction include levulinic, succinic, malonic, and glutaric acids. The presence of the lactone ring makes the compound labile to alkaline hydrolysis, and partial recyclization after acidifycation of the hydrolysis product has been reported.

Although few systematic studies have been carried out on the stability of the aflatoxins, the general experience would seem to indicate that some degradation takes place under several conditions. The compounds appear partially to decompose, for example, upon standing in methanolic solution, and this process is greatly accelerated in the presence of heat and light.

Substantial degradation also occurs on chromatograms exposed to air and ultraviolet or visible light. These processes may give rise to some of non aflatoxigenic fluorescent compounds typically seen in chromatograms of culture extracts.[34]

2.4 Formation of aflatoxins:

Aflatoxins constitute a family of acutely toxic, teratogenic, potent carcinogenic, and mutagenic metabolites produced by certain strains of common molds *A. flavus* and *A. parasiticus*. Recently, some strains of *A. nomius* and *A. tamarii* were also reported to produce aflatoxins. Sterigmatocystin (ST) is also a toxic and carcinogenic intermediate in the aflatoxin biosynthetic pathway but is not as potent as aflatoxins, and ST is produced by strains belonging to 20 species *Aspergillus.* Food contam- ination by aflatoxins and ST can seriously and adversely affect the health of animals and humans. The biosynthetic pathway of aflatoxins is mostly known, and (Figure 2.2) shows the latter part leading to formation of aflatoxins. B_1 (AFB_1), G_1 (AFG_1), B_2 (AFB_2), and G_2 (AFG_2) are major, naturally occurring substances. AFB_1 and AFG_1 contain dihydrobisfuran rings, and AFB_2 and AFG_2 contain tetrahydrobisfuran rings in their moiety. In the pathways, AFB_1 and AFG_1 are produced from demethylsterigmatocystin (DMST), and AFB_2 and AFG_2 are produced from dihydrodemethylsterigmatocystin (DHDMST). These different bisfuran rings are produced at the branching step between versicolorin B and versicolorin A, and common enzymes are suspected of being involved in independent pathways leading to the formation of AFB_1/AFG_1 and AFB_2/AFG_2. DMST and DHDMST have two hydroxyl groups in their molecules; the C-6-OH groups among them are first methylated by *O*-methyltransferase I (MT-I) to produce ST and dihydrosterigmatocystin (DHST), and the remaining C-7-OH groups are then methylated by *O*-methyltransferase II (MT-II) to make *O*-methylsterigmatocystin (OMST) and dihydro-*O*-methylsterigmatocystin (DHOMST). This methylation sequence is strictly determined by either enzyme substrate specificity.[35]

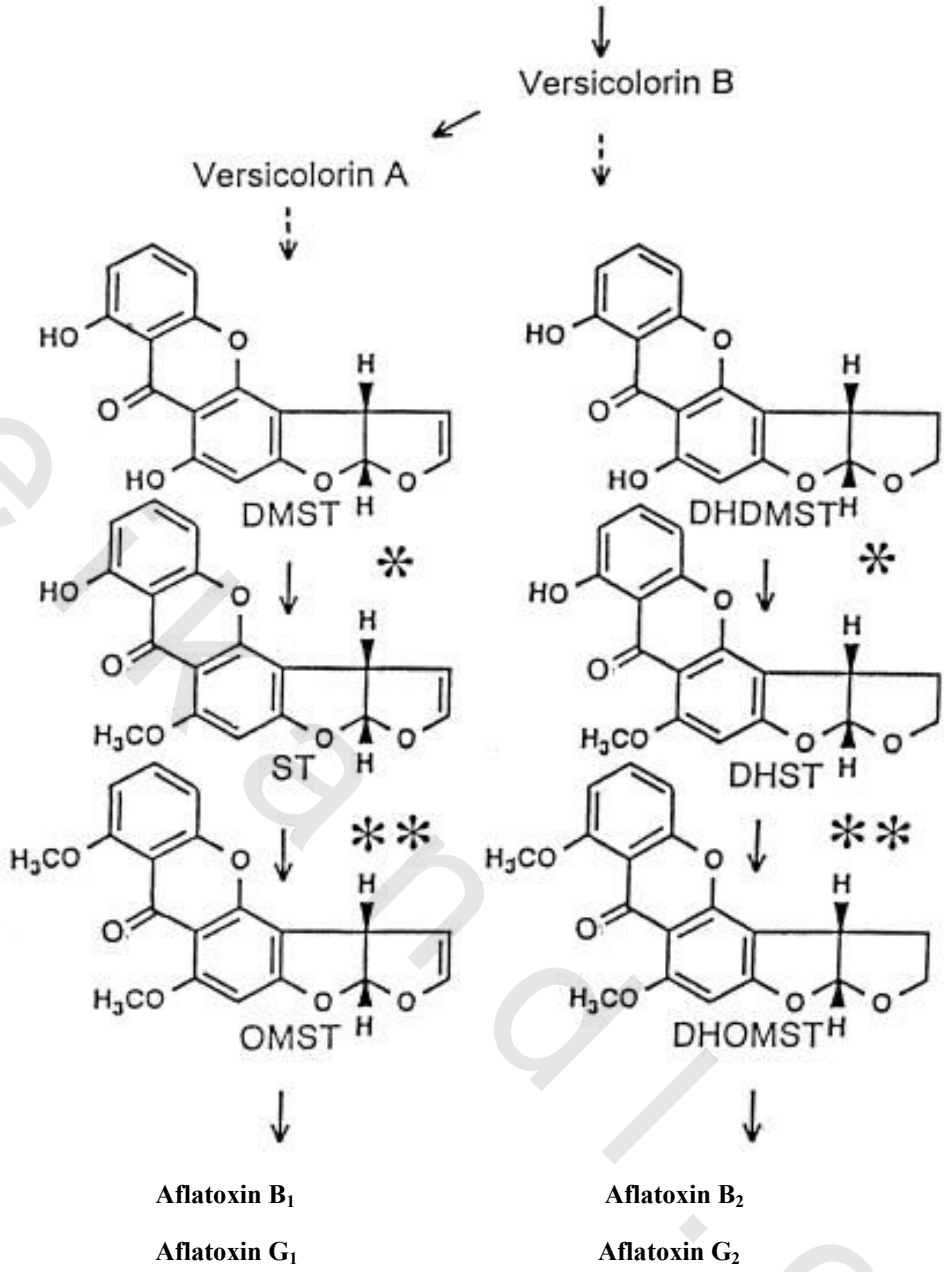


Figure 2.2: Metabolic scheme for aflatoxins (AFB_1, AFB_2, AFG_1, and AFG_2).
Biosynthesis showing structures of critical intermediates. Reactions catalyzed by MT-I are indicated by single asterisks, and those catalyzed by MT-II are indicated by double asterisks.

