CHAPTER I

INTRODUCTION
The science of air-hygiene encompasses the assay and control of radiological, chemical, biological and inert material as contaminants of air, particularly, to the toxicity of those contaminants on human health and wellbeing. Studies in the field of biological air analysis or detection of potential biohazards have not, until recently, had much stimulation. Biological contaminants in air include the fungi, viruses, bacteria, insects, vegetable dust, pollen grains, cysts, ova of parasites, etc. These contaminants persist in the air as aerosols, which are defined as solid or liquid particles suspended in air. Particulates in a biological aerosol usually vary in size from less than 1 microns to approximately 50 microns or even large. These particles may consist of a single unattached organism or may occur in the form of clump composed of a number of organisms. The organisms may adhere to a dust particle or may exist as a free floating particles.

Over 250 years ago, in 1713, Ramazzini had described the disease due to inhalation of grain dust among workers handling food, fodder and fiber crop. A number of epidemiological studies have been carried out on grain handlers and these studies have shown that employment as a grain handler or elevator operator is always associated with a variable increase in respiratory symptoms leading to an adverse effect on pulmonary function (DoPico, et al., 1984). It is reported that grain elevator dust and grain elevator dust extracts activate complement pathway and form a non-immunologic precipitate
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with human IgG (Broder, et al., 1981). These properties suggest the basis for a possible non-immunologic inflammatory process which may be activated when grain dust is inhaled into the lung.

It is now believed that grain dust per se does not cause any hypersensitivity disorder in lung, but something present in the dust act as aetiologic agents of a variety of extrinsic allergic alveolites (EAA) or hypersensitivity pneumonitis (HP). In this regard, fungi and thermophilic actinomycetes are being increasingly recognized throughout the world.

Besides other organic and inorganic matters, grain dust is also a good source for harbouring, not only micro-organisms which are disseminated and become airborne during its processing inside the factory, but to their toxins as well. Micro-organisms have an impact on human health and welfare in many beneficial and harmful ways. The agricultural workers are exposed to grain dust along with its micro-organisms in their day to day work. Simultaneously, they are also exposed to the spores and filaments of saprophytic or toxigenic strains which are capable of producing mycotoxins.

It is known since ages that micro-organisms have the ability to grow on the food and cause desirable changes like cheese and antibiotic production, unusual texture, flavours etc. and undesirable changes like food poisoning. Certain moulds under favourable conditions have the ability to accumulate toxic metabolites in large quantities. These metabolites cause undesirable changes in food due to toxin deposition and are harmful to human beings and animals when
consumed. These toxic metabolites are produced by different groups of fungi and are collectively termed as 'Mycotoxins'. Aflatoxins, zearalenone, ochratoxin, citrinin, tricothecenes, patulin, penicillic acid, ergot alkaloids etc. are some of the toxins produced by fungi.

**Different Types of Mycotoxins**

Since the discovery of Turkey X disease, which resulted in death of thousands of Turkey poult in England due to consumption of contaminated peanuts from Brazil, changed their attitude towards mould infestation on food and feeds (Blount, 1961), and the strain of *A. flavus* was soon isolated from the contaminated Brazilian peanuts and the toxin isolated was named as 'Aflatoxin'. Later, many other mycotoxins were discovered and isolated from the naturally contaminated agricultural commodity.

Mycotoxin contamination occurs in various foodstuffs and vary with geographical location, production and storage method. The environmental factors like humidity, temperature, water activity ($a_w$), etc. play important role in mycotoxin production. Some of the food or feed products are more suitable for fungal growth than the others, and they are more congenial for mycotoxin production. WHO (1979, 1990) has evaluated five classes of mycotoxins which are associated with health risk. These are aflatoxin, ochratoxin, zearalenone, tricothecones and ergots.

