REVIEW OF LITERATURE

1. Monitoring of aflatoxins in peanut and feed stuffs

Peanut kernels are a good substrate for growth and subsequent aflatoxin production by aflatoxigenic molds. Low-level aflatoxin contamination of peanuts may be unavoidable (Wilson and Flowers, 1978).

In edible nuts contamination by aflatoxins produced by toxigenic strains of *Aspergillus flavus* Link ex Fries and *A. parasiticus* Speare, is a potential problem (Ministry of Agriculture Fisheries and Food, 1980).

Kershaw (1985) detected aflatoxins contamination in peanut, almonds, brazil nut and walnut.

Sabino (1989) collected as part of a National Monitoring and Control Program on Mycotoxins in Brazil, samples during harvest in the groundnut-producing regions of Sao Paulo State. Of the samples (313) collected from rainy-season groundnut crops from west and northeast regions 48.0-73.5% contained aflatoxin B_1 at levels between 5 and 22 500 micro g/kg.

A survey of groundnuts from Egypt showed 19.5% of unshelled and 49.0% of shelled samples to contain low levels of aflatoxins B_1, B_2, G_1 and G_2; only B_1 was detected in roasted samples (3.5%). However, 60% of *Aspergillus flavus* strains from Egypt produced one or more aflatoxin, and 40% of toxigenic strains produced high quantities (5000-20 000micro g/kg) of B aflatoxins; the levels found in groundnuts were

therefore lower than expected. Studies on the effects of storage temperature and RH on groundnut mycoflora and aflatoxin development showed an RH of 95% to be optimum for aflatoxin production; since the RH in Egypt is lower than this, this was considered a factor contributing to the low levels encountered. Inoculation of Egyptian groundnut cv. Giza 1 with different strains of *A. flavus* showed the shells to act as a barrier towards invasion; this was considered the main reason for low aflatoxin production. Consequently, it is considered imperative to discard groundnuts with cracked shells before storage (El-Khadem, 1990).

Haydar *et al.* (1990) investigated various Syrian foods for aflatoxins and found high levels of contamination in peanut .More recently in survey carried out in UK, Patel *et al.* (1996) reported occurrence of aflatoxins in various ethnic foods.

Chiou and Tsao (1997) analyzed discolored kernels sampled over 3 consecutive years in Taiwan. The highest and average aflatoxin contents in 60 kernels sampled each year were 1.930 and 150 ppb in 1994, 4.040 and 160 ppb in 1995, and 410 and 32 ppb in 1996, respectively. In 1994, 1995 and 1996, 66.7, 85.0 and 61.7%, respectively, of the kernels tested contained aflatoxins; 16.7, 13.3 and 6.7% contained 50-100 ppb and 16.7, 15.0 and 10.0% contained more than 100 ppb.

Abdulkadar *et al.* (2000) analyzed edible nuts imported in Qatar from June 1997to December 1998 for aflatoxins. Eighty-one nut samples were analyzed in the second half of 1997 and contamination was detected in 19 samples with total aflatoxin level varied from a low

of about 0.53 to a high of 289μg/kg. Aflatoxin contamination was detected in pistachios and peanuts (groundnuts), while other nuts such as almond, cashew nut, walnut and hazelnut were found free from aflatoxins.

Itoh *et al.* (2001a) surveyed aflatoxin B_1 contamination in Virginia, large-type (5595 lots) and Spanish-Runner small-type (19 863 lots) raw shelled peanuts [groundnuts], imported to Japan during September 1972 to December 1991. The regulation level of aflatoxin B_1 in food is 10 ppb in Japan. Aflatoxin B_1 was detected in 0.4% (24 lots of 5595) of large-type raw shelled groundnut samples, and 0.1% (5 lots) of the samples were rejected as having above the regulation level of aflatoxin B_1. Aflatoxin B_1 contamination of small-type raw shelled groundnuts was detected in exports from 20 of 31 countries. Aflatoxin B_1 was detected in 3.4% (670 of 19 863 lots) of small-type raw shelled groundnut samples and 1.4% (269 lots) were rejected as having above the regulation level of aflatoxin B_1. The mean aflatoxin B_1 levels for large- and small-type raw shelled groundnuts were 56 ppb (range 0.3-608 ppb) and 202ppb (range 0.1-8065 ppb), respectively.

Itoh *et al.* (2001b) determined the presence of aflatoxin B_1 in commercial groundnut butter and groundnut products in Japan between November 1970 and February 1971. It was detected in 49% of the groundnut butter and in 88% of the groundnut products. The aflatoxin B_1 levels in the groundnut butter and groundnut products were in the range of 3-44 ppb and 3-9 ppb, respectively. Between March 1971 and August 1972, aflatoxin B_1 contamination was detected in 4.6% of raw

shelled groundnuts and 3.9% of groundnut butter, which were rejected due to contamination exceeding the regulation level in Japan (10 ppb).

Abdulkadar *et al.* (2002) investigated the occurrence of aflatoxin in commodities imported into Qatar from 1999 to 2000. During the 4 years, 351 samples of susceptible commodities were analysed. They found that peanut (groundnut) and peanut products (nine of 42 analysed) were found contaminated with low levels (0.1-20 micro g/ kg) of aflatoxins.

Caldas *et al.* (2002) detected of aflatoxins in 19.6% of the samples: raw groundnuts and its products, pop corn, maize and Brazilian nuts (>2 μg /kg). Groundnuts and its products showed the highest levels of aflatoxin contamination (34.7%) with up to 1280 μg/kg of AFB_1+AFG_1 and 1706 μg /kg of total aflatoxins. Of the positive samples, AFB_1 was detected in 98.5%, AFB_2 in 93%, AFG_1 in 66.7% and AFG_2 in 65.4%.

Batatinha *et al.* (2003) conducted a study to evaluate the contamination of groundnut and groundnut products by aflatoxins (B_1, B_2 and G_2) commercialized in Salvador and some cities of Bahia and Brazil. 100 samples were analysed. Of 33 groundnut samples analysed, 31 (93.9%) were positively contaminated; from these 19 (57.58%) exhibited contamination by aflatoxins (B_1 + B_2 + G_2) higher or equal to 20 micro g/kg. From the 67 samples of groundnut products, only 4 (5.98%) presented contamination by aflatoxins (B_1 + B_2 + G_2) higher or equal to 20 micro g/kg. 20 (80%) of the whole samples (25) collected at the cities of Bahia and 38 (50.7%) of the 75 samples collected in Salvador were positive.

Shundo *et al.* (2003) analysed eighty seven samples of groundnuts and groundnut products commercialized in the region of Marilia, Sao Paulo, Brazil between 1999 and 2001 to identify and quantify aflatoxin B_1 and G_1, using thin layer chromatography. Aflatoxins were found in 56 (64.4%) of the samples and 34 (39.1%) exceeded the limits of the Brazilian legislation valid in that period, which for aflatoxin B_1 + G_1 was 30 micro g/kg. The aflatoxin concentration in the samples varied from 3 to 1659 micro g/kg and the average of positive samples was 306micro g/kg. In 31 (35.6%) samples, aflatoxins were not detected. High levels of aflatoxins were observed during high temperature and rainfall levels. Results show that aflatoxin contamination exist mainly due to conditions of temperature and humidity prevalent in the region of Marilia that are favourable for fungal growth.

Vaąmonde *et al.* (2003) screened *Aspergillus* section *flavi* strains isolated from groundnuts, wheat and soyabean grown in Argentina for aflatoxins (type B and G) and cyclopiazonic acid (CPA) production. *Aspergillus flavus* was the predominant species in all substrates, although there was almost the same proportion of *A. flavus* and *Aspergillus parasiticus* in groundnuts. *Aspergillus nomius* was not found. Incidence of aflatoxigenic *A. flavus* strains was higher in groundnuts (69%) than in wheat (13%) or soybeans (5%),while the ratio of CPA producers *A. flavus* isolated from all substrates was very high (94% in groundnuts, 93% in wheat and 73% in soybeans). Isolates of *A. flavus* able to produce simultaneously aflatoxins type B and CPA were detected in all substrates, suggesting the possibility of co-

occurrence of these toxins. Almost all isolates of *A. parasiticus* resulted aflatoxins (type B and G) producers but did not produce CPA. Five of sixty-seven strains isolated from groundnuts showed an unusual pattern of mycotoxin production (aflatoxins type B and G simultaneously with CPA). These strains also produced numerous small sclerotia like S strains of *A. flavus* detected in cottonseed in Arizona and in soils of Thailand and West Africa. The atypical strains are not widely distributed in Argentina and were found uniquely in groundnuts.

Younis and Malik (2003) carried out study in order to assess the level of aflatoxin contamination on locally marketed groundnuts and groundnut products collected over 2 seasons from different sites in the Central Region, Sudan. Samples were analysed using high performance liquid chromatography (HPLC) and thin layer chromatography (TLC), with emphasis on examination of the degree of precision of the 2 techniques. The mean percentage aflatoxin recovery was 95% for HPLC and 79% for TLC. The lowest limits of detection, for total aflatoxins, on spiked groundnuts were 0.4 and 0.8 micro g/kg for HPLC and TLC, respectively. Among the 400 samples of each groundnut type expected to be naturally contaminated, the percentage aflatoxin contamination was 2, 64, 14 and 11% for groundnut kernels, groundnut butter, groundnut cake and roasted groundnuts, respectively. The highest aflatoxin levels were detected in groundnut butter (32-54 micro g/kg) and the lowest in groundnut kernels (3-8 micro g/kg), whereas groundnut cakes and roasted groundnuts showed moderate levels of contamination (7-10 and 4-12 micro g/kg, respectively). Within all contaminated samples and irrespective of the groundnut type, the

presence of aflatoxin B_1 was always predominant, followed by G_1, B_2 and G_2.

Dharmaputra *et al.* (2004) found the highest *Aspergillus flavus* infection and aflatoxin contamination in raw kernels of groundnuts collected from retailers in traditional markets. Post harvest handling methods prior to groundnuts being delivered to retailers and especially at the retailer level in traditional markets severely impact on the level of aflatoxin contamination in the Indonesian food chain. Some potential initiatives to minimize aflatoxin contamination, both at the pre and post harvest stages, are discussed in this paper. Critical to the further development of this work is a concentrated effort to monitor post harvest handling methods carried out by farmers, collectors and retailers in traditional markets and identify the critical control points for potential changes needed in their procedures.