2.5 Aflatoxin in cereals:

Cereals are the fruits of cultivated grasses and members of the grass family *Graminae* (*Poaceae*). The cereals crops like wheat (*Triticum aestivum* L.), maize (*Zea mays*), and rice (Oryza sativa L) are the major plant foods in developing country.[36] Cereal grains are plant seeds and as such contain a large centrally located starch endosperm which is rich in protein, a protective outer coat consisting of two or three layers of fibrous tissue and an embryo or germ usually located near the bottom of the seed. Most cereals contain the essential amino acids required by man as well as vitamins and minerals.[37]

Cereals are rich in carbohydrates, oils, minerals, vitamins, and proteins but when refined the majority of the nutrients are lost leaving mostly carbohydrates and are therefore grown mainly for energy. Grains as they are sometimes called provide more food energy worldwide than any other crop and thus are staples. They are staple for two third of the earth's population, providing 85% of the world's food energy and protein intake. Cereal consumption is moderate in developed countries however in Africa and Asia, it is a daily sustenance. In Africa, cereals contribute 46% of the total energy intake; however, this could be as high as 78% in some African countries. The most cultivated and hence consumed grains worldwide in order of decreasing production are maize, rice, wheat, barley, sorghum and millet, and of these major grains maize, wheat and rice together account for 87% of all cereal production worldwide and 43% of all food calories because of their rich nutrient composition. Cereals support fungal growth and mycotoxin production excellently on the farm, during storage and after processing into foods and feeds. Since these ideal substrates for mycotoxin contamination are highly consumed globally, they constitute the most remarkable sources of mycotoxins (especially the most prevalent of mycotoxins; AF) to animals and humans.[38] Cereals have been important crops for thousands of years, indeed the successful production, storage and use of cereals have contributed in the development of modern civilization.[39]

2.6 Factors enhancing the prevalence of aflatoxigenic fungi and aflatoxins:

Before AF contamination, the food must be infected with the fungus that has the genetic capacity to synthesize and deposit the toxins on the foods and feeds before or after harvest. Only species of the genus *Aspergillus* have 23 genes responsible for the synthesis of AF. Members belonging to this genus are most abundant in the tropical region and are the major food spoilage agents in warmer climates. This genus is metabolically versatile producing over twenty mycotoxins. Of the over 180 species of *Aspergillus*, only a few are aflatoxigenic. From all these known AF producing fungi particularly *A. flavus* are common and widespread in nature, and have been shown as fungal contaminants of foods and feeds.[40-43]

Despite the fact that a strain of mould has the genetic potential to produce a particular mycotoxin, the production level would be influenced by the nutrients available, Moulds require a source of energy in the form of carbohydrates or vegetable oils in addition to a source of nitrogen either organic or inorganic, trace elements and available moisture for growth and toxin production. Cereals particularly oil seeds provide all these nutrients and so are considered ideal substrates for growth of the fungi and toxin synthesis.[44] Mycotoxins contamination varies according to size and integrity of seed; small and compact grains such as wheat, rice, oat, sorghum and those encapsulated in hard seed coats such as beans and soybeans being less susceptible to fungal infection and mycotoxin formation than larger grains such as maize.[45]

Hot and humid conditions are the two most important environmental factors suitable for mould growth and AF production. Although the optimum temperature and moisture content for growth and toxin production for the various aflatoxigenic fungi varies, many of them achieve best growth and toxin synthesis between 24°C and 28°C [46] and seed moisture content of at least 17.5%.[47] These conditions approximate the ambient climatic conditions in most parts of Egypt and hence account for the high prevalence of the toxins.

Soil is another natural factor that influences the incidence of fungal growth. Crops grown in different soil types may have significantly different levels of AF contamination. For example, peanuts grown in light sandy soils support rapid growth of the fungi, particularly under dry conditions, while heavier soils result in less contamination of peanuts due to their high water holding capacity which helps the plant to prevent drought stress.(48)

Much more than in other parts of the world, insects, termites, rodents and birds constitute a major problem to food safety and availability in Africa. These pests and animals transmit spores from other plants and environmental surfaces to inoculate the already defective kernels and help to distribute moulds widely throughout a bulk mass of grains or feed. The metabolic activities of pests especially insect larvae also produce metabolic water and heat that are beneficial for mould growth.(49)

Harvesting methods that enhance seed breakage would also increase the degree of mycotoxin formation. This is in agreement with the suggestion that certain modern agricultural management practices may create unique ecological niches which select toxigenic fungi.(50) Although Africa is experiencing a boost in mechanized farming, there are no available control measures to reduce the negative impact of this agricultural revolution on mycotoxin contamination.

2.6.1 Factors enhancing aflatoxin production in Egypt:

Most regions in Egypt extended in African Sahara as a result of this drought is a major problem and lack of water is a large issue. Some steps are being taken to help with the limited water supply. The Ministry of Agriculture and Land Reclamation is looking at 1.4 million hectares in the Sahara that they intend to reclaim by 2017. They have made progress on this plan and it is well on its way. They also have intentions to further expand the Nile Delta, the Southern Valley, East Owaynat, and the Suez Canal region. With this the government is pushing for better irrigation practices to conserve the limited water supply. Even once these projects have been finished, there will not be enough water to maintain all of the production necessary in Egypt. These water projects may help ease the burden of aflatoxin poisoning caused by drought stressed crops, but they will not prevent it from occurring, so we need another way to withstand this dangerous problem.

2.7 Health risk and economic impact of aflatoxin contamination:

A. flavus can cause diseases in animals and human beings. The diseases caused by fungal invasion into animal or human hosts are collectively called "mycoses," while the diseases or symptoms caused by the toxic fungal metabolites are collectively called "mycotoxicoses." The diseases caused by the *Aspergillus* species including *A. flavus*, is called "aspergillosis." *A. flavus* is the second leading cause of invasive and noninvasive aspergillosis in humans and animals next to *A. fumigates.*(51,52)

Due to the spread of AIDS and organ transplantation, the incidence of aspergillosis in immune-compromised patients is rising. There is no effective antifungal drug available on the market to control fungal growth in human patients and so invasive aspergillosis is often fatal.(53)

A. flavus is a weak opportunistic plant pathogen that causes diseases of many agricultural crops such as maize (corn) (Appendix 3), cotton, groundnuts (peanuts), and tree nuts. Few plant pathogenic fungi such as *A. flavus* have such a broad host range that it can attack seeds of both monocots and dicots, and to infect seeds produced both above and below the ground. It infects corn cars, cotton bolls, and peanut pods after insect or mechanical damages occurs.(54) The post-harvest aflatoxin contamination is sometime problematic if food grain storage is poorly managed. Aflatoxin is associated with both toxicity and carcinogenicity in human and animal populations. Aflatoxicosis is poisoning resulting from ingestion of moderate to high levels of aflatoxins in contaminated food or feed. Toxicological studies demonstrate that ducklings, hamsters, rats, trout, rabbits, and a number of other vertebrates are all susceptible to aflatoxin poisoning. (55)

Acute aflatoxicosis results in rapid progressive jaundice, edema of the limbs, pain, vomiting, necrosis, cirrhosis, or in severe cases, acute liver failure and death.(56-58)Outbreaks of acute aflatoxicosis from contaminated food in humans have been documented in Kenya, India, Malaysia, and Thailand.(59) However, the most widely spread outbreak of aflatoxicosis in humans occurred in more than 150 villages in western India in 1974 where 397 persons were affected and 108 persons died. As recent as in July 2004, an incident of all toxin poisoning in Kenya had occurred involving 317 cases and 125 deaths due to consumption of aflatoxin contaminated maize (corn), the largest and most severe outbreaks of acute aflatoxicosis documented worldwide.(57,59)

The numbers of cases of acute poisoning are not large relative to the populations at risk, probably because people usually avoid obviously moldy foods, however in times of food scarcity or under condition of poverty, people usually have no option but to use lower-priced, poorer-quality food, which commonly is contaminated.