**Aflatoxin:**

The term "Aflatoxin" refers to a group of secondary fungal metabolites that are produced by two species of the genus
Aspergillus, viz. *A. flavus* Link ex Fries (Sargeant et al. 1961; Boiler and Schroeder, 1966; Lillehoj, et al., 1976) and *A. parasiticus* Speare (Boiler and Schroeder, 1973). Aflatoxins are highly oxygenated naturally occurring heterocyclic compounds and have closely related structures. There are four major types of aflatoxins, viz., $B_1$, $B_2$, $G_1$ and $G_2$ denoting their blue and green characteristic fluorescent. Aflatoxins are acutely toxic, mutagenic, teratogenic and carcinogenic in nature (Newberne, 1965; IARC, 1983).

Aflatoxins are found to produce acute and chronic effects on animals and human beings depending upon the level of aflatoxin present in foods and feeds. Liver is the primary target organ. Besides liver, kidney and the immune system are also affected by aflatoxin (WHO, 1979).

Ochratoxin:

The ochratoxins are produced by *A. ochraceus* including several other species of *Aspergillus* and *Penicillium*. Ochratoxins are a group of closely-related derivatives of isocoumarin linked to L-phenylalanine. Cereals and some beans (coffee beans, soyabeans, coco beans) are the major agricultural commodities to be infested with ochratoxin A. The major target organ for ochratoxin A is the kidney, while liver and immune systems are reported as minor target organs. The teratogenic and carcinogenic effect of ochratoxin have been reported in animals (WHO, 1990). Ochratoxin A has been found in various countries like Australia, Europe and North America. Occurrence of ochratoxin B is rare.
In human being, Balkan endemic nephropathy disorder which is supposed to be caused by ochratoxin A has been observed in rural areas of Bulgaria, Romania and Yugoslavia (WHO, 1990).

Tricothecenes:

Tricothecenes are produced by a group of fungi - Fusarium, Trichoderma, Trichothecium, Myrothecium and Stachybotrys. So far 148 types of tricothecenes have been isolated from fungal cultures and plants. The most frequent contaminants of foods and feeds are T-2 toxin, diacetoxyscirphenol (DAS), 4-deoxynivalenol (DON) and nivalenol (NIV). They occur mostly in cereal grains.

The tricothecenes are highly toxic and potent dermal irritants. T-2 toxin is cytotoxic in nature. Alimentary toxic aleukia (ATA) in the U.S.S.R. and toxicity due to contaminated wheat in Japan and Korea have been associated with the consumption of grain invaded by Fusarium. Food consumption containing tricothecenes from India and China resulted in gastro-intestinal symptoms and throat irritation (WHO, 1990).

Zearalenone:

Zearalenone is produced by different species of the same genus Fusarium, viz., F.roseum, F.tricinctum, F.oxysporum and F.moniliforme. Zearalenone is found in cereal especially maize, and in feedstuffs. It has been detected from U.S.A., Europe and Africa. It is estrogenic in nature. The primary target organs in animals are urino-genital tract, mammary glands and testicular tissue. No reports concerning zearalenone toxicity to human being have been available in
literature (WHO, 1979).

Ergot:

Ergot poisoning has been known to occur since Biblical times (Bové, 1970). Ergot is the name given to sclerotia of fungal species within the genus *Clavipes* - *C. purpurea* and *C. fusiformis*. These sclerotia contaminated foods and feeds, when consumed by man and animals, result in the development of toxicoses due to the alkaloids present in sclerotia. More than 40 ergot alkaloids have been isolated. Cereals, especially wheat, rye and barley, oats and sorghum, are common for the occurrence of ergot. Clavine-type ergot outbreak due to *C. fusiformis* contaminated pearl millet has been reported from India at various time intervals.

International Association for Research on Cancer (IARC), World Health Organization (WHO), Food and Drug Administration (FDA) and other organizations have evaluated, and observed that aflatoxins are the most potent toxin among all the mycotoxins. The level of aflatoxins in foods and feeds has been set up. Aflatoxins are considered as the chemical pollutants of biological origin and pose the most serious biohazards to animals and human beings. Thus, aflatoxin among all the mycotoxins warrants more attention.

