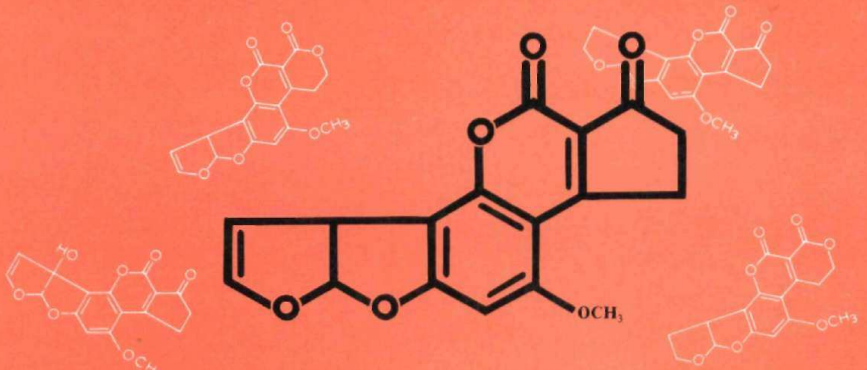
The Groundnut Aflatoxin Problem

Review and Literature Database



International Crops Research Institute for the Semi-Arid Tropics

Abstract

Citation: Mehan. V.K., McDonald, D., Haravu, L.J., and Jayanthi, S. 1991. The groundnut aflatoxin problem : review and literature database. Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Contains reviews of important aspects of the groundnut aflatoxin problem together with annotated bibliographies. Aspects covered include aflatoxicosis in animals and humans, research on aflatoxin contamination of groundnuts, aflatoxins in groundnut and groundnut products, limits and regulations on aflatoxins, methods for aflatoxin analysis, and management of aflatoxin contamination.

Résumé

Référence: Mehan, V.K., McDonald, D., Haravu, L.J., et Jayanthi, S. 1991. Le problème de l'aflatoxine chez l'arachide : revue et base de données bibliographiques. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

Cet ouvrage présente différentes revues traitant des aspects importants du problème d'aflatoxine sur l'arachide, accompagnées de revues bibliographiques annotées. Les thèmes abordés sont, entre autres : l'aflatoxicose chez l'animal et l'homme, les recherches sur la contamination de l'arachide, les aflatoxines dans l'arachide et dans ses produits dérivés, les seuils de toxicité et la réglementation concernant les aflatoxines, les méthodes d'analyse et le contrôle de la contamination.

Resumen

Citación: Mehan, V.K., McDonald, D., Haravu, L.J. y Jayanthi, S. 1991. El problema de aflatoxinas en maní : revista y datos literarios. Patancheru, A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Contiene una recopilación de los problemas mas importantes relacionados con las aflatoxinas en el maní, junto con una detallada compilación de datos bibliográficos. Los aspectos considerados incluyen aflatoxicosis en animales y humanos, investigaciones sobre contaminación con aflatoxinas en maní, aflatoxinas en productos obtenidos de maní, límites y reglamentaciones en aflatoxinas, metodos de análisis en aflatoxinas y manejo en caso de contaminación con aflatoxinas.

The Groundnut Aflatoxin Problem
Review and Literature Database

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ICRISAT

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1991

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FOREWORD

Aflatoxins can cause serious animal and human health problems and when present in groundnuts reduce their quality and value. They have become a subject for concern in agriculture on a global scale. Many countries have assigned high priority to research to find a solution to aflatoxin contamination of groundnut. This has resulted in rapid proliferation of literature both in conventional and nonconventional forms, and researchers are finding it increasingly difficult to keep track of this information which is essential for their research work. This problem was discussed in the International Workshop on Aflatoxin Contamination of Groundnut held at 1CRISAT in October 1987, and it was recommended that ICRISAT should collect and review the literature on the subject and produce a bibliography. The present publication is in response to that workshop recommendation. It provides a comprehensive overview of literature published in the last 30 years and an annotated bibliography. The data collected for this publication are also available in machine-readable form utilizing the Micro CDS/ISIS application.

1 compliment the authors for their efforts in bringing out this useful publication and hope it will facilitate research efforts towards solving the aflatoxin problem in groundnut.

Y. L. Nene
Deputy Director General
ICRISAT

PREFACE

This review and bibliography of the groundnut aflatoxin problem should be of particular value to scientists in the developing world who may not have ready access to the special journals and reports in which much of the research on aflatoxins has been documented.

Each aspect of the groundnut aflatoxin problem is reviewed in a separate section, and each review is followed by an annotated bibliography. Citations are arranged numerically in alphabetical order according to the name of the senior author. The same publication may be listed in several different sections, depending upon its contents. The period 1960 to 1990 is covered and 1450 references are given. The authors' original abstracts or summaries are used wherever possible, but some editing has been done to provide consistency of style and economy of space. Where no abstracts or summaries were provided in the original publications/reports, the authors have prepared them. In a publication of this nature omissions are inevitable and the authors would appreciate having these brought to their notice.

ACKNOWLEDGMENTS

This publication was funded in part by the International Development Research Center (IDRC), Ottawa, Canada, to support the Semi-Arid Tropical Crops Information Service (SATCRIS), and in part by the ICRISAT Legumes Program.

The authors greatly appreciate the support given by Drs L.D. Swindale and Y.L. **Nene,** Director General and Deputy Director General of ICRISAT.

Assistance in literature search provided by Mr P.K. Sinha, Senior Documentation Officer, and Mr P.S. Jadhav, Senior Library Officer of ICRISAT is gratefully acknowledged. The help provided by the British Library Document Supply Centre, Boston Spa, West Yorkshire, United Kingdom, and the Commonwealth Agricultural Bureaux International, Wallingford, United Kingdom, is much appreciated.

The authors thank Drs D.H. Smith, S.B. King, L.K. Mughogho, D.V.R. Reddy, and R. Jambunathan of ICRISAT; Dr R.V. Bhat of the National Institute of Nutrition, Hyderabad. India, and Dr T. Shantha of the Central Food Technological Research Institute, Mysore, India, for reviewing the various sections and for giving thoughtful, constructive, and helpful criticism.

We are grateful to all those who contributed copies of reprints and provided translations when necessary.

The authors thank the following ICRISAT staff members for their help in the compilation and production of this book :

J.B. Wills, Head, Information Services; S.D. Hall, Research Editor
G.K. Guglani for art direction.
Sheila Bhatnagar for cover design.
T.R. Kapoor for composition.

NOTES

1. *Arachis hypogaea* L., the cultivated groundnut (peanut), is referred to in this book as "groundnut". However, in proper names of organizations e.g., Peanut CRSP or in widely recognized names for groundnut products e.g., peanut butter, the term peanut is used.

2. Different authors have expressed concentrations of aflatoxins in groundnuts and groundnut products in different ways. Initially it was common practice to use parts per million (ppm), later parts per billion (ppb) were used.

In order to achieve greater uniformity, aflatoxin contents are expressed in micrograms per kilogram (ug kg^{-1}) or per gram (*ug* g') or in picograms **per gram** (pg g).

INTRODUCTION

The aflatoxin problem was first recognized following outbreaks of Turkey 'X' disease in the United Kingdom in 1960. The common factor in the outbreaks was the inclusion of groundnut meal from Brazil in the turkeys' diets. Research revealed that the disease was caused by toxins produced by strains of the fungus *Aspergillus flavus* which had grown in the groundnuts. The toxins were named aflatoxins. Aflatoxins are now known to be hepatotoxic, carcinogenic, and teratogenic in many animal species.

The cultivated groundnut *(Arachis hypogaea L.)* is the most important oilseed in the developing world. It is a valuable source of protein for human and animal nutrition, and provides a high quality cooking oil. The crop is widely grown in tropical and subtropical regions and is important to the economies of several developing countries. Groundnuts are eaten raw, boiled, or roasted, and made into confectionery and snack foods, and arc added to soups and other dishes. In the USA, the Philippines, and some other countries groundnuts are commonly processed into peanut butter. In the developing world a significant segment of the groundnut produce goes for oil extraction, while the expeller oilcake is used primarily for animal feed. High protein groundnut flour has been produced to enhance diets in areas where protein malnutrition among children is common.

The aflatoxin contamination problem is of obvious importance as it affects the utilization of, and trade in groundnuts and groundnut products, and affects animal and human health.

The high priority given to the solution of this problem in many countries has resulted in large numbers of research publications and repons on a wide range of subjects involving agriculture, the food and feeds industries, human and animal health, and trade legislation. This literature explosion that has occurred over the past 30 years has made it difficult for an individual scientist to obtain an overview of all areas of the subject and to access relevant information from disciplines other than his own.

In October 1987 an International Workshop on Aflatoxin Contamination of Groundnut was held at ICRISAT to discuss ways of evaluating and managing the aflatoxin contamination problem in groundnuts around the world, and to develop plans for disseminating information useful to researchers, groundnut growers, processors, users, advisory services, and policy makers. A major Workshop recommendation was "to prepare a database on literature on the subject".

After considerable thought we decided to use the computer software package - Mini-Micro CDS\SIS - developed by UNESCO for organizing an annotated bibliography incorporating all available publications both formal and informal. A computerized database and soft-ware package has been completed for the period 1960-1990 and may be obtained, together with a User's Manual, from ICRISAT's Information Services. This database provided the foundation for this book which presents in hard copy the annotated bibliography together with reviews on the major aspects of the groundnut aflatoxin problem.

We hope that this publication will be useful to workers in different fields who wish to have an overview of the groundnut aflatoxin problem and an introduction to the available literature on research carried out over the **last thirty years.**

1. AFLATOXICOSIS FROM INGESTION OF TOXIC GROUNDNUTS AND GROUNDNUT PRODUCTS

1.1 REVIEW

Aflatoxicosis is the disease condition caused by the action of aflatoxin. As it was first recognized and described in farm animals, it is appropriate that we first deal with the condition in several important groups of livestock. We shall then consider clinical and pathological effects of aflatoxin ingestion in laboratory animals and in livestock experiments. Finally, we shall deal with the effects of aflatoxins on primates including man.

1.1.1 Aflatoxicosis in Livestock

Poultry

The aflatoxin story began in 1960 with the report of an outbreak of disease in turkey poults in England. Since the aetiology of the disease was obscure it was called Turkey X disease (Blount 1961). Affected birds lost appetite, became lethargic, and died within 7 days after the onset of symptoms. Livers of diseased turkeys were severely damaged. A similar disease of ducklings and young pheasants was reported from England (Asplin and Carnaghan 1961). A common factor in all disease outbreaks was the inclusion of Brazilian groundnut meal in the affected birds' diets (Blount 1961, Asplin and Carnaghan 1961). Test birds that ingested this groundnut meal developed symptoms typical of Turkey X disease. No known poisonous agent was found in the meal (Blount 1961).

A similar disease of ducklings was reported from Kenya. The ducklings' feed ration contained a groundnut meal produced in eastern Africa, indicating that the problem was not solely associated with Brazilian groundnut meal (Allcroft and Carnaghan 1962).

A few outbreaks of the disease were diagnosed among chickens. Experimental feeding trials showed that chickens were much less susceptible to the effects of aflatoxin poisoning than were turkey poults, ducklings or pheasant chicks (Asplin and Carnaghan 1961).

Later in 1960. outbreaks of disease occurred in pigs and calves, apparently caused by an unknown toxic factor in Brazilian groundnut meal contained in animal rations (Loosmore and Harding 1961. Loosmore and Markson 1961).

The discovery that the toxic groundnut meals were contaminated with a mold provided an important clue to the aetiology of Turkey X disease (Austwick and Ayerst 1963. Sargeant et al. 1961). Sargeant et al. (1961) demonstrated that an isolate of the common mold *Aspergillus flavus* Link ex Fries was in fact the responsible agent. The disease was caused by toxins produced by strains of the fungus *Aspergillus flavus* when growing on the meal, and hence these toxins, in view of their origin, were named aflatoxins.

There were fewer reports of aflatoxicosis in farm animals from other European countries probably because on the continent groundnut meal is used mainly in the diets of older animals (Allcroft and Carnaghan 1962). However, the disease in poultry was reported from Spain (Carnaghan and Allcroft 1962), Austria (Kohler and Swaboda 1962), and Hungary (Derzsy et al. 1962).

Several outbreaks of aflatoxicosis in poultry have been reported from India (Char et al. 1982, Choudary and Rao 1982, Gopal et al. 1969, Kishan Rao 1980). In 1962, a heavy mortality occurred among ducklings in Tamil Nadu State (Bhat et al. 1978). The feed used for the ducklings contained groundnut meal with a total aflatoxin content of 6200 ug kg^{1}. Another outbreak of the disease in ducklings was recorded in Kerala State (Sivadas 1968).

Gopal et al. (1969) reported an outbreak of aflatoxicosis in fowls in Kerala State. The disease symptoms included severe and sudden anorexia, loss of weight, staggering gait and convulsive movements. Subacute and chronic cases showed petechial haemorrhage, entertis, ascites and histopathological changes typical of aflatoxicosis such as yellow liver, fatty changes and biliary epithelial hyperplasia.