Chronic aflatoxicosis is due to long term exposure of moderate to low aflatoxin concentration and results in cancer, immune suppression, and other "slow" pathological conditions. The liver is the primary target organ when mammalian species are fed with aflatoxins. In farm and laboratory animals, chronic exposure to aflatoxins compromises immunity and interferes with protein metabolism and multiple micronutrients that are critical to health.(60)

Immuno-suppression is due to the reactivity of aflatoxins with T-cells, decrease in Vitamin K activities, and a decrease in phagocytic activity in macrophages. These immuno suppressive effects of aflatoxins predispose the animals to many secondary infections due to other fungi, bacteria and viruses.(61)

The danger of AF lies in their mode of action by inhibiting the incorporation of precursors for the synthesis of DNA, RNA and proteins; they also block the action of some enzymes that are responsible for the synthesis of nucleic acids, causing centrilobular necrosis in the liver, polymorphonuclear infiltration and fatty degeneration. AF toxicity depends on the dose, the exposure degree, the age, the nutritional status of the animal and the possible synergic effects of the chemical agents to which they are exposed.(62)

Some secondary metabolites produced by Aspergillus species are harmful for animals too. That's the case of cyclopiazonic acid (CPA), which causes necrosis of liver or gastrointestinal tissue and necrotic changes in skeletal muscle and kidney.(63,64)

In developing countries, food safety is the major problem where detection and decontamination policies are impractical. In those countries where populations are facing starvation routine ingestion of aflatoxin-contaminated food may occur. Statistics indicates that worldwide liver cancer incidence rates are 2 to 10 times higher in developing countries than in developed countries.[65] This is because, in the developed countries the toxic contaminants are monitored and regulated. The maximum allowable amount of aflatoxin in food and feed for human consumption and for livestocks has been controlled by laws. A guideline of 20 parts aflatoxin per billion parts of food or feed substrate (ppb) is the maximum allowable limit (for total aflatoxins) imposed by the U.S. Food and Drug Administration. The European Union has a maximum level of 2 ppb for aflatoxin B_1 and 4 ppb for total aflatoxins. The aflatoxin contaminated harvest is destroyed resulting yearly in billion dollar losses worldwide. Thus, aflatoxin contamination is not only a serious food safety concern, but has significant economic impact in agriculture worldwide.[66]

Even non-mouldy foods or raw materials may contain AF. Spores can be transferred by insects especially flies, wasps and bees or by birds to foods where the spores germinate, produce mycelium, and AF are excreted. Seeds can contain AF by infection of the egg-cells of the flowering plants. The spores of *A. flavus* and *A. parasiticus* can germinate on the stigma surfaces of plants, then the germ tube penetrates to the developing embryo mimicking pollen germ tubes. The mycelium can establish an endotrophic relationship which is not harmful for the healthy plant. However, if the plant is under drought stress, then significant levels of AF may be produced in the plant tissue during growth in the field. Under these circumstances food commodities may already be contaminated at harvest and, although the con-centrations are never as high as those formed in stored commodities, they can be economically significant.[67]

2.7.1 Cancer– based estimates of aflatoxin exposure:

For humans, aflatoxin is an agent promoting liver cancer, although lung cancer is also a risk among workers handling contaminated grain.[68]

The increased risk of hepatomas is caused by deletion mutations in the P53 tumor–suppressing gene and by activation of dominant onco-genes.[69] The risk of cancers due to exposure to the various forms of aflatoxin is well established and is based on the cumulative lifetime dose.[70]

The International Cancer Research Institute identifies aflatoxin as a Class1 carcinogen, however, in many developing countries, epidemics of HBV and HCV affect 20% of the population. A strong synergy is observed between aflatoxin and these biological agents for liver cancer. In hepatitis B surface antigen–positive subjects, aflatoxin is 30 times more potent than in persons without the virus.[71] HBV patients increases from 5 with only HBV infection to 60 when HBV infection and aflatoxin exposure are combined.[72]

In some areas where aflatoxin contamination and HBV occur together, hepatomas are the predominant cancer 64% of cancers,[73] and they may be a predominant cause of death. Thus, to minimize the risk of liver cancer, it is critically important that exposure of HBV- and HCV-infected persons to aflatoxin is minimzed. The use of the incidence of cancer to indicate aflatoxin exposure is supported by the extensive studies on risk associated with exposure, but this approach provides only circumstantial evidence for

exposure, and liver cancer is not caused solely by aflatoxicosis. Two major factors aflatoxin and HBV, which commonly occur in the same populations, influence the risk of liver cancer. Independently, each factor significantly increases the relative risk of cancer, and most studies report them, together, to be synergistic,[74] increasing the risk of cancer 25–30-fold; there is, however, one epidemiologic study that contradicts those findings.[75]The suggested mechanism for this synergy is that aflatoxin suppresses DNA repair mechanisms that help limit the development of cancer from HBV, and HBV prevents detoxification, but it is also possible that immunotoxicity of aflatoxin interfere with the suppression of cancer.[76]

2.7.2. Epidemiology of liver cancer in Egypt:

In Egypt, several attempts were made to establish cancer registries. Among these attempts in 1998, the Egyptian Ministry of Health and Population in collaboration with several partners established a population-based Cancer Registry (NCR) in Gharbiah Governorate, in addition to a multi-institutional cancer statistics collected in collaboration with the National Cancer Institute of Cairo University. This was intended to estimate the size of the problem nationally. The NCR data confirmed the high incidence of HCC in Egypt and the change in the trends during the last decade as shown in (Figure 2.3). Liver tumors as seen in (Figure 2.4), were mostly HCC 70.48%, while hepatoblastoma constituted 10.24%, non-Hodgkin's lymphoma 44.21% of hepatic malignancies and adenocarcinoma unspecified 9.03%.[2]

In El Zayadi's study in (2005)[77] the pattern changes of HCC in Egypt over the period between1993-2002 as well as the possible risk factors were studied. The duration of the study was divided into two periods of 5 years each: period I (1993–1997) and period II (1998–2002) over this decade, 1328 HCC patients (5.9%) out of 22,450 chronic liver disease (CLD) patients were diagnosed. The annual proportion of HCC showed a significant rising from 4.0% in 1993 to 7.2% in 2002. Kafrawy et al, (2005)[78] documented the presence of p53 codon 249 mutations associated with aflatoxin exposure in a sample of HCC tumor tissues analyzed by gene chip analysis in Egypt. Hifnawy et al, (2004)[79] suggested that the progressive nature of HCV-related liver diseases was influenced by aflatoxin exposure. A positive correlation was found between aflatoxin and positive HCV-PCR together with liver disease progression to stage G3S, that was indicative of HCC.