**Occurrence of Aflatoxin**

The incidence of aflatoxin contamination is closely associated with climatic conditions. Aflatoxin is found in most of the tropical and sub-tropical countries, which favour the mould growth. The mould, *A. flavus* Link ex Fries and *A. parasiticus* Speare have the
ability to produce aflatoxin in large quantities on a variety of natural substrates such as cereal grains, oilseeds, tree nuts, tubers, fruits, crude vegetable oil, etc.

Cereal Grains:

Cereal grains appear to be good substrates for aflatoxin production than any other commodity, probably because of high content of carbohydrate. Maize, rice, wheat and sorghum are well known common agricultural commodities on which A. flavus and A. parasiticus grow luxuriantly, and produce significant aflatoxin contamination.

Maize: Maize is a major world crop and is an important commodity in international trade (FAO, 1976). The distribution and occurrence of aflatoxin in maize appear to be world-wide. The first A. flavus infection in corn was reported by Taubenhaus in 1920.

Since 1965, a systematic survey of aflatoxin contamination in U.S.A. has been conducted by Shotwell and his colleagues (1969a,b, 1971; 1973; 1977a,b). They have reported the presence of aflatoxigenic strain of A. flavus and aflatoxin at low levels except from Southeast 1973 crop. Lillehoj, et al. (1975a,b; 1977) detected toxigenic strain from Southeast U.S.A. and Iowa. In Costa Rica (Central America) mouldy maize were found to contain aflatoxin level above 20 ug/kg (F.A.O., 1979). About 50% of samples analysed during the time of harvest in North Carolina were contaminated with aflatoxin (Hesseltine, et al.,1981). Sinha (1987) has reported aflatoxin in 70% of Indian corn samples in flooded areas of
Aflatoxin has been reported to be a natural contaminant in corn samples analysed from Mozambique (Van Rensburg, et al. 1975); Kenya (Peers and Linsell, 1973); Uganda (Alpert, et al., 1971); Thailand and Hong Kong (Shank, et al., 1972a) etc.

Rice: Rice is cultivated in tropical and sub-tropical regions where heavy rainfall and humidity are common. It is a staple food in many countries of South-East Asia including India.

In Central and South America, aflatoxin was detected in rice samples (FAO, 1979). Patel, et al. (1981) reported 50% of rice samples obtained from feeds were positive. Parboiled Indian rice from cyclone affected area had aflatoxin contamination (Tulpule, et al., 1982). Aflatoxin contaminated rice was found to be associated with liver cancer in Thailand (Shank, et al. 1972b; FAO 1979). Rice samples were reported to be contaminated with aflatoxin from Uganda (Alpert, et al., 1971); Mozambique (Van Rensburg, et al., 1975); Vietnam (Lucas, et al., 1971), etc.

Other Cereals - In wheat and sorghum, the incidence and level of aflatoxin contamination are low. Shotwell, et al. (1969a) detected low levels of aflatoxin from both the cereals of U.S. origin. In flooded areas of Pakistan, wheat were found to be contaminated with aflatoxin (FAO, 1979). Patel, et al. (1981) reported that 50% of Indian sorghum samples were contaminated with aflatoxin.

Occurrences of aflatoxin have been reported in major oilseeds like groundnuts, cottonseeds, soyabean, etc. (FAO, 1979).
Pulses, tree nuts, root crops, animal products, vegetable products, beverages, fermented food etc. were also reported for aflatoxin contamination.