Gurses and Erdogan (2004) collected 22 groundnuts, 13 pistachio and 9 almond samples from retail market in Erzurum, Turkey, and analysed for aflatoxin B_1 using thin layer chromatography (TLC) method. It was determined that 5 (22.7%) of groundnut samples, 3 (23.1%) of pistachio samples and 2 (22.2%) of almond samples contained aflatoxin B_1. The presence of aflatoxin B_1 was detected in concentrations ranging between 1.1 and 11.3 ppb of dry fruits. The highest-level of aflatoxin B_1 (11.3 ppb) was determined in a sample of groundnuts. It was determined that the aflatoxin is found in low levels in the contaminated samples.

Hifnawy *et al.* (2004) mentioned that aflatoxins, particularly aflatoxin B_1 (AFB_1) have been recognized as one of the most potent

chemical carcinogen. In Egypt, HCV is prevalent. The progressive nature of HCV-related liver diseases was found to be influenced by other factors. In this paper, the role of aflatoxin contamination in the onset of liver cancer in HCV-infected patients was studied. The quantitative identification of possible aflatoxin contamination in six urban and eleven rural areas in Al-Sharkia Governorate, Egypt, using high performance liquid chromatography technique, revealed that corn, wheat, peanut, lupine "termis", white rice, cowpea "lobiya", faba beans, and brown rice were contaminated, with a prevalence rate of 64.7%, 53%, 53%, 47%, 47%, 41%, 29.4% and 29.4%, respectively, for each food item. A positive correlation was found between aflatoxin and positive HCV-PCR together with liver disease progression to G3S3, which is indicative of hepatocellular carcinoma. Such correlation was not fully understood, but the oncogene amplification caused by HCV-infection may be aggravated by the consumption of aflatoxin-contaminated raw food materials or their products.

Mphande *et al.* (2004) examined raw groundnuts for fungi and mycotoxin (aflatoxins and cyclopiazonic acid) contamination. Zygomycetes were the most common fungi isolated; they accounted for 41% of all the isolates and were found on 98% of the groundnut samples. Among the Zygomycetes, *Absidia corymbifera* and *Rhizopus stolonifer* were the most common. *Aspergillus spp.* accounted for 35% of all the isolates, with *Aspergillus niger* being the most prevalent (20.4%). *A. flavus* and *A. parasiticus* were also present and accounted for 8.5% of all the isolates, with *A. flavus* accounting for the majority of the *A. flavus* and *A. parasiticus* identified. Of the 32 isolates of *A.*

flavus screened for mycotoxin production, 11 did not produce detectable aflatoxins, 8 produced only aflatoxins B_1 and B_2, and 13 produced all four aflatoxins (B_1, B_2, G_1, and G_2) in varying amounts. Only 6 of the *A. flavus* isolates produced cyclopiazonic acid at concentrations ranging from 1 to 55micro g/kg. The one *A. parasiticus* isolate screened also produced all the four aflatoxins (1200 micro g/kg) but did not produce cyclopiazonic acid. When the raw groundnut samples (n=120) were analysed for total aflatoxins, 78% contained aflatoxins at concentrations ranging from 12 to 329 micro g/kg. Many of the samples (49%) contained total aflatoxins at concentrations above the 20 micro g/kg limit set by the World Health Organization. Only 21% (n=83) of the samples contained cyclopiazonic acid with concentrations ranging from 1 to 10 micro g/kg. The results show that mycotoxins and toxinogenic fungi are common contaminants of groundnuts sold at retail in Botswana.

Razzazi-Fazeli *et al.* (2004) evaluated the level of total aflatoxin (AfT) and aflatoxin B_1 (AfB_1) contamination of baby foods, groundnut products, and maize products, which were purchased from traditional markets and supermarkets in Indonesia during the year 2001-2002. Eighty-two groundnut products, 12 baby foods products, and 11 maize products from different brands were analysed for AfT and AfB_1 using Enzyme-Linked Immunosorbent Assay (ELISA) method. Of the brands analysed, 35% of the groundnut products were contaminated with aflatoxins at various levels (range 5 to 870 micro g/kg). Groundnut-chilli sauces had the highest percentage of AfT contamination 9/12 (75%), which was followed by traditional snacks 5/11 (45%),

groundnut butter 4/11 (40%), flour egg coated groundnut 6/16 (37%), and groundnut cake 3/10 (30%). Fried groundnuts and roasted groundnuts were found to contain aflatoxin at relatively lower percentages of 9% and 8%, respectively. From the 12 analysed baby food samples, on the other hand, no sample was found to be contaminated with aflatoxins. Two of 11 samples (18%) of maize-based products were contaminated with AfT, ranging between 5.8 and 12.4 micro g/kg. Additionally, 30 selected samples in different concentration ranges were further analysed to verify the correlation between ELISA and HPLC techniques and results were compared.

Eizendeher *et al.* (2005) analysed a total of 44 samples of groundnut sweets (n=38) and natural groundnuts (n=6) collected in 21 districts of Parana, Brazil during 2002, for aflatoxin content. Results showed that 73.68% of samples of groundnut sweets were contaminated with aflatoxins. Aflatoxin content ranged from 25.95 to 350.02 μg/kg for total aflatoxins B_1 and G_1. 36.84% of samples had levels above30 μg/kg and 15.79% had levels above 20μg/kg for all aflatoxins (B_1, B_2, G_1 and G_2). 26.31% of samples had levels of aflatoxin G_1 ranging from 15.87 to 127.87μg/kg. Of the natural groundnut samples, 16.67% were contaminated with aflatoxins; the total level of contamination with aflatoxins B_1, B_2, G_1 and G_2 was 3420.84μg/kg.

2. Toxicity and carcinogenicity of aflatoxins

Aflatoxins are a group of potently hepatotoxic, teratogenic, mutagenic, and carcinogenic mycotoxins produced mainly by

Aspergillus flavus and *Aspergillus parasiticus* in a number of foods and feeds, particularly in corn, peanuts, and edible nuts (IARC, 1993).

Aflatoxins B_1 (AFB_1), B_2 (AFB_2), G1 (AFG_1) and G_2 (AFG_2) are toxic metabolites produced by the food spoilage fungi *Aspergillus* particularly *A. flavus* and *A. parasiticus*. The AFB_1 is listed as a carcinogen of group I by the International Agency for Research on Cancer (WHO, 1987).

Groundnut is frequently infected with fungi that produce mycotoxins during and after harvesting, which affect the quality and safety of human food (Martin *et al.,* 1999). The prevalence of human exposure to aflatoxin has been shown to be over 98% in West Africa, including Nigeria (Wild, 1996). In the World Bank Report, Investing in Health (1993), mycotoxin- induced diseases led to a reduced life expectancy in the developing countries (Miller, 1996). Aflatoxin is a very powerful hepatocarcinogen, and naturally-occurring mixtures of aflatoxins have been classified as class1 human carcinogens (IARC, 1993). It has been linked with the high incidence of liver cancer in Africa (Oettle, 1964). Aflatoxin synergises with other agents, such as hepatitis B, in the causation of liver cancer (Henry *et al.,* 1998). Though, the etiology and pathogenesis of kwashiorkor still remain obscure, much higher aflatoxins have been found in the blood, urine and livers of children in Nigeria with the disease than similar age-matched children (Hendrickse, 1983), and the presence of the toxin was established in the autopsy brain tissue of some Nigerian children (Oyelami *et al.,* 1996). Aflatoxins have also been proven to be immunotoxic (Turner *et al.,* 2003), and a recent epidemiological study

reveals a striking association between exposure to aflatoxins and growth stunting (Gong *et al.,* 2002).

Epidemiological studies have demonstrated a strong association between exposure to aflatoxin B_1 and an increased incidence of human hepatocellular carcinoma in Africa, Thailand and The Philippines (Busby and Wogan, 1979 and Bulatao-Jayme *et al.,* 1982).The pathological effects of aflatoxin exposure may be acute, especially in the young, as described in a Ugandan report of a 15-year-old boy weighing 36 Kg who died after eating cassava contaminated with 1.7 mg/Kg aflatoxin (Serck-Hansen, 1970).A paper from India reported the death of 100 people from acute aflatoxicosis (Krishnamachari *et al.,*1975).Another report from Malaysia related how 13 children between the ages of 2.5 and 11 years died as a result of eating contaminated noodles in the state of Perak (Cheng, 1992). Aflatoxins are also reported to be immunosuppressive (Mocchegiani *et al.,* 1998 and Fernandez *et al.,* 2000) and to reduce growth in experimental animals (Butler and Wigglesworth, 1966). Aflatoxins also produce chronic effects with reports of liver damage and hepatocarcinoma (Wogan, 1992 and Jackson and Groopman, 1999). Bruce (1994) studied this extensively and Hoseyni (1992) estimated that the risk of death from liver cancer in people ingesting aflatoxin B_1-contaminated food increased by 0.05% per ng/Kg body weight/day exposure to aflatoxin over those not exposed. In 1993 a WHO document accepted evidence of the carcinogenicity of aflatoxin B_1 (IARC, 1993).

In an Egyptian study, AFB_1 was detected in the sera of 17% of patients with hepatocellular carcinoma and in 9.4% of healthy controls attending Cairo Liver Center (Rahman *et al.,* 2001)

Hepatocellular carcinoma (HCC) accounts for 5.5% of all cancer cases world wide (Kensler *et al.,* 2003) and is one of the most common cancers in Asia, Africa and in groups of Asian- and Hispanic-Americans. HCC attacks people at an early age in high risk zones. The highest occurrence and the youngest people with this disease are in the hyper endemic areas of China, Taiwan, Thailand and sub-Saharan Africa. There appears to be an increasing trend of HCC in these regions in recent years. For instance data from death certificates in Thailand from 1993 to 2003 reveal that liver cancer mortality in Sa Kaeo Province of Thailand increased from 3.1 to 26.1 per 100,000 populations between 1993 and 2003. In Thailand overall rates increased from 9.0 to 19.8 per 100,000 population between 1996 and 2003. According to electronic hospital records, the total number of patient encounters (in-patient admissions and out-patient visits) for liver cancer in the two main hospitals in Sa Kaeo Province increased by 56% (14% annually) between 1999 and 2003. The number of cases of HCC increased from 42 in 2001 to 73 in 2003 (Amon *et al.,* 2005).

Epidemiological studies have identified chronic infection with hepatitis B virus (HBV) and dietary aflatoxin exposure as two major etiological risk factors for the development of HCC (Kew, 2003). The synergistic interaction between HBV and aflatoxins especially aflatoxin B_1 (AFB_1) has been observed in both animals (Bannasch *et al.,* 1995) and humans (Lunn *et al.,* 1997 and Wang *et al.,* 2001).

