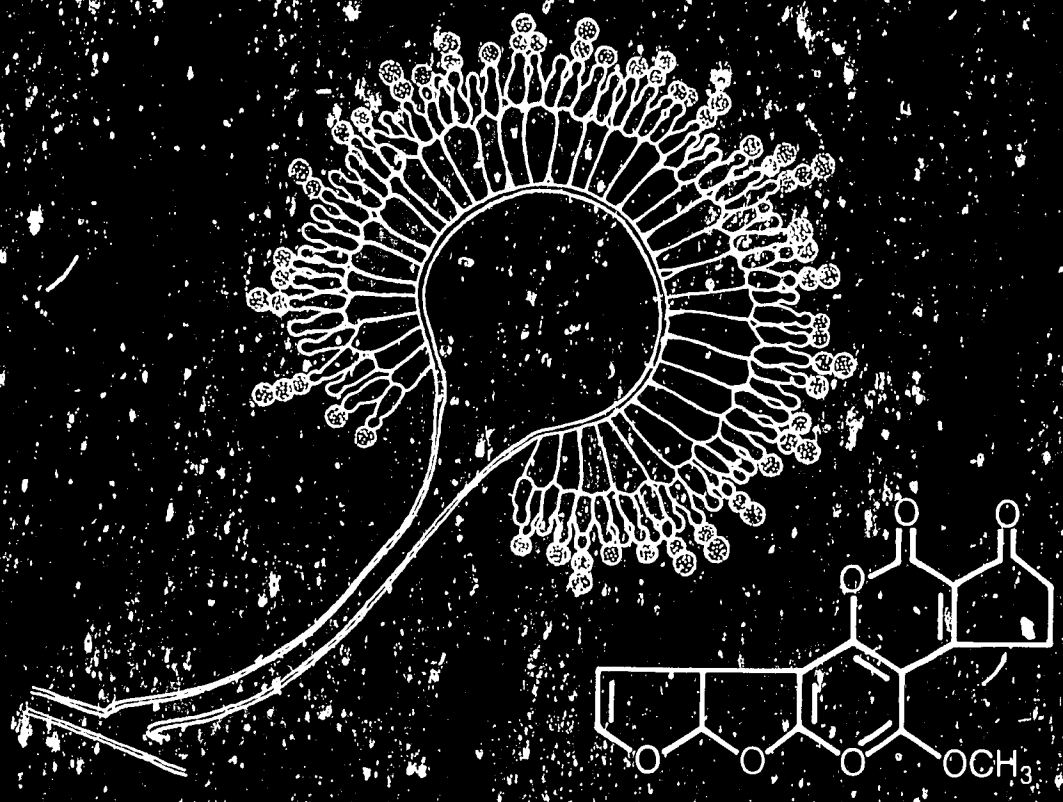
Summary and Recommendations of the International Workshop on Aflatoxin Contamination of Groundnut

6-9 Oct 1987
ICRISAT Center, India



International Crops Research Institute for the Semi-Arid Tropics

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Introduction

A General Overview of the Problem of Aflatoxin Contamination of Groundnut

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The cultivated groundnut (*Arachis hypogaea* L.) is the most important oilseed in the developing world, and is a valuable source of protein for human and animal nutrition. According to the Food and Agriculture Organization of the United Nations (FAO) in 1985 nearly 19 million hectares were sown to groundnut worldwide, and 21 million tonnes of dried pods were harvested, a little better than one tonne per hectare. About 80% of the world production is from developing countries, and approximately 67% from the semi-arid tropics which is ICRISAT's mandate area. We have been charged by the Consultative Group on International Agricultural Research (CGIAR) to carry out research on groundnut, particularly aimed at small farmers, since small farmers in the semi-arid tropics are indeed the major producers of the crop. Research on groundnuts started in ICRISAT in 1976, and specific mention was made in the report that led to this program's initiation that it would be necessary to tackle the problem of aflatoxin contamination.

The problem was first recognized following outbreaks of Turkey 'X' disease in the United Kingdom in 1960. The common factor in the outbreaks was that the turkeys' diets contained groundnut meal from Brazil. Research in the United Kingdom revealed that the disease was caused by toxins produced by strains of the fungus *Aspergillus flavus* when growing on the meal, and hence these toxins were named aflatoxins.

The Tropical Products Institute, London, now part of the Overseas Development Natural Resources Institute (ODNRI) and the Central Veterinary Laboratory, Weybridge, UK, played leading parts in the extraction, purification, and identification of the toxins, and in the development of biological and physicochemical methods for identifying and quantifying toxins in groundnut and other commodities. This laid the foundation for rapidly expanding research worldwide to determine which commodities in addition to groundnut could be contaminated with aflatoxins, and which species of livestock in addition to poultry were subject to aflatoxicosis. Several important oilseeds, cereals, legumes, and spice crops were found to be naturally contaminated with aflatoxins, and a wide range of livestock were affected to a greater or lesser extent. Of the other four ICRISAT mandate crops only sorghum is likely to become contaminated with aflatoxins, and of the cereal crops the most important

contamination by far occurs in maize. Maize and groundnuts are common items in the diet of many people in the tropics and are common ingredients in livestock feeds in developed countries. The most common of the aflatoxins, aflatoxin B_1, is also the most toxic.

As evidence accumulated on the actual and potential importance of aflatoxicosis in relation to farm animals, there was increasing concern expressed as to the possible hazard to human health. This concern was greatly intensified when it was shown that rats fed on aflatoxin-contaminated groundnut meal developed liver cancer. Over the past 25 years much data have been obtained on the occurrence of aflatoxins in the diet in particular regions of the world in relation to the incidence of liver cancer in those regions, and there are strong indications that the two are related.

The possible presence of such acutely toxic and carcinogenic substances in foods and in animal feeds has had a profound effect on the utilization of and trade in groundnuts and groundnut products. Processors and importing countries have placed limits on the levels of aflatoxins permissible in groundnuts and groundnut products. In a paper at the recent FAO WHO UNEP International Conference on Mycotoxins, van Egmond stated that some 50 countries have enforced or proposed aflatoxin regulations for foodstuffs. The maximum limits range from zero detectable to 50 $\mu g\ kg^{-1}$. There has been a tendency for regulations to become increasingly stringent as methods of detection have improved. It would naturally be preferred that no aflatoxins are present, but this has not proved to be practicable. It is not only groundnuts for direct human consumption that are subject to restriction. When mammals ingest aflatoxin B_1, the toxin can be passed through to the milk where it occurs in a slightly changed form called aflatoxin M_1. In August 1981 the Ministry of Agriculture in the United Kingdom banned the feeding of groundnut products to dairy cows because of the possible hazard to the health of milk-drinkers. The milk toxin is thought to be particularly important as young animals, and presumably children also, are more susceptible to aflatoxicosis and the carcinogenic effects of aflatoxins than are adults.

The human and livestock health hazards from ingestion of aflatoxin-contaminated food are much greater in the developing than in the developed world. Most developing countries lie within the tropics where temperatures and relative humidities often favor mold growth on these products. Also, conditions for storage often leave much to be desired. In many countries there are only limited or no facilities for monitoring groundnuts and groundnut products for this contamination. There are also possible synergistic interactions between aflatoxin and infectious hepatitis virus B and there is evidence that the effects of ingestion of aflatoxin are much more severe in the case of children suffering from severe protein malnutrition, an unfortunately common condition in some countries where aflatoxins occur. Where groundnut is a cash export crop there has been a tendency to concentrate efforts on ensuring the acceptability of the commodity to the importing country, while little attention has been paid to aflatoxin levels in produce for local consumption. It is, of course, appreciated that loss of export income can be of great importance, and it is also appreciated that it is difficult for some groundnut-producing countries to meet the stringent regulations currently imposed by importing countries. Nevertheless, local problems are important. What can be done to eliminate or reduce aflatoxin contamination of groundnuts and groundnut prod-

ucts? There is at present no single practice that can prevent aflatoxin contamination of the commodity. Of course, if we could breed varieties of groundnut upon which the toxigenic *A. flavus* could not grow, or on which it could grow but could not produce toxins, then the problem would be solved. Unfortunately we have not done that.

What can be done at the farm level is to grow varieties that have the highest available resistance to pod and seed invasion by *A. flavus* and also follow cultural practices that minimize damage to pods. Late-season drought stress should be avoided, and the crop should be harvested as soon as the majority of pods are mature. Postharvest drying should be rapid but not so fast as to lead to seed damage, and storage should be under clean, dry, pest-free conditions. Produce should be monitored for aflatoxin contamination as it leaves the farm or on arrival at buying stations or processing plants, and lots with aflatoxin levels above those permissible should be diverted to nonfood use, or be subjected to some detoxification process before use as food or livestock feed. Refined oil from groundnuts processed in modern solvent-extraction plants should be free from aflatoxin, but oil produced in more primitive, village-level crushing plants may contain significant levels and require additional treatment to render it safe for human consumption. Even when the groundnut product reaches the consumer, the risk of aflatoxin contamination is not over. Spores of *A. flavus* are common in the air and in water in tropical and warm temperate regions so exposed food may be colonized and aflatoxins produced if environmental conditions and the constitution of the food are suitable.

It is evident that efforts to prevent aflatoxin contamination of groundnuts must start during crop growth and continue until the product is consumed. While many of the practices recommended for prevention of contamination are simple and easy to apply, they have to be adapted to particular agroecological conditions and some may not be feasible in less-developed countries where facilities may be minimal or nonexistent. Many different groups have to be involved and must work together to tackle the problem.