Reports of aflatoxicosis in Australia have described acute disease in poultry fed imported groundnut meal (Gardiner and Oldroyd 1965, Hart 1965).

Cole (1986) suggested that cyclopiazonic acid (CPA) could have had a role in the aetiology of Turkey X disease. This is based on the fact that some clinical signs of Turkey X disease described by Blount (1960) and by Siller and Ostler (1961) such as the characteristic posture of affected turkey poults (arched neck, head drawn back, and legs stretched fully backwards) result from an acute dose of CPA and not from an acute dose of aflatoxin. These atypical effects of aflatoxicosis could be explained by the presence of CPA which is commonly produced by *A. flavus* along with aflatoxins (Cole 1986). It would be interesting to examine the effects of CPA in relation to aflatoxicosis in other animal species.

Several species of farm animals arc considered **to be particularly susceptible** to aflatoxicosis.

Pigs

Loosmore and Harding (1961) first described natural outbreaks of allatoxicosis **in** pigs associated with ingestion of toxic Brazilian groundnut meal. The carcasses were jaundiced and gross haemorrhage was present in many pans of the body. Experimentally induced allatoxicosis showed thai changes in the liver progressed through steatosis, ductule proliferation and pericellular fibrosis to karyomegaly, dissecting fibrosis and finally nodular hyperplasia (Harding et al. 1963). Abrams (1965) stated that seneciosis can easily be confused with aflatoxicosis in pigs and cattle. However, this confusion did not appear to exist in pigs since Harding et al. (1964) found dial the disease in pigs poisoned by *Senecio jacobaea* (ragwort) differed clinically, anatomically and histologically from that caused by ingestion of aflatoxin-contaminated groundnut meal.

Sivadas (1968), in India, reported a disease of pigs in Kerala State. The affected pigs were stunted, and had considerable liver damage. The groundnut cake implicated **in** the suspected aflatoxicosis of the pigs had aflatoxin levels estimated at over 20000 fi.g kg'. However, this study did not definitively establish aflatoxin as the causative factor.

Ketterer et al. (1982) reported five cases of aflatoxicosis in pigs in southern Queensland, Australia. Of these, two cases of acute toxicity were caused by feeding groundnut screenings containing high levels of aflatoxins (8600 - 44000 |ig kg^{-1}). The disease syndrome included severe depression, vomiting, abortion and deaths. Nine pigs died; 2 died within 12 h, another 6 died in the next 12 h and one died a week after ingesting the toxic material. Some of these symptoms, e.g., abortion, were probably not caused by aflatoxins. Other **mycotoxins may have been** present raising possibilities of synergistic interactions.

Cattle

Several researchers in the U.K. have reported outbreaks of aflatoxicosis **in** cattle (Loosmore and Markson 1961, dray 1961, Allcroft and Lewis 1963). The lesions were confined mainly to the liver, showing degenerative changes with biliary proliferation and finally leading to diffuse cirrhosis. Allcroft and Lewis (1963) described the effects of experimental feeding of rations containing a highly toxic-groundnut meal to catde of different ages. The first symptomatic effect of continuous ingestion of toxic groundnut meal in calves (about 6 weeks of age) was a reduction in growth rate followed by unthriftiness, and loss of appetite. After 16 to 25 weeks on the toxic meal, terminal symptoms, characterized by severe tenesmus, occurred 2 to 4 days before death. Post-mortem examination showed fibrosis of the liver, ascites and visceral oedema. Similar lesions were described by Loosmore and Markson (1961).

In India, Sastry et al. (1965) reported outbreaks of aflatoxicosis in Murrah buffaloes in Andhra Pradesh State. This disease was similar to **that reported in cattle** in the U.K. by Loosmore and Markson (1961). The disease was attributed to **the** inclusion of groundnut cake (20%) in the animals' feed rations, but no data **were** provided on aflatoxin contamination.

Groundnut meal in Livestock Feeds

The **previously cited publications clearly establish the role of aflatoxin-contaminated** groundnut meal in outbreaks of aflatoxicosis **in** livestock. Groundnut meal has traditionally been an important component of poultry and other livestock feeds both in groundnut-producing and importing countries. The economies of some developing countries, e.g., Senegal, arc strongly dependent on export of groundnuts and groundnut products, and no alternative crop is al present available. However, ii should be emphasized that oilier agricultural commodities used in livestock feeds, e.g., cottonseed and maize, can also contain **high levels** of aflatoxins and have **been** implicated in outbreaks of allatoxicosis.

In several developing countries groundnuts are very important, both for home consumption and as a cash export crop. Every effort should be made to reduce aflatoxin contamination and so maintain trade in groundnuts and groundnut products. Until aflatoxins can be eliminated from groundnut products the feed industry and livestock producers should pay close attention to regulations concerning the inclusion of aflatoxin-contaminated groundnut meal in rations for different animal species and livestocks categories. Different species of animals and animals of different ages vary considerably in susceptibility to aflatoxicosis. This has permitted the establishment of codes of practice with regard to permissible levels of aflatoxin in formulation of feeds for particular categories of livestock. Readers can refer to lhe section on "Regulation and Limits for Anatoxins" for information on this **aspect.**

Effects of aflatoxins on animals

Estimates of the susceptibility of most farm and laboratory animals have **been** derived from feeding aflatoxin-containing groundnut meal. Effects of aflatoxins **on** these animals are discussed from three points of view: (1) acute toxicity associated with ingestion of a lethal dose, (2) subacute toxicity associated with consumption of small amounts of aflatoxin, and (3) carcinogenicity of aflatoxins.

Acute toxicity

Aflatoxins are acutely toxic to most animal species. Many experimental studies and observations on natural outbreaks of aflatoxicosis suggest that ducklings are the species most susceptible to acute poisoning by aflatoxins. The LD_{50} of a-day-old duckling is approximately 0.3 mg kg^{1} bodyweight, considerably lower than LD_{50}'s

for rats and hamsters. Some studies indicate that rainbow trout, rabbit, and guinea-pig all have LD_{50} values similar to that of ducklings (Ashley et al. 1964, Wogan 1966). Single dose LD_{50} values of aflatoxin B, in various animals have been obtained by several researchers (Butler 1964, 1966, Wogan 1966, 1968). These values provide useful index of species susceptibility.

The distinct acute aflatoxicosis in ducklings has been put lo good account by using this species for bioassay of aflatoxin.

Chickens are much less susceptible than ducklings and turkey poults. In field outbreaks of aflatoxicosis, mortality was low and reduced growth rate during the first few weeks of age was the main clinical effect (Asplin and Carnaghan 1961; Allcroft and Carnaghan 1963). Carnaghan el al. (1966) confirmed the high tolerance of Rhode Island Red chicks to the effects of aflatoxin. When the chicks were continuously fed with a ration containing 15% of a highly toxic meal, there was only one death and the main effect was retardation of growth rate.

Experimental and field observations have shown that calves from 1 to 6 months are highly susceptible to aflatoxin (Allcroft and Carnaghan 1963, Allcroft and Lewis 1963, Allcroft 1965). They become more tolerant to aflatoxin with increased age. Long-term feeding studies have shown that 3- to 4-year-old heifers became clinically affected after continuous feeding on concentrated rations containing 20% of a highly toxic groundnut meal, while 8- to 10-year-old cows under the same conditions showed no adverse clinical effects. In older cows, no effects on conception or gestation were noted (Allcroft and Carnaghan 1963, Allcroft 1965).

Pigs from 3 to 12 weeks of age are the most susceptible large farm animals. Of the mature swine, pregnant sows are the most commonly affected (Harding et al. **1963).**

Sheep do not appear to be susceptible to aflatoxin as no outbreaks of aflatoxicosis have been reported in this species. When 3-month-old sheep were fed rations containing 20% highly toxic groundnut meal for 3 years they did not show any obvious clinical effects other than a slight growth retardation. No liver damage was observed after 2 years on this ration (Allcroft and Carnaghan 1963, Allcroft and Lewis 1963). Abrams (1965) reported that sheep were not susceptible to amounts of aflatoxin usually encountered in rations, but were affected when given 2 ounces of highly toxic groundnut meal containing 60000 ug allatoxin kg^{-1} twice weekly for 4 to 6 weeks. The symptoms were not described.

Dogs are susceptible to aflatoxin (Newberne et al. 1966). Holding (1964) reported that post-mortem examination of a dog that died with acute haemorrhagic **gastroenteritis, fever,** and jaundice revealed a friable, bright yellow liver and haemorrhages in many other organs. Aflatoxin was detected in the food, which contained groundnut meal.

Rainbow trout are highly susceptible to anatoxins. Feeding of crude allatoxin extracts to rainbow trout resulted in liver lesions similar to those observed in ducklings, consisting of massive haemorrhagic necrosis of the liver parenchyma **with** little or no bile duct proliferation (Halvcr 1965). Based on these experiments **the** $LD_{V)}$ in a 10-day test with a 50 g rainbow trout was 1 to 3 mg of crude allatoxin kg' body weight, which would represent 0.5 to 1 mg of aflatoxins B, and G, kg^{-1} of body weight. Considerable liver damage was observed when 0.2 mg of pure aflatoxin kg^{-1} of body weight was administered.

Guinea-pigs are very susceptible to acute toxicity of aflatoxins (Butler and Barnes 1963). When fed with 20% toxic groundnut meal, all animals succumbed in 14 **to** 28 days.

Mice are resistant, to a certain extent, to aflatoxins (Allcroft et al. 1961, Allcroft and Carnaghan 1962, 1963). Platonow (1964) showed that continued feeding of toxic groundnut meal to mice for at least 3 months had no deleterious effects. Levels of aflatoxins B, and G, in the diet were 4500 ug kg^{-1}, and of aflatoxins B_2 and G_2 600 ug kg^{-1}.

Rats are comparatively more resistant to aflatoxin. They were able lo survive short-term feeding experiments with a diet containing as much as 50% of **toxic** groundnut meal (Butler and Barnes 1963). However, prolonged feeding of a 20% toxic meal diet reduced the growth rate of rats and their food intake, and prolonged feeding beyond a few weeks' time resulted in further liver lesions and hepatomas (Lancaster et al. 1961). Butler and Barnes (1963) showed that pregnancy increased the susceptibility of the animals to aflatoxin. When high doses of aflatoxin were given halfway through pregnancy the litter was stunted and some animals died. The mothers also exhibited pronounced liver changes.

It is emphasized that the acute toxicity of aflatoxin for any given species of animal is influenced by such factors as age, sex. breed, condition of animal, and composition of diet. Therefore, a comparison of LD_{50} values should be used only as a rough guide.

Young animals lend to be more sensitive than mature animals. Different species may metabolize and excrete aflatoxin in different ways and/or at different rates (Hsieh et al. 1977, Stoloff 1989).

In most cases, death occurs within 72 h of the toxin being administered.

Post-mortem examination reveals gross liver damage and occasional haemorrhaging in the intestinal tract and peritoneal cavity.

Subacute toxicity

Animals which consume sub-lethal quantities of aflatoxin for several days or weeks develop a subacute toxicity syndrome which commonly includes moderate to severe liver damage. Several types of liver lesions have been observed in different animals (Table 1). Biliary hyperplasia is the lesion most commonly observed in all species except sheep (Wogan 1966). Lethal dietary aflatoxin levels in domestic animals (cattle, swine, turkey, chickens, ducks) range from 0.3 rng kg' in the ducklings to 2.2 mg kg' in calves (Allcroft 1965). Similar results have been obtained in laboratory animals, including the guinea-pig and rat, which develop toxicity symptoms at 0.7 mg kg^{1} and 3-4 mg kg^{1}, respectively (Butler 1964b, 1966b).

Subacute toxic effects of aflatoxins in monkeys have also been reported (Tulpule et al. 1964). Young Rhesus monkeys (1.5 to 2.0 kg) were fed either 1.0 mg of aflatoxin per day, or 0.5 mg per day for the first 18 days followed by 1.0 mg per day. All animals lost their appetite and died in 14 to 28 days. The principal observations on autopsy were of liver lesions similar to those seen in ducklings and which were suggestive of liver cirrhosis.

Table 1. Liver lesions in animals fed aflatoxin-contaminated groundnut meal.

Liver lesions	Calves	Cattle	Swine	Sheep	Duckling	Duck	Chick
Bile duct hyperplasia	+	+	+	-	+	+	±
Enlarged hepatic cells	+	+	+	-	+	+	-
Acute necrosis and haemorrhage	-	-	+	-	+	-	-
Chronic fibrosis	+	+	+	-	-	+	-
Regeneration nodules	-	+	+	-	±	+	-
Veno-occlusive disease	+	+	-	-	-	-	-

Source : Wogan (1966)

The production of liver lesions does not necessarily result in death, and recovery can occur if a wholesome diet is introduced. Factors that influence the acute toxicity of aflatoxin also affect its chronic toxicity.