Of the aflatoxins, AFB_1 is the most prevalent, the most occurring and also the most potent. Acute dietary exposure to AFB_1 has been implicated in epidemics of acute hepatic injury (Sudakin, 2003). The liver is the primary site of biotransformation of ingested AFB_1. The predominant human CYP450 isoforms involved in human metabolism of AFB_1 are CYP 3A4 and CYP 1A2. Both enzymes catalyze the biotransformation of AFB_1 to the highly reactive *exo*-8, 9-epoxide of AFB_1 (Guengerich *et al.,* 1998). CYP 1A2 is also capable of catalyzing the epoxidation of AFB_1 to yield a high proportion of *endo* epoxide and hydroxylation of AFB_1 to form aflatoxin M_1 (AFM_1), which is a poor substrate for epoxidation (Guengerich *et al.,* 1998), less potent than AFB_1 (Wild and Turner, 2002) and generally considered detoxification metabolite, while CYP 3A4 can also form AFQ_1 a less toxic detoxification metabolite. CYP 3A5 metabolizes AFB_1 mainly to the *exo* epoxide and some AFQ_1 (Wang *et al.,* 1998). Polymorphism studies with CYP 3A5 reveal that this isoform is not expressed by most people and in particular about 40% of African- Americans do not express this enzyme (Wild and Turner, 2002). Studies with Gambian children reveal that aflatoxin can cross the placenta and be transported into the new born (Wild *et al.,* 1993). Thus CYP 3A7 a major cytochrome P450 in human fetal liver, has the capacity to activate AFB_1 to the 8, 9- epoxide (Kitada *et al.,* 1989).

Epoxidation of AFB_1 to the *exo*-8, 9-epoxide is a critical step in the genotoxic pathway of this carcinogen. The epoxide is highly unstable and binds with high affinity to guanine bases in DNA to form afltoxin-N7- guanine (Guengerich, 2001). The afltoxin-N7-guanine has

been shown to be capable of forming guanine (purine) to thymine (pyrimidine) transversion mutations in DNA (Bailey *et al.,* 1996). Studies *in vitro* and animal models as well as epidemiological studies have revealed a high incidence of this transversion mutation occurring at codon 249 of the p53 tumor suppressor gene (Li *et al.*, 1993 and Mace *et al.,* 1997) a region corresponding to the DNA binding domain of the corresponding protein (Sudakin, 2003).

Glutathione pathway has been shown to play a major role in the detoxification of AFB_1 (Johnson *et al.*, 1997 and Farombi *et al.,* 2005). The AFB_1 8, 9 *exo* and *endo* epoxides can be conjugated with glutathione resulting in the formation of AFB-mercapturate catalyzed by glutathione S-transferase (GST) (Johnson *et a*l., 1997). The *exo* and *endo* epoxide can also be converted nonenzymatically to AFB_1-8, 9-dihydrodiol which in turn can slowly undergo a base-catalysed ring opening reaction to a dialdehyde phenolate ion (Guengerich *et al.*, 1998). AFB_1 dialdehyde can form Schiff bases with lysine residues in serum albumin forming aflatoxin-albumin complex (Sabbioni and Wild, 1991). Furthermore, aflatoxin dialdehyde can be reduced to a dialcohol in a NADPH-dependent catalyzed reaction by aflatoxin aldehyde reductase (AFAR) (Hayes *et al.*, 1991 and Knight *et al.*, 1999).

Aflatoxin contamination of foods is considered as major risk factors in HCC (Kensler *et al.,* 2003).

A synergistic interaction between AFB_1 exposure and HBV infection on HCC risk has been reported in several epidemiological studies (Ross *et al.,* 1992 and Wang *et al.,* 1996). Studies on HCC in

Swaziland and Guangxi Province of China have revealed the possible synergistic interaction between AFB_1 and HBV (Peers *et al,* 1987 and Yeh *et al.,* 1989). In animal model, interaction of AFB_1 and HBV has also been demonstrated. In a transgenic mice over expressing the large envelope polypeptide of HBV and fed with AFB_1 model, Sell *et al.* (1991) showed that these mice produced more rapid and extensive hepatocyte dysplasia and HCCs than mice unexposed to these agents. Further evidence for a more synergistic interaction between AFB_1 and HBV came from the studies of interaction between AFB_2 and another member of *Hepadnaviridae* family, the woodchuck hepatitis virus (WHV) (Bannasch *et al.,* 1995). De Flora *et al.* (1989) showed that infection of woodchucks with WHV enhanced the activation of AFB_1 to the reactive AFB_1-8, 9- epoxide. Furthermore using urinary AFB_1 metabolite and aflatoxin-albumin adducts as biomarkers, studies in Shangai, China and Taiwan showed a synergistic interaction between exposure to AFB_1 and HBV carrier state (Kew, 2003). Chen *et al.* (2001) in a study involving adolescents in Taiwan demonstrated a positive association between hepatitis B surface antigen (Hbs- AG) status and AFB_1-albumin supporting the synergistic interaction between HBV and AFB_1. However, other studies failed to find an association between HbsAg status and albumin adducts (Wang *et al.*, 1996 and Wild *et al.*, 2000).

Genetic polymorphism seems to play an important role in the interaction between HBV and AFB_1. GST M_1 and T_1 phase II detoxification genes involved in the detoxification of AFB1-8, 9-epoxide has been identified. In a cohort study in Taiwan, Sun *et al.*

(2001) found a statistically significant relationship between detectable levels of AFB1-albumin adducts in serum and risk of HCC among chronic HBsAg carriers. In addition they found that the effect of aflatoxin exposure on HCC risk was more pronounced among chronic HBsAg carriers with the $GSTT_1$ null genotype than those who were non-null The interaction between serum AFB_1-albumin adduct level and $GSTT_1$ genotype was also significant.

The tumor suppressor gene p53 has been implicated in the synergistic interaction between AFB_1 and HBV. P53 is the most commonly mutated gene in human cancers. A guanine (G) to thymine (T) transversion at the third position of codon 249 of the p53 gene (249^{ser}) is commonly found in HCC from patients in regions with dietary aflatoxin exposure. *In vitro* studies have supported this finding demonstrating that AFB1 can induce this mutation (Puisieux *et al.,* 1991 and Aguilar *et al.,* 1993). In a study in China, all the patients with 249^{ser} mutations showed evidence of chromic HBV infection (Ming *et al.,* 2002). Similarly in Taiwanese patients with HCC, all the 249^{ser} mutations occurred in patients positive for HBsAg (Lunn *et al.,* 1997). On the contrary, Stern *et al.* (2001) in a study involving Guangxi, People's Republic of China found little evidence for an HBV-aflatoxin interaction modulating the presence of the p53 249^{ser} mutation or any type of p53 mutation.

AFB_1, the most potent of the aflatoxins, has been implicated in the aetiology of HCC by numerous studies. Studies have also demonstrated that the concurrent infection with HBV during aflatoxin

exposure increased the risk of HCC. In mechanistic terms, a number of molecular pathways have been proposed linking AFB_1 with HBV.

It has been proposed that HBV infection directly or indirectly may induce the specific CYP that metabolise AFB_1 to the reactive metabolite. In transgenic mice model, the induction of phase I enzymes was demonstrated (Gechu-Hatewu *et al.,* 1997). In addition, it was reported that Gambian children and adolescents chronically infected with HBV have higher concentration of AFB_1 adducts than uninfected individuals (Turner *et al.,* 2000 and Chen *et al.,* 2001). Furthermore, induction of phase 2 detoxification enzymes such as the GST families has been described in AFB_1 and HBV hepatocarcinogenesis (Yu *et al.,* 1997 and Sun *et al.,* 2001).

As an alternative mechanism to the formation of 8, 9- epoxide from activation of AFB_1, formation of ROS has been demonstrated in several models (Shen *et al.,* 1996; Yang *et al.,* 2000 and Lee *et al.,* 2005). ROS has been shown to be mutagenic and may thus contribute to the process of cancer formation. HBV has also been shown to generate ROS (Liu *et al.,* 1994). Synergistic interaction of both AFB_1 and HBV via ROS formation may be a major mechanism by which they induce HCC. Furthermore, carcinogens have been shown to activate $NF\text{-}_kB$ via ROS production. It may be possible that AFB_1 induces liver cancer via ROS-induced activation of $NF\text{-}_kB$. Experimental studies to test this hypothesis are necessary.

3. Effects of heat treatments on aflatoxins in peanut

Attempts have been made by Lee *et al.* (1968) to apply dry roasting on a means of detoxifying aflatoxin- contaminated peanuts.

The work however did not attempt to predict the variation in temperature and time.

Fukal *et al.* (1987) showed that 239 of 410 samples of roasted groundnuts contained aflatoxin below the detection limit. Only 1.9% of all samples had aflatoxin more than 5 mu g/kg. The highest concentrations of aflatoxin were in a raw sample (202.1 mu g/kg) and in a roasted sample (32.6 mu g/kg).

Pluyer *et al.* (1987) studied the effects of oven and microwave roasting on aflatoxin-contaminated groundnuts. In artificially contaminated groundnuts, oven-roasting for 30 minutes at 150 degrees C or microwave-roasting for 8.5 minutes at 0.7 kW were equally effective in destroying 30-45% of aflatoxin B_1 (AFB_1). In naturally contaminated groundnuts, both oven- and microwave-roasting were equally effective in destroying 48-61% of $AFB_1$and 32-40% of aflatoxin G_1.

Hag-Elamin *et al.*(1988)investigated the aflatoxin contamination of groundnuts in Sudan .They found that Groundnut paste and grey roasted nuts accumulated less aflatoxin than red roasted nuts.

Singh *et al.* (1989) found that samples collected from the markets in Khartoum and Wad Medani contained aflatoxin up to 945micro g/kg in raw groundnuts, up to 517micro g/kg in roasted groundnuts and up to 994micro g/kg in groundnut paste. Groundnut paste prepared after careful sorting and cleaning had only 39 micro g/kg aflatoxin. Analyses of 145 samples in Jamaica and St. Vincent in 1984 indicated only 8 samples containing >20 micro g/kg aflatoxins. Roasted groundnuts and peanut butter samples collected from markets

in Jamaica and Trinidad did not contain detectable amounts of aflatoxins. However, groundnut products collected from St. Vincent had very high levels of aflatoxins varying from 1 to 469micro g/kg.

Oliveira *et al.* (1991) found that 34.6% of 104 retail samples of roasted groundnuts examined by TLC, contained aflatoxins B_1 and G_1 at >30 micro g/kg in Brazil.