This Workshop has brought together agricultural research and extension workers from 26 developing and developed countries, and from various international and regional institutions. Invited representatives from marketing and processing units and government trade interests, and medical and veterinary representatives have attended to provide a comprehensive coverage of the problem. The major objectives of the Workshop are to make an up-to-date evaluation of the problem, and to assess recent and ongoing research, with a view to providing the best possible advice to all concerned with the production of groundnuts on how to reduce, if not eliminate, contamination with aflatoxins.

ICRISAT's own approach to aflatoxin research concentrates on developing groundnuts that do not tolerate invasion by *A. flavus*, or that prohibit the development of the toxin. Related studies deal with developing in vitro methods to detect seed resistance, detecting the toxins themselves, and studying the environmental factors affecting resistance. This may seem to be a somewhat limited approach to the problem but ICRISAT is an international agricultural research and training institute that conducts most of its groundnut research in three host countries: India, Niger, and Malawi. It works, moreover, for the small farmers of all regions of the tropics where

groundnut is grown. It is not appropriate for ICRISAT to undertake extensive research on postharvest aflatoxin problems since that is the clear responsibility of national scientific establishments, and it would not be appropriate for ICRISAT to undertake work that would reflect upon their ability to deal with such problems. It is, however, necessary to remember that the target group of small farmers does not, for the most part, have either the education, the information, or the means to control aflatoxin levels by sophisticated management practices. We do what it is best for us to do and we believe that our research on *A. flavus* and its toxins will enable small farmers of the tropics to obtain more profitable returns from groundnut production. Our work, however, is but a small contribution to a large problem.

It is hoped that the Workshop and the Proceedings from it will assist in presenting progress made on all fronts in dealing with the serious problem of aflatoxin contamination of groundnuts and will show how the various research achievements contribute to a greater whole.

It is also hoped that by widely distributing both the Summary and full Proceedings of this Workshop, we can bring the problem to the attention of relevant government authorities and policy makers so that they can take appropriate action to minimize the health risks to both humans and livestock from consuming contaminated groundnuts and groundnut products, and also to improve the quality of traded groundnuts, and thus increase export earnings.

Objectives and Structure of the Workshop

Objectives

The main objectives of the Workshop were to:

- Bring together research workers and others concerned with the many different aspects of the groundnut aflatoxin problem to exchange the latest information,
- Evaluate the status of research on aflatoxins in different countries/regions,
- Identify areas for collaborative research,
- Discuss ways of evaluating and managing the aflatoxin contamination problem in groundnuts around the world,
- Identify specific training needs and organizations that can offer training, and
- Develop plans for disseminating information useful to groundnut growers, processors, users, advisory services, and policy makers.

Structure

In order to cover the wide range of topics and disciplines represented at the Workshop, papers were arranged in sessions that were ordered to move from general aspects of the problem to specific research topics. Summaries of all presented papers are included in this Proceedings. With over 40 papers presented, discussions at the end of each were of necessity brief, but the program allowed for the participants to break into groups and hold in-depth discussions within these groups. At a final plenary session each group chairman presented a report and recommendations that are included in this Proceedings.

The recommendations of each group were considered during the plenary session by all participants and the final recommendations of the Workshop have been formulated from these deliberations.

A major recommendation, and a continuing theme throughout presentations and discussions was the need to increase awareness of the problem of aflatoxin contamination at all levels, from the general public and farmers, to food policy makers and representatives of trade and industry. In recognition of this a decision was made to prepare this Summary Proceedings and to distribute it as widely as possible. A full Proceedings that includes full texts of presented papers is in production and will be available from ICRISAT later in 1988.

Throughout this document the term groundnut is used for *Arachis hypogea* L., except in proper names of organizations e.g., Peanut CRSP or in widely recognized names for groundnut products e.g., peanut butter.

In order to achieve uniformity in reporting all aflatoxin contents are expressed in micrograms per gram ($\mu g\ g^{-1}$) or per kilogram ($\mu g\ kg^{-1}$).

Summaries of Presented Papers

Importance of Aflatoxins

Risk to Human Health Associated with Consumption of Groundnuts Contaminated with Aflatoxins

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Acute and chronic effects of aflatoxins in man are well documented. The reported outbreaks of aflatoxicosis in man were due to the consumption of staple foods such as maize and not to the consumption of groundnut. Circumstantial evidence has implicated groundnut meal containing aflatoxin as causing Indian childhood cirrhosis. Dietary intake of aflatoxin through groundnut has been implicated in the development of liver cancer in certain parts of the developing world. The incidence of liver cancer associated with the ingestion of aflatoxin from groundnuts is low in developed countries such as the USA.

Food consumption surveys in India have indicated that the consumption of nuts, (mostly groundnuts) varies from 2 to 35 g per consumption unit per person per day depending on the region and season. Data from the Indian Multicentric Food Contamination Monitoring Program have indicated that though aflatoxins could be detected in 13% of the groundnut samples they tested, the toxins exceeded the official permissible limit of 30 $\mu g\ kg^{-1}$ in only 2.6% of the samples. Studies carried out in Thailand, the Philippines, and the USA have indicated that the dietary intake of aflatoxins from groundnut is lower than that from maize.

The aflatoxin regulatory actions taken by the European Economic Community (EEC), Japan, and other developed countries on importing groundnut and its derivatives have resulted in safeguarding exports rather than minimizing health hazards in the developing countries.

Hazards to Livestock of Consuming Aflatoxin-contaminated Groundnut Meal in Africa

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The outbreak of Turkey 'X' disease in England led to the discovery of aflatoxicosis, caused by feeding groundnut meal contaminated by *Aspergillus flavus* to livestock. The high content of aflatoxins in groundnut meal in African countries has serious implications for livestock feeding. The risks depend on the level and type of aflatoxin in the diet, the strain of animal, and its nutritional status. Subclinical aflatoxicosis is characterized by reduced feed intake and poor productivity, but may not be associated with overt clinical symptoms. Chronic problems occur when aflatoxins are present in the diet at less than 1000 $\mu g\ kg^{-1}$ but the lower limits for effects on productivity are not certain.

The principal lesions of aflatoxicosis occur in the liver and may be classified as toxic hepatitis. Natural cases usually result from repeated ingestion of the toxin. One of the most constant responses to aflatoxin B_1 is bile ductule hyperplasia at the periphery of hepatic lobules. Changes in hepatocytes (vacuolization, fatty change) leading to necrosis, are usually localized in one part of the hepatic lobule, depending on the species. Hepatic veno-occlusive lesions are also common.

The immunosuppresive effect of aflatoxin, coupled with high exposure to diseases and poor nutrition, are detrimental to increased livestock production in Africa.

Aflatoxins and Trade in Groundnuts

Groundnut Trade in India and with the World: Implications of Aflatoxin Contamination

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Although India is the largest producer of groundnuts, her share in the world edible groundnut trade has declined sharply in the last 10 years. A persistent deficit in oilseeds production resulting in high groundnut prices in India compared to those from other producing countries has made Indian groundnuts more expensive and less attractive to world trade. Apprehensions of aflatoxin contamination of groundnuts have done much less damage to the Indian groundnut trade than have vacillating

government policies. In India selection of edible groundnuts is still done manually because of the high cost of mechanization and investment risk. Yet, Indian graders are capable of supplying groundnuts of internationally acceptable quality. In the present setting, the impact of aflatoxin incidence in groundnuts is at best marginal for India. Government support to ensure larger exports of edible groundnuts from India at competitive prices is bound to bring greater awareness and motivation amongst graders to prepare aflatoxin-free high quality groundnuts for the world market.

The Problem of Aflatoxin Contamination of Groundnut and Groundnut Products as seen by the African Groundnut Council

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In member states of the African Groundnut Council (AGC) groundnuts are an important traditional and economic crop. For the past 25 years, the problem of aflatoxin has confronted the groundnut industry and AGC. The economic and commercial problems worsen each year. Aflatoxin is a serious constraint to exports particularly in groundnut cake and meal destined for the traditional western European markets. The nature of the aflatoxin problem is indisputable, but its solution involves matters of trade and politics as well as scientific research.

On the basis of scientific information on *Aspergillus flavus* and other mycotoxin-producing fungi, and despite the controversies, the AGC launched an aflatoxin control program in 1975. Phases I and II have been completed with financial aid from the European Economic Community (EEC) and the United Nations Development Programme (UNDP) and with technical assistance from the Food and Agriculture Organization of the United Nations (FAO). The following results have been acheived: (1) staff have been trained to survey and monitor aflatoxin in fields and laboratories; (2) laboratories for aflatoxin analysis have been established and equipped; (3) control measures have been identified; and (4) two pilot detoxification plants have been constructed to supplement cultural control measures.

The AGC monitors contacts with EEC representatives and exporters of groundnut products on relevant legislation and standards and their application.

A scientific solution is not sufficient in itself, it can only be implemented by the combined efforts, goodwill, and initiatives of trade, industry, and politicians.

Aflatoxins in Groundnut: Monitoring and Action at National Level

Aflatoxin Contamination of Stored Groundnuts in Zimbabwe

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Aflatoxins were analyzed in groundnuts stored for either local or export sales in the period 1982/83 to 1986/87. Four hundred and forty-one samples of seven groundnut varieties were collected for analysis. Sixty-eight percent of the samples had total concentrations of aflatoxin B_1 and G_1 of up to 25 $\mu g\ kg^{-1}$. In the 1986/87 groundnut crop, the Flamingo and Makulu Red varieties, which constitute the bulk of export sales, had up to 25 $\mu g\ kg^{-1}$ aflatoxin B_1 in 83% of the samples, plus aflatoxin G_1 in 61% of the samples. Overall, the Egret variety was the most susceptible to aflatoxin contamination during this period.