Carcinogenicity of aflatoxin

The chronic effect of aflatoxin which has aroused most interest is the production of liver tumors in fish, ducks, rats and non-human primates. Prolonged administration of the toxin at subacute levels results in the formation of liver tumors which are cancerous. This was observed in early investigations on the feeding of toxic groundnut meal to rats (Lancaster et al. 1961). In these experiments, aflatoxin-contaminated Brazilian groundnut meal (which was highly toxic to poultry) was fed to rats at levels that did not cause acute toxic symptoms. After 24 weeks of feeding, 81% of the rats developed malignant liver tumors. This was confirmed by several other investigators using aflatoxin-contaminated groundnut meals (Butler and Barnes 1963, Newberne et al. 1964, Salmon and Newberne 1963. Schoental 1961). Aflatoxin levels as low as 100 µg kg^{-1} caused significant incidence of liver tumors in rats when fed for as long as 73 weeks (Salmon and Newberne 1963).

Aflatoxin-contaminated groundnut meals have also been shown to be carcinogenic to ducks and rainbow trout (Carnaghan 1965, Ashley et al. 1965).

Some animal species such as mice are relatively resistant to carcinogenic effects of aflatoxin (Platonow 1964, Allcroft 1965).

Several studies with rats have demonstrated a positive correlation between liver tumor incidence and dietary aflatoxin in the range of 60 to 1800 µg kg^{-1} (Newberne et al. 1964, Wogan 1966). Administration of 1800 µg aflatoxin kg^{-1} for 370 days resulted in a tumor incidence of more than 90%.

The rainbow trout is considerably more sensitive than the rat to the carcinogenic effects of aflatoxin; this fish develops liver tumors when fed purified diets containing 0.5 to 2.0 µg aflatoxin kg^{-1} (Ashley et al. 1964, Ashley et al. 1965).

Clinical and Pathological Effects

Early clinical signs of aflatoxicosis in livestock are reduction in feed intake, and weight loss, this is often rapidly followed by death. The most important pathological effect is liver damage. Non-specific but characteristic clinical and pathological

effects can be seen for each species. Several review papers have described in detail the clinical and pathological effects of aflatoxin ingestion in various animal species (Allcroft 1969. Wogan 1966).

The principal lesions occur in the liver, and may be classified as toxic hepatitis. One of the most constant responses to aflatoxin B, 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 (Allcroft 1969, Wogan 1966).

Aflatoxins also impair the effectiveness of native defence mechanisms and immunogenesis. The major effect of aflatoxin is on the cell-mediated immune system as has been shown in poultry with fowl cholera and in swine erysipelas. Aflatoxin ingestion increases susceptibility of poultry to salmonellosis, candidosis, and coccidiosis, and of calves to fascioliasis (Pier el al. 1979).

Role of Nutrition in Aflatoxin Toxicity

Many researchers have investigated the influence of malnutrition on aflatoxin toxicity in various animals. Madhavan et al. (1965) first observed that reduced protein intake significantly increased the susceptibility of monkeys to aflatoxin. Newbeme et al. (1966) reported that rats given diets containing low level of protein and exposed to aflatoxin B, over 3 weeks suffered a higher incidence of liver tumors in a shorter period of time than did rats receiving diets containing a normal level of protein. A high-protein diet precluded severe injury/toxicity from aflatoxin (Madhavan and Gopalan 1968).

Foy et al. (1966) has suggested that the high incidence of liver cirrhosis in baboons, and liver carcinoma in human populations in Africa could result from diets containing aflatoxins and deficient in pyridoxine.

Many laboratory studies with rats have indicated that marginal deficiencies of lipotropes may significantly influence the response to aflatoxin (Newberne et al. 1966, Rogers and Newbeme 1969). Interactions of vitamin A deficiency with aflatoxin have been investigated by a few workers, but no definite results were obtained (Reddy et al. 1973).

Newberne and Gross (1977) comprehensively reviewed nutritional factors that might influence responses of animals and humans to aflatoxin.

As the situation is by no means clear, it is important that further studies should be made in those least developed countries where malnutrition, infectious diseases and risk of aflatoxicosis coexist.

1.1.2 Aflatoxicosis in Man

During the past two decades, there have been several attempts to correlate the consumption of foodstuffs contaminated with aflatoxins with human diseases. Investigators have relied on natural outbreaks of suspected aflatoxicosis and upon cases of accidental consumption of aflatoxin-contaminated foodstuffs. There have also been extrapolations regarding toxicity and carcinogenicity of aflatoxins from in vitro experiments with primates and other animals.

Association between aflatoxin ingestion and liver cancer

Epidemiological studies have been carried out to sec if aflatoxin ingestion might be a factor in the high incidence of liver cancer in some areas of Africa. Asia, and the USA. Several early epidemiological studies in Indonesia, Kenya, Mozambique, South Africa. Swaziland. Thailand, and Uganda found a positive correlation between aflatoxin ingestion by humans and liver cancer incidence (Husaini et al. 1974, Peers et al. 1976, Van Rensburg et al. 1974, Keen and Martin 1971, Shank et al. 1972, Alpert et al. 1969). There appears to be a trend for percentages of aflatoxin-contaminated food samples to be higher at low altitudes than in cooler, high altitude areas. This parallels the trend for liver cancer in Swaziland and Kenya (Keen and Martin 1971, Peers and Linsell 1973). However, it could not definitely be concluded from these studies that aflatoxin caused liver cancer, also there had been no attempt to consider other possible causes of liver cancer. Several researchers have highlighted the strong relationship between hepatitis B virus (HBV) infection and incidence of liver cancer (Beasley et al. 1981. Beasley 1982). All epidemiological studies of aflatoxin and liver cancer conducted in Africa and Asia involved populations subjected to HBV infection.

Subsequent epidemiological studies included data on the prevalence of HBV infection (Autrup el al. 1987, Peers et al. 1987, Van Rensburg et al. 1985, Yeh et al. 1985. 1989). In a repeat study in Swaziland, Peers et al. (1987) found that virtually all the liver cancer cases had been exposed to HBV infection, but aflatoxin exposure was a more important factor in liver cancer than the prevalence of HBV infection. Other studies of populations with endemic HBV infection provided no convincing evidence to support a primary role for aflatoxin in the induction of human liver cancer, although an accessory role to HBV infection for aflatoxin could not be ruled out (Autrup et al. 1987, Van Rensburg et al. 1985, Yeh et al, 1985, 1989). In a recent study in China. Chen et al. (1990) found no association between liver cancer mortality rates and aflatoxin exposure (controlled for HBV infection) but confirmed the strong association of liver cancer mortality with HBV infection. In the USA, a study of liver cancer in relation to aflatoxin ingestion in populations free of HBV infection also ruled out any association between liver cancer and aflatoxin exposure (Stoloff 1983).

Several in vitro experiments with human liver tissue have indicated that it metabolizes aflaloxins in a similar fashion to the livers of species refractory to aflatoxin carcinogenesis (Stoloff 1989).

Stoloff (1989) has given an excellent review of the liver cancer/aflatoxin/HBV relationship. Based on published evidence, he concluded that aflatoxin is not a probable human carcinogen.

Aflatoxins and occupational diseases among groundnut workers

Workers engaged in harvesting, shelling, bagging, storage, marketing, and transport of groundnuts may be exposed to aflatoxin through the respiratory route. A chemical engineer involved in sterilizing Brazilian groundnut meal contaminated by *Aspergillus flavus* developed alveolar cell carcinoma and died within a year. Aflatoxin B, was detected in his lung tissue (Dvorackova et al. 1976). Three cases of pulmonary interstitial fibrosis were reported from Czechoslovakia, of whom two were agricultural workers; aflatoxin (10-54 ug Kg^{1}) was detected in lung samples of all the workers (Dvorackova and Pichova 1986).

In a Dutch groundnut oil factory workers were exposed to an estimated 0.039-2.5 ug aflatoxin per working week (45 h). An epidemiological study of 55 workers indicated that 11 developed various forms of cancer, mainly of the respiratory tract (Van Nieu Wenhuize et al. 1973).

A study conducted 18 years after initial exposure to aflatoxin-contaminated dust did not give a definite indication of cancer risk associated with respiratory exposure to aflatoxin, but it did suggest certain associations warranting further study (Hayes et al. 1984). There was no information on the extent to which dustborne aflatoxin enters the body, nor any explanation of the manner in which aflatoxin could be related to any of the observed cancers.

There can be considerable risk associated with exposure to dust from groundnut and other agricultural commodities contaminated with aflatoxin; however, the exact magnitude of the risk cannot be adequately estimated (Baxter et al. 1981).

Acute toxicity of aflatoxin in humans

Acute effects of aflatoxins in humans are well documented. There are two reports of aflatoxicosis in humans involving consumption of maize heavily contaminated with aflatoxins (Krishnamachari et al. 1975 a, b, Ngindu et al. 1982). In one of the incidents, in India, there were 272 hospital admissions with clinical symptoms of aflatoxicosis and a 27% monality. In the second incident, in Kenya, there were 20 hospital admissions with a 60% mortality.

A report (Willis et al. 1980) helps to establish a possible no effect level for aflatoxin B,. In an attempted suicide, a laboratory technician consumed 12 ug kg^{-1} body weight day^{1} over a 2 day period and 6 months later 11 ug kg^{1} body weight day^{1} over a 14 day period. Outside of transient rash, nausea and headache there were no immediate ill effects, and on a 14 year follow-up the physical examination and blood chemistry, including tests for liver function, gave normal results.

There is certainly a basis for concern about the acute toxicity of aflatoxin in humans from dietary intake of the toxin.

The long term follow up of patients who have survived outbreaks of aflatoxicosis for possible liver cancer should be of considerable importance.

Sublethal effects of aflatoxins

In Senegal, several children less than 1 year old each received 70-140 g of groundnut meal per day for 10 months as a treatment for kwashiorkor (Payet et al. 1966). The meal samples were later found to be contaminated with aflatoxin at 500-1000 ug kg^{1}, providing an aflatoxin intake of 35-140 ug day^{-1}. Two of these children were identified for liver biopsies 4 and 6 years after consumption of the contaminated meal. One child had gross abnormalities in the liver structure, persisting through the sixth year; the other child had minor liver abnormalities at 4 years and recovered to completely normal at 6 years.

Aflatoxin and Indian childhood cirrhosis

Ingestion of aflatoxin, viral diseases, and hereditary factors have all been suggested as possible aetiologic agents of Indian childhood cirrhosis (Bhat 1989). Amla et al. (1970. 1971) presented circumstantial evidence to indicate that children exposed to aflatoxin through breast milk and dietary items such as unrefined groundnut oil and parboiled rice may develop cirrhosis. They detected aflatoxin B, in 7% of urine samples from cirrhotic children. In another study (Amla et al. 1971), they reported that malnourished children in a pediatric ward of a hospital developed liver lesions similar to those observed in Indian childhood cirrhosis after accidentally consuming 30-50 g of groundnut protein flour contaminated with 300 ug kg^{1} of aflatoxin for periods ranging from 5 days to 1 month. They also observed similar skeletal muscle changes in cirrhotic children, in children who consumed afiatoxin-contaminated groundnut protein flour, and in rats fed aflatoxin. Other workers have also detected aflatoxins or aflatoxin-like substances in urine samples from cirrhotic children (Yadgiri et al. 1970, Parpia et al. 1972).

It is surprising that childhood cirrhosis has not been found in other countries where aflatoxin contamination of dietary items is fairly high.

Anatoxins and Kwashiorkor

Several epidemiological studies have provided circumstantial evidence for the involvement of aflatoxins in Kwashiorkor, particularly among malnourished children in the humid tropics. In several studies in the Sudan, Nigeria, Ghana, and South Africa, in both autopsied as well as biopsied livers from children with protein energy malnutrition, aflatoxins were detected in Kwashiorkor and marasmic Kwashiorkor, but not marasmus (Coulter et al. 1986, Hendrickse 1984). Groundnuts and peanut butter were the major source of aflatoxins among these populations (Hendrickse et al. 1982). Aflatoxins M, and M_2 have also been frequently detected in breast milk and neonatal cord blood of mothers (Coulter et al. 1984, Lamplugh and Hendrickse 1988). Based on these studies it is postulated that Kwashiorkor might result from chronic aflatoxin poisoning (Hendrickse 1984. Pearson 1990). These observations do not establish a cause- effect relationship between aflatoxin ingestion and kwashiorkor. Some typical diagnostic features of kwashiorkor, e.g., oedema, mild pathological damage to liver, and rapid response to dietary therapy, are not characteristic of aflatoxicosis. Some studies have reported that children with kwashiorkor have a reduced ability to metabolize and excrete aflatoxins compared with marasmic and healthy children (Coulter et al. 1986). This could explain the higher levels of anatoxins detected in kwashiorkor children. It appears that aflatoxins are not primarily responsible for kwashiorkor development, but malnourished children may show increased susceptibility to the toxic effects of aflatoxin.

Bhat (1989) critically reviewed the risks to human health associated with consumption of groundnuts and groundnut products contaminated with aflatoxins.

Aflatoxins and Reye's Syndrome

Several investigators have suggested allatoxin as an aetiologic agent of Reye's or Reye's-like syndromes in children in Thailand, New Zealand, and Czechoslovakia (Becroft and Webster 1972. Dvorackova' et al. 1977). This was based on aflatoxins being detected in serum and urine samples from the children suffering from Reye's syndrome, and the presence of liver lesions. There is as yet no conclusive evidence to show that Reye's syndrome is directly associated with consumption of aflatoxin-contaminated foodstuffs.