It is established that heating significantly reduces aflatoxins in contaminated samples (Kpodo, 1996). Thus, the level of contamination in the raw groundnuts used for the processing of the DRG would have been considerably higher than the levels obtained in the current work.

Njapau *et al.* (1998) found that roasting whole groundnut kernels reduced ($P<0.001$) the concentrations of toxins from raw kernels (AFB_1=8600 micro g/kg and AFG_1=6200 micro g/kg) to 1300 and 1200 micro g/kg, respectively.

Taha *et al.* (2001) evaluated the effect of microwave roasting on aflatoxin contents of naturally contaminated groundnuts. Aflatoxin contents were determined by the method of Soares and Rodriquez-Amaya, with a detection limit of 1 micro g/kg. Microwave roasting was done at medium power (0.525 kW) for 7, 9 and 11 minutes, with five repetitions for each time of roasting. Best results were obtained after roasting the contaminated groundnuts for 9 minutes. At this time, mean reduction in total aflatoxin content was 77.8% (range, 68.4%-88.5%). It is concluded that microwave roasting reduces the aflatoxin content of naturally contaminated groundnuts.

The levels of contamination of maize, roasted and raw groundnuts, and poultry feed were considerably high, with average

total AFB_1contents of 33, 13, 65, and 7 μg/kg, respectively, and maximum AFB_1 contents of 245, 79, 480, and 160 μg/kg, respectively (Dawlatana *et al.,* 2002).

Kumar-Harish *et al.* (2002) found that maximum (60.24%) reduction in AFB_1 was obtained in the infected groundnut kernels roasted at 130 degrees C for 15 minutes at 30% moisture and minimum (48%) reduction was at 150 degrees C for 10 minutes at 10% moisture. The samples treated at 15 psi for 90 minutes at 30% moisture exhibited maximum (62.5%) inactivation whereas minimum (24%) inactivation was found in those treated at 15 psi for 30 minutes at 10% moisture.

Thomas *et al.* (2003) evaluated the level of aflatoxin contamination in roasted, boiled and raw groundnuts *(Arachis hypogaea*) obtained from different locations in Lagos, Nigeria, using ultraviolet-visible spectrophotometry and high performance liquid chromatography. Results revealed that, generally, raw groundnuts showed the highest aflatoxin level, followed by boiled and roasted groundnuts. These results indicate that roasting of groundnuts reduces but not eliminate aflatoxin contamination, and cooking may not cause aflatoxin destruction .

Ogunsanwo *et al.* (2004) obtained a positive correlation between loss of aflatoxins in the products and the roasting conditions. Seeds dry- roasted at 140^{O}C for 40 minutes resulted in 58.8% and 64.5% reductions in AFB_1 and AFG_1; those roasted at 150^{0}C for 25 minutes resulted in 68.5% and 73.3% reductions in AFB_1 and AFG_1, respectively. Roasting at150^{O}C for 30minuts led to 70.0% and 79.8% reductions in AFB_1 and AFG_1, respectively.

Groundnut is consumed in the boiled or roasted form, and also as groundnut cake ('kulikuli'). The dry roasted groundnut (DRG) snack is presently the most widely consumed form of groundnut in Nigeria. It can be consumed alone or combined with dry roasted maize (popcorn), 'gari', coconut, bread or plantain. The traditional method of preparing DRG first involves sorting out the physically damaged and mouldy kernels of raw groundnuts, followed by soaking in water for about 20 min, salting with NaCl to taste, and then roasting by stirring the kernels in hot sand placed in an earthen ware pot on an open fire. On cooling, the roasted groundnuts are separated from the sand by a metal sieve (Bankole *et al.*, 2005).

Bankole *et al.* (2005) analysed samples of dry roasted groundnuts (DRG) purchased from street hawkers, markets and retail shops in southwestern Nigeria for moisture content, fungal populations and aflatoxin contamination. The moisture content varied from 2.1% to 3.6% while the mould counts, using the dilution plating method, ranged from 2.9×10^2 to 6.3×10^2 colony-forming units per gramme. Aflatoxin B_1 was found in 64.2% of samples with a mean of 25.5 ppb. Aflatoxins B_2, G_1 and G_2 were detected in 26.4%, 11.3% and 2.8% of the samples with mean levels of 10.7, 7.2 and 8 ppb, respectively, in contaminated samples. It is concluded that the regular consumption of DRG by Nigerians might present potential health hazards to consumers.

4. Biological evaluation of aflatoxins

a. Effects of aflatoxins on animals

Newberne and Rogers (1973) found that weanling rats fed low dietary concentration of aflatoxin B_1, isolated from ground-nut meal

diets causing hepatic necrosis, developed liver cell carcinoma after 12-18 months. Livers of exposed rats reveal a variety of biochemical changes related to alteration in nucleic acid and protein synthesis, particularly in the hepatocyte nucleus and nucleolus. Liver tumors induced by aflatoxin $B_1$are multicentric hepatocellular carcinomas without cirrhosis or significant fibrosis. The patterns of liver changes are similar whichever form of exposure is used. Signs and symptoms of aflatoxin B_1 -induced liver cell carcinoma in the rat are similar to uncomplicated primary hepatocarcinoma in man with the exception that the former has no cirrhosis.

Mainigi (1982) mentioned that in cases both of protein deficiency and oestrogen administration there was significant decrease in aflatoxin B_1 binding to high-Mr proteins in rat liver cytosol.

Venkitasubramanian and Saraswathy (1983) studied the biochemical changes associated with binding of aflatoxins with tissue constituents. Impaired nucleic acid metabolism due to inhibition of polymerases responsiblefortheir respective syntheses was demonstrated using *in vivo* exposed rats and *in vitro* rat liver cell cultures. The inhibitory effect on protein synthesis through incorporation studies as well as by changes in enzyme levels was studied. A significant reduction of liver glycogen was suggestive of a disturbance in carbohydrate metabolism. The aflatoxins were found to inhibit electron transport and also act as inhibitory uncouplers of oxidative phosphorylation. Since polyamine levels increase in various cancer patients, this was taken as a biochemical parameter to estimate changes at different stages of chemical carcinogenesis. The effect of

phenobarbitone on changes in liver polyamine levels caused by administration of aflatoxin B_1 to rats was studied. It is concluded that it is unlikely that the biological effects of aflatoxins are attributable to a single molecular event.

Okoye and Neal (1987) measured serum glutamic-oxaloacetic transaminase (SGOT), glutamic-pyruvate transaminase (SGPT) and serum glucose levels in weanling male Fischer F344 rats fed on aflatoxin B_1 (AFB_1)-contaminated protein deficient and sufficient diets, to determine whether they bear any relationship to pathological changes in the liver and whether protein status has any influence on observed variations. Both SGOT and SGPT levels were elevated in rats in both dietary groups with significant increases in rats fed low protein toxic diet ($p < 0.001$: Student's t test) by 4week. Serum glucose levels in rats on low protein toxic diet were significantly reduced by 4week suggesting a protein deficiency-induced change in the nature of serum glucose response to AFB_1 poisoning. Generally, the trends of change in the levels of these parameters correlated reasonably well with the changes observed in liver histology and morphology, the initial phases of the serum SGOT, SGPT and glucose (low protein toxic diet) profiles corresponding roughly to the stage of acute toxic response of the liver to dietary AFB_1 while the second phases corresponded to the stage of development and manifestations of neoplastic lesions.

Rati *et al.* (1991) fed sub acute doses of aflatoxin B_1 and ochratoxin A to weanling albino rats individually and in combination for 36 weeks. Rats were then maintained on toxin-free normal diet for a period of 24 weeks. Livers of rats were fatty whenever aflatoxin was

administered but enzyme activity did not show significant differences among the various groups. In a few individuals where livers were severely affected, higher concn of urine creatinine, liver RNA and DNA, and ALT enzyme activity were recorded. Histopathological examination showed various stages of hepatoma and hepatocarcinoma including nodular hyperplasia,hypertrophy,vacuolisation, degeneration, pseudolobulation, cellular infiltration and fibrosis of liver of rats fed with aflatoxin individually and in combination. Anaplastic cells in the corticomedullary region and nuclear enlargement of proximal tubular epithelium of kidney were found whenever combined toxins and ochratoxin alone were administered.

Youngman and Campbell (1991) examined the effects of successive administration, withdrawal and readministration of diets high in protein (20% casein) on the promotional growth, remodelling and regrowth of aflatoxin B_1-induced preneoplastic liver lesions (foci). Weanling male Fischer 344 rats were given 10 intragastric doses of aflatoxin B_1 at 250 micro g/kg body weights during a 2-week period (initiation). The subsequent 12-week period was subdivided into 4 feeding periods, each lasting 3 weeks (promotion). Two groups of rats were given a diet with 20 or 5% casein during all 4 periods; additional groups were alternately fed on these diets in different sequences. Switching from the high-protein diet to a low-protein diet (5% casein) resulted in marked remodelling (regression) of the growing lesions to a response level similar to that in rats that did not receive the initial promotional stimulus of high-protein feeding. Refeeding on the high-protein diet caused significant reappearance of these lesions. The

restimulated development of these remodelled lesions far exceeded lesion growth in rats given only the late promotional stimulus of high dietary protein. The results suggest that a second occurrence of high-protein feeding promotes the growth of remodelled foci, thus demonstrating their potential for future promotional growth.

Toskulkao *et al.* (1996) studied the effect of endurance exercise training on hepatotoxicity induced by aflatoxin B_1 (AFB_1) in rats. Rats were subjected to swimming with 1% body weight resistance for 30 minutes, 5 days/week for 14 weeks before administration of AFB_1. Endurance exercise training induced high physical fitness as shown by reduction in resting heart rate and increase in the activities of mitochondrial succinate dehydrogenase and citrate synthase in the gastrocnemius muscle. Water-immersed rats had similar basal physical fitness when compared with that of the untrained rats. Endurance exercise training as per the above schedule followed by a single intraperitoneal injection of AFB_1 (2 mg/kg) caused a significant increase in the activities of serum alanine aminotransferase (ALT) by 6.6-fold and aspartate aminotransferase (AST) by 1.8-fold and increased the severity of histopathological hepatic necrosis at 24 hours after AFB_1 administration. Endurance exercise training potentiated AFB_1-induced hepatotoxicity by increasing the activity of the hepatic monooxygenase enzymes aniline hydroxylase and p-nitroanisole-O-demethylase. It is suggested that potentiation of AFB_1-hepatotoxicity by endurance exercise training may be due to an increase in the metabolic formation of AFB_1-8,9-oxide which, in turn, causes a marked increase in AFB_1 binding to hepatic DNA and proteins.