Aflatoxin and *Aspergillus flavus* Contamination Problems of Groundnuts in Zambia

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In Zambia, groundnut kernels meant for export are routinely monitored for aflatoxin contamination. Since 1979, 6.3% of the 28 410 samples analyzed had contamination levels of more than 5 μg aflatoxin kg^{-1}. A 2-year study with promising varieties revealed the variability of *A. flavus* seed infection. Seed treatment with Benlate®or Labilite® at 3 g kg^{-1} seed was found to control *A. flavus* in groundnut seed and can improve crop stand.

Aflatoxin Contamination of Groundnuts: Control Strategies in Malawi

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In Malawi the rains start in October and finish in April so that long-duration groundnut cultivars are harvested under dry conditions. These dry conditions favor rapid postharvest drying of groundnut pods thus limiting the opportunity for seed invasion by *Aspergillus flavus* and *A. parasiticus* and aflatoxin contamination. Aflatoxin contamination of groundnuts is not a problem in the country. However, certain practices used by smallholder farmers to process groundnuts in readiness for sale create conditions that favor the rapid development of *A. flavus* and *A. parasiticus* and possible aflatoxin contamination of groundnuts. These practices include moistening groundnut pods in order to soften the shell for ease of handshelling. The Agricultural Development and Marketing Corporation (ADMARC) purchases shelled and graded nuts from smallholder farmers, and electronically sorts and tests the nuts for aflatoxin contamination at the Liwonde Groundnut Factory. The process of handshelling and handgrading of groundnuts by smallholder farmers, followed by re-grading, and aflatoxin testing of the nuts has earned Malawi a reputation as a source of high-quality groundnuts for the confectionery trade. Research needs to be done to incorporate resistance to *A. flavus* and *A. parasiticus* and aflatoxin contamination in the already established commercial cultivars, coupled with education for farmers on proper handling methods for processing groundnuts prior to marketing.

Status and Management of Aflatoxin in Groundnuts in Nigeria

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A committee to coordinate action on the aflatoxin problem in Nigeria was constituted in 1961 with representatives from four ministries, the Institute for Agricultural Research (IAR), Zaria, the Nigerian Stored Products Research Institute (NSPRI), and the Northern Nigeria Marketing Board. This committee was charged with the responsibility of assessing the extent of the aflatoxin problem in groundnut in the country and of initiating and coordinating all actions leading towards its elimination. IAR was to investigate the aflatoxin contamination of the groundnut crop up to the stage where the produce was sold by farmers, while NSPRI was to look at the problem from the time of storage until produce was exported or consumed. NSPRI, therefore, routinely monitored groundnuts in storage pyramids to determine aflatoxin levels before export. Meanwhile, IAR investigated the time of invasion of groundnut kernels

by *Aspergillus flavus*, and when, and under what conditions it produced aflatoxins. An interplay of temperature, relative humidity, drought, and erratic rainfall situations, and maturity of the crop at lifting was found to affect seed invasion by *A. flavus* and aflatoxin contamination of groundnut in the field and store. In the wetter areas of the Southern Guinea Savanna which have long rainy seasons, aflatoxin contamination of groundnuts is mainly a postharvest problem; whereas in the major groundnut-growing areas that lie in the drier Northern Guinea and Sudan Savanna the problem is more important preharvest. Insect infestations and wetting of stored groundnuts increase aflatoxin contamination.

Research information from IAR and NSPRI still provides the basis for recommendations on the handling of groundnut to minimize or prevent aflatoxin contamination. Vegetable oil and feed mill companies routinely submit their groundnut and other feed materials for aflatoxin analysis as there is great awareness among the companies, people, and governments of Nigeria of the dangers posed by aflatoxin to poultry, livestock, and humans. Nigeria is a consignatory to the African Groundnut Council's resolution to export only groundnuts whose aflatoxin content does not exceed the maximum permissible limit of 200 $\mu g\ kg^{-1}$ set by the European Economic Community (EEC). None of the commercially grown groundnut cultivars in Nigeria is resistant to *A. flavus* invasion and aflatoxin contamination of seeds. Breeding materials from both domestic and exotic sources are being screened for resistance while other improved management practices are being used or researched.

Groundnut Aflatoxin Problems in Tanzania

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Groundnuts are grown in most parts of Tanzania but the bulk of the crop is grown in the southeast of the country. The crop is exclusively grown by small-scale farmers mainly for local markets. Groundnut research in Tanzania started at Nachingwea in the late 1940s. In early 1970 with assistance from the Overseas Development Administration (ODA), UK, groundnut research work was transferred to Naliendele, Mtwara in southeast Tanzania. Apart from a little research at Sokoine University of Agriculture, Morogoro, most of the research work on groundnut breeding, agronomy, and crop protection is done at Naliendele.

Earlier efforts on crop protection were devoted to foliage diseases and insects. Recently it was realized that aflatoxin contamination of groundnut was one of the major factors reducing groundnut quality in the country. The National Groundnut Improvement Program has decided to start research on the problem to develop effective control measures.

Present Status and Perspectives of Aflatoxin Research in Mozambique

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In Mozambique, there is a high correlation between the incidence of primary liver cancer and the consumption of aflatoxin-contaminated food. Some work has been done to assess and minimize the aflatoxin problem.

Institutions such as the Instituto Nacional de Investiganao Veterinaria (INIV) and the Laborotorio Nacional Para a Higiene de Agua e Alimentos (LNHAA) are involved in the analysis of food products, for both animals and humans. The main technique used for determination and quantification of aflatoxins is thin layer chromatography (TLC) although high performance liquid chromatography (HPLC) is also available at the LNHAA.

In 1981, 17 food products, comprising a total of 313 samples were tested and it was found that 16 samples were contaminated with aflatoxin B_1, 10 with B_2, 4 with G_1, and 3 with G_2. It was found that 87-100% of the groundnut, beer, rice, and maize samples tested were contaminated. The aflatoxin contamination levels in the groundnut samples ranged from 3 to 5500 $\mu g\ kg^{-1}$, aflatoxin B_1, being the main contaminant.

An analysis program is investigating the possible correlation between the consumption of contaminated food and the possible presence of aflatoxin M_1 in human breast milk.

The possibility of further work involving the INIV, LNHAA, and the Faculdade de Agronomia, Universidade Eduardo Mondlane, Groundnut Improvement Project is being studied to include an agronomic component and to formulate recommendations for small farmers and traders.

Research on Aflatoxin Contamination in Groundnut in the People's Republic of China

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During 1973-77, 1690 samples of groundnut kernels and 1172 samples of groundnut oil from 24 provinces of the People's Republic of China were analyzed for contamination with aflatoxin B_1. The percentages of samples containing the toxin were 26.3 for kernels and 47.3 for oil. Across the country as a whole the percentage of contaminated

samples decreased with increase in latitude, being high in southern China, moderate in the Yangtze valley, and low in northern China. The key to prevention of aflatoxin contamination was rapid drying of groundnuts to below 10% moisture content.

Aflatoxin Contamination in Groundnuts at the Post-production Level of Operation in the Philippines

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The results of surveys in the Philippines have shown that the farm level aflatoxin significantly increased from harvest to farm storage during the main cropping season. At harvest, groundnuts contained, on average, 3.16 $\mu g\ kg^{-1}$ aflatoxin. During windrowing, aflatoxin levels increased at the rate of 1.5 $\mu g\ kg^{-1}$ per day. In farm storage aflatoxin contamination continued to increase at the rate of 1.4 $\mu g\ kg^{-1}$ per day. Aflatoxin contamination was significantly higher during the main cropping season than the second cropping season.

At the traders' level, groundnut samples taken from various middlemen contained 35.0 $\mu g\ kg^{-1}$ aflatoxin. On the other hand, samples taken from the wholesalers' newly procured groundnuts contained 188 $\mu g\ kg^{-1}$ aflatoxin. Groundnuts that had been in the wholesalers' warehouse for more than 3 months contained 275 $\mu g\ kg^{-1}$ aflatoxin.

At the processors' level, raw materials for confectionery groundnuts (roasted and fried) contained 7.73 μg aflatoxin kg^{-1}, groundnuts intended for peanut butter contained 17.13 $\mu g\ kg^{-1}$, and rejected groundnuts had 120.6 $\mu g\ kg^{-1}$.

Aflatoxin contamination could start during harvest. Aflatoxin content climbed to a significantly high level during trade and processing. This continued increase was attributed to insufficient drying of groundnuts after harvest.

Aflatoxin Contamination of Groundnuts in Pakistan

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Groundnut samples from various parts of Pakistan were analyzed for aflatoxin content and no fresh samples contained the toxin. However, 6 — 15% of the roasted peanuts from areas other than Khuzdar were contaminated. The aflatoxin content of the contaminated samples varied from 24 to 800 $\mu g\ kg^{-1}$. All the tested samples of roasted peanuts from Khuzdar were contaminated with aflatoxins.

National Monitoring and Control Program on Mycotoxins in Brazil

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The west of São Paulo State is a region of high temperature and humidity and is the principal groundnut-growing area of Brazil. Survey results of groundnut samples collected in the rainy season (313 samples) and dry season (83 samples) in that State showed that on an average 48 - 74% of the samples collected from the west and northeast regions contained 5 - 22 500 μg aflatoxin B_1 kg^{-1}.

This survey reconfirmed the extent and level of occurrence of aflatoxins in groundnut in Brazil and showed that a mycotoxin problem exists. Suggestions and recommendations were made to the relevant authorities as a result of the survey.