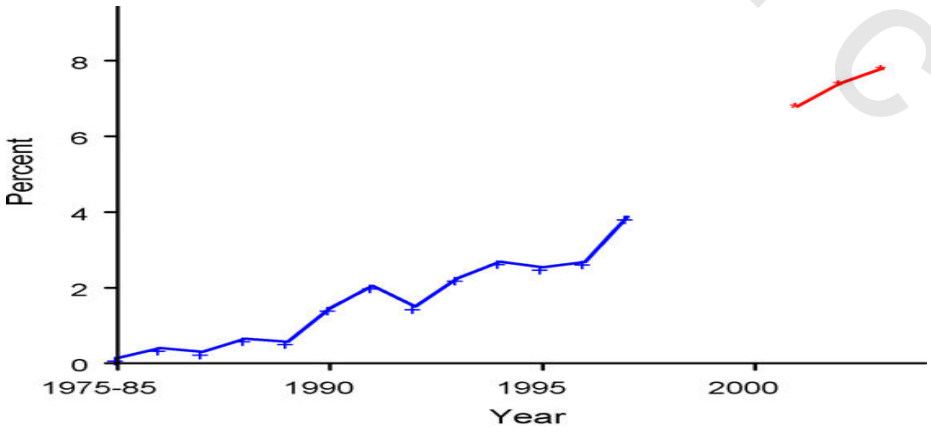


Figure 2.3: Trends in frequency of liver cancer, in Egypt according to the National Cancer Institute records, NCI 1975–2003.

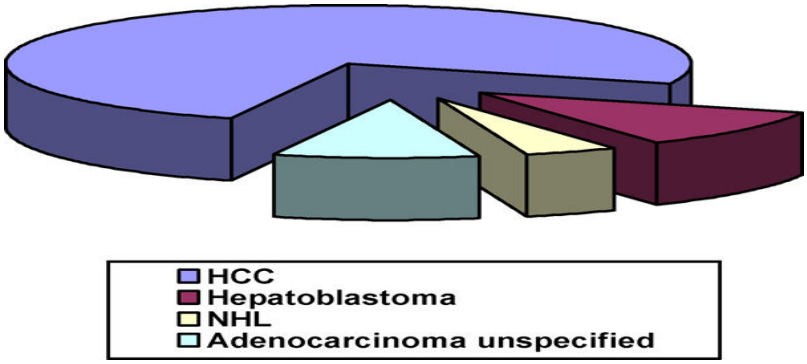


Figure 2.4: Proportion of types of malignant liver tumors in Egypt.

2.7.3 Aflatoxin and children:

Fetal and childhood environment, including the nutritional status of the pregnant mother and the infant, are considered critical for growth and risk of disease in earlier life. Mal-nutrition is one of the common problems in developing countries. Apart from these, they are also exposed to high levels of mycotoxins. Aflatoxins are the major among these. It has been proved that these aflatoxins are immunogenic, teratogenic, and they retard the growth among experimental animals. In the developing countries the environmental conditions favor their production. High exposure to aflatoxins occurs throughout these regions. A study in West Africa showed a significant correlation among the aflatoxin exposure and stunted growth in children who are exposed to aflatoxin right for neonatal stages.[80] Apart from that due to the capacity of aflatoxins to cross the placental barrier, aflatoxins can cause genetic defects at fetal stages itself.[81]

In a study to screen for biomarkers of aflatoxin exposure in Egypt, Polychronaki et al, (2006)[82] assessed the level and frequency of breast milk AFM_1 as a biomarker of maternal exposure. Breast milk samples were collected from a selected group of 388 Egyptian lactating mothers of children attending the New El-Qalyub Hospital, Qalyubiyah governorate, Egypt, during May–September 2003. Approximately 36% of mothers tested positive for AFM_1. Non-working status, obesity, high corn oil consumption, number of children, and early lactation stage (<1 month), contributed to the occurrence of AF in breast milk. The same research group continued their study following up with 50 of those women who were AFM_1 positive at baseline; they were revisited monthly for 12 months to assess the temporal variation in breast milk AFM_1. In a multilevel regression model of the data there was a highly significant ($p < 0.001$) effect of month of sampling on the frequency of AFM_1 detection with summer months having the highest frequency (>80%) and winter months the lowest frequency (<20%) of detection. The duration of lactation and peanut consumption also contributed to the model. The identification and understanding of factors determining the presence of toxicants in human milk is important and may provide a knowledge driven basis for controlling the transfer of chemicals to infant.

Hassan et al (2006)[83], assessed the presence of aflatoxin (AFM_1) in both mothers' milk and the infants' sera. Fifty healthy breast lactating mothers and their infants who were exclusively breast fed for at least 4 months were included. Twenty-four mothers (48%) and their infants had detectable levels of aflatoxin in their serum.

2.8 Aflatoxin B_1 metabolism:

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 B_1 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. When AFB_1 is ingested, once inside the body, it is absorbed by the intestine and carried to the liver. There, AFB_1 is activated and metabolized by cytochromes p450 (CYP) of hepatocytes to AFB_1-8,9-exo-epoxide and AFB_1-8,9-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 AFB_1 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 inter-individual 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 AFB_1 to 8,9-epoxide.[84]

AFB_1-8,9-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-AFB_1 (AFB1–N7-Gua) adduct. AFB_1–N7-Gua adduct confers the mutagenic properties of the compound. This may be the most important product from the carcinogenic point of view. 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.[72]Indeed, there is evidence that a dose-dependent relationship between dietary aflatoxin B_1 intake and codon 249ser p53 mutations was observed in hepatocellular carcinoma from Asia, Africa and North America Cytochrome p450 3A4 can activate and detoxicate AFB_1. Only the 8,9-exoepoxide appears to be mutagenic and others are detoxification products. The putative AFB_1 epoxide is generally accepted as the active electrophilic form of AFB_1 that may attack nucleophilic nitrogen, oxygen and sulphur heteroatoms in cellular constituents of 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 AFB_1-8,9-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 dinucleotidephosphate (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.[84]

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. In addition to errors in DNA transcription due to its binding to AFB_1 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 AFB_1. AFM_1 also been detected in urine, indicating that this toxin is also capable of reacting with DNA and form adducts. 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. In a next phase, the challenge is to stabilize and inactivate the epoxide, hydrolyzing and conjugating it with glutathione to form AFB_1-Glutathione (AFB-SG) that will be excreted in urine. In this metabolic stage are also originated three major hydroxylated metabolites: AFQ_1, AFP_1 and AFM_1, which begin to distribute systemically and can be found in milk, eggs and tissues from intoxicated animals. Another important derivative from AFB_1 metabolism is aflatoxicol, which extends the presence of AFB_1 in the organism; it comes from reducing AFB_1, and it can be re-oxidized back to AFB_1 by NADHP.[84]In an extensive review made by Verma in (2004)[85], 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 (B_1, B_2, G_1 and G_2) and metabolized forms (aflatoxicol, M_1 and M_2) 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 B_1 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.[84] Some of these informations illustrated in (Figure 2.5) and (Figure 2.6).