Gyamfi and Aniya (1998) found that administration of AFB_1 resulted in significant increases in serum alanine aminotransferase (ALT) and glutathione S-transferase (GST) levels and a significant decrease in aniline hydroxylase activity in liver microsomes. When *T. sanguinea* (5 ml/kg) was intraperitoneally administered to rats 12 hours and 1 hour before AFB_1, liver injury was significantly reduced as seen in the decreased levels of serum ALT and serum GST. However, the decrease in aniline hydroxylase activity by AFB_1 was not recovered but enhanced by *T. sanguinea* pre-treatment. Kinetic analysis of cytochrome P450 activity of rat liver microsomes *in vitro* demonstrated that *T. sanguinea* inhibited aniline hydroxylase non-competitively, indicating depressionof biotransformation of AFB_1 to toxic metabolites.

Pozzi *et al.* (2000) evaluated the effects of prolonged oral administration (21 days) of aflatoxin B_1 (AFB_1) on male wistar rats. The results showed that treated animals presented differences in body weight and absolute/relative weights of liver and kidney as well as altered hepatic function and cholesterol blood levels. Rats fed with the greatest doses of AFB_1 gained less weight (2.79 g/day) at the end of the experimental period; their blood concentrations of liver enzymes aspartate aminotransferase (AST) and alkaline phosphatase (AP) were above control levels (130.35 μ/l and 471.00 μ/l, respectively.

Celik (2001)mentioned that the toxic effects ofaflatoxins include mutagenesis, carcinogenesis, teratogenesis, and immunosuppression. The liver is the principal organ affected. High doses of aflatoxins cause severe hepatocellular necrosis. Prolonged low dosages cause reduced growth and liver enlargement. For diagnosis of aflatoxicosis, the

presence and levels of aflatoxins in the feed must be determined. In this article, biochemical, histological and carcinogenic effects of aflatoxins and alternative treatments are discussed.

Preetha *et al.* (2006) mentioned that aflatoxins are potent hepatotoxic and hepatocarcinogenic agents. Reactive oxygen species and consequent peroxidative damage caused by aflatoxin are considered to be the main mechanisms leading to hepatotoxicity. Lactate dehydrogenase, alkaline phosphatase, alanine and aspartate aminotransferases were found to be significantly increased in the serum and decreased in the liver of AFB_1 administered (1 mg/kg body mass, orally) rats, suggesting hepatic damage. Marked increase in the lipid peroxide levels and a concomitant decrease in the enzymic (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase and glutathione-S-transferase) and nonenzymic (reduced glutathione, vitaminCand vitamin E) antioxidants in the hepatic tissue were observed in AFB_1 administered rats. Pretreatment with lupeol (100 mg/kg body mass, orally) and silymarin (100 mg/kg body mass, orally) for 7 days reverted the condition to near normalcy. Hepatoprotection by lupeol is further substantiated by the normal histologic findings as against degenerative changes in the AFB_1 administered rats. The results of this study indicate that lupeol is a potent hepatoprotectant as silymarin.

Saleh *et al.* (2007) studied the ability of whey protein concentrate (WPC) to protect against aflatoxicosis. Three groups of Sprague-Dawley male rats were used. The control (a) was fed on casein diet; the control (b) received the same diet contaminated with aflatoxins

(AFT; 2.5 mg AFB_1 kg^{-1}). The AFT-contaminated diet was supplemented with WPC and fed to the third group for 9 weeks. Compared with control (a) rats, those fed the contaminated diet (control b) showed significant drop ($p<0.05$) in body weight gains, food efficiency ratio (FER), an increase in the liver function enzymes, malondialdehyde level (MDA) and a decrease in blood glutathione (GSH), plasma calcium, magnesium and potassium. Addition of WPC to the AFT-contaminated diet realized better growth rate and FER and improved the above biochemical parameters. This study indicates that WPC supplementation appears beneficial to compensate AFT toxicity.

b. Dietary intake of aflatoxins

Food represents an unavoidable source of human exposure to certain mycotoxins and data from many developing countries show that a wide range of dietary staples and agricultural products are contaminated with a significant proportion of these toxins, particularly AFB_1. Monitoring and regulatory programs have been put in place by many countries in view of the potential hazard of aflatoxins to human health. Levels ranging from zero to 50 ppb have been set as permissible levels for aflatoxin content in foods and feeds (Patterson, 1983).

The daily aflatoxin B_1 intake is estimated to be between 3.60 and 998.40μg (mean = 184.08μg) for adults consuming foods made from 400g of corn. The estimated PDI of aflatoxin B_1 is between 0.07 and 19.96 μg /kg of body weight (bw) /day (mean = 3.68μg /kg of bw/day), 0.06 – 17.40 (average = 3.20) times the estimated TD_{50} of aflatoxin B_1 in the rat (1.15 μg /kg of bw/day), an indicator of carcinogenic potency of the dose at which 50% of the animals would

have developed the tumors (Kuiper-Goodman, 1990). It would certainly be considerably higher than the tolerable daily intake (TDI) for aflatoxin B_1. On the basis of the data stated above, it can be estimated that the annual human exposure to aflatoxin B_1 from corn is in the range of 1.31-364.40 mg (mean = 67.19mg). Thus, human exposure to aflatoxin B_1 in Chongzuo County, either the highest or the average level, was much higher than described in other human aflatoxin investigations (Yeh and Shen, 1986 and Groopman *et al.*, 1992).

Aflatoxins have been found in several foods (Ellis *et al.*, 1991)being peanut and their derivative products (Selim *et al.*, 1996; Ali *et al.* 1999; Whitaker *et al.*, 1999; Candlish *et al.*, 2001; Sobolev and Dorner, 2002 and Blesa *et al.*, 2003) the main commodities to have high aflatoxin level. To protect the health of the consumers, the European Union established legal directives to control their levels in peanuts through the maximum tolerated levels that are 2 and 4 ng/g for AFB_1 and total aflatoxins, respectively (Anonymous, 2001).

Permitted contamination limits are established in different countries according to the balance between economic and health interests and the technological advance of analytical methods (FAO, 1991). The MERCOSUR has established as permitted contamination limits for peanuts and peanut paste 20 $\mu g\ kg^{-1}$ for total afltoxins and 5 $\mu g\ kg^{-1}$ for afltoxin B_1 (Codigo Alimentario Argentino, 1995).

Nigeria was listed as regulating aflatoxin B_1 at 20 ppb in all classes of food, while most countries regulate total aflatoxins at 20 ppb (FAO, 1997).

Since 1 February 1999 the European Economic Community has established permitted contamination limits in food of 2 μg kg $^{-1}$ for afltoxin B_1 and 4 μg kg $^{-1}$ for the total concentration of the four aflatoxins (Burdaspal and Legarda, 1998). For overall sanitary precaution, the European Union has enacted, in 1998, very severe aflatoxin tolerance standards of 2 ppb aflatoxin B_1 and 4 ppb total aflatoxins in dry groundnuts for human consumption (European Commission, 1998). Thus, by the Nigerian regulations, the present study indicates that aflatoxin contamination is occurring at unacceptable levels in 31.1% of the DRG being consumed while, by international standards.

The maximum levels of aflatoxin B_1 and the sum of total aflatoxins are 2 and 4 μg kg^{-1}, respectively (Commission Regulation 466/2001/EC). The sampling method and the criteria for the analytical method are described in commission Directive 98/53/EC (European Commission, 1998).

The joint FAO/WHO Expert Committee on Food Additives (JECFA, 1998) evaluated aflatoxins (B and G forms) the last time at its 49th meeting in 1998. Aflatoxins are considered to be human liver carcinogens, aflatoxin B_1, being the most potent in the aflatoxin group (B_1, B_2, G1, G_2). The potency of aflatoxins in hepatitis B carriers is substantially higher (about 30 times) than the potency in non-carriers. Since aflatoxins are genotoxic carcinogens, no tolerable daily intake (TDI) can be allocated. However, the JECFA (Joint Expert Committee on Food Additives) estimated the carcinogenic potency of aflatoxin B_1 (per ng kg^{-1} body weight/ day) to be about 0.01 cancers per year per

100000 persons in non-carriers, and about 30 times higher in hepatitis B carriers.

Li *et al.* (2001) found that the average daily intake of aflatoxin B_1 from corn in the high-risk area was 184.1μg/, and the probable daily intake is estimated to be 3.68μg/kg of body weight/day, 3.20 times the TD_{50} in rats.

Thuvander *et al.* (2001) estimated the average daily intake of aflatoxins consumption of Brazil nuts by using data on consumption from a minor survey (frequency questionnaire) incorporating 200 people and a limited number of nut samples (n = 17). In that study, the intake of Brazil nuts was approximately 0.3 g day^{-1} for both the average and the 95th percentile consumer, which is slightly lower than in the example above. The aflatoxin intakes estimated by Thuvander *et al.* (2001) were based on the assumption that the consumer ate all and did not discard any of the nuts and reached 0.6 and 0.7 ng kg^{-1} body weight day^{-1} for the mean and 95th percentile consumer, respectively. Corresponding daily intakes estimated from this study (assuming a consumption of 300g Brazil nuts during Christmas time) would be 0.002 and 0.3 ng kg^{-1} body weight day^{-1}, respectively, if smoothed over a full year.

The dietary intake of aflatoxins by the Swedish population has been estimated by examining the concentration of aflatoxin in retail products from shops in the country's four largest cities (Thuvander *et al.,* 2001). In this study, the daily intake of aflatoxins from consumption of Brazil nuts was estimated as 0.6 and 0.7 ng kg^{-1} body

weight and day^{-1} (95th percentile) for average and high consumers, respectively.

Most countries including the USA have a regulatory level around 20 ppb in foods; however in 1999 European Economic Community established a lower limit of 2.0 ppb for AFB_1 and 4.0 ppb for total aflatoxins (Mishra and Chitrangada, 2003).

Adams and Whitaker (2004) mentioned that The European Union (EU) reviewed the U.S. Origin Certification Program (OCP) to test U.S. export groundnuts for aflatoxin at origin and indicated that the OCP provides a similar level of assurance as the EU Directive concerning aflatoxin testing in various commodities. EU member countries that choose to use the OCP are not precluded from conducting random testing of lots for aflatoxin at the port of entry. For domestic use, the U.S. Department of Agriculture (USDA) requires three 22 kg laboratory samples to average less than 15 total ng/g for acceptance. The EU requires one 30 kg laboratory sample to test less than 15 total ng/g ($8B_1$) for raw groundnuts destined for further processing and three 10 kg laboratory samples to all tests less than 4 total ng/g ($2B_1$) for consumer-ready groundnuts sold for direct human consumption. The U.S. proposal to the EU was to use the official USDA 22 kg sample for raw groundnuts or divide the USDA 22 kg sample into three 7.3 kg samples for consumer-ready groundnuts.