Removal of Aflatoxins

Control of Aflatoxin in Groundnut Products with Emphasis on Sampling, Analysis, and Detoxification

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The control of the occurrence of aflatoxin in groundnut products requires a combination of quality control and decontamination procedures. Recent work at the Overseas Development Natural Resources Institute (ODNRI) has focussed upon the development of efficient sampling, sample preparation, aflatoxin assay, and chemical detoxification procedures.

The use of selected mathematical models to describe the distribution of aflatoxin in groundnut kernels, roasted peanuts, peanut butter, and groundnut cake has been investigated in order to facilitate the design of statistically sound sampling plans for these commodities. A subsampling mill has been developed, in collaboration with a UK company, which enables representative, comminuted subsamples to be rapidly produced from large samples of groundnut kernels.

Methods have been elaborated for the accurate analysis of the aflatoxin content of groundnut products utilizing bonded-phase cleanup procedures in combination with high performance liquid chromatography (HPLC) and high performance thin-layer chromatography (HPTLC) quantification methods. The application of enzyme-linked immunosorbent assay (ELISA) methods to the analysis of peanut butter has also been extensively examined.

A procedure for the detoxification of groundnut cake using ammonia gas at high temperatures and moderate pressures has been developed, and preliminary toxicity trials have been completed using ammoniated material generated by a 50-kg capacity reaction vessel. A 1-t h^{-1} capacity reaction vessel is under construction and trials will begin in India in 1988.

Removal of Aflatoxin Contamination from the Australian Groundnut Crop

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The Australian groundnut crop is significantly affected by aflatoxin in some years because of preharvest drought stress. By a process of selective segregation and sorting, aflatoxin-containing kernels are removed from contaminated lots to satisfy a 15 μg kg^{-1}(total) regulatory limit. This sorting is made possible by the characteristic discoloration of groundnut flesh caused by *Aspergillus* spp growth and the small percentage of aflatoxin-containing kernels. The variance contributions of sampling, sample preparation, and analysis are quoted. Even with very high standards of sampling and analysis, uncertainty in aflatoxin control is significant.

Removal of Aflatoxin B_1 from Peanut Milk by *Flavobacterium aurantiacum*

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The potential for using *Flavobacterium aurantiacum* to remove aflatoxin B_1 from peanut milk was evaluated. Preliminary experiments determined that this bacterium grew in both nondefatted peanut milk (NDPM) and partially defatted peanut milk (PDPM). In neither case was the growth inhibited by the presence of aflatoxin B_1. Other experiments were designed to assess the ability of 10^9 resting (stationary) cells of *F. aurantiacum* to remove aflatoxin B_1 from phosphate buffer (PB), NDPM, and PDPM. After 24 h at 30° C, *F. aurantiacum* decreased aflatoxin B_1 by 40% in PB, 23% in NDPM, and 70% in PDPM. Proteolysis of PDPM before inoculation with *F. aurantiacum* increased recovery of toxin by about 30% over nonproteolyzed samples. This increase in recovery was not observed when NDPM samples were proteolyzed, suggesting that some of the toxin may be bound to the groundnut protein and not be available for removal by *F. aurantiacum*.

Detoxification of Groundnut Seed and Products in India

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The exposure of groundnut oil contaminated with aflatoxin B_1 to bright sunlight for a given period of time completely destroys the toxin. The safety and shelf life of the sunlight-exposed oil have been confirmed. Aflatoxin is present in finely suspended solids in the oil and most of it can be removed by filtration or by extraction with 10% NaCl. Aflatoxin-contaminated groundnut kernels (0.5-mm thick flakes) can be partially detoxified if exposed to sunlight for 14 h. To remove aflatoxin from groundnut cake, 50% alcohol, acetone, methanol, or 1% calcium chloride can be used. Treating the protein isolate with hydrogen peroxide, and groundnuts in the form of thin flakes or powder with urea and soyaflour, with or without formaldehyde can destroy 90% of their aflatoxin content.

Methods for Aflatoxin Analysis

Current Immunochemical Methods for Analysis of Aflatoxin in Groundnuts and Groundnut Products

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With the availability of specific monoclonal and polyclonal antibodies against mycotoxins in recent years, simple, sensitive and specific radio-immunoassays (RIA) and enzyme-linked immunosorbent assays (ELISA) of mycotoxins have been developed. The sensitivities of RIA were in the range of 0.1-0.5 ng and of ELISA 2.5-25 pg assay^{-1}. Simple and quick immunoassay protocols (ELISA) for monitoring aflatoxin B_1 in groundnuts and groundnut products, that require less than 1 h to complete, have been developed and successfully tested in naturally contaminated groundnut samples at levels about 5 to 10 μg g^{-1}. In addition, antibodies against mycotoxins have been used as an immunohistochemical tool to monitor mycotoxins in tissues and for the preparation of immunoaffinity columns that were then used, either for aflatoxin determination in groundnuts, or as a cleanup tool for aflatoxin analysis. Details of recent progress on the production of antibodies, antibody specificity, and the advantages and disadvantages of different immunoassays, as well as problems associated with immunochemical research on mycotoxins, with emphasis on aflatoxin, are reviewed. Emphasis is centered on the immunoassays of aflatoxin in groundnut products.

Methods for the Analysis of Aflatoxins in Groundnut and Other Agricultural Commodities

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Methods for aflatoxin analysis using thin-layer chromatography (TLC) and fluorescence detection were developed in the 1960s and are still widely used. In the late 1970s, several applications of high performance liquid chromatography (HPLC) were developed and as they were generally more sensitive than the TLC methods, they are now popular for aflatoxin analysis when a high degree of accuracy is required. For some test purposes convenience and rapidity of analysis are more important than accuracy, and the bright greenish yellow fluorescence (BGYF) and minicolumn methods were evolved with this in mind. More recently, radio-immunoassay (RIA) methods have been applied and recently several enzyme-linked immunosorbent assay (ELISA) systems kits for aflatoxin analysis have been examined, and some of them found suitable for the kind of testing now carried out using the BGYF and minicolumn methods. Gas chromatography can also be used for aflatoxin analysis under certain conditions. From the range of aflatoxin analysis methods now available it should be possible to choose methods suitable for specific purposes.

Enzyme-Linked Immunosorbent Assay (ELISA) for Aflatoxin B_1 Estimation in Groundnuts

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The commercially available hapten, afla B_1-oxime-bovine serum albumin, was used to produce an antiserum in rabbits. The same hapten was coupled with alkaline phosphatase (hapten-BSA-ALP) and used in the competitive direct enzyme-linked immunosorbent assay (ELISA) for the detection of aflatoxin B_1. Aflatoxin B_1 was extracted in methanol from naturally contaminated or 'spiked' groundnut seed samples.

Wells of a polystyrene microtitre plate were coated with the antiserum, the plates were washed in PBS-Tween, aflatoxin B_1 standards or groundnut sample extracts, and hapten-BSA-ALP conjugate were added and the plates incubated. The plates were again washed, and the amount of conjugate bound to the antibody was determined after addition of the substrate, p-nitrophenylphosphate.

The hapten-BSA-ALP conjugate has advantages in stability, simplicity of preparation, and high specificity over the conventional toxin-enzyme conjugate in direct competitive ELISA. The assay method is more rapid and less expensive than the physico-chemical methods of aflatoxin analysis and it can detect levels of aflatoxin B_1 as low as 50 picograms.

Aflatoxin Analytical Methods for Groundnuts

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Aflatoxin determination in groundnuts can be approached in several ways. Groundnuts are often contaminated with aflatoxin B_1 and B_2 and less often with aflatoxins B_1, B_2 and G_1, and G_2 so it is important to have analytical values that represent the total aflatoxin content. Some countries are only interested in B_1 content and others are interested in the total aflatoxin content. It is essential to safely handle all experimental materials associated with aflatoxin analyses or the aflatoxigenic fungi. Visual screening of suspect groundnut lots, based on the presence of conidial heads of the *Aspergillus flavus* group, is not a chemical test and may allow aflatoxin-contaminated lots into commerce. Minicolumn screening techniques can be useful but they should always be used in conjunction with a quantitative method. Several thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC) methods are suitable for quantification and are in general use. The newer immunochemical methods such as the enzyme-linked immunosorbent assay (ELISA) or affinity column methods are being rapidly developed. ELISA methods are available for screening as well as quantification, but these methods are temperature-sensitive and they should only be used with proper controls. The affinity column method is less temperature-sensitive and can be used for either screening or quantification. The chemical and immunochemical methods are reliable if care is taken and personnel are well trained. All analytical laboratories should stress safety and include suitable analytical validation procedures.

Research on Aflatoxin Contamination of Groundnut: General

Aflatoxin Research in the Peanut CRSP: An Overview

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The global problem of aflatoxin is being pursued by the Peanut Collaborative Research Support Program (Peanut CRSP) through: (1) development of cultivars resistant to invasion by aflatoxin-producing fungi; (2) cultural practices to minimize insect damage which facilitates fungal invasion; (3) detoxification of contaminated

nuts and their products; and (4) separation of contaminated nuts. The dimensions of the problem appear to indicate that a substantial portion of the crop must be sorted out to eliminate aflatoxin. Progress in each of these areas gives promise of the elimination of aflatoxin from food-grade groundnuts.

Queensland Department of Primary Industries' Involvement with Aflatoxin in Groundnuts in Australia and Indonesia

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Rainfed groundnut production in Queensland, Australia is often severely affected by aflatoxin contamination. The Queensland Department of Primary Industries (QDPI) provides extension and research services to groundnut producers, and has addressed this problem in a variety of ways since becoming aware of it.