Risks through dietary intake of contaminated groundnuts and groundnut products

Outbreaks of aflatoxicosis in man have been attributed to ingestion of staple foods such as maize highly contaminated with aflatoxins. Although high levels of aflatoxins have been reported in groundnuts and groundnut products these pose less of a hazard to human health than contaminated maize products because of the smaller quantities consumed in most diets liowever, dietary intake of groundnuts and/or groundnut products can be substantial in some developing countries, e.g., Mozambique. Senegal, the Sudan. One must consider the possibility of chronic effects of aflatoxin following continuous exposure to relatively low levels of the toxins through ingestion of groundnuts and groundnut products. Studies in several groundnut-producing countries of Africa and Asia have attempted to link aflatoxin ingestion with liver cancer. Although the causal relationship has not yet been definitively established, it is obviously important to reduce dietary intake of aflatoxins. In developed countries there are procedures for monitoring levels of aflatoxins in foodstuffs, but in most developing countries only limited or no protection of this kind is available. Substantial exposure to aflatoxin may result from consumption of contaminated peanut butter, use of unrefined oil, and snack foods from reject trade groundnuts. These risks are much greater in developing than in developed countries. Particular care should be taken to ensure that groundnut products commonly consumed by children should be free from aflatoxin. Protein concentrates used in treatment of malnourished children should be carefully checked to ensure that they are free from aflatoxin.

Although there are some doubts as to the toxic and carcinogenic effects of aflatoxins on man, one should not underestimate their possible hazards to human health, particularly in the presence of nutritional disorders and other diseases.

1.2 BIBLIOGRAPHY

1.2.1 AFLATOXICOSIS FROM INGESTION OF TOXIC GROUNDNUTS AND GROUNDNUT PRODUCTS

1. **Blaney,** B.J. 1985. Mycotoxins in crops grown in different climatic regions of Queensland. Pages 97-108 in Trichothecenes and other mycotoxins (Lacey, J., ed.). John Wiley & Sons Ltd. : UK.

This paper considers climatic conditions in relation to mycotoxin contamination in different crops in Queensland, Australia. Queensland has a history of recurring drought, and drought stress is an important factor in preharvest aflatoxin contamination of the groundnut and maize crop, particularly in the drier Burnett region. Conversely, while drought depresses yields, dry conditions usually limit the extent of fungal damage to wheat, barley and sorghum. However, the 1983 season was exceptional in that persistent heavy rain produced extensive mold damage to all crops. Mycotoxins produced by *Fusarium* spp. have never been implicated in widespread mycotoxicosis in Queensland, although they may contaminate maize and sorghum in regions with high rainfall during the growing season, such as the tablelands in Far North Queensland. Mycotoxicosis of man is unlikely to occur in Queensland, but mycotoxicosis of livestock occurs occasionally. It is usually associated with feeding materials molded from being stored damp. In addition, cases are likely to occur in drought seasons when feeds are not only scarce, but also likely to be of poorer quality.

2. Edds, G.T. 1973. Acute aflatoxicosis : A review. Journal of American Veterinary Medical Association 162(4): 304-309.

The factors influencing aflatoxicosis, clinical signs and gross and microscopical lesions of the condition in fowl, duck, turkey, swine, sheep, monkey, rat, mouse and man, and biochemical changes produced are discussed.

3. Fong, L.Y.Y., and Chan, W.C. 1981. Long-term effects of feeding aflatoxin-contaminated market peanut oil to Sprague- Dawley rats. Food and Cosmetics Toxicology 19(2): 179-183.

Groundnut oil obtained from Hong Kong markets was frequently contaminated with aflatoxins. A purified diet in which aflatoxin- contaminated market groundnut oil (aflatoxin B, 110 ug kg ') was used as the fat source was given to Sprague-Dawley rats for 22 months from weaning; its estimated aflatoxin Bl content was 5 to 7 ug kg^{1}. Controls were given a diet of identical composition except that Manzola maize oil (aflatoxin-free) was used. Of 76 rats given aflatoxin, 3 had sarcomat, one in the liver, one in the wall of the colon and one in the subcutaneous tissue of the groin; 18 rats given groundnut oil showed parenchymal liver damage and different degrees of fatty change and one showed premalignant changes in liver cells. Of 90 control rats, none developed malignant tumours. The liver-to-body weight ratios for experimental and control rats were 2.93 and 2.62. respectively. The difference between those values was significant, reflecting the degree of fatty change in the livers of experimental rats compared with that in the controls. Over 90 % of Hong Kong households use groundnut oils for cooking purposes, and these results indicate a possible health hazard in the use of contaminated groundnut oil.

4. **Hertrampf,** J. 1978. [Groundnut meal and its problems], Erdnusssehrot und seine probleme. Muhle + Mischfuttertechnik 115(3): 36.

Problems arising from the contamination of groundnut meal with aflatoxin are reviewed. Progress in the detection of related compounds and knowledge of their toxic properties are summarized, with details of acute toxicity on monkeys, and indications of the chronic effects of very small doses on liver and kidneys of children. Transmission of the toxic constituents from animal feeds to cow's milk and pig liver and kidneys is also reported, although there are no apparent indications of progressive accumulation. Aflatoxin in imported groundnut meal is discussed and the limits imposed by the EEC on feeds of different kinds are tabulated. The practical impliations of stringent limits on aflatoxin contents of meal imported into European and other countries are discussed, with particular reference to the danger that more heavily contaminated batches may be retained in the developing countries where the population relies heavily on groundnut meal as a source of protein. The possibility of detoxification with ammonia is briefly discussed, and it is noted that tests with rats indicate that the protein quality if thereby decreased by about 10 %.

5. Hesseltine, C.W. 1976. Mycotoxin research in India. Mycopathologia 58(3): 157-163.

This paper briefly reviews mycotoxin research in India. Research on various aspects of the groundnut aflatoxin problem, and on other mycotoxins is highlighted. Aspects discussed include occurrence of aflatoxins in various agricultural commodities, toxicity of aflatoxins to humans, resistance to aflatoxin production in natural substrates, detoxification, and mycology of aflatoxin-producing strains *of Aspergillus flavus,*

6. Martin, P.M.D., and Gilman, G.A. 1976. A consideration of the mycotoxin hypothesis with special reference to the mycoflora or maize, sorghum, wheat and groundnuts. Tropical Products Institute Report; Series G (UK) no. 105, 119 pp.

This review attempts to trace the connection between the mycology of foodstuffs and the onset of disease due to the toxins that various fungi produce within those foodstuffs. Particular emphasis is placed on the practical side of the problem, especially with reference to the physiological interactions among fungi, the incidence of fungi and their toxins in various substrates, the ecology of mycotoxin formation, and the acute and chronic effects of mycotoxicosis.

7. Maselli, J.A. 1977. Controlling aflatoxin in your plant. Manufacturing Confectioner 57: 35-38,40-41.

The clinical effects of aflatoxin exposure are briefly discussed, and the current US FDA regulations and sampling procedure for control of aflatoxins in groundnuts are discussed. Some quality control procedures to ensure minimal quantities of toxin persisting into the finished product are also outlined.

8. McDonald, D. 1976. Aflatoxins : Poisonous substances that can be present in Nigerian groundnuts. Samaru Miscellaneous Paper 53, Institute for Agricultural Research, Samaru, Ahmadu Bello University, Zaria, Nigeria, 14 pp.

This paper outlines events leading up to the discovery of aflatoxin, describes briefly research done on the groundnut aflatoxin problem in northern states of Nigeria, and considers the implications of aflatoxin in relation to animal and human health, and discusses measures for elimination of aflatoxin from Nigerian groundnuts, or to at least greatly reduce incidence of aflatoxin.

9. Peers, F.G. 1967. Aflatoxin - A summary of recent work. Tropical Science **IX(4):** 186-203.

The important published information on aflatoxin during the period January 1964 to March 1967 is summarized. The topics covered include (a) assay of aflatoxins. (b) toxicology of aflatoxins, (c) biosynthesis of aflatoxins, (d) biochemical and intercellular effects of aflatoxins. (e) metabolism of aflatoxins in animals, (f) incidence, control and detoxification, and (g) implication in man.

10. **Pruthi,** J.S. 1978. Mycotoxins in foods and feeds - their detection, estimation, preventive and curative measures. Bulletin of Grain Technology 16(1): 51-68.

This review covers several aspects including a survey of aflatoxin contamination of food grains and of groundnut oil, human and animal health hazards from mycotoxins, techniques for detection and estimation of aflatoxins, factors affecting aflatoxin formation, preventive or control measures.

11. Saito, M., and Singh, R.B. 1976. Reports on study of mycotoxins in foods in relation to liver diseases in Malaysia and Thailand. Institute of Medical Science, University of Tokyo, Tokyo, Japan, 85 pp.

Part I of this report deals with a survey of mycotoxins contamination in food **and** its relation to hepatoma in Malaysia. The topics covered include : (a) Biostatical aspect of liver cancer in Malaysia, (b) A semi-quantitative study on frequency of food intake in Sekinchan, (c) Results of mycological survey of Malaysian foods, and (d) Analysis of mycotoxins in foods in Malaysia. The incidence of liver cancer is higher in Malaysia than in Japan and is highest in the Chinese male population. In the three communities rice, oil, fat and vegetables are the main foods. The Malays frequently eat fresh fish, beans and bawang and use chili kering; the Chinese frequently eat bawang meats, fresh fish and beans; the Indians frequently use milk and its products and dry curry powder. *Aspergillus* spp. were the major fungal species found, especially in milled rice. *Aspergillus candidus* was most frequent in rice (52 % of samples) and *A. niger* moderately abundant. *A. flavus* was isolated from 13-21 % of rice samples and *A. fumigatus* from 3-21 %. Curry powder was highly contaminated with *A. flavus; A. niger, Penicillium citrinum* and mucoraceous fungi were also dominant. *Aspergillus flavus* was found in groundnuts, cereal powders, beans and dried fish and *A. niger* was widespread in some foodstuffs. No mycotoxins were detected in rice, moldy soybeans or tempeh. Aflatoxin was detected in groundnut samples. Extracts of rice, beans and tempeh samples showed varying toxicity to HeLa cells. All except one groundnut sample showed low toxicity. Of 30 strains of *A. flavus* isolated, 8 produced aflatoxin, and 3 of 4 strains of A. *versicolor* produced sterigmatocystin. All 91 strains of *A. candidus* examined produced terphenyllin but not xanthoascin. Part II, on studies in Thailand, includes (i) Mycological survey on market foods of Thailand, (ii) Distribution of aflatoxin-producing fungi in agricultural soils of Southeast Asia, and (iii) A glimpse into Reye syndrome in Khon Kaen, a north-eastern district of Thailand. The dominant fungi isolated from 36 samples of eight types of foods were : *A. candidus, A. flavus, A. niger, Eurotium* and *Penicillium* spp. from rice; *A. flavus, A. niger, Mucor* and *Rhizopus* spp. from groundnuts; *Fusarium* spp. from beans and maize; *A. niger* from pepper; *A. niger* and *Fusarium* spp. from cassava starch; *A. flavus* **and** *A. niger* from chilli, chilli powder, shrimp and shell fish. *A. flavus* was isolated from 15 of 50 soil samples from Malaysia and 29 of 106 from Thailand. Of these 44 strains, 16 produced aflatoxin.

12. **Schmidt,** F.R., **and** Esser, K. 1985. Aflatoxins : medical, economic impact, and prospects for control. Process Biochemistry 20(**6): 167-174.**

Current knowledge about mode of action of aflatoxins, their medical and economic importance and prospects for tlicir control are reviewed, considering the following aspects : structure, nomenclature and biosynthesis; mode of action; health risks and legislative regulation; postharvest control (segregation, decontamination, **storage);**

and preharvest control (pesticides, and biological control by breeding or microbial interactions, particularly repression of aflatoxin synthesis by *Aspergillus flavus* following infection with a virus from *Penicillium* chrysogenum).

13. Spensley, P.C. 1963. Aflatoxin, the active principle in turkey 'X' disease. Endeavour 22: 75-79.

Mold infection of oil-seed cakes has long been suspected as a possible cause of ill-health among farm animals, but only within the last three years has the problem been closely investigated. Groundnuts may become infected with strains of the fungus *Aspergillus flavus* that produce a group of highly toxic substances known collectively as aflatoxin. This has in the past caused the deaths of large numbers of farm animals, especially young animals. There is, however, no evidence that human beings have suffered, and with recognition of the nature of the hazard, steps can be taken to ensure that there need be no danger from the substance in future.

1.2.2 Aflatoxicosis in Livestock

14. Alleroft, R. 1969. Aflatoxicosis in farm animals. Pages 237-264 in Aflatoxin : Scientific background, control and implications (Goldblatt, L.A., ed). New York, USA : Academic Press, 472 pp.

Aflatoxin toxicity problems in farm animals are reviewed. Clinical and pathological effects in animals are described. Metabolism and excretion of aflatoxin in animals are also discussed.