Gachomo *et al.* (2004) found that the threat of aflatoxin contamination in food commodities and its association with health risks in both animals and humans continues to raise increasing concern over years. In this report, fungal species found in association with peanuts in

storage and their potential to produce aflatoxin in collected samples was determined.

Iqbal *et al.* (2004) found that the aflatoxin content was within the safe limit (50micro g/kg as recommended by FAO). The aflatoxin content of inoculated seeds significantly ($P<0.05$) increased over control (un- inoculate seeds). This was positively related ($r=0.65$) to moisture content of the seeds. However, negative correlation ($r=-0.50$) existed between aflatoxin and ash content of the seeds. Protein, fat and total carbohydrate (NFE) contents were not significantly related to the inoculum and its toxin production.

Pitt (2004) showed the steps used to set maximum limits for aflatoxins in foods in international trade. These steps are applied to the development of a food safety objective for aflatoxins in groundnuts in correlation with the incidence of liver cancer and hepatitis B. It is recommended that the maximum permitted level for aflatoxins in groundnuts in international trade is 15micro g/kg.

Scussel (2004) mentioned that South America (SA) is predominantly a tropical and subtropical continent and provides environmental conditions favourable for fungus growth on food crops, especially the species *Aspergillus flavus* and *A. parasiticus*. Depending on the grain and weather conditions in certain regions of SA, high levels of aflatoxins (AFLs) can be produced during harvesting or storage. That is a real problem in most of the continent. South American economies rely upon government policies to address issues of food safety. As expected, the exporting countries must comply with the standards and regulations that are implemented by the importing

country. Thus, the highest quality and safest commodity food products are sold internationally. Conversely, food for internal consumption does not meet the same high quality standards compared with exports. Some SA governments have established food safety guidelines and regulations for AFL control in national food supplies.

Kladpan *et al.* (2005) mentioned that aflatoxin which is produced by several *Aspergillus sp.* is known to have carcinogenic compounds and affects humans and animals. It contaminates nuts and cereals particularly groundnuts. At present, the Ministry of Public Health requires that the maximum aflatoxin content in foods should not be >20 ppb.

El-Sawi (2006) found that estimated daily intakes (EDI) of aflatoxin B_1 for corn calculated from mean, median and maximum were 0.097, 0.013 and 1.051 (ng/kg body weight/day), respectively, which contribute 64.7%, 20.7% and 700.7% from their respective provisional maximum daily intake (PMDI) seated by CSHPF (1999) Consell Superieur Pubique de France (CSHPF). While, for maize EDI of aflatoxin B_1 were 11.093, 11.632 and 98.331 (ng/kg body weight/day), respectively, which contribute 7395.3%, 7754.7% and 65554%, respectively.

Saleemullah *et al.* (2006) mentioned that almond, walnut and peanut varied from 5 to 17micro g/kg. The aflatoxin content was within the safe limit (50 micro g/kg) recommended by FAO.

5. Decontamination and detoxification of aflatoxins

Numerous strategies are evolving for the management and field-practical control of mycotoxins. Some clearly are more practical and

effective than others. Novel genetic and molecular approaches to prevent or minimize the occurrence of aflatoxins and other mycotoxins in grains and modification of toxicity and carcinogenicity in animals through diverse chemical and dietary interventions show great promise.

Because mycotoxin contamination often is unavoidable, developing and implementing efficient detoxification methods become very important. Guidelines for evaluating mycotoxin detoxification and decontamination procedures have been established. The process should (1) inactivate, destroy, or remove the mycotoxin; (2) not result in the deposition of toxic substances, metabolites, or byproducts in the food or feed; (3) retain nutrient value and feed acceptability of the product or commodity; (4) not result in significant alterations of the product's technological properties; and, if possible, (5) destroy fungal spores (Park *et al.*, 1988). In addition to these criteria, the process (es) should be readily available, easily utilized, and inexpensive. These latter attributes facilitate administration of a comprehensive decontamination program for affected feed and foodstuffs and are more applicable in regions where less-stringent protocols exist.

Detoxification efforts for mycotoxins have focused primarily on the aflatoxins (Phillips *et al.*, 1994 and Phillips, 1999); many strategies to decontaminate aflatoxin- contaminated crops and products have been reported and reviewed (Goldblatt and Dollear, 1977 and 1979; Marth and Doyle, 1979; Council for Agricultural Science and Technology, 1979, 1989; Anderson, 1983; Palmgren and Hayes, 1987; Cole, 1989; Phillips *et al.*, 1994 and 1995 and Phillips, 1999). Although much more limited in scope, methods to detoxify mycotoxins other than the

aflatoxins also have been reported. As the impact of these hazardous toxins is being recognized, successfully removing them from the food supply represents an emerging area of interest and research. Various approaches to the detoxification, inactivation, degradation, or decrease of aflatoxin and other mycotoxins and their applications are outlined in the following sections.

a. Physical methods of separation

1. Mechanical separation

Toxin levels decrease as clean product is physically separated from contaminated product in corn and wheat (for example); ultimately, this method is not very practical due to incomplete removal of mycotoxin- tainted grains and the converse removal of clean grain (Natarajan *et al.*, 1975 and Phillips *et al.*, 1994). Significant decreases in aflatoxin levels from electronic- and hand-sorted peanuts have been reported and is commonly utilized (Dickens and Whitaker, 1975 and Natarajan *et al.*, 1975). Even though complete removal of all residual contamination cannot be expected with a variety of mechanical methods of separation, aflatoxin concentrations may be markedly decreased following rigorous treatment strategies. Interestingly, in cheese manufacturing, aflatoxin M_1 occurs predominantly with the casein, which results in a higher concentration in the curd than the whey. Consequently, aflatoxin levels in cheese can be significantly enriched, i.e., by a factor of 2.5 to 3.3 in soft cheeses versus 3.9 to 5.8 in hard cheeses (Yousef and Marth, 1989 and Van Egmond, 1994). Aflatoxin M_1 apparently is not significantly deactivated during the ripening of cheese.

2. Density segregation

Density segregation of contaminated grain and oilseeds involves sorting and delineating good versus contaminated kernels by flotation; importantly, this method can notably decrease aflatoxin concentrations (Huff, 1980; Huff and Hagler, 1982 and Cole, 1989). Peanuts that are contaminated with aflatoxin respond positively to density segregation by floating in tap water. This procedure may be compatible with current wet milling practices and alkaline processing of corn. It should be noted, however, that the appearance and weight of a particular kernel do not always indicate the presence or absence of mycotoxin. The removal of damaged grain by density segregation also has been shown to achieve decreases in deoxynivalenol and zearalenone in corn and wheat (Jackson and Bullerman, 1999). Combinations of methods involving hand picking and density segregation of contaminated or moldy grains resulted in a decrease of 70 to 90% of aflatoxin and fumonisins (Vasanthi and Bhat, 1998). Also, a combination of rinsing and density segregation of floating material was shown to be effective as a practical method for diminishing the toxicity to pigs from moldy corn containing deoxynivalenol and zearalenone (Rotter *et al.,* 1995). An overall decrease of total fumonisins (as FB_1 and FB_2 by HPLC analysis) of 60% was obtained by screening and gravity separating corn being discharged from a storage silo (Malone *et al.,* 1998).

b. Physical methods of detoxification

1. Thermal inactivation

Because aflatoxins are heat stable, they are not completely destroyed by heat treatments, e.g., boiling water, autoclaving (Christensen *et al.,* 1977). Partial destruction of aflatoxin may be accomplished by oil roasting or dry roasting peanuts and oilseed meals (Marth and Doyle, 1979) or roasting corn (Conway et *al.,* 1978). In a study by Lee (1989), roasting conditions and initial aflatoxin concentration in raw peanuts determined the degree of mycotoxin reduction, with decreases ranging from 45 to 83%. Other studies using roasting demonstrated that aflatoxin concentrations could be decreased in nuts and oilseed meals and in corn (Conway *et al.,* 1978). Aflatoxin destruction, however, is not complete (nor uniform) and is affected by the commodity's temperature, heating interval, and moisture content (Mann *et al.,* 1967). In contrast to aflatoxins, thermal processing is usually ineffective for decreasing the content of fumonisins and zearalenone in foods (Jackson and Bullerman, 1999).

Aflatoxin levels in dough are not significantly altered by baking temperatures (Reiss, 1978), and aflatoxin in contaminated wheat shows only partial destruction, exhibiting a similar resistance to heat and other processes involved in the bread making process.

Aflatoxin M_1 apparently was stable in raw milk and was resistant to pasteurization and processing (Stoloff, 1975 and 1980). A wide range of decrease in aflatoxin M_1 concentration during the preparation of freeze-dried milk has been reported (Purchase *et al.,*

1972). These steps include pasteurization, sterilization, evaporation, roller drying, and spray drying.

Cardenas *et al.* (1986) found that in mechanically sorted groundnuts, aflatoxin B_1 in 42.4% of the samples, 22.8% of this containing > 100 micro g/kg. The maximum Concentration was 3638 micro g, manual sorting improved the quality of groundnuts by eliminating damaged and spotted nuts. No direct correlation was noted between groundnut moisture and contamination. Aflatoxins were not completely eliminated by roasting or frying. In groundnuts for animal consumption, 85% of the samples from the 1first crop contained 50-3810 micro g/kg of aflatoxins B_1, B_2, G_1 and G_2. In the 2second crop, 43% of the samples contained > 50 micro g/kg, the maximum being 2417 micro g/kg.

Galvez *et al.* (2003) developed a manual sorting procedure to eliminate aflatoxin contamination from peanuts. The efficiency of the sorting process in eliminating aflatoxin-contaminated kernels from lots of raw peanuts was verified. The blanching of 20 kg of peanuts at 140°C for 25 minutes in preheated roasters facilitated the manual sorting of aflatoxin-contaminated kernels after desk inning. The manual sorting of raw materials with initially high aflatoxin contents (300 ppb) resulted in aflatoxin-free peanuts (i.e., peanuts in which no aflatoxin was detected). Verification procedures showed that the sorted sound peanuts contained no aflatoxin or contained low levels (<15 ppb) of aflatoxin.