Extension activities have attempted to improve producers' understanding of the causes of aflatoxin formation in groundnuts and of the management methods available at the farm level and at the shelling plant.

Scientific support has been made available to: assist the groundnut industry establish its own quality-control facilities; help define some of the local factors important in aflatoxin development; conduct an Australian site for the International Groundnut *Aspergillus flavus* Nursery; and to collaborate with industry, the Commonwealth Scientific and Industrial Research Organization (CSIRO), and the Australian National University (ANU) in aflatoxin research activities.

The Australian Centre for International Agricultural Research (ACIAR)-funded groundnut project in Indonesia conducted in collaboration by scientists from QDPI and the Agency for Agricultural Research and Development (AARD) may, in future, consider the inclusion of research on production aspects of aflatoxin contamination.

Groundnut Aflatoxin Problems in Indonesia

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Aflatoxin research in Indonesia was initiated in 1969. Sixty to eighty percent of the marketable groundnuts were contaminated with aflatoxin at levels from 40 to 4100 μg kg^{-1} seeds; retail groundnuts being the most highly contaminated.

Processing raw groundnut seeds into other products, such as peanut butter and fermented groundnut press cake significantly reduced aflatoxin contamination. Clini-

cal studies suggested a positive correlation between aflatoxin ingestion and human hepatic cancer.

More research is needed on the role of preharvest fungal infection on postharvest aflatoxin contamination, the control of storage contamination, and on fungi × groundnut × environment interactions favoring aflatoxin production.

Field Studies on *Aspergillus flavus* and Aflatoxins in Australian Groundnuts

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Aflatoxins have been a serious problem in Australian groundnuts in the past decade. With the aid of government and industrial funding, the Commonwealth Scientific and Industrial Research Organization (CSIRO), Division of Food Research has carried out an ongoing project for most of this period, with emphasis on studies under commercial field conditions.

Research has been primarily concerned with understanding the variables that influence the invasion of groundnuts by *Aspergillus flavus* and *A. parasiticus*, and the subsequent production of aflatoxins. Factors studied include: levels of *A. flavus* in soils, environmental factors; farm management practices affecting *A. flavus* invasion; and the influence of drying and storage procedures on aflatoxin development.

Most groundnut soils in Kingaroy have been found to contain detectable levels of *A. flavus*, while surrounding virgin soils usually do not. Levels of *A. flavus* in groundnut soils vary widely, from less than 10^2 g^{-1} to as high as 10^5 g^{-1}; high levels are much more likely to lead to invasion. Some fields contained consistently high levels over several years. The *A. flavus* : *A. parasiticus* ratio also varies widely from farm to farm, and may influence invasion and toxin production.

Investigations have shown that invasion of groundnuts by *A. flavus* takes place before groundnuts are harvested. Invasion will not occur subsequently, neither will aflatoxin be produced, even under the least effective drying procedures. In all but exceptionally dry seasons, little aflatoxin is produced while groundnuts are in the ground, i.e., most aflatoxin is produced postharvest. Under the most favorable conditions, groundnuts require 6 to 10 days to dry in the field after harvest, a period sufficiently long for aflatoxin to reach unacceptable levels. Field drying cannot be sufficiently rapid, even in dry seasons, to ensure aflatoxin-free nuts at intake to shellers.

The perceived importance of preharvest invasion as the necessary condition for the production of unacceptable aflatoxin levels has led to attempts to predict aflatoxin levels at shelling intake from *A. flavus* levels at harvest. Success rates have been encouraging but are not yet of practical utility.

Aflatoxin Research at the Indian National Research Centre for Groundnut

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The potential of *Aspergillus flavus* isolates to produce aflatoxins, and the toxicity of their culture filtrates to germinating seeds and seedlings were studied. Isolates NRRL 3000 and V 3734 10 produced high levels of aflatoxins in culture. Culture filtrates from these isolates and from NRCG AFA were most toxic to seeds and seedlings. Commercial cultivars, advanced breeding lines, and wild *Arachis* species were screened for resistance to in vitro colonization of seeds by *A. flavus* isolates, and to aflatoxin production. Genotypes CGC 2, 1-4, CGC 7, S 230, derivatives of S230 × PI 337394F, Latur 33 × PI 337394F and the wild species *A. cardenasii* and *A. duranensis* were resistant to seed colonization by *A. flavus*. All genotypes of groundnut and three wild *Arachis* species supported high production of aflatoxins, but only trace levels were produced in *A. cardenasii* and *A. duranensis*.

Aflatoxins were found (27-146 $\mu g\ kg^{-1}$) in commercial groundnut cake and in deoiled cake. Moisture intake capacity, levels of seed coat phenols, and protein content of seeds were considered to influence aflatoxin contamination levels. Soaking seeds in various organic and inorganic substances was found to influence the degree of seed invasion by *A. flavus* and of aflatoxin production in in vitro inoculation tests. Several detoxification methods were examined.

Aflatoxin Contamination of Groundnuts with Special Reference to Sudan and some Caribbean Countries

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Based on analyses using the Velasco and the Tropical Development Research Institute (TDRI) methods, none of the samples collected from farm households in two regions of Sudan, a rainfed area (El Obeid), and an irrigated area (Wad Medani) for the crop year 1983 84, contained more than 15 $\mu g\ kg^{-1}$ of aflatoxin. However, samples collected from the markets in Khartoum and Wad Medani contained aflatoxin up to 945 $\mu g\ kg^{-1}$ in raw groundnuts, up to 517 $\mu g\ kg^{-1}$ in roasted peanuts, and up to 994 $\mu g\ kg^{-1}$ in groundnut paste . Groundnut paste prepared after a careful sorting and cleaning had only 19 μg aflatoxin kg^{-1}. Analyses of 145 samples in Jamaica and St. Vincent in 1984 indicated only eight samples containing more than 20 $\mu g\ kg^{-1}$ of aflatoxins.

Roasted peanuts 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 469 $\mu g\ kg^{-1}$.

Aspergillus flavus Colonization and Aflatoxin Contamination of Groundnut in Sudan

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The effects of irrigation regimes and date of harvesting on preharvest infection by *Aspergillus flavus* and aflatoxin contamination of seed of four commercial and two other groundnut cultivars were studied. Groundnuts watered at 1-, 2-, and 3-week intervals and harvested at the normal time, and at 1 week before and 1 week after this time were free from *A. flavus* colonization and aflatoxin contamination. *Aspergillus flavus* colonized a low percentage (2.7-7%) of groundnuts left in the soil for 6 weeks after harvest. However, no aflatoxin contamination was detected. Wilt diseases and insect damage, mainly by white grubs and termites, predisposed seeds to preharvest *A. flavus* infection (56.4-69.8%) and aflatoxin contamination (18-21 $\mu g\ kg^{-1}$). Groundnuts stored for 3 months in a well-ventilated room with an average temperature of 15°C were colonized at a low level but with no aflatoxin contamination. Infection increased with time in storage. Groundnuts harvested 1 week before maturity were most affected by *A. flavus* infection of seeds in storage, but there were no differences between genotypes. Groundnuts stacked in sacks at shelling sites were sampled, 4% were contaminated, with an average aflatoxin content of 11 $\mu g\ kg^{-1}$. Those sampled at oil mill sites were 15% contaminated with an average aflatoxin content of 20 $\mu g\ kg^{-1}$. Groundnuts left in the soil for 2-3 weeks after harvest in trials on the Gezira and Rahad irrigation schemes had 12% of samples contaminated, with an average aflatoxin content of 10 $\mu g\ kg^{-1}$. This produce is usually allocated for local processing.

Traditional Groundnut Storage and Aflatoxin Problems in Côte d'Ivoire: Ecological Approaches

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Groundnut storage problems were studied in Côte d'Ivoire during two successive storage periods (1985–1986 and 1986–1987) and from three different aspects: insect pests; mold damage; and contamination with aflatoxins.

Samples were taken periodically from traditional producers' fields throughout the groundnut-growing areas of the survey and from town and village markets.

Generally, locally stored samples were a little less infested than samples taken from markets. With few exceptions, all the locally sampled material was contaminated with measurable levels of aflatoxin. Over the 2-year survey period, 7.9% of the 434 local stocks examined exceeded the toxicity level threshold of 250 $\mu g\ kg^{-1}$, with 4.4% above 1000 $\mu g\ kg^{-1}$. It was also found that 73% of these samples were above the European Economic Community (EEC) safety level of 10 $\mu g\ kg^{-1}$.

Significant correlations were found between aflatoxin contamination and different storage and metereological variables. These included physical characteristics and age of the pods, and the influence of the prevailing atmospheric conditions.

Engineering Aspects of Aflatoxin Research in Groundnuts: Evolution of an Environmental Control Plot Facility

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In 1980, an environmental control plot facility was designed and built at the United States Department of Agriculture (USDA), Agricultural Research Service (ARS), National Peanut Research Laboratory to study the preharvest invasion of groundnuts by *Aspergillus flavus* and subsequent aflatoxin production. Requirements for the planned research included the ability to induce drought and to manipulate soil temperature. Initially, the facility consisted of six 12.2-m long × 5.5-m wide × 1.8-m deep, isolated plots with electric-motor-powered roofs for rainfall exclusion as required. Geocarposphere temperature manipulation was accomplished with ther-

mostatically controlled, electrically heated cables; and cooling coils supplied with chilled water. Environmental data were collected using a microprocessor-based, digital data acquisition system that recorded conditions every 2 h during experiments. The facility was recently expanded to investigate the potential of the separate roles of plant stress and pod stress in aflatoxin contamination using two ancillary plots in which pod and root locations in the soil are separated and independently controlled. A microcomputer-based temperature control alarm system has been designed and installed to replace manual controls for soil temperature manipulation. The functional performance of the facility has to date been adequate to provide a wide variety of required environmental conditions for research.