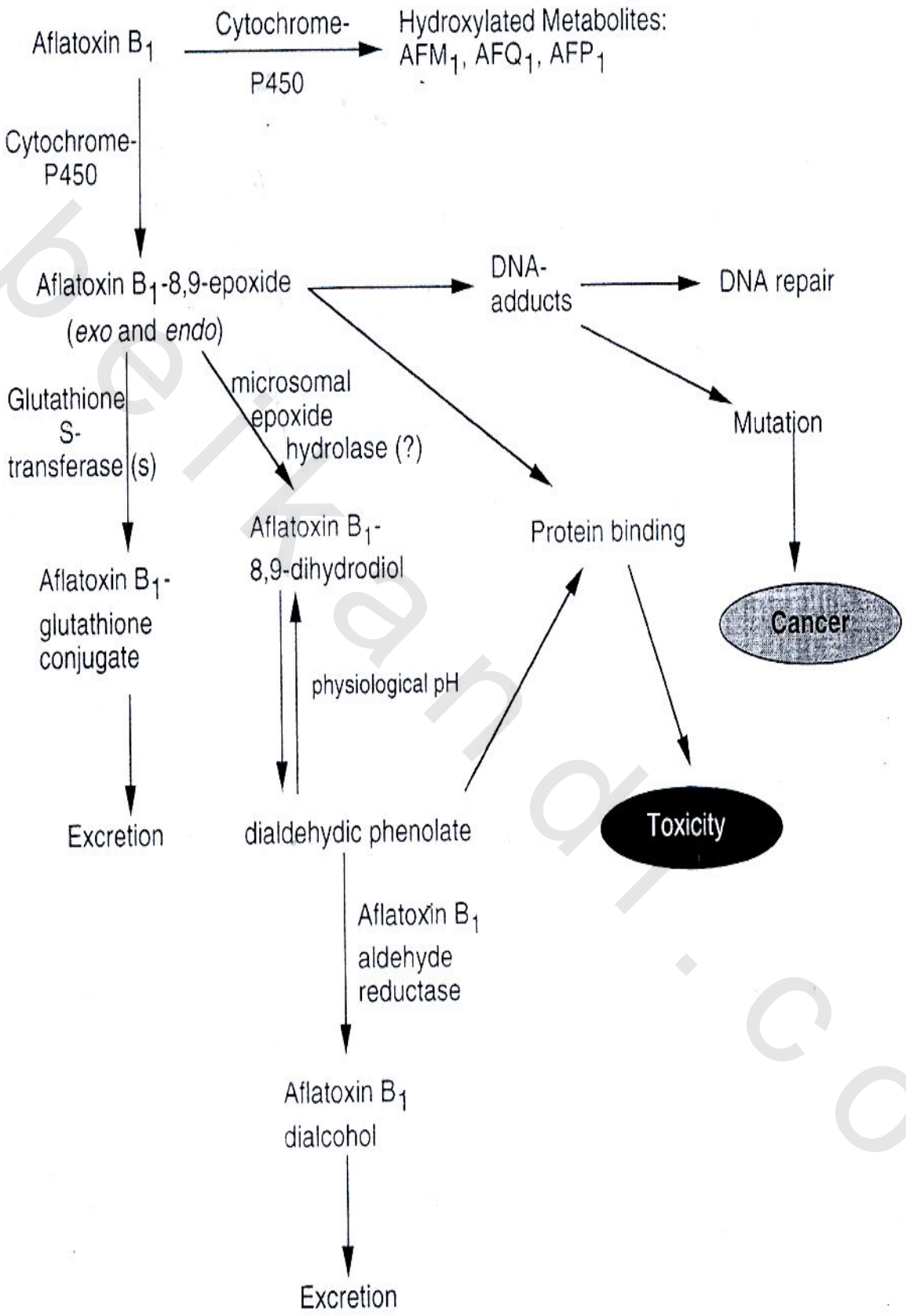


Figure 2.5: Aflatoxin B_1 metabolic pathways.

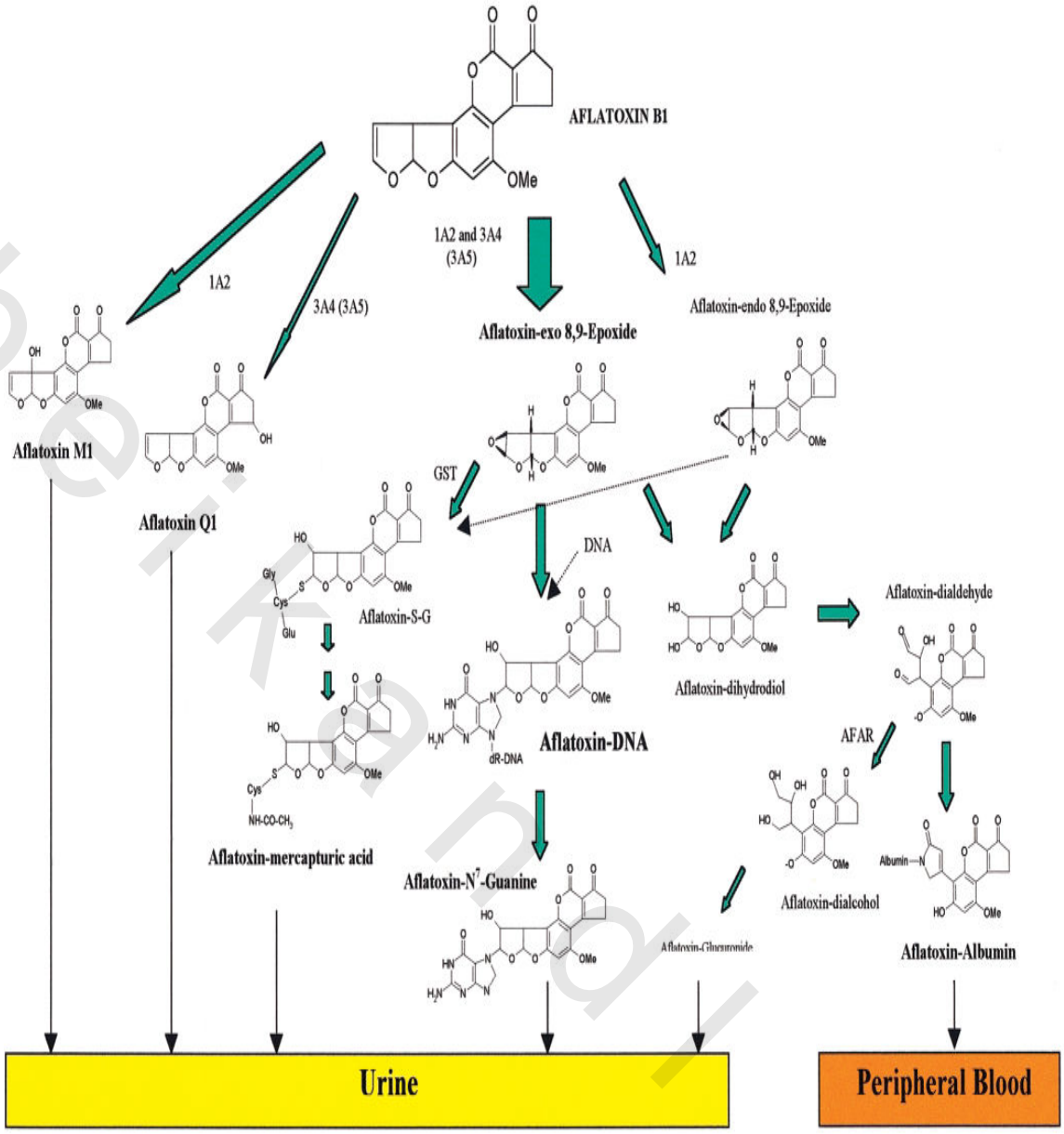


Figure 2.6: Metabolic pathways of aflatoxins illustrated by structures.
GST, (glutathione-*S*-transferase), AFAR, (aflatoxin aldehyde reductase), Aflatoxin-S-G, (aflatoxin–glutathione conjugate).