2. Irradiation

Exposing peanut oil to shortwave and long wave UV light has been reported to decrease aflatoxin levels (Shantha and Sreenivasa, 1977). Other studies have shown that exposure to gamma irradiation (2.5 rad) does not degrade aflatoxin in contaminated peanut meal, while UV light produced no observable change in fluorescence or toxicity (Feuell, 1977). Sunlight (14- hour exposure) destroyed between 77 and 90% of the aflatoxin B_1 added to groundnut flakes, although only half the toxin was destroyed in the naturally contaminated product (Shantha, 1987). Exposure of aflatoxins to UV light has been reported to activate these chemicals to mutagens (Stark *et al.,* 1990). Applying UV light for 20 minutes at 25°C decreased the concentration of aflatoxin M_1 in contaminated milk by 89.1% in the presence of 0.05% peroxide, compared to 60.7% without peroxide (Yousef and Marth, 1989). There is concern by some that this treatment could cause peroxidation leading to more toxic products. Microwave irradiation also has been suggested as a method for the detoxification of certain mycotoxins in model systems and in foodstuffs. Farag *et al.* (1996) report that aflatoxins B_1, B_2, G_1, and G_2 respond to microwave treatment in both model and food systems; the rate of mycotoxin destruction was positively correlated with the power setting and exposure time.

Prado *et al.* (2003) investigated the effect of gamma irradiation on aflatoxin B_1 levels and fungal infection in samples of peanuts cv. Tatu Vermelho. The results showed that at a radiation dose of 10 KGy, mould growth was completely inhibited. Doses of 15, 20, 25, and 30 KGy were sufficient for destruction of aflatoxin B_1 by 55-74%. The

results suggest that decontamination of peanuts by irradiation is the most acceptable method for preservation of peanuts.

Prado *et al.* (2005) determined the effect of different doses of gamma -irradiation on aflatoxin B_1 production by *A. flavus* IMI 190443 in peanut, Tatu Vermelho variety grown during 2001-2002 and 2002-2003. Samples were submitted to two tests. In one assay, peanut samples were irradiated first before inoculation and in another assay; peanuts were inoculated first with the aflatoxigenic strain of *A. flavus* before irradiation. After irradiation, *Aspergillus flavus* IMI 190443 was eradicated. Aflatoxin B_1 was extracted with a mixture of methanol and 4% potassium chloride (270+30, v/v), followed by clarification with 10% cupric sulfate and partition with chloroform. Quantification was done by thin layer chromatography and fluorescent areas in the samples were measured using a densitometer. Previously irradiated peanuts inoculated with the aflatoxigenic strain had high levels of aflatoxin B_1. Gamma-irradiation doses of 25 and 30 kGy were necessary to inactivate *A. flavus* IMI 190443.

Analysis of grains revealed the occurrence of aflatoxin B_1 ochratoxin A, cycolopiazonic acid and citrinin. Of the 90 samples, 67 were positive for one or more mycotoxin. Irradiation of grains at dose of 2.0 and 4.0 kGy decreased significantly the total fungal counts compared with unirradiated controls. After 100 days of storage at room temperature, the unirradiated grains were contaminated with high concentrations of mycotoxins as compared with irradiated 4.0-kGy samples. Mycotoxin production in grains decreased with increasing

irradiation doses and was not detected at 6.0 kGy over 100 days of storage (Aziz *et al.,* 2006).

3. Solvent extraction

Aflatoxins can be extracted efficiently from contaminated grains using carefully selected solvent mixtures, including binary and tertiary systems; importantly, this toxin reduction method has a minimal effect on protein content and nutritional value of the contaminated commodity (Rayner *et al.,* 1977 and Goldblatt and Dollear, 1979). Examples include 95% ethanol, 90% aqueous acetone, 80% isopropanol, hexane-ethanol, hexane-methanol, hexane-acetone-water, and hexane-ethanol-water combinations. Although effective, such treatment is considered cost prohibitive and impractical for most applications (Shantha, 1987).

c. Biological methods of inactivation microorganisms

Strategies that shift the focus from product decontamination (post-harvest) to prevention of aflatoxin production (preharvest) using biological controls have been reported. Nontoxigenic strains of *Aspergillus flavus* and *A. parasiticus* may compete with (and exclude) toxigenic (wild-type) strains and significantly decrease aflatoxin contamination in peanuts and cottonseed (Cole and Cotty, 1990). Microorganisms, e.g., yeasts, molds, and bacteria, have been screened for their ability to modify or inactivate aflatoxin. *Flavobacterium aurantiacum* (NRRL B-184) was shown to significantly remove aflatoxin from a liquid medium without producing toxic byproducts or metabolites (Ciegler *et al.,* 1966). These same investigators also determined that certain acid-producing molds could catalyze hydration

of aflatoxin B_1 to B_2a (a less-toxic product). Applications of microbial detoxification of aflatoxins have been reviewed (Ciegler, 1978 and Marth and Doyle, 1979). Hao *et al.* (1987) reported that *F. aurantiacum* removed aflatoxin B_1 from peanut milk. This bacterium grew in both defatted and partially defatted peanut milk and was not inhibited by aflatoxin. Aflatoxins in contaminated grains are degraded by fermentation (Dam *et al.,* 1977) but ensiling contaminated high-moisture corn was not as effective. A variety of microorganisms have been shown to interfere with aflatoxin production, and this interference is thought to occur by competition for nutrients and space or through the production of substances that interfere with toxigenesis (Bhatnagar *et al.,* 1994).

In the case of the trichothecenes, it is known that the 1213-epoxide ring is responsible for their toxic activity, and removal of this epoxide group entails a significant loss of toxicity. Several authors described this de-epoxidation reaction of ruminal or intestinal flora (Yoshizawa *et al.,* 1983; He *et al.,* 1992 and Kollarczik *et al.,* 1994). Active, DON-transforming mixed cultures could be isolated from bovine rumen content, using anaerobic techniques described by Hungate (1969) and media according to Caldwell and Bryant (1966), which were modified for screening purposes. Through variation of medium components (energy source, minerals and antibiotics) and subsequent sub cultivation in dilution series and highly active enriched cultures, an isolate finally was obtained. Molecular biological analyses as well as physiological characteristics indicated a new species of the genus *Eubacterium*, referred to as BBSH 797. For the use of BBSH 797

as a feed additive, the fermentation and stabilization processes were optimized with respect to fast growth of the microbe and high biotransformation activity of the resulting product. For enhancement of stability during storage and within the GI tract, a three-step encapsulation process was implemented.

For optimizing the cell count of the final product, *in vitro* experiments using pieces of pig intestine were carried out: within 24 hour, 31% of 50 ppm DON were transformed at a bacterial concentration of 3.55 X 10^4 CFU/g, while after 48 hour, the total amount of DON was de-epoxidized. Cell counts of 3.55 X 10^5 CFU/g resulted in 83% transformation, while 3.55 X 10^6 CFU/g metabolized 100% within 24 hour. Through observing the mechanism of microbial detoxification, two important reactions were detected (Fuchs *et al.*, 1999): besides the transformation of the epoxide into a double bond, hydrolyzation of ester groups into corresponding hydroxyl groups occurred in some cases. While a direct de-epoxidation occurred in the case of nivalenol, fusarenon X was first transformed into nivalenol and then de-epoxidated. The same could be observed with3- and 15-acetyl-DON, which also were deacetylated prior to de-epoxylation. A partial deacetylation of Atrichothecenes was found prior to or together with deepoxidation.

The efficiency of the final formulation (i.e., stabilized bacteria) was tested in feeding trials, which were conducted under the surveillance of the University for Veterinary Medicine, Vienna, Austria. Highly significant results ($P < 0.001$) were obtained in piglet feeding trials with a contamination of 2.5 ppm DON. The trial started

on the 25th live day of the animals that weighed 6.7 kg. After 45 days, the animals that were fed contaminated feed gained up to 16.4 kg at a feed conversion rate (FCR) of 2.0, while those groups that had received uncontaminated feed plus different concentrations of BBSH 797 weighed between 22.3 and 23.6 kg and a FCR of 1.6.

It could be shown *in vitro* that the bacterial isolate BBSH 797 is able to biotransform trichothecenes into less toxic de-epoxy metabolites. Field trial results also suggest that GGSH 797 can alleviate performance decreasing effects of DON in growing piglets and broilers under field conditions and may prove beneficial in the preventive management of such mycotoxin contaminations.

d. Modification of biological effects of aflatoxins (chemoprotection)

Dietary factors that influence the toxicity of aflatoxin B_1 have been widely reported and continue to be an area of active research. These factors can be classified into two categories: nutrient and no nutrient food components. Of the former, attention has been directed to the presence of methyl donors such as choline and methionine (often in conjunction with folate) in the diet as ameliorating factors in induction of hepatic preneoplastic foci. Protein deficiency has been shown to affect the incidence of these foci. Dietary fat content (as well as effects of saturated versus unsaturated fats), vitamins (e.g., vitamin A, folic acid and carotene), and trace minerals such as selenium appear to modulate the hepatocarcinogenicity of aflatoxin B_1. Examples of dietary components that can be classified as no nutrients include butylated hydrotoluene, butylated hydroxyanisole, ellagic acid (a plant

phenolic), indole 3-carbinol (a component of cruciferous vegetables), various garlic extracts, and capsaicins. For a thorough discussion, the reader is directed to the following reviews (Cullen and Newberne, 1994; Eaton *et al.*, 1994; Kensler *et al.*, 1994; Rogers, 1994 and Galvano *et al.*, 2001).

Asuzu *et al.* (1988) investigated the interactions of aflatoxin B_1 (AFB_1) with vitamin K, phenylbutazone, and sulfamethoxine in albino rats. Vitamin K (5 mg/kg) was able to completely suppress the increase in whole blood clotting time caused by AFB_1 (25microg/kg). Phenylbutazone (50 mg/kg) and sulfamethoxine (50 mg/kg) also significantly ($P<0.05$) lowered the increased clotting time caused by AFB_1. Equilibrium dialysis was performed on rat plasma (4 mg/ml protein content) to investigate the displacement of AFB_1 (3 micro g) from its bound form by vitamin K (250 micro g), phenylbutazone (2500 micro g), and sulfamethoxine (2500 micro g). Phenylbutazone and sulfamethoxine significantly ($P<0.05$) displaced AFB_1 from rat plasma protein. Histopathological examinations performed on the liver, kidneys, and spleen of control and treated rats showed that none of the drugs used appeared to offer any significant organ protection against AFB_1 except in the spleen.