Environmental Conditions Required to Induce Preharvest Aflatoxin Contamination of Groundnuts: Summary of Seven Years' Research

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Environmental conditions necessary for preharvest aflatoxin contamination of visibly sound groundnuts are reviewed on the basis of studies conducted at Dawson, Georgia, USA during 7 consecutive crop years using six environmentally controlled plots. The role of temperature and moisture in preharvest aflatoxin contamination of groundnuts was established. Preventive measures, including the use of so-called 'resistant' varieties, calcium nutrition, and irrigation, were evaluated using environmentally controlled plots. The studies showed that groundnuts do not become contaminated with aflatoxins in the absence of severe and prolonged drought stress in spite of invasion levels of up to 80% by the aflatoxin-producing fungi, *Aspergillus flavus* and *A. parasiticus*. Also, larger, more mature groundnut kernels require considerably more drought stress to become contaminated than do smaller, more immature kernels. Phytoalexin-based resistance can readily explain the broader-based resistance observed in the larger, more mature kernels. Studies during 1983 supported the hypothesis that preharvest contamination with aflatoxin originates mainly from the soil and not from the air via floral invasion.

Research on Aflatoxin Contamination of Groundnut: Genetic Resistance

Screening Groundnut Cultivars for Resistance to *Aspergillus flavus*, *Aspergillus parasiticus*, and Aflatoxin Contamination

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Screening groundnut cultivars for aflatoxin resistance involves a consideration of the environmental conditions that favor activity by the *Aspergillus flavus* group of fungi. The plant tissues penetrated, time of penetration, and the physical and biochemical factors that restrict invasion and aflatoxin formation also require consideration. *Aspergillus*-invaded cotyledonary leaves may be a primary source of inoculum.

Developing shells of all cultivars examined were easily invaded; but penetration through the shell into the pod cavity varied with cultivar. Pods that formed lignified sclerenchyma bands early in their development were less susceptible to hyphal penetration than those without such bands. Kernel invasion is influenced by features of the hilum and seed coat. Small, covered hila, and compact seed coats with a thick wax deposition are important in relation to resistance. The content of the seed coats and pods varied among cultivars. Inhibitory compounds in the cotyledons slow fungal growth or inhibit aflatoxin formation. Tannin-like compounds (umbelliferone and methyl catechol) found in some groundnut seed coats were found to inhibit *A. flavus* growth and aflatoxin formation. Electrophoretic separation under sodium dodecyl sulfate denaturation has revealed the presence of 20 polypeptides that vary among cultivars differing in susceptibility to *A. flavus*. Isolation of various plant constituents to detect the presence of specific proteins, tannin-like compounds, lignins, phytoalexins, and other compounds may correlate with levels of resistance and should be helpful in screening cultivars.

Resistance of Groundnut Varieties to *Aspergillus flavus* in Senegal

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In four rainy seasons (1977-1980) some 40 groundnut genotypes were screened for field resistance to seed invasion by the aflatoxigenic fungus, *Aspergillus flavus* in trials

at Bambey and Darou research stations in Senegal. Significant varietal differences were observed at harvest in respect of levels of naturally occurring seed infestation by *A. flavus*. Field resistances were positively correlated with previously measured resistance to in vitro seed colonization by *A. flavus* in laboratory inoculation tests.

The commercially grown variety 55-437 had high levels of resistance to *A. flavus* in both field and laboratory screening, while two other varieties (73-30 and 73-33) also grown in Senegal had moderate levels of resistance.

In associated investigations it was found that genotypes with seed resistance to *A. flavus* had a lower proportion of *A. flavus* in their rhizosphere mycoflorae than had genotypes susceptible to seed invasion by this fungus. Varieties, through their effects on rhizosphere mycoflorae may influence the composition of the soil mycoflora of groundnut fields.

Occurrence of Aflatoxins and Aflatoxin-producing Strains of *Aspergillus flavus* in Groundnut Cultivars in Egypt

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The use of groundnut cultivars resistant to seed invasion and colonization by *Aspergillus flavus* is a possible means of preventing or reducing contamination by aflatoxin. Twenty-one groundnut cultivars obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, and one cultivar (Giza 4) from Egypt included as a local susceptible control, were tested for their ability to support aflatoxin production. All the cultivars supported production of aflatoxins B_1 and B_2, although the amounts produced differed between cultivars, the lowest level of total aflatoxin production being 19180 $\mu g\ kg^{-1}$ seed in cultivar Ah 7223, and the highest 44290 $\mu g\ kg^{-1}$ seed in cultivar Giza 4.

The ICRISAT Approach to Research on the Groundnut Aflatoxin Problem

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Research in a number of countries in the 1960s and early 1970s provided an excellent understanding of the effects of cultural practices, produce handling, and storage conditions on aflatoxin contamination in groundnuts and groundnut products. But

the recommendations for management of the problem evolved from the early research, while readily adopted by progressive farmers in countries with advanced agriculture, were not being taken up by the majority of small-scale groundnut farmers in developing countries. This influenced the decision of groundnut scientists at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) to concentrate on identification and utilization of genetic resistance to seed invasion by *Aspergillus flavus* and to production of aflatoxins.

Screening Groundnuts for Resistance to Seed Invasion by *Aspergillus flavus* and to Aflatoxin Production

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Research in several countries into evaluation of responses of groundnuts to seed colonization and infection by *Aspergillus flavus* and/or aflatoxin production is reviewed, and progress made in this field at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is summarized. Several laboratory and field screening procedures have been developed to screen groundnuts for resistance to *A. flavus* infection and/or aflatoxin production. Research on the effects of environmental factors on pod and seed invasion by *A. flavus* has produced information useful in the development of field screening methods. For instance, imposed drought stress has been used to improve large-scale field screening of groundnut genotypes for resistance to preharvest infection of seeds by *A. flavus*. Several genotypes were found resistant to infection, and some of them were also resistant to in vitro seed colonization by *A. flavus* in laboratory inoculation tests. Two genotypes supported only very low levels of aflatoxin B_1 production when seeds were colonized by an aflatoxin-producing strain of *A. flavus*.

Screening Groundnuts for Seed Resistance to *Aspergillus flavus*: Statistical Approaches to Data Evaluation

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Environmental factors influence the degree of groundnut seed infection by *Aspergillus flavus* and other fungi. This complicates resistance screening over seasons and locations as levels of infection can vary considerably within a genotype. Statistical methods were used to separate genotypes into different resistance/susceptibility cate-

gories and to ensure a stable basis for comparisons of control cultivar and test genotypes across environments. An approach was also adopted for comparing the degree and distribution of resistance in spanish and valencia type groundnuts. The establishment of such procedures would facilitate interpretation of screening data from different environments.

Aspergillus flavus Resistance Breeding in Groundnut: Progress made at ICRISAT Center

M.J. Vasudeva Rao, S.N. Nigam, V.K. Mehan, and D.McDonald

Groundnut Breeder, Principal Groundnut Breeder, Groundnut Pathologist, and Principal Groundnut Pathologist, Legumes Program, ICRISAT.

Progress worldwide in breeding groundnuts resistant to seed colonization by *Aspergillus flavus* and aflatoxin contamination is summarized, and research at ICRISAT described. Resistance to *A. flavus* infection may occur at various levels, but efforts to breed for resistance have concentrated on the utilization of the resistance in the testae of mature seeds. At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), genotypes identified as resistant to in vitro seed colonization by *A. flavus* have been crossed with susceptible cultivars of good agronomic character, and several breeding lines with stable resistance to seed colonization and with acceptable yield and quality have been produced. The genetics of inheritance of testa resistance is discussed. It is important that when breeding for resistance to *A. flavus* and aflatoxin production, breeders incorporate other resistance traits.

Polyphenols in Groundnut Genotypes Resistant and Susceptible to Seed Colonization by *Aspergillus flavus*

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Thirteen groundnut genotypes, eight resistant and five susceptible to in vitro seed colonization by *Aspergillus flavus* were grown in replicated trials at three locations in Andhra Pradesh, India. Seed coats of these genotypes were analyzed for polyphenols using different methods. No significant correlation was observed between seed colonization and polyphenol content, which corroborates earlier observations on many genotypes using a single method for polyphenol estimation.

The Geocarposphere Mycoflora and Resistance of Groundnut to *Aspergillus flavus*

S. Nahdi

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Four groundnut genotypes, two resistant and two susceptible to in vitro seed colonization by *Aspergillus flavus* (IVSCAF), were grown in field trials at ICRISAT Center in the 1984 and 1985 rainy seasons. Geocarposphere mycoflorae were examined and significant quantitative and qualitative differences were observed between genotypes. Populations of *A. flavus* were higher in the geocarpospheres of the IVSCAF-susceptible genotypes than in those of the IVSCAF-resistant genotypes.

Genotypes were also evaluated at the time of harvest for levels of seed infection by *A. flavus*. The IVSCAF-susceptible genotypes had higher levels of infection in seed from nondamaged mature pods than had seed from IVSCAF-resistant genotypes.

In a greenhouse experiment, exudates were collected from pods of the four genotypes. Exudates from the two IVSCAF-resistant genotypes inhibited in vitro germination of *A. flavus* spores to a greater degree than did exudates from pods of IVSCAF-susceptible genotypes.