15. Allcroft, R., and **Carnaghan, R.B.A.** 1962. Groundnut toxicity. *Aspergillus flavus* toxin (aflatoxin) in animal producis : preliminary communication. Veterinary Record 74: 863-864.

Whole milk of cows given toxic groundnuts as 20 % of the diet, an extract of that milk, and the fraction precipitated by rennet were toxic to ducklings. The degree of toxicity of the milk was related to that of the groundnut given. The toxin was not destroyed by pasteurisation. In 19 samples of National dried full cream and separated milk and proprietary infant foods there was no evidence of toxicity. No toxin was found in the liver of 1 of the cows given toxic groundnut meal or the eggs of pullets given 15 % toxic groundnut meat in their diets.

16. Allcroft, R., and Carnaghan, R.B.A. 1963a. Toxic products in groundnuts. Biological effects. Chemistry and Industry (London) 2 : 50-53.

Biological effects of toxic groundnut meal (meal containing aflatoxin) in various birds and animals are reviewed. Ducklings are the most susceptible to the toxin. They are suitable for bioassay of aflatoxin. Turkey poults are less susceptible, while chickens are comparatively resistant. Among the larger farm animals, pigs are most susceptible. Calves from 1 to 6 months of age are highly susceptible, becoming tolerant with age, and sheep are comparatively resistant.

17. Allcroft, R., and Carnaghan, R.B.A. 1963b. Groundnut toxicity : An examination for toxin in human food products from animals fed toxic groundnut meal. Veterinary Record 75: 259-263.

Cows fed rations containing toxic groundnut meal excreted in the milk a toxic factor having a biological effect in ducklings similar to that caused by aflatoxin. Precipitation of protein fractions of the milk showed that the toxin was present only in the rennet-precipitated casein fraction which also included the fat; none was found in the protein-free filtrate. Its presence was not detected in samples of bulked milk supplies from collection centres in Britain; nor was it found in liver from a cow or a pig, or clotted blood and serum from a cow, or pullet eggs, from animals fed rations containing toxic groundnut meal.

18. Alleroft, R., and Lewis, G. 1963. Groundnut toxicity in cattle : Experimental poisoning of calves and a report on clinical effects in older cattle. Veterinary Record 75: 487-494.

The effects of feeding rations containing 20 % of a highly toxic groundnut meal to calves, first-calving heifers, and older cows are described. The first abnormality in the calves was a reduction in growth rate followed by unthriftiness, and loss of appetite. After 16 to 25 weeks on the toxic diet, terminal symptoms, characterized by severe tenesmus, occurred 2 to 4 days before death. Fibrosis of the liver, ascites and visceral oedema were the most important post-mortem findings. The most notable biochemical changes in the calves were : (1) an increase in serum alkaline phosphatase activity up to the 12th week followed by a decline to normal values during the terminal phase; (2) almost complete absence of vitamin A in the livers at death. The main histological changes in serial-biopsy and post mortem liver specimens consisted of hepatic centro-lobular necrosis, ductal cell hyperplasia and veno-occlusive disease. Loss of condition occurred in the heifers after seven months. No clinical abnormality was noted in the older animals apart from a Jersey cow which developed clinical Johne's disease at calving after 13 months on toxic-groundnut meal. In a group of eight cows a significant fall in milk yield resulted from feeding a concentrate ration containing 15 % of a very highly toxic groundnut meal.

19. Allcroft, R., and Loosmore, R.M. 1963. Toxic effects associated with the

feeding of groundnuts. Pages 175-178 in Proceedings of the XVIIth World Veterinary Congress, Hanover, 1963.

The clinical and pathological effects of a disease in poultry, pigs, cattle and laboratory animals associated with the feeding of toxic batches of groundnut meal are described. The toxic factor is a hepatotoxin and is produced by infection of groundnuts by a toxigenic strain of *Aspergillus flavus* which has been found in some batches of groundnuts from all major groundnut-producing countries. In cattle, the disease closely resembles seneciosis; in rats, multiple liver tumors, some malignant, have been produced. Cows fed rations containing toxic groundnut meal excrete the toxin in their milk.

20. Allcroft, R., and Roberts, B.A. 1968. Toxic groundnut meal : the relationship between aflatoxin B, intake by cows and excretion of aflatoxin M, in milk. Veterinary Record 82(4): 116-118.

Dairy cows were fed diets containing aflatoxin B_1-contaminated groundnut meal and their milk was tested alter 4, 5 and 6 days of feeding. Assays of liquid and dried milk samples indicated a simple linear relationship between the amount of aflatoxin B, ingested and the concentration of aflatoxin M, excreted in the milk ($P < 0.001$). The lowest aflatoxin B, intake to give detectable amounts of aflatoxin M, was 0.6-0.9 mg day^{1}.

21. Allcroft, R., Roberts, B.A., and Lloyd, M.K. 1968. Excretion of aflatoxin in a lactating cow. Food and Cosmetics Toxicology 6: 619-625.

A cow of 600 kg liveweight was given a single oral dose (300 mg) of a mixture of aflatoxins (B, 44 %; G, 44 %; B2 2 %), equivalent to 0.5 mg kg^{1} bodyweight. The toxins were detected in milk, urine and faeces over a period of 9 days. About 85 % of the total aflatoxin was detected in milk and urine in the first 48 hours. No aflatoxin was delected in milk after 4 days and in urine and faeces alter 6 days. Only 4.52 % of the total dose of 300 mg was detected, the milk containing 0.18 %, urine 1.55 % and faeces 2.79 %. The only form of aflatoxin detected in milk was aflatoxin M_1, and this represented 0.35 % of aflatoxin B, given to the animal. Aflatoxin M_1 was also the main form found in urine. Aflatoxin G, was found in both urine and faeces. Aflatoxin B, was present in greatest amounts in faeces. The pattern of excretion was compared with that in a ewe given 1.0 mg of a similar mixture kg^{1}. The pattern was similar in the 2 species, but excretion of unchanged aflatoxin B, was greater in the cow.

22. Allcroft, R., Rogers, H., Lewis, G., Nabney, J., and Best, P.E. 1966. Metabolism of aflatoxin in sheep : excretion of the "milk toxin". Nature 209: 154-155.

A mixture of aflatoxins B_1, G_1, B_2 and G_2, 1 mg kg^{1} bodyweight, was given to 3 yearling sheep by stomach tube or by intraperitoneal injection. The sheep were killed 2 h later. Aflatoxin and the "milk toxin" were estimated in liver and kidney and in urine before the toxin was given, after 1 h, and at death. The "milk toxin" was more intense in kidney and urine than the aflatoxins, but the reverse was **true** for the liver. It is suggested that tests on urine may be useful for investigating ingestion of aflatoxin by man or animals. The name aflatoxin M is proposed for **the** "milk toxin".

23. Amaral, L.B.S. 1962. Mortality in pigs fed groundnut meal. Biologico 27(3): 61.

Occurrence of aflatoxicosis in pigs is reported. The disease was associated with feeding toxic groundnut meal.

24. Anonymous. 1960. Disease of turkey poults (news and comment). Veterinary Record 72(35): 710.

Outbreaks of a new disease of turkey poults in England are reported. Disease outbreaks were associated with certain feeds. Disease symptoms are described.

25. Anonymous. 1983. Aflatoxin-contaminated feed not sale for dairy cattle. Feedstuffs 55(44): 20.

Dairy producers were advised against feeding aflatoxin-contaminated feeds to dairy cattle. Aflatoxin toxicity problems in dairy cattle were highlighted. Guidelines for usage of contaminated feeds and for avoiding problems were given.

26. Archibald, R.Mc.G., Smith, H.J., and Smith, J.D. 1962. Brazilian groundnut toxicosis in Canadian broiler chickens. Canadian Veterinary Journal 3(10): 322-325.

Examination of chickens after death showed liver damage typical of groundnut poisoning. Birds less than 5 weeks old were more affected than older ones, and their mortality was higher. It was confirmed that diets of all affected chickens contained 5 % of Brazilian groundnut meal.

27. Asplin, F.D., and Carnaghan, R.B.A. 1961. The toxicity of certain groundnut meals for poultry with special reference **to their effect on ducklings and chickens.** Veterinary Record 73(46): 1215-1219.

Evidence of the susceptibility of ducklings and chickens to the toxic principle in certain samples of groundnut meal is presented. Ducklings were highly susceptible to the toxic principle in these meals, and it was suggested that they are eminently

suitable for screening suspected samples of groundnut meal and for other experimental work connected with this type of toxicity. A toxic agent was found in certain Brazilian and East African groundnut meals, and evidence is presented which suggests that the toxic principle in these meals is identical. The gross and microscopic lesions in ducklings and chickens fed on toxic groundnut meals are described and the similarities and differences between the lesions in these birds and turkeys and in large animals arc discussed.

28. Balaraman, N., and Arora, S.P. 1986. Effect of aflatoxin on growth in Karan Swiss calves and assessment of safe level. Indian Journal of Animal Nutrition 3(4): 223-228.

In a controlled experiment, male Karan Swiss calves (3-4 months old) were given feeds contaminated with aflatoxin at levels of 0.5, 1.0 and 1.5 ug g^{1} over a period of 16 weeks. For calves given aflatoxin-contaminated feed, weight gains were significantly reduced from 3rd week onwards. Feeding aflatoxin at levels of 0.26 ug $kg^{"1}$ did not adversely affect growth.

29. Bhatt, P.C. 1980. Aflatoxins in poultry management. Poultry Guide 17(4): 69-71.

Aflatoxin toxicity in poultry is described. Effects of ingestion of aflatoxins on young chicks and layers are discussed. Preventive measures for avoiding aflatoxicosis in poultry are given.

30. Blount, W.P. 1960a. A new turkey disease problem in England characterized by heavy mortality. Quaterly Poultry Bulletin 27: 1-3.

Outbreaks of a new disease of turkey poults in England are reported. Disease symptoms are described. Disease outbreaks were associated with certain feeds.

31. Blount, W.P. 1960b. Disease of turkey poults (Letter). Veterinary Record 72(38): 786.

Some comments are given on the implication of rations in turkey X disease. Outbreaks of turkey X disease were not associated with rations containing milo.

32. Blount, W.P. 1961a. Turkey "X" disease and the labelling of poultry foods (Letter). Veterinary Record 73(9): 227.

This letter emphasizes the importance of labelling of poultry foods in relation to turkey X disease.

33. Blount, W.P. 1961b. Turkey "X" disease. Turkeys (Journal of British Turkey Federation) 9(2): 52,55-58,61.

This paper is a report upon turkey X disease occurring in the United Kingdom with information on its aetiology.

34. Bryden, W.L., Lloyd, A.B., and Cumming, R.B. 1980. Aflatoxin contamination of Australian animal feeds and suspected cases of mycotoxicosis. Australian Veterinary Journal 56(4): 176-180.

Aflatoxin B, was detected in 23 of 55 feedstuff's known to be either water-damaged or visibly moldy. The highest level of aflatoxin was 700 u*g* kg' of feed, and the mean concentration was 140 |ig kg^{1}. Of 36 feedstuffs purchased from local manufacturers, only groundnut meal contained aflatoxin B, (500 ug kg'). Eleven feedstuffs were associated with field outbreaks of animal disease and seven of these contained aflatoxin. but not in sufficient quantity to account for the described disease symptoms.

35. Carnaghan, R.B.A. 1961. The toxicity of certain groundnut meals to poultry (Letter). Veterinary Record 73(29): 726-727.

Various outbreaks of Turkey X disease in ducklings and pheasant chicks in the U.K. during 1961 are described. Indian groundnut meal in the birds' rations was associated with many of these outbreaks. Results of feeding experiments suggested that certain consignments of Indian groundnut meal contained a toxic principle similar to that found in Brazilian and East African samples in 1960. The toxicity of the Indian groundnut meal samples tested was considerably less than any of the toxic Brazilian or East African groundnut meals examined.

36. Carnaghan, R.B.A., and Alleroft, R. 1962. Groundnut toxicity (Letter). Veterinary Record 74(34): 925-926.

This letter highlights the possible hazards of incorporation of toxic groundnut meal in animal feeds.

37. Carnaghan, R.B.A., and Crawford, M. 1964. Relationship between ingestion of aflatoxin and primary liver cancer. British Veterinary Journal 120: 201-204.

Literature on the occurrence of cancer in livers of domestic and laboratory animals fed on groundnut meals infected with *Aspergillus flavus* is reviewed. The possible role of mycotoxins in production of liver tumours in man in Africa and Asia appears to warrant investigation.

38. Carnaghan, R.B.A., and Sargeant, K. 1961. The toxicity of certain groundnut meals to poultry. Veterinary Record 73: 726- 727.

Day-old ducklings in groups of six were given two turkey diets which had been associated with outbreaks of Turkey "X" disease. The diets had about 6 % Indian groundnut meal. Other groups were given similar amounts of Indian groundnut meal known to be non-toxic. Those given the toxic meal did not grow well and five in each group died within 5 weeks. Gross and microscopical lesions, similar to those produced by toxic Brazilian and East African groundnut meals, were found. Extracts of the Indian meals in amounts equivalent to 100, 200 and 750 g in 5, 5 and 11 days did not kill day-old ducklings, but liver lesions were found post- mortem.