**Chemical Properties of Aflatoxin**

The name 'Aflatoxin' comes from their generic origin of *A. flavus* toxin. Aflatoxins were first isolated and characterised by Hartley, et al. (1963). Of the four major types, B₁ is usually found in large quantity, followed by G₁, while B₂ and G₂ are usually present in lower quantity. The structure of Aflatoxin B₁ and G₁ are determined by Asao, et al. (1963, 1965) and B₂ by Chang, et al. (1963). Aflatoxin B₂ and G₂ are dihydroderivatives of AFB₁ and G₁ respectively. These structures were established on the basis of ultraviolet penetration, infrared spectra, nuclear magnetic resonance and mass spectra analysis (Asao, et al., 1965). It was later confirmed by X-ray crystallography.

**Aflatoxin B₁ (AFB₁):**

Aflatoxin B₁ is the most potent toxin and a well known hepatocarcinogen. It is highly unsaturated molecule. The B series contain dihydrofuran moiety which makes them more potent than G series. It has an olefinic double bond and a carbonyl group in the molecule. The ketone function is attached to C₃ position of the coumarin nucleus and forms a part of a 5 membered carbocyclic ring. Detroy and Hesseltine (1970) observed that the reduction of ketone moiety of the terminal cyclopentene ring of AFB₁ to form aflatoxicol thus causes loss in potency. The AFM₁ is the 4 hydroxy derivative
of aflatoxin B₁. This reduces the carcinogenicity (Wogan and Paglia-lunga, 1974) and mutagenicity (Wong and Hsieh, 1976). The abolition of toxicity and mutagenicity is observed due to hydroxylation of aflatoxin molecule at other positions as in AFP₁ and aflatoxicol H₁ (Wong and Hsieh, 1976).

Aflatoxin B₁ (AFB₁):

AFB₁ is relatively less toxic, carcinogenic and mutagenic than AFB₂. AFB₂ has similar blue fluorescent, but the Rf value is lower than AFB₁. The partial catalytic hydrogenation of AFB₁ with one hydrogen molecule gives AFB₂ (Chang, et al., 1963; Van der Merwe, et al., 1963). The AFB₂ is dihydro-aflatoxin B₁.

Molecular Formula C₁₇H₁₂O₆

Aflatoxin G₁ (AFG₁):

The G series contain lactone ring attached to the coumarin nucleus which makes them less potent than B series. de Iongh, et al. (1962) noted that the presence of a lactone ring in the aflatoxin
molecule makes them susceptible to alkaline hydrolysis.

**Molecular Formula C\textsubscript{17}H\textsubscript{12}O\textsubscript{7}**

![Molecular Structure of Aflatoxin G\textsubscript{2}}

**Aflatoxin G\textsubscript{2} (AFG\textsubscript{2}):**

AFG\textsubscript{2} is the dihydroderivative of AFG\textsubscript{1} (Van der Merwe, et al., 1963). AFG\textsubscript{1} and AFG\textsubscript{2} share the same relationship between them like AFB\textsubscript{1} and AFB\textsubscript{2}.

**Molecular Formula C\textsubscript{17}H\textsubscript{14}O\textsubscript{7}**

![Molecular Structure of Aflatoxin G\textsubscript{2}]

Other than these four major types, there are several other metabolites and derivatives of aflatoxin found in the contaminated commodities. They are Aflatoxin M\textsubscript{1}, Aflatoxin M\textsubscript{2}, Aflatoxin M\textsubscript{2a}, Aflatoxin GM\textsubscript{2a}, Aflatoxin Q\textsubscript{1}, Aflatoxin P\textsubscript{1}, etc.

**Evaluation of Health Risk Associated with Exposure to Aflatoxin**

Aflatoxin contaminated foods and feeds when consumed by human and animals have been reported to cause acute and chronic effects. Their toxicity varies with different species depending upon the individual susceptibility. Human beings and animals are exposed
Agar Medium (CAM), etc.

(d) To study the surface morphology of environmental dust samples by using SEM.

(e) To detect airborne aflatoxin from the work environment of maize and rice processing industries using ELISA technique.

(f) To screen the toxicity of the airborne dust extract on bacterial test system like Microtox™.