Once the toxin has entered the liver cell, it causes tissue injury in particular animal species which is detected 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. [86]

2.9 Regulatory Limits:

Because mycotoxins are toxic and carcinogenic in animals, many countries regulate the maximum level that can occur in foods and feeds. Most regulations are concerned with controlling aflatoxin because it is considered the most toxic and carcinogenic of the naturally occurring mycotoxins. A recent FAO/WHO survey indicated that almost 100 countries regulate aflatoxin and several other mycotoxins in foods and feeds However, maximum levels differ widely from country to country because of a lack of agreement on what constitutes a safe maximum level for humans. Some of the maximum limits found in the FAO/WHO survey for aflatoxin are shown below in Table 2.3.

Table 2.3: Examples of aflatoxins legal limits found in some counties.

country	Aflatoxin B_1 (ng/g)	Total aflatoxin (ng/g)
Egypt for maize	5	20
Egypt for peanut		20
Nigeria	20	10
South Africa		10
Australia		15
Canada		15
EU for peanut (for processing)	8	15
Philippine		20
US		20

Because of differences in regulatory limits for mycotoxins, FAO and WHO, working through the CODEX system, are attempting to harmonize international maximum limits and sampling plans for mycotoxins to promote world trade and protect the consumer.[87]

2.10 Mycotoxin Units:

Mycotoxins are measured in concentration units or a ratio of the mass of the mycotoxin to the mass of the commodity. Units of measurement are usually grams of mycotoxin divided by the grams of commodity. Because the mass of a mycotoxin is usually very small, the units are reported in parts per billion (ppb) or parts per million (ppm). One ppb is 1 nanogram (ng) of mycotoxin per 1 g of commodity or 1 ng/g.[87]

3. Control of aflatoxin.

Talking about Aflatoxins is not a new issue. Aflatoxins are a big problem that day by day turns more important due to their implication in crop production, food quality and

human and animal health. Aflatoxins (AF) affect almost everything we eat: cereals (maize, wheat and rice principally) and their derivates; oilseeds (cotton, peanut, rapeseed, coconut, sunflowers and others), cassava, nuts, dry fruits, spices, legumes, fruits, milk and milk derivatives,[88]and even chocolates.[89] In order to find a solution for this problem, some organizations and institutions have purposed prevention strategies in order to reduce the risks given by this public problem especially in low-income countries, but those strategies are not enough to give a real solution to this worldwide daily problem. AF are very stable and persistent, so they are difficult to remove. Due to they are contained in many crops that are consumed by animals, AF have turned into a serious animal problem too. AF can be transmitted from animals to human food (by eggs, meat and dairy) with the consequent risk to human health.[90]

3.1 Pre-harvest aflatoxins prevention:

It was established in about 1970 that fungal contamination could start in the field before harvest.[63] Although the highest levels of AF are undoubtedly associated with post-harvest spoilage of food commodities stored under inappropriate conditions of water activity and temperature.[67]

Factors that influence the incidence of fungal infection and subsequent toxin development include invertebrate vectors, grain damage, oxygen and carbon dioxide levels, inoculum load, substrate composition, fungal infection levels, prevalence of toxigenic strains and microbiological interactions. Insect damage on crops allows fungi to access in them, increasing the chances of AF contamination, especially when loose-husked maize hybrids are used.[91,92]

Controlling or reducing infection by regulating the factors that increase the risk of AF contamination in the field contributes extensively in managing AF. Management practices that reduce the incidence of AF contamination in the field include timely planting, maintaining optimal plant densities, proper plant nutrition, avoiding drought stress, controlling other plant pathogens, weeds and insect pests and proper harvesting.[93]

Pre-harvest measures that are efficient in reducing AF levels are the same as those that will enhance yields. Crop rotation and management of crop residues also are important in controlling *A. flavus* infection in the field. Tillage practices, fertilizer application, weed control, late season rainfall, irrigation, wind and pest vectors affect the source and level of fungal inoculum, maintaining a disease cycle in crops like maize.[91] Lime app- lication, use of farm yard manure and cereal crop residues as soil amendments have shown to be effective in reducing *A. flavus* contamination as well as AF levels by 50-90%. Calcium, which is part of lime, thickens the cell wall and accelerates pod filling, while manure facilitates growth of microorganisms that suppress soil infections.[94]

In order to minimize the levels of AF and mycotoxins in general, the National Institute of Agricultural Technology of Argentina (INTA), recommends to make early plantings, to plant resistant genotypes, to do good farming practices, to avoid stress conditions, to minimize insect damage, to harvest early in order to avoid delays, to avoid damaged kernels and to storage at less of 13% moisture in a clean, fresh and airy place with no insects. As mentioned before, it is important to avoid product moisture, high temperatures (between 25 and 32°C) and high relative humidity in storage and seeds preservation. Weeds have to be removed and crop rotation should be done routinely. Prior to the preparation of the ground, dead organic matter has to be disabled

or burned; product mechanical damage has to be avoided; crops have to be collected at full maturity; storage places should be dry and the entry of water has not to be allowed; storage health standards have to be fulfilled (pallets, proper humidity levels, adequate ventilation and lighting, etc.), and periodic inspection of the stored product should be done. To avoid risks to human and animal health, INTA also suggests to avoid feeding animals with crops in poor condition (especially corn), not to use fractions of discarded corn fodder, and to make good manufacturing practices.[95]

3.2 Post harvest regulations:

Since AF have been recognized as a significant worldwide problem, researches have proposed some ways of detoxification. AF detoxification refers to those post-harvest treatments directed to eliminate or diminish the toxic effects of toxins. Those strategies can be divided into three different groups: natural methods, physical methods and chemical methods. These methods are focused on destroying, modifying or adsorbing AF. There is variety of tools such as post-harvest drying (which is economically accessible), adequate storage, shelling, dehulling, product sorting, early harvest, regionally adjusted planting dates, and insect control. However, even when storage conditions are generally good, AF frequently form prior to harvest while the crop is maturing and/or a waiting harvest, which can result in significant losses.[23]