39. Char, N.L., Rao, P., Khan, I., and Sarma, D.R. 1982. An outbreak of aflatoxicosis in poultry. Poultry Adviser, Bangalore, India 15(3): 57-58.

Heavy mortality in chicks in Chittoor district of Andhra Pradesh State, India, due to aflatoxicosis is reported. Groundnut cake was implicated in the aflatoxicosis; groundnut cake was contaminated with aflatoxin at a level of 3590 ug kg'.

40. Chen, C, Pearson, A.M., Coleman, T.H., Gray, J.I., and Wolzak, A.M. 1985. Broiler aflatoxicosis with recovery after replacement of the contaminated diet. British Poultry Science 26(1): 65-71.

Broiler chickens were fed a diet containing 2057 and 1323 *ug* kg ' feed of aflatoxins B, and B,, respectively, for 35 days. Effects of aflatoxins on growth, feed consumption, efficiency of feed use and manifestations of aflatoxicosis were compared with control birds at the end of the feeding trial and at 1, 2, 4, 8 and 16 days after replacing the contaminated feed. No differences in feed consumption were observed between the controls and the aflatoxin-fed chickens, but efficiency of feed use was decreased from 2.2 for the controls to 2.4 g feed g ' gain for the group fed aflatoxins. Aflatoxins caused depressed growth and enlargement of the kidney, liver, heart and gall bladder. Haemorrhagic spots were present on the surface of the muscles and some of the livers. Most livers from aflatoxin-fed birds were pale and infiltrated with lipid. After withdrawal of the feed containing aflatoxins, all apparent gross lesions of aflatoxicosis disappeared, with no evidence of any lesions 8 days alter removal of the contaminated diet.

41. Choudary, C. 1986. An outbreak of "fatty liver syndrome" in commercial layer farms. Poultry Adviser 19(7): 59-60.

Egg production dropped from 85 to 40 % during an outbreak of aflatoxicosis in poultry during October 1985 in and around Warangal, Andhra Pradesh, India. Clinical symptoms included loss of appetite, unthriftiness, pale combs and lameness. Post-mortem examination of dead birds revealed liver lesions of varying severity in all birds examined. Aflatoxin (600 ug kg^{1}) was detected in feed samples. Histopathological examination of liver tissues revealed fatty changes leading to total necrosis and diffuse haemorrhages. No mortality was observed after the feed was changed and egg production increased gradually.

42. Choudary, C, and Rao, M.R.K.M. 1982. An outbreak of aflatoxicosis in commercial poultry farms. Poultry Adviser, Bangalore, India 16(6): 75-76.

Cyanotic combs, loss of appetite and listlessness, with a fall in egg production from 80 to 20 % and 100 % mortality occurred in poultry farms in and around Chittoor (Andhra Pradesh). India. Post-mortem examination revealed hydropericardium, ascites and large, friable haemorrhagic livers, some of which had ruptured. Aflatoxins (1400-3600 ug kg') were found in samples of maize and groundnut cake fed to the birds during the outbreak. The crops used for the feed had been harvested in heavy rain.

43. Christopher, J., Rao, P.R., Narayana, J.V., and Sastry, G.A. 1968. Neoplasma of ducks in Andhra Pradesh, (ii) A report of four intra-hepatic tumours. Indian Veterinary Journal 45: 7-9.

Hepatomas in four ducks, two involving the parenchymatous cells and two the bile-duct epithelium, are reported.

44. Clegg, F.G., and Bryson, H. 1962. An outbreak of poisoning in store cattle attributed to Brazilian groundnut meal. Veterinary Record 74: 992-994.

All 16 of a herd of fattening bullocks and heifers from 18 to 22 months of age lost condition when they were given 2 lb Brazilian groundnut meal daily. They ate the meal unwillingly. After 6 weeks 4 were killed, and liver damage typical of groundnut poisoning was found. Even after 6 months without the meal the remaining cattle did not grow well. Liver damage was found in all animals. An extract of the meal produced typical damage in livers of ducklings and guinea pigs.

45. Cole, R.J. 1986. Etiology of turkey "X" disease in retrospect : A case for the involvement of cyclopiazonic acid. Mycotoxin Research 2 : 3-7.

The etiology of the classical turkey "X" disease syndrome is reappraised based on original reports in conjunction with current information. The clinical signs described in those original reports cannot be totally explained as being typical for aflatoxicosis. The unexplained effects of the disease can be resolved by the proposed presence of the mycotoxin cyclopiazonic acid which is frequently produced by *Aspergillus flavus* along with aflatoxins.

46. Commercial Research Group. 1964. A summary of recent pig and cattle experiments with toxic groundnut meal. Veterinary Record 76: 498-501.

Experiments were conducted to determine the precise levels of'aflatoxin that would produce adverse effects in pigs and cattle in long- and short-term feeding trials. Five groups of pigs and 2 groups of cattle were used in feeding experiments. Feeds were designed to contain equal amounts of groundnut meal but various proportions of control and toxic meal. Acute toxicity did not occur when moderate levels of groundnut meals containing substantial levels of aflatoxin were given to growing and finishing pigs and cattle. Unthriftiness was noted only in pigs. Dietary aflatoxin B, concentrations up to 140 ug kg' were tolerated by growing pigs, and up to 690 ug kg' B, by pigs in the finishing stage, without significant effects on performance or anything exceeding minimal microscopic liver changes. Moderate levels did not appear to affect performance or induce significant liver damage in store or fattening cattle. Post-mortem examination of carcases, livers and kidneys showed no gross macroscopic changes attributable to toxic groundnut meal in either calves or store cattle. Microscopic liver lesions were present in calves which received 220 and 440 ug kg' aflatoxin B,, the incidence of lesions increasing with the increase in toxic groundnut meal. Two of 8 store cattle given the highest level of toxic meal (660 ug kg' aflatoxin B,) also showed liver lesions.

47. de Iongh, H., Beerthuis, R.K., Vles, R.O., Barrett, C.B., and Ord, W.O. 1962. Investigation of the factor in groundnut meal responsible for "turkey X disease". Biochimica et Biophysica Acta 65 : 548-551.

Toxin-containing extracts of either toxic groundnut meals or *Aspergillus flavus* cultures were resolved by thin-layer chromatography into several zones which were fluorescent when viewed in ultraviolet light. The separated fluorescent materials were administered to ducklings. The fraction B, was toxic to ducklings. The combined fractions (B, and B_2) showed greater toxicity than B, alone, indicating some toxicity also due to B_2. The B, fraction from mold cultures and B, from extracts of toxic groundnut meals had the same R, values, and identical ultraviolet-absorption spectra. It is concluded that the extracts from cultures of *A. flavus* contained at least two substances toxic to ducklings.

48. de Iongh, H., Vies, R.O., and Van Pelt, J.G. 1964. Milk of mammals fed aflatoxin-containing diet. Nature 202: 466-467.

Thin-layer chromatography of the toxic milk from cows fed on groundnut meal containing aflatoxin revealed a violet-fluorescent spot with an R_f similar to that of a minor component of an extract *of Aspergillus flavus.* By chromatography on silica gel columns a fraction which contained this material and induced proliferation of bile duct in ducklings was obtained. Studies showed that the lactating rat could convert aflatoxin B, into the 'milk toxin'.

49. Department of Agriculture for Northern Ireland. 1982. Annual Report on research and technical work, 1980. Belfast, Northern Ireland, U.K., pp. 300.

The Veterinary Research Laboratories report (pages 201-202) an outbreak of aflatoxicosis among 60 bucket fed calves which had been weaned onto a farm-mixed concentrate ration containing groundnut meal imported from the Gambia. Three died after showing nervous signs, blindness and constipation for 4-10 days, while others were dull and unthrifty. Autopsies revealed liver cirrhosis. Analysis revealed high levels of aflatoxins B_1, B_2, G_1 and G_2 in the groundnut meal. This is the first reported outbreak of the disease in the UK since the mid-1960s.

50. Derzsy, I)., Meszaros, J., Prokopovitsch, L., and Toth-Baranyi, 1. 1962. Virus hepatitis and toxic liver damage in ducks.. A kacsak virusos es toxikus majgyulladasa. Magyar Allatorvosok Lapja 17(2): 49-53.

In 1961 two diseases, virus hepatitis and toxic liver damage caused by feeding groundnut meal caused considerable losses of ducks. The preventive inoculation of ducklings on infected premises with hyperimmune serum completely prevented losses caused by virus hepatitis. Food mashes containing groundnut meal imported from Brazil, Africa and India caused heavy losses among young ducks but intoxications of the same origin occurred also among young chickens and turkeys. In young ducklings such a meal of high toxicity caused an acute liver degeneration, but usually the condition was of a subacute or chronic character. Pathologically an inclination to regeneration was characteristic for the condition, but in more adult ducks cirrhotic livers were often encountered. The only possibility of the control of this condition is to stop the feeding of the toxic groundnut meal immediately after loss of appetite was observed. Loss of appetite may usually be observed in such cases several days before the first deaths occurred.

51. Doerr, G.T., **Huff,** W.E., Wabeck, C.J., Chaloupka, (G.W., May, J.D., **and** Merkley, J.W. 1983. Effects of low level chronic aflatoxicosis in broiler chickens. Poultry Science 62: 1971-1977.

Studies were conducted to determine if low level exposure to aflatoxin from day-old through market age would induce detectable changes in performance, yield, or carcass grade of broiler chickens at processing. In Trial 1, the treatments were control, 0.75, 0.225, and 0.675 ug aflatoxin g ' of feed, and in Trial 2 control, 0.3, 0.9, and 2.7 ug toxin g' feed. All aflatoxin dose levels in Trial 1 significantly ($P < 0.05$) decreased live, dressed, and chilled eviscerated weight, whereas only 2.7 |ig g ' significantly ($P < 0.05$) decreased live and dressed weight in Trial 2, with chilled eviscerated weight being significantly ($P < 0.05$) decreased at 0.3 and 2.7 ug g^{-1} aflatoxin in Trial 2. Parts weights and dimension measurements reflected the aflatoxin-induced decrease in dressed weight. Breast yield (%) was significantly ($P < 0.05$) decreased by aflatoxin while back, wing, drum, and high yields were significantly increased by aflatoxin. No effect of aflatoxin was seen on the incidence of crooked keel, feather follicle infection, breast blisters, or conformation. A hypocarotenoidemia and hepatic hyperlipemia were clearly a result of chronic

aflatoxicosis in these broiler chickens. These data demonstrate that the toxicity of aflatoxin is dependent on the environment in which broiler chickens are exposed. In general, reduced growth, poor pigmentation and fatty livers can result from chronic low level aflatoxicosis.

52. Edds, G.T., Nair, K.P.C., and Simpson, C.F. 1973. Effect of aflatoxin B, on resistance in poultry against cecal coccidiosis and Marek's disease. American Journal of Veterinary Research 34: 819-826.

Previous exposure to aflatoxin B, at 0.2 mg kg' commercial feed increased susceptibility to and mortality from cecal coccidiosis in chickens but did not interfere with the protection afforded by a coccidiostat. Chickens vaccinated against Marek's disease (MD) and exposed to aflatoxin B, seemed more resistant against challenge exposure to the MD virus than did nonvaccinated control chickens. Vaccinated and nonvaccinated groups of chickens given aflatoxin B, and subsequently exposed to cecal coccidiosis were more susceptible to challenge inoculation with MD virus than were similar groups of chickens not given aflatoxin, as judged from the severity of lesions observed.

53. Ferrando, R., Palisse-Roussel, M., and Jacquot, L. 1984. [Relay toxicity of aflatoxin M, in dried milk. A medium term study with ducklings.). Toxicite de relais de l'aflatoxine M, de la poudre de lait. Etude a moyen terme sur le Cancton. Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences, III. Sciences de la Vie 298(13): 355-358.

Three cows were given for 6 weeks a ration containing groundnut cake (mean aflatoxin B, content 1710 ug kg') and supplying daily 6840 u*g* aflatoxin B,. Milk contained afltoxin M, as 6-12 % of aflatoxin ingested. Dried milk given as 30 % of diet to ducklings had no toxic effect in 69 days whereas diets with aflatoxin B, 80, 240 and 280 ug kg'^{1} supplied by groundnut cake produced liver lesions in 41, 41 and 28 days, respectively, and caused weight loss.

54. Fremy, J.M., Gautier, J.P., Herrv, M.P., Terrier, C, and Calet, C. 1988. Effects of ammoniation on the 'carry-over' of allatoxins into bovine milk. Food Additives and Contaminants 5(1): 39-44.

Lactating cows were led a diet containing aflatoxin B,-contaminated groundnut cakes, and then on cakes treated with ammonia gas. Between the two periods the diet contained noncontaminated soyabean meals. With the nontreated groundnut meal containing aflatoxin B, at 1100 ug kg', total excretion of aflatoxin M, was 2.6 % of total ingested aflatoxin B,. With the treated groundnut meal, aflatoxin M, content of milk was below 0.1 ug L^{1}. In a second experiment 50 lactating cows were fed for 16 montlis on mixed feeds containing 30 % ammoniated groundnut cake containing aflatoxin B, less than 10 ug kg'. Aflatoxin M, residue in milk was not above 0.1 ug L '.