The Semi-Arid Tropical Crops Information Service (SATCRIS) and the Aflatoxin Database

L.J. Haravu

Manager, Library and Documentation Services, ICRISAT.

A description of the SATCRIS Project at ICRISAT Center, the characteristics of the SATCRIS database, and its information retrieval and dissemination services for groundnut, and the specialized database on aflatoxin.

Group Discussion Reports

Group I: Evaluation and Monitoring of Aflatoxin Contamination of Groundnuts and Groundnut Products

Participants

Name	Institution	Country
D. McDonald (Chairman)	ICRISAT	India
P. Subrahmanyam (Cochairman)	ICRISAT	India
A. Bockelée-Morvan	IRHO	France
B. Coulibaly	AGC	Nigeria
R.D. Coker	ODNRI	UK
S. Nahdi	ICRISAT NIN	India
J. Kannaiyan	Msekera RRS	Zambia
C.L. Kisyombe	Chitedze ARS	Malawi
A. Pollet	ORSTOM	France
R. Quitco	NAP HIRI	The Philippines
P.S. Reddy	NRCG	India
M. Sabino	Instituto Adolfo Lutz	Brazil
B. Singh	Peanut CRSP	USA

The group was concerned largely with the country approach to the aflatoxin problem in groundnut. Participants agreed that there was a definite need in many countries to alert producers, processors, and consumers of groundnuts and groundnut products of the hazards to livestock, and the likely hazards to humans from ingesting aflatoxin-contaminated groundnuts and groundnut products.

Groundnuts are rarely consumed as an independent item in human diets or in animal feeds, and it was felt that agriculturalists and others concerned with such crops as maize, sorghum, and cotton should take some responsibility for publicizing the harmful effects of aflatoxin-contaminated foods and feeds.

It was recommended that groundnut-producing countries set up working groups comprising representatives of:

- Agricultural research and extension institutions
- Veterinary and animal production institutes
- Medical research and public health institutes
- Marketing organizations
- Producers' associations
- Processors' associations
- Economists, etc.

Such a group could endeavor to establish a coordinated approach to the aflatoxin problem, inform policy makers, and send representatives to regional or international meetings on relevant topics.

The group felt that each country should develop a system to evaluate and continuously monitor the aflatoxin problem at all levels, including export-oriented and local consumption segments.

Well-proven, and statistically acceptable standardized sampling and aflatoxin assay methods should be selected on the basis of available technology and personnel and the degree of accuracy required. Some laboratories set up in the developing countries over the past 25 years have limited facilities, and are only able to carry out specific methods. The need to maintain these laboratories, upgrade their facilities, and train their staff in new techniques, was recognized. These laboratories are often the only facilities available in a country and they should not be discarded as obsolete until viable replacements are available.

Training in sampling methods and in aflatoxin analysis is a critical requirement. Regional training courses were advocated to facilitate this upgrading.

It was agreed that the most effective way to avoid contamination of produce was to prevent infection of the groundnuts and their products by *Aspergillus flavus* at all stages in production, storage, and processing.

It was recommended that emphasis be given to development of effective detoxification systems for (a) large-scale industrial plants, and (b) small-scale plants for village level operation, to cover both cake and oil production.

While it was agreed that there were reasonable prospects of prevention or removal of aflatoxin contamination from groundnuts produced for export markets, there was little optimism as to the likelihood of immediate improvements in the important local consumption segment.

It was noted that several speakers during the Workshop had highlighted the danger of contaminated nuts segregated from export produce being diverted into the local market. It was therefore advocated that those concerned with segregation should ensure that highly toxic material does not get back into the food chain. Where facilities exist, oil may be extracted from such material and detoxified, but the cake would presumably only be suitable for use as organic fertilizer.

The activities of such organizations as Peanut CRSP and ODNRI in carrying out studies of local systems of processing groundnuts for human consumption were commended. Advice on methods for such studies would enable socio-economists and home economists in developing countries to make recommendations to processors and consumers based on reliable and comprehensive data.

The idea of a literature database was welcomed by the group. The proposal to produce information bulletins or handbooks on aflatoxin management in groundnuts and groundnut products was strongly supported.

The need to have meetings on a regular basis to ensure information exchange and cooperation in research was expressed. This may be provided by holding special meetings as satellites to international and regional workshops as was done when a mycotoxin meeting was held in conjunction with the International Plant Pathology Congress in Australia.

Group II: Analytical Methods for Aflatoxins in Groundnuts and Groundnut Products

Participants

Name	Institution	Country
F.S. Chu (Chairman)	University of Wisconsin	USA
D.V.R. Reddy (Cochairman)	ICRISAT	India
R.J. Cole	USDA-ARS	USA
Xiao Dazen	CAAS	China
T. Goto	NFRI	Japan
S. Moody	RSBS	Australia
J.D. Reed	ILCA	Ethiopia
L.A. Rana	NARC	Pakistan
A.H. Siwela	CSRI	Zimbabwe
M. Somabhi	FCRI	Thailand
R. Jambunathan	ICRISAT	India
V. Anjaiah	ICRISAT	India
S. Jayanthi	ICRISAT	India

The group considered that sample size and sampling techniques should be appropriate to the objectives of the study. For surveillance studies, and where cultivars are to be screened for resistance, etc., large samples are recommended. For purposes of quality control and regulation, official standardized sampling methods should be followed, such as the Overseas Development Natural Resources Institute (ODNRI) (adequate up to 30 $\mu g\ kg^{-1}$ level) and the USA sampling methods (prefered at the 20 $\mu g\ kg^{-1}$ level). Subsampling procedures and sample treatment are also important. Care should be taken to avoid recontamination after sampling by storing samples under cool and dry conditions.

Though several speakers and participants felt that while ELISA is a powerful tool to screen for aflatoxin in groundnuts, such well-established methods as the minicolumn should not be discarded. It was generally felt that ELISA could be adopted only after further collaborative study, and acceptance by the Association of Analytical Chemists (AOAC). However, others were of the opinion that rapid ELISA was a better approach than, for instance, the rapid ELISA (Quick-card) test.

It was noted that, while aflatoxin standards are commercially available, the purity of the materials should be checked by the thin layer chromatography (TLC) method and aflatoxin concentrations determined spectrophotometrically. It was suggested that aflatoxin standards be made available through "International" efforts. Groundnut samples containing specified amounts of aflatoxin (available from the European Economic Community, EEC) could be used as a control to check each laboratory's analytical capability and performance. Participation in the international mycotoxin

check sample programme organized by the International Agency for Research against Cancer, Lyon, France was mentioned as a point of interaction. It was recommended that regional and national check sample programmes be organized.

The group agreed that only those methods which had been subjected to a collaborative study and have been adopted by a sponsoring agency such as the AOAC or the EEC, should be followed for quality control and regulatory measurement. For research purposes, other methods which have been shown to be comparable with the official methods could also be used.

The group agreed that ELISA is a simple, sensitive, and specific method for mycotoxin analysis, with the potential for use in quantitative analysis of aflatoxin in groundnuts, and as a screen test at lower detection limits of 5 to 10 $\mu g\ kg^{-1}$. It could be automated for screening a large number of samples. The high cost of commercially available ELISA kits as well as the availability and stability of reagents may limit its use in developing countries. Though studies have shown that results obtained from ELISA are comparable to TLC and high performance liquid chromatography (HPLC) methods, more comparative studies are needed. Currently, collaborative studies on two ELISA methods to screen aflatoxin in agricultural commodities including groundnuts are underway. If good results are obtained these two methods are likely to be adopted for use by the AOAC. It should be pointed out that collaborative studies on ELISA only evaluate ELISA protocols and serve to establish a set of standards for ELISA. The efficiency of each commercially available kit will have to be tested by the users. The group recommended that the following criteria be established for the evaluation of protocols in the collaborative studies:

1. standard range and limits of detection (sensitivity);
2. flexibility of using different extraction solvents;
3. limits for signal/noise ratio;
4. specificity (cross-relativity);
5. reproducibility (CV); and
6. avoidance of interference of sample matrix.

The group strongly recommended that regional training workshops on analytical methodology be established. Such workshops should include both lectures and laboratory demonstrations on general analytical methodology as well as ELISA methods for mycotoxins. Trainees with relevant background and experience would be drawn from developing countries. It was recommended that an ad hoc committee be established for this purpose.

Group III: Research on On-farm Control of Aflatoxin Contamination

Participants

Name	Institution	Country
K.J. Middleton (Chairman)	QDPI	Australia
R.C. Nageswara Rao (Cochairman)	ICRISAT	India
M. Arora	University College, London	UK
V.Ramanatha Rao	ICRISAT	India
M.J. Freire	UEM ICRISAT	Mozambique
J.I Pitt	CSIRO	Australia
M.J. Vasudeva Rao	ICRISAT	India
F. Waliyar	ICRISAT	India
S.N.Nigam	ICRISAT	India
P.D. Blankenship	USDA-ARS	USA
R.J. Cole	USDA-ARS	USA
V.K. Mehan	ICRISAT	India
R.S. Sandhu	FAO	Zambia
K.K. Shrestha	Department of Agriculture	Nepal
R.F. Pettit	Texas A & M University	USA
M.D. Raya	Department of Agriculture	Tanzania
J.H. Williams	ICRISAT	India

The group considered the on-farm control of aflatoxin contamination of groundnut under two main headings: firstly the development of packages of practices relevant for small farmers' use in the SAT, and secondly the identification of important deficiencies in the understanding of the factors that determine whether or not a groundnut is invaded by the toxigenic fungi with subsequent contamination with aflatoxin.