3.2.1. Natural methods:

The natural methods used to avoid AF are principally: seed cleaning, sorting and seed division by screening and extrusion. Nevertheless, those techniques are neither practical nor efficient at all, and food micronutrients content get diminished. Since 1989, the FAO has supported some decontamination processes like the UK-Thai Project (UTP) System, which showed to reliably produce low AF-content maize during the rainy season. With the UTP system, maize is first field dried on the stalk for one to two weeks before harvesting to reduce moisture content to 20%. It is next shelled within 24 to 48 hours of harvest, and loaded into a drier within 12 hours of shelling. Thus, within 48 hours, it is dried to 14% moisture content, with no part exceeding 15%. AF content is monitored rapidly by a special adaptation of the bright greenish-yellow fluorescence (BGYF) test. Maize dried to 14% moisture content by the UTP system can be safely stored for a minimum of two months with no increase in AF content. By the other hand, cleaning of stores before loading in the new harvests has been correlated with reduction in AF levels. Separating heavily damaged ears (those having greater than 10% ear damage) also reduces AF levels in crops like maize. Wild hosts, which constitute a major source of infestation for storage pests, should also be removed from the vicinity of stores.[96]

AF are unevenly distributed in a seed lot and may be concentrated in a very small percentage of the product. Sorting out of physically damaged and infected grains (known from colorations, odd shapes and size) from the intact commodity can result in 40-80% reduction in AF levels.[91]

3.2.2 Chemical methods:

Chemical AF control methods are principally those which involve the use of chemical reagents for different purposes. Most investigators are looking for new sources of materials to control spoilage caused by fungi in food. However, the application of synthetic preservatives has led to a number of environmental and health problems because

they are themselves carcinogenic, teratogenic, and highly toxic with long degradation periods.[17]

Insecticides and fumigants were the first chemicals to be used to deal with aflatoxigenic fungi. The DOA Division of Plant Pathology and Micro- biology screened since several decades ago, seven reagents in the laboratory for effectiveness in preventing or reducing AF contamination of maize. Only three of the reagents were found to be effective: sodium bisulphite, amm- onia, and propionic acid. Sodium bisulphite and ammonia treatments resulted in grain with a strong residual odor; the ammonia treatment also produced darker grain. The most promising regent was the propionic acid-based fungicide formulation, which effectively controlled both mould growth (*A. flavus*) and AF formation, while not adversely affecting the physical quality of the grain.[96] Nowadays, the use of insecticides for this purpose has been abandoned due to the toxic residues that they generate.[88]

About fumigants, only two were in common use in the last decade: methyl bromide and phosphine. Methyl bromide has been identified as a major contributor to ozone depletion, which casts a doubt on its future use in pest control. There have been repeated indications that certain insects have developed resistance to phosphine, so its use is now doubtful.[97,98]

It has also been reported that propionic acid, sodium propionate, benzoic acid, ammonia, urea and citric acid are the best anti-fungal chemical compounds tested in feeds.[99]

Organic solvents can be used to remove AF in food because mycotoxins have the physicochemical characteristic to be soluble in them. Combinations such as hexane-acetone-water or isopropanol-water have been reported to be effective mycotoxins draggers. Some acids such as hydro- chloric acid, sulfuric acid and their derivatives have the capability to react with the lactone groups of AFB_1, AFG_1, and with non-aromatic double bonds present in AF. Toxicologically, the addition reaction of the acids with the double bonds structures appears to be most effective in terms of detoxification because the reaction products are polar substances that can be eliminated in the urine. Alkalis like monoethylmethylamine, hydroxide and calcium chloride, sodium hydroxide and ammonium carbonate, are reactive with the lactone group of AF. Oxidant agents such as ozone, peroxides and permanganates in alkaline solutions are reactive with non-conjugated double bonds of AF. The ozonolysis reaction leads to the creation of smaller molecules, but some of the obtained products could be toxic. The glycosylation reaction results in the creation of two hydroxyl groups that can subsequently form hydrogen bonds; nevertheless although this mechanism is effective for AF detoxification, it should be used in combination with polymers or silicates capable of adsorbing physically AF through hydrogen bonds. Adsorption of mycotoxin molecules has been studied recently. It can be done by different inert chemicals, such as some complex indigestible carbohydrates (cellulose, polysaccharides in the cell walls of yeast and bacteria like glucomannans, peptidoglycans and others), synthetic polymers (such as cholestyramine and polyvinylpyrrolidone), humic acid and vege- table fibers, and clays or synthetic silicates, which can sequester mycotoxins but more studies is needed to evaluate these methods.[97]

3.2.3 Physical methods:

Although natural methods are cost-effective and chemical methods leave toxic residues so we need other methods to avoid these problems.[100,101] Physical food processing procedures like dehulling, roasting, baking, frying, extrusion cooking and nixtamalization have been studied. In this work we are concerned with radiation with γ-radiation and its possible effect in the reduction of aflatoxin B_1 in food and the effect of γ-radiation on the nutritive values of food.

4. Food irradiation.

4.1 The history of food irradiation:

The history of food irradiation dates to the discovery of x-ray by Roentgen in 1895 and radioactive substance by Becquerel 1896 following these discoveries there was much research examining the effect of these radiations on biological organisms. As a result become recognized in the early decades of 20^{th} century that ionizing radiation could have beneficial food irradiation applications however the technology for delivering the required dose at affordable cost was not available. Comparative research in late 40s and early 50s examined the utility of five different radiations (ultra-violet, x-ray, electrons, neutrons and alpha particles) for food preservation, from these studies it was concluded that only electrons had the necessary characteristic of efficiency safety and practically. The penetration depth for ultraviolet and alpha particles is too limited and x-ray considered impractical because of low generation efficiency. While neutrons have good penetration and are quite effective in destroying bacteria, they were eliminated from further consideration because of potential for inducing radioactivity. The first practical sources of ionizing radiation were particle accelerators that produced electron beams with energies up to 24 Mev. Also in the late 40s, man made radionuclides such as ^{60}Co and ^{137}Cs which emit penetrating gamma rays become available through development of atomic energy with the availability of these sources of ionizing radiation, research was focused not only on issues associated with food preservation and safety, but also on the development of commercial food irradiation technology. This work has now result in the world wide availability of reliable commercially available food irradiation equipment, processors and facilities capable of contributing significantly to the supply of safe and wholesome food at low costs.[102]

4.2 Significance of food irradiation:

Although a patent was issued in 1929 for the use of radiation as a mean of preserving and protecting food it was until shortly after world war ɪɪ that this method of food protection received any serious consideration. While the application of food irradiation has been somewhat slow in reaching its maximum potential use, the full application of this method presents some interesting challenges to food microbiologist and food scientist. Food irradiation may be defined as the emission and propagation of energy through space or through material medium. The type of radiation of primary interest in food preservation is electromagnetic. The various of radiations are separated on the basis of their wave lengths with the shorter wave length being the most damaging to microorganisms. The electromagnetic spectrum may be further divided as follows with respect to the radiations of interest in food preservation (microwaves, ultraviolet rays, x-rays and gamma rays). The

radiations of primary interest in food are ionizing radiations defined as those radiations that have wave lengths 2000A° or less their quanta contain enough energy to ionize molecules in their paths. The Joint FAO/IAEA/WHO expert committee on food irradiation held in Geneva in 1980 concluded that irradiation of food at dose level up to 10 KGy does not cause any toxicological risk to the food and does not affect its nutritional value.(103)

In considering the application of radiations to food, there are several useful concepts that should be indicated.