55. Gardiner, M.R., and Oldroyd, B. 1965. Avian aflatoxicosis. Australian Veterinary Journal 41: 272-276.

In 1964 in Western Australia, aflatoxicosis was diagnosed in broiler flocks fed on rations containing 14 % groundnut meal. Post-mortem examination revealed very pale, sometimes almost white livers and kidneys which were firmer than normal. Aflatoxin B, content of three groundnut meal samples was assayed at 2800, 2200 and 2500 ug kg'. The pathology in ducklings, chickens and laying hens fed the original ration, or synthetic rations with added groundnut meal, is described. In chickens after 21 days feeding on the toxic ration, pale areas were noticed in skeletal muscle, which were seen to be large areas of necrosis with a diffuse increase in sarcolemmal nuclei. Toxigenic strains *of Aspergillus flavus* were isolated from the aflatoxin-contaminated samples of groundnut meal.

56. Gibson, W.W.C. 1961. Turkey "X" disease and the labelling of poultry foods. Veterinary Record 73: 150-151.

Outbreaks of Turkey "X" disease have not always been associated with the inclusion of groundnut meal in the diet. It is not considered that listing of ingredients of proprietary foods would serve any practical purpose.

57. Gibson, W.W.C. 1962. Toxicity of groundnut meal. Veterinary Record 74(3): 99-100.

The danger of implicating groundnut meal in all otherwise unexplained liver damage is stressed. Three ducklings sent for examination were all reported as having signs of groundnut poisoning; 1 of them had received no groundnut meal.

58. Gibson, E.A., and Harris, A.H. 1961. Disease of turkey poults (Letter). Veterinary Record 73: 150.

It is considered that there is sufficient evidence that recent heavy losses in flocks of turkeys in the United Kingdom were caused by poisoning by Brazilian groundnut meal.

59. Gopal, T., Zaki, S., Narayanaswamy, M., and Premlata, S. 1968. Aflatoxicosis in dairy cattle. Indian Veterinary Journal 45(9): 707-712.

A natural outbreak of aflatoxicosis in dairy cattle is reported. The condition was tentatively diagnosed as aflatoxicosis becuase of histopathological changes in the liver, and was subsequently confirmed by the presence of aflatoxin in the groundnut

cake fed to the dairy cattle. Feeding extracts of the feed samples to day-old ducklings induced characteristic lesions of aflatoxicosis.

60. Gopal, T., Zaki, S., Narayanaswamy, ML, and Premlata, S. 1969. Aflatoxicosis in fowls. Indian Veterinary Journal 46: 348-349.

Occurrence of aflatoxicosis in poultry in Mysore State, India, is reported. The disease was first recognised at the Government Poultry Breeding Unit, Hebbal, Bangalore in 1966 wherein 2219 chicks died in one week. Subsequently, several sporadic incidences were found in various poultry farms in the State. The disease was predominent in younger stocks, possibly due to the increased percentage of protein in the form of toxic groundnut cake.

61. Gopalan, C. 1966. Studies on aflatoxin. Nutrition Document : Aflatoxin/19. P.A.G. (WHO/FAO/UNICEF) August 1966 Meeting - Geneva. 7 pp.

Research on aflatoxins carried out at the National Research Laboratories, Hyderabad (India), is presented. Aflatoxicosis was found in buffaloes and ducks at government farms near Hyderabad. Aflatoxin contamination was found in the livestock feeds. Biological effects of aflatoxins in ducklings and rats are described. Of 60 varieties of groundnut screened for resistance to aflatoxin production by a toxigenic strain of *Aspergillus flavus,* one variety "U.S. 26" did not support aflatoxin production.

62. Gray, W.V. 1961. Groundnut toxicity. Veterinary Record 73: 865.

Deaths are reported in calves up to 3 months old which had been given Brazilian groundnut meal in the feed from 3 weeks of age. At first a respiratory disease and lead poisoning was suspected. Several calves died up to a week after withdrawal of groundnut meal from the feed; one calf responded to treatment with vitamin A. The same meal had been given to the dairy herd, without any apparent ill effect.

63. Hart, L. 1965. Avian aflatoxicosis. Australian Veterinary Journal 41: 395-396.

In an outbreak of poisoning in turkeys the feed had 5 % of groundnut meal. The groundnut meal had more than 2000 ug kg' aflatoxin B, estimated by thin-layer chromatography, and water extracts were poisonous to ducklings. Other samples of groundnut meal imported into Australia had 1600 to 2000 ug kg' aflatoxin and a local product had 2700 to 3300 ug kg ' aflatoxin.

64. Holding. A.S. 1964. Veterinary Bulletin 34: 327.

Occurrence of aflatoxicosis in a dog is reported. Postmortem examination of a dog **that** died with acute haemorrhagic gastroenteritis, fever, and jaundice showed a friable, bright yellow liver. Aflatoxin was found in the food, which contained groundnut meal.

65. Hornby, R.B., Miller, J.C., and Dabell, J.S. 1962. Toxicity of groundnut meal. Veterinary Record 74(1): 52.

Two cases **are reported of poisoning** of **pigs, the first in 2 store pigs of a group of** 6, and the second in 2 sows which refused to eat but otherwise **appeared normal.** Groundnut meal toxicity was suspected, and lesions in the liver and other organs and tissues of the younger pigs were similar to those described by Loosmore and Harding (1961). The difficulty of diagnosing groundnut poisoning in pigs is stressed.

66. Horrocks, D., Burt, A.W.A., Thomas, D.C., **and Lancaster,** M.C. 1965. Effects of groundnut meal containing aflatoxin in cattle diets. Animal Production 7: 253-261.

Ayrshire bull calves in 3 groups of 8 were given weaning meal with no toxic-groundnut meal or with 4 or 8 % of a toxic meal with 5000 to 6000 pg kg' aflatoxin B_1; all diets had a total of 16 % groundnut meal, the balance being made up with non-toxic meal with 20 to 30 pg kg ' aflatoxin B_1. The diets were given to appetite as meal mixtures to maximum 6 lb per day. Calves got the diets until they were 116 days old when they were killed. For 32 Aberdeen-Angus crossbred bullocks and heifers about 2 years old, the meal mixtures had 0, 4, 8 or 12 % toxic groundnut meal. Rations were 9 lb meal daily in the first week rising to 14 lb from 8 to 20 weeks and all the cattle had access to silage and hay. In the diets of calves 4 or 8 % toxic meal significantly depressed liveweight gain and intake of feed for 3 months; feed utilisation was impaired. As 4 to 12 % of the diet toxic meal had no effect on the performance of fattening cattle. There were enlarged hepatic cell nuclei in the calves, and the incidence of these slight lesions was related to intake of toxic meal; incidence of similar lesions was small in fattening cattle given 12 % toxic-meal.

67. Hurter, L.R. 1966. Some field observations on aflatoxicosis in the Potgietersus Veterinary area. Journal of South African Veterinary and Medical Association 37: 77-78.

In 24 samples of poisonous groundnuts aflatoxin was present at levels of 2000 to 300000 pg kg' (mean 66000 pg kg '). *Aspergillus flavus, A. awamori* and 4 named species of *l'enicillium* were isolated from the samples. Deaths of 10 pigs, 3 cattle, 2 goats and a sheep were investigated. In 8 samples of groundnut hay there was none to 16000 pg kg ' of aflatoxin (mean 4000 pg kg'). Only 3 samples had over 2000 pg kg'. It was thought unlikely that normal groundnut hay would poison **animals.**

68. Jones, M.G.S., and Ewart, J.M. 1979. Effects on milk production associated with consumption of decorticated extracted groundnut meal contaminated with aflatoxin. Veterinary Record 105(21): 492-493.

Two samples of groundnut meal were collected from a dairy farm where milk production had fallen to 76 % of normal, and many of the cows were in poor condition, with reduced appetites and scouring. Anatoxins were demonstrated at a level which could give about 50 ng kg-1 (dry matter) in the complete diet. In 2 samples of groundnut meal the ailatoxin B, levels were 750 and 620, of B_2 40 and 30, of G, 60 and 50, and of G: up to 10 ng kg-1. Within 3-4 days of withdrawal of the meal, scouring ceased and appetite improved. Milk production rose again, though not to its predicted level.

69. Kettercr, P.J., Blaney, B.J., Moore, CJ., McInnes, I.S., and Cook, P.W. 1982. Field cases of aflatoxicosis in pigs. Australian Veterinary Journal 59: 113-117.

Five cases of aflatoxicosis in pigs in Southern Queensland arc described. One peracute case where aflatoxin concentration of up to 5000 ng aflatoxin B, kg' was demonstrated in stomach contents was presumed to be caused by consumption of moldy bread. High levels of toxins were also present in the livers Two cases of acute toxicity were caused by feeding moldy groundnut screenings containing 22000 Mg aflatoxin B, kg"[1]. One case of subacute, and one of chronic toxicity were caused by sorghum grain based rations with lower aflatoxin levels (4640 and 255 (ig kg"[1]). Peracute toxicity caused collapse and deaths within a few hours, acute toxicity caused deaths within 12 h, and subacute toxicity caused deaths after 3 weeks on the toxic rations. Anorexia and ill thrift affecting only growing animals were seen with chronic toxicity. Extensive centrilobular liver necrosis and haemorrhage occurred with peracute toxicity, and in cases of acute poisoning there was hepatic centrilobular cellular infiltration, hepatocyte swelling and bile stasis. With subacute toxicity hepatocyte vacuolation together with bile stasis and bile ductule hyperplasia were seen.

70. Keyl, A.C., and Booth, A.N. 1971. Aflatoxin effects in livestock. Journal of the American Oil Chemists" Society 48: 599-604.

Feeding trials were conducted with swine, beef cattle, dairy cattle and poultry to determine adverse effects, if any, of graded levels of anatoxins in rations. In addition, samples of meat, eggs and milk from these animals were analyzed chemically to determine if aflatoxin was transmitted into these products. In growing-fattening swine, no evidence of toxic effects was observed when the aflatoxin level fed was 233 (ig kg ' or less. In a swine reproduction experiment, no adverse effects were detected in pigs produced from sows led 450 ug kg ' aflatoxin. No toxic effects were observed at levels of 300 ug kg^{1} or lower in cross-bred beef steers fed rations containing aflatoxin for 4.5 months. Using recognized chemical methods, no aflatoxin was detected in meat from swine and cattle fed rations containing 800 and 1000 mg kg^{-1} of aflatoxin, respectively. In dairy cows, weekly intakes of 67 to 200 mg of aflatoxin B, per cow produced 70 to 154 ug kg^{-1} aflatoxin M, in lyophilized milk. Rapid disappearance of aflatoxin M, in the milk took place after withdrawal of aflatoxin from the ration. No adverse effects were discernible in broilers fed from one day to eight weeks of age a ration containing 400 ug kg^{-1} aflatoxin. Lyophilized meat from broilers fed 1600 ug kg^{-1} aflatoxin for eight weeks contained no detectable aflatoxin. Striking differences in aflatoxin susceptibility were observed in 17 different breeds and strains of poultry and game birds fed from two to six weeks of age a ration containing 800 ug kg-1 aflatoxin B,. New Hampshire chicks and turkey poults were highly susceptible to aflatoxin in contrast to the resistance of Barred Rock and Australop chickens and guinea fowls. Hybrid chicks from a New Hampshire-White Leghom cross were highly resistant to aflatoxin. Eggs and meal from White Leghorn hens fed a ration containing 2700 ug kg' aflatoxin contained no detectable aflatoxin.

71. Kishan Rao, D. 1980. *Aflatoxicosis* in and *around* Hyderabad. Poultry Guide 17(4): 29-30.

Aflatoxin toxicity problems in poultry are described. Information on outbreaks of aflatoxicosis in poultry in Hyderabad, India, are presented. Preventive measures for avoiding toxicity problems are suggested.

72. Lewis, G., Markson, L., and Allcroft, R. 1967. The effect of feeding toxic groundnut meal to sheep over a period of five **years.** Veterinary Record 80: 312-314.

A group of 5 Kerry crossbred lambs 3 months old at the start were fed for 5 years on hay and a concentrate with 20 % highly toxic groundnut meal, containing 1750 (ig kg ' aflatoxin during the first 3-5 years, and 1000 (ig kg' since then. Lambs bom were added to the experimental group. A control group, for the first 3 years, received concentrate with fishmeal and maize gluten supplying protein. In the group given aflatoxin, fertility was lower and growth during the first 18 months was also retarded. One lamb was killed after 5 months and others died during the experiment. Damage to the liver was found in only one, and there were no typical signs of groundnut poisoning. In 2 sheep there were nasal chondromata; the rarity of this disorder and the possibility of its being related to the feed are discussed.

73. Loosmore, R.M., and Harding, J.D.J. 1961. A toxic factor in Brazilian groundnut causing liver damage in pigs. Veterinary Record 73: 1362-1364.