The development of hepatocellular, putatively preneoplastic, gamma -glutamyl transpeptidase positive (GGT+) foci and tumours induced by aflatoxin B_1 (AFB_1) has been shown to be reduced in male F344 rats fed a diet containing 6% protein (as casein). This reduction occurs despite increased energy intake, when compared with animals fed a diet containing 22% protein. Among its many effects, low protein

intake is known to increase the proportion of energy intake expended in the form of heat (thermogenesis), therefore the association between the development of GGT+ foci and alterations in indices of thermogenesis induced by feeding varying levels of dietary protein was examined. Five days following the completion of AFB_1 dosing, animals were assigned to groups fed 4, 8, 12, 16 or 20% dietary protein for 6 weeks. Foci development (% liver volume occupied) was markedly reduced in animals fed the low-protein diet (4, 8%), yet calorie consumption per 100 g body weight was greater. A modest negative linear trend was observed in oxygen consumption with increasing levels of dietary protein intake. Neither Urinary nor epinephrine levels were elevated in the groups fed 4 and 8% protein; urinary dopamine and nor epinephrine turnover rates in brown adipose tissue were highest in animals fed 4% protein. It is suggested that GGT+ foci development occurs when a "critical level" (approx. 12%) of dietary protein intake is reached. Inhibition of foci development at lower levels of protein intake is associated with several indicators of increased thermogenesis Horio et *al.* (1991).

Choi *et al.* (1995) studied the effects of vitamin E and Se supplementation to diets containing aflatoxin B_1 on the contents of liver lipids and various blood parameters. Two levels of dietary aflatoxin (0 and 1 ppm), 3 levels of vitamin E (30, 60 and 120 IU/kg) and 3 levels of Se (0.1, 1 and 2 ppm) were used to design a 2 x 3 x 3 factorial experiment. Rats, weighing approx. 200 g, were randomly allotted to 18 cages, 5rats/cage. The aflatoxin significantly ($P<0.05$) decreased growth rate, feed intake and feed efficiency. Aflatoxin increased the

glucose level and decreased the cholesterol level in blood significantly. Levels of blood triglyceride, total protein and albumin were not affected by aflatoxin, vitamin E or Se. Activities of blood alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were significantly increased by aflatoxin; however, the glutathione peroxidase (GSH-Px) activity in the blood was decreased by aflatoxin even in the presence of Se. The vitamin E supplementation decreased the AST activity significantly, while GSH-Px activity increased significantly as the levels of dietary Se increased. The levels of total cholesterol and free cholesterol in the liver were significantly lower in rats receiving aflatoxin, while the extra vitamin E supplementation increased these hepatic cholesterol levels. It is concluded that the extra dietary vitamin E or Se supplementation might partially alleviate some of the harmful effects of aflatoxin in rats.

Karakilcik *et al.* (2004) mentioned that hepatotoxic substances such as aflatoxin B_1 (AFB_1) produce free radical reactions during biotransformation damage to liver cells vitamins C and E are important natural antioxidants suppressing free radical. This study investigated the effects of vitamins C and E on liver enzymes and other biochemical parameters in rabbits experimentally exposed to AFB_1. The first group was control and fed the diet with dimethyl sulfoxide; the second group received 0.1mgAFB_1/kg diet; the third group received vitamin C (100 mg L-ascorbic acid/kg diet): the fourth group received vitamin E (100 mg α tocopherol /kg diet): and the fifth group received vitamin C + vitamin E(100 mg L-ascorbic acid/kg diet+100 mg α tocopherol /kg diet).Diets of the second, third, fourth and fifth groups were mixed

with 0.1 mg AFB_1/kg diet) and feedings were continued for 10 W levels of aspartate transaminase; alanine transaminase, alkaline phosphatase, creatine phosphkinase and lactate dehydrogenase after receiving AFB_1 were significantly increased, While activities of aspartate transaminase, alanine transaminase, amylase, creatine phosphokinase and lactate dehydrogenase in groups receiving AFB_1+vitamins C, E or C+E were significantly lower than that of the AFB_1-alon group. Although of the activity of alkaline phosphatase increased with AFB_1 exposure, it decreased with vitamin C administration. levels of urea, triglyceride, cholesterol and albumin were affected by $AFB_1$and AFB_1+vitamin C. AFB_1 affected some liver enzymes and other biochemical parameters, but vitamins C, E and C+E partially prevented an increase in these liver enzymes and some the biochemical parameters induced by AFB_1.

e. Chemical methods of detoxification
structural degradation following chemical treatment

Numerous chemicals have been tested for their ability to degrade or detoxify aflatoxin; these include acids, bases, aldehydes, bisulfite, oxidizing agents and various gases (Feuell, 1966; Trager and Stoloff, 1967; Mann *et al.*, 1970; Goldblatt and Dollear, 1977 and 1979; Anderson, 1983; Park *et al.*, 1988; Hagler, 1991; Samarajeewa *et al.*, 1991 and Phillips *et al.*, 1994). Although many proposed treatments may successfully destroy aflatoxin, they may be impractical or potentially harmful due to generation of toxic byproducts and/or significant alteration of product quality. Numerous chemical strategies (which may be practical as well as effective) for the aflatoxins include

ammoniation, ozonation, and reaction with food-grade additives such as sodium bisulfite. This is not the case for the fumonisins, however, which have been shown to be very resistant to degradation and detoxification by a variety of methods including milling, fermentation, ammoniation, and ozonation. In recent studies (Lemke *et al.*, 2001), fumonisin B_1 was significantly deaminated in aqueous solution under conditions of acidic pH and low temperature by the addition of NaNO2. Diazotization of fumonisins makes use of common, relatively inexpensive components (sodium nitrite [NaNO2] and hydrochloric acid [HCl]) whose concentrations can be optimized in larger-scale processes to maximize HONO production.

1. Ammoniation

Degradation using ammonia is purportedly a feasible method to detoxify aflatoxin-contaminated products. Ammoniation involves use of gaseous ammonia or ammonium hydroxide and, when performed under appropriate conditions, has been shown in some cases to decrease aflatoxin levels by more than 99% (Dollear *et al.*, 1968; Masri *et al.*, 1969; Gardner *et al.*, 1971; Brekke *et al.*, 1977 and 1979; Park *et al.*, 1984 and Phillips *et al.*, 1994).

Ammoniation purportedly results in the conversion of aflatoxin B_1 to less toxic products, including aflatoxin D_1 and a derivative with molecular weight 206. Because the initial step is reversible and the lactone ring may reform, it is "extremely important" to allow the reaction to proceed to completion.

Two different ammoniation procedures are currently utilized: a high-pressureandhigh-temperatureprocess (HP/HT) and an atmospheric

pressure and ambient temperature procedure (AP/AT). The HP/HT process frequently is used to decontaminate whole cottonseed and cottonseed meal and AP/AT is used mainly for whole cottonseed. Arizona and California have approved ammoniation to decontaminate cottonseed products; Texas, North Carolina, Georgia, and Alabama permit ammoniation of aflatoxin-contaminated corn. Internationally, this procedure is an accepted decontamination practice in Mexico, Sudan, South Africa, Senegal, and Brazil. Aflatoxin detoxification using ammonia and other chemical treatment methods has been extensively reviewed (Goldblatt and Dollear, 1979; Anderson, 1983; Palmgren and Hayes, 1987 and Park *et al.*, 1988).

Coker (1989) mentioned that aflatoxin assays and detoxification by ammoniation is summarized.The commodities investigated included groundnut kernels, in-shell groundnuts, roasted groundnuts, groundnut butter and groundnut cake and groundnut meal.

2. Treatment with bisulfite and other food additives

Promising results have been achieved in aflatoxin detoxification using accepted food additives; sodium bisulfite has received particular attention. Sodium bisulfite reacts with aflatoxins B_1, G_1, M_1, and aflatoxicol to form water-soluble products (Doyle and Marth, 1978a and 1978b; Moerck *et al.*, 1980 and Hagler *et al.*, 1982). Information on the chemistry of the reaction can be found in studies by Yagen *et al.* (1989) and Phillips *et al.* (1994).

Sommartya *et al.* (1988) collected groundnut kernels from north-eastern plantations in Kalasin province, Thailand, in August. 1987 were roasted, split open and the seed coat, cotyledon and embryo

were analysed for aflatoxins via a Velasco aflatoxin meter. Results indicated accumulation of aflatoxin in embryos (274 ppb) and ground cotyledon (275 ppb). It is reported that among chemicals which were tested for detoxification of aflatoxins in groundnuts, the bisulfite salts of sodium performed best.

3. Ozonization

Another effective method of mycotoxin degradation is based on reaction with ozone (O3) gas, a powerful oxidant with a preference for double bonds. Several studies indicate that ozone gas (generated by corona discharge) degrades aflatoxins in corn and cottonseed meals (Dollear *et al.*, 1968 and Dwarakanath *et al.*, 1968) and in aqueous solution (Maeba *et al.*, 1988). This procedure has been shown to degrade other mycotoxins such as DON (Young, 1986 and Young *et al.*, 1986) and moniliformin (Zhang and Li, 1994). Studies by Maeba *et al.* (1988) report that ozone gas chemically degrades and detoxifies aflatoxins B_1, G_1, B_2, and G_2 (present as pure standards) *in vitro*.

A novel (electrochemical) method of ozone production couples anodic decomposition of water (hydrolysis) at the water/porous anode interface and utilizes a proton exchange membrane in an electrolysis cell to produce up to 20% by weight (242.0 mg/ L air) O3 gas (Rogers *et al.*, 1992), as compared to a maximum of 6% by weight O3 in dried, oxygen-fed systems(Foller and Tobias, 1982). This concentrated ozone has been used to degrade and detoxify numerous mycotoxins *in vitro*, including the four commonly occurring aflatoxins, cyclopiazonic acid, ochratoxin A, patulin, secalonic acid D, and zearalenone (McKenzie *et al.*, 1997). Fumonisin B_1 was degraded to a keto-substituted analog of

FB_1 (3k-FB_1), although detoxification was not demonstrated by two separate bioassays. In another study, electrolytically generated ozone gas significantly degraded high concentrations of aflatoxins present in naturally contaminated, toxic corn. Importantly, ozone treatment of this contaminated corn significantly protected young turkeys from the deleterious effects of aflatoxins (McKenzie *et al.*, 1998).

Ozone is a fairly stable gas, but in an aqueous environment, its half-life drops to approximately 20 minutes. Ozone decomposes to form oxygen and, therefore, can be classified as a nonpersistent chemical; however, it must be generated at the location of its intended use. Ozonization may help remediate bulk quantities of corn at a minimal cost with minimal destruction of important nutrients. These findings indicate a potentially practical approach to the remediation of unprocessed corn contaminated with aflatoxin (McKenzie *et al.*, 1998).