A Roentgen: Is a unit of measure used for expressing an exposure dose of x-ray or gamma ray.

A milliroentgen: Is equal to 1/1000 of roentgen.

A curie: Is a quantity of radioactive substance in which $3.7x10^{10}$ radioactive disintegrations occur per second.

For practical purpose 1g of pure radium possesses the radioactivity of 1curie of radium. The unit for a curie is the Becquerel (Bq).

A rad: Is a unit equivalent to the absorption of 100 erglg of matter.

A kilo rad (K rad) is equal1000 rad and mega rad is equal 1million rad. The newer unit of absorbed dose is gray (Gy), 1Gy=100 rad =11 Joule/Kg.

$1KGy=10^5$ rads.

Electron volt: The energy gained by an electron in moving through 1V is designed (ev).

A mev is equal 1 million electron volts. Both (rad) and (ev) are measurements of intensity of radiation.(103)

4.3 Advantages and disadvantages of food irradiation:

Agriculture provides the economic backbone of developing countries. These countries have to produce a sufficient food supply for their population and export any excess to earn foreign exchange. However, post harvest loss of food is still a great problem affecting the food supply and the economies of these countries. Food and agricultural products contribute significantly to the overall volume of international trade. The export of these products is hampered by strict quality regulations and quarantine restrictions imposed by the importing countries. Developed countries on the other hand, are facing an increasing demand for safe and convenient food. Food-borne diseases are on the increase in both developed and developing countries. A need exists to address some of the above-mentioned problems in order to reduce post harvest food losses, to meet quarantine restrictions and to improve the safety and hygienic quality of foods. Hence, food science and technology continually strive to develop and provide mankind with adequate food which is safe, wholesome and of better quality, meeting the demand of ready availability, variety and convenience. Food irradiation is a physical method of processing food (e.g. freezing and canning). It is recognized as a safe and wholesome method. It has the potential both of disinfecting dried food to reduce storage losses and disinfecting fruits and

vegetables to meet quarantine requirements for export trade. One of the most important advantages of food irradiation processing is that it is cold process which does not significantly alter phsico-chemical characters of the treated product. It can be applied to food after its final packaging. Similar to other physical processes of food processing (e.g. canning, freezing), irradiation is a capital intensive process. Thus, adequate product volume must be made available in order to maximize the use of the facility and minimize the unit cost of treatment. Lack of the harmonization of regulations among the countries which have approved irradiated foods hampers the introduction of this technique for international trade. Action at the international level has to be taken in order to remedy this situation. One of the important limitations of food irradiation processing is its slow acceptance by consumers, due inter alia to a perceived association with radioactivity. The food industry tends to be reluctant to use the technology in view of uncertainties regarding consumer acceptance of treated foods. Several market testing and consumer acceptance studies have been carried out on food irradiation in recent years. These studies showed that, if the safety and benefits of food irradiation were properly explained, the consumers were willing to accept irradiated foods. Considering its potential role in the reduction of post harvest losses, providing safe supply of food and overcoming quarantine barriers, food irradiation has received wider government approval during the last decade. There is also a trend towards increased commercialization of irradiated food.(104)

4.4 Chracterestic of radiations of interest of food preservation:

4.4.1 Ultraviolet light (UV):

Ultraviolet light is a powerful bactericidal agent with the most effective wave length being about 2600A°. It is non-ionizing and is absorbed by proteins and nucleic acids in which photochemical changes are produced that may lead to cell death. The poor penetrative capacities of UV light limit its food use to surface applications, where it may catalyze oxidative changes that lead to rancidity, discoloration and other reactions small quantities of ozone may also be produced when UV light is used for surface treatment of certain food.(103)

4.4.2 Beta rays:

May be defined as streams of electrons emitted from radioactive substance, cathode rays are the same except that they are emitted from the cathode of an evacuated tube .These rays posses poor penetration power. Among the commercial source of cathode ray are Varde Groaf generator and linear accelerator. The latter seem better suited for food protection use. There is some concern over the upper limit of energy level of cathode rays that can be employed without inducing radioactivity in certain constituent of food.(103)

4.4.3 X- ray:

The key elements in this facility include accelerator system to deliver the energetic beam, a scanning system to provide uniform beam coverage of the product and material handling system that move the product through the beam in a precisely controlled manner, Auxiliary equipment for the accelerator system includes vacuum and cooling sub-systems. extensive shi- elding is necessary to reduce the external radiation exposure rates to acceptable levels, and a safety system is necessary to prevent accidental exposure of personnel during accelerator operation to avoid the any issues associated with nuclear activation of foods the kinetic energy of the electrons produced by the accelerator system is

limited by regulation to 5 or 7.5 Mev for indirect radiation using x-rays where electron beam doesn't interact with the product. Rather, the beam is scanned across an x-ray converter made a high atomic number metal such as tantalum or tungsten. When energetic electrons interact with the converter material they generate x-ray radiation by a process known as bremsstrahlung.(102)

When fast electrons hit matter they lose energy by collision with electrons of the target material and by deflection by nuclei. In the collision process the electrons of the target material are excited or even completely ejected from the atom, at the expense of some energy from the incident high-energy electron. The emitting radiation appears as electromagnetic radiation of a wide and almost continuous range of wavelengths superimposed on a few characteristic lines. Since each element has distinctive energy levels, these characteristic x-rays are specific for the element bombarded. The fact that x-rays consists of a wide variety of wave lengths is the main difference from gamma radiation. At the present time the use of x-rays for the treatment of food on an industrial scale is not proposed, but the principle involved is the same as that in the employment of gamma radiation.(104)

4.4.4 Gamma irradiation:

Gamma emission is produced by the nuclear disintegration of certain radioactive materials. A radioactive transformation is the change of an atom from one element to another by involvement of particulate radiation (alpha, beta, neutrons). The gamma radiation released during some radioactive transformations is probably due to the transition of the daughter nucleus from a higher energy level (excited state) to the ground state and it consists of electromagnetic radiation of sharply defined wave-lengths. The least expensive sources of radiation for food preservation are gamma rays from the nuclides cobalt-60 and cesium-137. These radioactive elements are either byproducts of atomic fission or waste products of the atomic industry. ^{60}Co is a radioactive isotope of cobalt, prepared artificially by bombarding natural cobalt (^{59}Co) with neutrons in a nuclear reactor.^{60}Co has a half-life of 5.3 years and emits gamma rays of 1.17 and 1.33MeV. ^{137}Cs is a radioactive isotope of cesium which is obtained as a fission product from uranium and other elements in a nucler reactor. ^{137}Cs has a half-life of 30 years and emits gamma rays with an energy of 0.66 MeV. The use of ^{60}Co is much more common since this isotope is more easily available and safer to use. The supply of ^{137}Cs seems to be more limited because of regulatory restrictions imposed on the processing of spent reactor fuel. Since radioactive cesium is actually manufactured as its chloride salt, there is also a risk associated with the use of a water-soluble compound in the event of an accidental leakage through the protective stainless steel envelope.(105)