The group felt that there was already considerable information and advice available as to how the small farmers of the SAT could avoid, or at least greatly reduce, the risk of their groundnuts being contaminated with aflatoxins. The problem was considered to be lack of awareness on the part of farmers of the need to follow the recommended practices for control of fungal infection of seeds both before harvest and during postharvest curing and drying. Several countries have provided excellent aflatoxin control recommendations through their extension agencies and their example should be followed by others. Factors that should be stressed in preparing recommended practices include: the importance of late-season drought, because pods on plants that go into permanent wilt within the last 2-3 weeks of the crop maturation are very likely to contain aflatoxins; the role of soil insects in predisposing pods and seeds to invasion by the toxigenic fungi; the possible role of calcium deficiency in relation to fungal infection of pods; the importance of timely lifting of the crop to reduce the proportions of overmature and excessively immature pods in the harvested produce; avoidance of

damage to pods during threshing, picking, and drying; and the importance of providing dry, well-ventilated, on-farm storage. Prospects of village level or on-farm detoxification of groundnut oil were also considered, the use of clays, sunlight, etc. being suggested. Inputs are required from health, nutrition, and home economics specialists in addition to agricultural extension staff if hazards of aflatoxin contamination are to be reduced at the farm and village levels. The need for training of such staff to better prepare them for the extension of aflatoxin control procedures was expressed, and the production of an information bulletin or handbook on control of aflatoxin in groundnuts was recommended.

Gaps in our knowledge of how the toxigenic *A. flavus* and *A. parasiticus* invade groundnut pods and seeds, and of environmental factors influencing this process, were considered. It is important to study the survival of the two fungi in the soil of groundnut fields and discover how soil type, cropping systems, and temperature and moisture conditions affect invasion. Production of sclerotia may be important. Seed transmission of *A. flavus* may be involved in building up the inoculum of the fungus in groundnut soils. It has been suggested that groundnuts found to contain moderately high levels of aflatoxin could be used for sowing. This was agreed to be a dangerous practice as such groundnuts could well contain viable mycelium of *A. flavus* and, given conditions unfavorable for germination, this could result in a complete emergence failure from aflatoxin disease. A suggestion had been made that aflatoxin contaminated groundnuts or groundnut cake could be used as organic fertilizer. This may be feasible for cake where the heating during oil extraction would have killed any fungal mycelium present, but adding seeds that contain viable *A. flavus* to the soil could greatly boost the population of this fungus and exacerbate the contamination problem in succeeding groundnut crops. There is little definite information on the mechanisms of resistance in peg, pod, and seed to invasion by *A. flavus* and *A. parasiticus*. Further studies in resistant and susceptible cultivars should be carried out to examine possible infection and colonization of pods and seeds. Chemical resistance in the shell would be preferable to having such resistance in the seed in case protective chemicals have any antinutritional or toxic effects when ingested by humans or livestock. There was interest in research into phytoalexin production in groundnut seed, and it would be useful if a cultivar could be bred in which seeds retained the capacity to produce phytoalexins until they are mature.

Definitive information on the mechanisms of resistance to fungal invasion of pods and seeds would greatly facilitate the breeding of resistant cultivars. The contributions of different mechanisms to the overall resistance could be assessed and resistance screening techniques could be improved. Resistance breeding is an important long-term objective.

It was felt that a global survey of the occurrence of toxigenic and nontoxigenic strains of *A. flavus* and *A. parasiticus* would be of value. This would require precise identification of the two species and careful evaluation of their populations in the soil mycoflorae.

The need for training in resistance screening methods and in techniques for handling soil mycofloral analysis, etc. was expressed. Information on screening methods could be included in the proposed information bulletin.

Group IV: Research on Control of Aflatoxin Contamination with Reference to Storage, Transit, Processing, etc.

Participants

Name	Institution	Country
T.O.M. Nakayama (Chairman)	Peanut CRSP	USA
J.A. Wightman (Cochairman)	ICRISAT	India
R.V. Bhat	NIN	India
G. Chandrashekhar	IOPEA	India
C.L.L. Gowda	ICRISAT-AGLN	India
M. Machmud	BORIF	Indonesia
G.V. Ranga Rao	ICRISAT	India
M. Read	PMB	Australia
T. Shantha	CFTRI	India
D.B.T. Wijeratne	Directorate of Agriculture	Sri Lanka
H. Amra	NRC	Egypt
N.E. Ahmed	Gezira ARS	Sudan

The discussions covered problems of aflatoxin contamination during postharvest handling, storage, and processing of groundnuts. It was appreciated that growth of *Aspergillus flavus* and production of aflatoxin in groundnut produce could occur at all stages, and that with conidia of the fungus being almost universally present, the most important factor determining contamination was the moisture content of the groundnuts or groundnut products. The need to dry groundnuts to a safe level of seed moisture content was emphasized. Stored seed may acquire sufficient moisture to enable growth of *A. flavus* to occur if insect infestation of pods or seeds occurs, or if ambient conditions of high relative humidity prevail for several weeks. Methods of monitoring and controlling insect infestations are already well developed and are in common use in well-organized storage depots. Siting of groundnut stores in areas of high relative humidity should be avoided if possible. It was suggested that ICRISAT's Agroclimatology Unit could be asked to compile relative humidity data for major groundnut-growing regions of different countries during the months following harvesting of the crops. The point was made that where groundnuts were an export crop the produce is likely to be stored under very humid conditions in, or close to, seaports while awaiting shipment overseas. Problems of condensation in the holds of ships carrying produce from the tropics to temperate regions could also lead to wetting of groundnuts and growth of the toxigenic fungi. This supported the need for aflatoxin analysis of groundnut shipments on arrival at their destinations, and also indicated that detoxification should also be carried out in the area/country where the produce is to be processed unless suitable safeguards were instituted to avoid recontamination of the materials.

There was discussion on the efficiency of sampling of groundnuts and groundnut products and work at Overseas Development Natural Resources Institute (ODNRI) to refine the Tropical Production Institute (TPI) plan was described. This involves computer simulation and research on appropriate mathematical models to illustrate the distribution of aflatoxin in sample lots. The need to use reliable, standardized methods of aflatoxin analysis was also stressed.

The group strongly supported the need for further research into detoxification of groundnuts and groundnut products. Contamination of lots of whole seeds may be reduced by removal of visible mold-damaged or discolored seeds. However, there may be a problem concerning the disposal of the rejected seeds. Such rejects are likely to have high levels of aflatoxin contamination and should not be used in foods or animal feeds. They could be crushed for oil, but the oil would probably have higher than usual levels of free fatty acids, and would probably also require special treatment for removal of aflatoxins. Detoxification of groundnut cake and meal was discussed in depth, with special reference to the use of ammonia. It was felt that further research was required to elucidate the possible toxicology of detoxified groundnut products. It was suggested that groundnut products could be detoxified at ports of discharge, there being legal provisions for warehousing and in-bond processing in importing countries.

The need for training in relation to postharvest handling of groundnuts, storage procedures including pest control and avoidance of wetting or hydration of produce, sampling and aflatoxin analysis, and detoxification processes, was agreed. It was suggested that ICRISAT could inform national programs and institutions of courses available worldwide on various aspects of the aflatoxin problem, and provide organizers of such courses with contacts in client countries to suggest names of potential participants. Specific mention was made of ODNRI's annual 13-week training course in aflatoxin analysis. The main objective of this course is to train potential trainers who can in turn impart training in their own countries. ICRISAT's proposal to produce an information bulletin on aflatoxin in groundnut giving up-to-date information on control procedures was strongly supported. Increasing awareness of the importance of aflatoxin could lead to increased demand for general and specialist training, necessitating effective collaboration among international and regional organizations concerned with the problem to meet such a demand.

Recommendations

The Workshop identified various areas of concern about aflatoxin contamination of groundnut and made recommendations that are covered in detail in the Group Discussions. The overall recommendations of the Workshop are as follows.

Information and Training

The Workshop emphasized the need to increase awareness of the dangers of aflatoxin contamination of groundnuts and groundnut products among international groups, national governments, the groundnut industry, the producers, and ultimately the consumers. Organizations such as FAO, WHO, the EEC, etc could do more in this respect and could cover the problem of aflatoxins in all commodities. National governments are likely to take more notice of advice from FAO and WHO than from other organizations.

The need to make information on the groundnut aflatoxin problem more readily available was stressed. ICRISAT was encouraged to proceed with the preparation of a database on literature on the subject, and to organize the production of a handbook or information bulletins on sampling and analytical methods, and on management practices for control of aflatoxin in groundnut. It was also suggested that ICRISAT could act as a clearing house to inform all concerned with the aflatoxin problem of proposed training courses, workshops, etc.

Strategies

The concerned groups, AGC, EEC, FAO, and Codex Alimentarius, should continue to work towards a standard international legislation on regulatory levels of aflatoxin in groundnuts and groundnut products for human and animal consumption. At the national level, countries are recommended to set up interdisciplinary working groups to coordinate the evaluation of the aflatoxin problem in their country, identify a responsible agency, organize monitoring of aflatoxin levels in foods and feeds, and initiate and coordinate research with a view to preparing recommendations for control at all levels. Particular attention was directed to ensuring that control measures for reduction of aflatoxin levels in groundnuts destined for export should not further exacerbate the problem in groundnuts for local consumption.

Research Needs

Research needs should be clearly defined in the light of each country's problems and capabilities, and work should be carried out using the most appropriate technologies and by the most relevant organizations. Training of staff in new techniques will be required in many countries if rapid progress is to be made. The need for cooperation in training and research, both nationally and internationally, was recognized.