High mortality is reported in young pigs in a herd given Brazilian groundnut meal (17.5 % in the starter pellets and 8.75 % in the sow and weaner meal). Pigs of the

same herd transferred to another farm were not affected. Sows lost appetite and tended to subsist on grass. Liver lesions in acute, subacute and chronic types of poisoning are described. There were some similarities to chronic copper poisoning. There was no difference in toxicity between solvent-and expeller-processed meals. Experimental poisoning was also studied. The suspected diet was given to 2 pigs for 4 weeks and 5 others got a meal with 20 % Brazilian groundnut meal. All developed signs of poisoning, with typical acute or subacute liver damage. Pigs given another commercial meal or 20 % Nigerian groundnut meal were not poisoned.

74. Loosmore, R.M., and Markson, L.M. 1961. Poisoning of cattle by Brazilian groundnut meal. Veterinary Record 73: 813-814.

Over 11 instances of calves dying after eating feeds containing groundnut meals are reported. The meals comprised 10 to 15 % of the feeds given from the age of 3 days onwards; it was estimated that from 40 to 85 lb of the meal was eaten in 6 to 16 weeks. Signs of poisoning developed from 6 weeks to 4 months alter the start of feeding. The calves affected were 3 to 9 months old but mainly 4 to 6 months. All test animals which showed clinical signs died, usually within 2 days of severe signs appearing. Clinical signs are described. Typical lesions in the liver were found. On 2 farms concentrates with 15 to 20 % groundnut meal had been given to dairy cows for 3 to 4 months. There were loss of appetite and reduced milk yields, and 3 cows had liver lesions typical of groundnut poisoning. When the meal was withdrawn from the feed there was no further sign of poisoning. One outbreak is reported among store bullocks aged 18 to 22 months, which had received 2 lb of groundnut meal daily for 3 months. In all cases signs were indistinguishable from those of Senecio poisoning. The total dose of the toxic substance seems to be more important than the rate of consumption of the groundnut meal. Susceptibility to poisoning decreases with age,

75. McKenzie, R.A., Blaney, B.J., Connote, M.D.. and Fitzpatrick, L.A. 1981. Acute aflatoxicosis in calves fed peanut hay. Australian Veterinary Journal 57(6): 284-286.

Acute aflatoxicosis was believed to be the cause of death in 12 of 90 Hereford calves, fed groundnut hay during drought in Queensland. They developed jaundice, photosensitization, diarrhoea, anorexia, and depression. Increase in serum levels of enzymes of hepatic origin and bilirubin content were higher than normal. Haemorrhage, hepatocyte damage and bile duct proliferation were seen in groundnut hay-fed dead calves. The groundnut hay contained up to 2230 ug kg ' aflatoxin. **with** most toxin concentrated in the nut-in-shell.

76. Mehrotra, M.L., and Khanna, R.S. 1973. Aflatoxicosis in Angora rabbits. Indian Veterinary Journal 50(7): 620-622.

On a commerical rabbit farm with 7000 rabbits in the Kulu valley in Himachal Pradesh State of India, 4000 rabbits died, and mortality was not prevented by antibiotics. In 15 rabbits examined post-mortem, damage to livers similar to that seen in guineapigs poisoned by aflatoxin was noted. The rabbits were fed on green feed to appetite and a pelleted concentrate, with somewhat high moisture content and including 20 % groundnut meal, which was prepared in bulk and stored before use. Aflatoxin was detected in the feed.

77. Minne, J.A., Adelaar, T.F., Terblanche, M., and Smit, J.D. 1964 Groundnut poisoning due to aflatoxin in slock in South Africa. Journal of the South African Veterinary Medical Association 35: 7-8.

After eating moldy groundnuts, 4 pigs and 2 goats died within 24 hours. Investigations showed that the moldy material contained aflatoxin in high concentration produced by the fungus *Aspergillus flavus.* In various experimental animals, except the rat, it caused acute liver necrosis and generalized haemorrhages.

78. Newberne, P.M. 1965. Carcinogenicity of aflatoxin contaminated peanut meals. Pages 187-208 **in** Mycotoxins in Foodstuffs (Wogan, G.N., ed.). Cambridge, Massachussets : MIT Press.

A high incidence of hepatocellular carcinomas was induced in rats fed aflatoxin-contaminated groundnut meal grown and processed in the United States; the incidence of tumors paralleled the aflatoxin content. Carcinogenicity for rats **was** reduced progressively by extracting the groundnut meal with petroleum solvent (n-hexane), methanol and chloroform. Widespread bile duct hyperplasia and nodular regeneration of the liver was induced in ducklings fed the toxic groundnut meal for 30 days. Ducks held on experiment for 16 months developed hepatic cirrhosis and hepatomas which appeared nonmalignant histologically. Mice fed toxic groundnut meal for 16 months developed bizarre cell forms throughout the liver and a low incidence (15 %) of hepatomas which appeared to be nonmalignant. It is concluded that aflatoxins were the major contributing factor in liver tumors observed in rats fed groundnut meal in the United States.

79. **Patterson, D.S.P., and Roberts, B.A.** 1980. **Aflatoxin B, in dairy** concentrates and other animal feedstuffs. Veterinary Record 107(11): 249-252.

Over 13 years, 740 samples of animal feeds were analyzed, about 500 of which were suspected of causing disease in farm animals. Aflatoxin B, was detected in **only** 13.6 % of the samples. Groundnut meal samples nearly always contained the **toxin.** Where mycotoxicosis was suspected in dairy cattle, 27 % of the samples proved positive, as compared with 9 % of other dairy feed samples.

80. Patterson, D.S.P., Roberts, B.A., Shreeve, B.J., Wrathall, A.E., and Gitter,

M. 1977. Aflatoxin, ochratoxin, and zearalenone in animal feedstuffs : some clinical and experimental observations.

Only 10 % of food samples examined during 1973-1975 contained mycotoxins, viz. aflatoxins B, and G„ ochratoxin A, sterigmatocystin and zearalenone. Aflatoxin B, (and sometimes G,) was present up to 2000 ug kg' in groundnut meal obtained from farms with production disease in cattle. III thrift occurred in calves, and milk production was lower. Ochratoxin A was occasionally found in stored barley and it has been suggested that it may cause foetal resorption in farm animals, but experimental studies in pregnant sows did not confirm this when they were fed ochratoxin A or B. Ochratoxin A accumulated in the body tissues of the dam. Zearalenone has been implicated in the aetiology of sprayleg piglets, but only 1 such case was seen among 63 piglets from 7 gilts fed naturally contaminated wheat. However, when such wheat was fed to laying hens, some newly-hatched chicks suffered leg weakness.

81. Platonow, N. 1965. Investigation of the possibility of the presence of aflatoxin in meat and liver of chickens fed toxic groundnut meal. Veterinary Record 77: 1028.

Groups of 10 White Leghorn chickens (4 weeks old) were fed for 1, 2, 4 and 6 weeks on diets with 30 % toxic groundnut meal, to supply 3100 ug kg' aflatoxin in the diet. There was no residue of aflatoxin or its **fluorescent metabolites** in **liver,** breast or leg meat of chickens killed at any age.

82. Ray, A.C., **Abbitt. B., Cotter, S.R., Murphy, MJ., Reagor, J.(., Robinson, R.M., West, J.E., and Whitford, H.W.** 1986. Bovine abortion and death associated with consumption of aflatoxin-contaminated peanuts. Journal of the American Veterinary Medical Association 188(10): 1187-1188.

When an estimated 10-14 pregnant cows in a 68-cow herd were given moldy groundnuts as a supplementary feed, constituting a large proportion of their diet for 4 days, most of them aborted on or soon after the fifth day. The cows were recumbent and unable to rise; in one, there was a low rectal temperature (99 C) and a tremor of the head. All of the cows that aborted were in the third trimester of pregnancy, and all died within 8 days. Biochemical analyses performed on 3 cows revealed hepatic dysfunction, indicated by high values for lactate dehydrogenase, aspartate transaminase and total bilirubin; there was also evidence of mild dehydration; no fetuses were examined. The groundnuts contianed 77 ug g' of aflatoxin B„ which was also found (5 ng g') when a liver extract of one cow was analyzed for mycotoxins. Cows fed groundnut hay from the same field as the groundnuts were not affected; the nuts had been kept in the open at freezing temperatures. Abortion preceding fatal mycotoxicosis, as occurred in at least 8 of **these cows, is an unusual feature of acute aflatoxicosis,**

83. Richir, C, Toury, J., Martineaud, M., and Dupin, H. 1964. | Fungus intoxications in the rearing of ducklings.J. Observations sur des accidents de toxicoses fungiques survenus dans des elevages de canetons. Nutritio et Dieta 6: 229-233.

Four cases are reported of outbreaks of poisoning in ducklings given commercial feeds with 10 or 9 % groundnut meal. By tests of fluorescence those meals were found to have 4000 and 2000 ug aflatoxin kg^{-1}, but in one outbreak deaths ceased when another meal without groundnut was given. This meal contained 5000 |ig aflatoxin kg^{-1}. The ducklings which died in these outbreaks (more than half of flocks of 400 in 2 of the outbreaks) had lesions in the liver which justified the diagnosis of poisoning by groundnut.

84. Sargeant, K., **and Carnaghan, R.B.A.** 1963. Groundnut toxicity in poultry : Experimental and chemical aspects. British Veterinary Journal 119(4): 178-184.

Work on groundnut toxicity to various animals is reviewed. Procedures for isolation and detection of aflatoxins from toxic groundnut meal and fungal cultures are described.

85. Sargeant, K., O'Kelly, J., Carnaghan, R.B.A., and Allcroft, R. 1961. **The assay** of **a** toxic principle in certain groundnut meals. Veterinary Record 73: 1219-1223.

A method for the fractionation of Brazilian groundnut meal is described. All the toxic material, 0.4 % by weight of the original, was found in the fraction insoluble in methanol and water, extracted with chloroform and separated with petrol and water. A suspension in water was made so that 1 mL was equivalent in toxicity to 40 g meal. Ducklings were killed in under 24 h by 1 mL of that suspension and it was lethal at lower concentrations. The equivalent of 0.5 g meal caused liver damage. The corresponding fraction from Indian groundnut meal was not toxic. Turkey poults were less susceptible than were ducklings and the mortality and lesions were identical to those in field outbreaks of Turkey "X" disease. It was confirmed that the toxic substance is neither a pyrrolizidine alkaloid nor the N-oxide of such an alkaloid. It may be derived from a micro-organism. A similar toxic substance has since been found in samples of groundnuts from India, Uganda and Tanganyika, French West Africa, Nigeria, the Gambia and Ghana.

86. Sargeant, K., Sheridan, A., O'Kelly, J., and Carnaghan, R.B.A. 1961. Toxicity associated with certain samples of groundnuts. Nature 192: 1096-1097.

The toxic extract of a Brazilian groundnut meal was further purified **and a** fluorescent method of identification after chromatographic separation was devised. **The toxic substance was isolated from a** fungus, *Aspergillus flavus.* When the fungus

was grown on sterilized groundnuts and fed to ducklings, it **resulted in typical diver** lesions in ducklings.

87. Sastry, G.A., Narayana, J.V., Rama Rao, P., Christopher, K.J., and **Hill,** K.R. 1965. A report of the groundnut toxicity in Murrah buffaloes in Andhra Pradesh (India). Indian Veterinary Journal 42: 79-82.

A suspected outbreak of groundnut poisoning affecting 24 Murrah buffaloes is described with details of the clinical course, post-mortem lesions and histology of **the** liver. Investigations eliminated poisonous plants, parasites, bacteria or viruses as agents of the disease. The concentrate mixture contained 20 % groundnut cake. This is the first record of groundnut toxicity in India although frequent cases of liver damage in Murrah buffaloes have been recorded in certain areas. Six buffaloes died in this outbreak.

88. Sharma, K.S., Chawla, J.S., and Ichhpoaani, J.S. 1989. Studies on the Improvement in the nutritive value of fungal infested groundnut cake. Indian Journal of Poultry Science 24(3): 179-185.

For 75 days laying hens were given diets containing groundnut cake (GNC). aflatoxin-contaminated GNC (AGNC), or AGNC treated wim 2 % ammonia, 0.1 % gentian violet, 0.5 % propionic acid or defatted with hexanc without or with supplementary copper sulphate or vitamins. Egg production was decreased ($p < 0.05$) with AGNC. Copper sulphate did not improve egg production but vitamin supplementation increased ($p < 0.05$) egg production compared with AGNC alone. Treating AGNC with ammonia, gentian violet or propionic acid improved egg production. Defatting AGNC improved ($p < 0.05$) egg production. Feed intake was not affected by treatment. Average egg weight, feed intake kg^{-1} egg weight, daily protein intake per hen, protein intake per kg egg weight and percent protein efficiency were affected ($p < 0.05$) by treatment. Weight (g 100^{-1} body weight) of liver, heart and pancreas were affected ($p < 0.05$), but weight of spleen was unaffected by treatment. Liver DM was not affected, but protein content of liver decreased ($p < 0.05$) accompanied by the increased lipid content with AGNC.

89. Sharma, U.K., and Singh, N. 1971. Haemorrhagic syndrome in poultry. Indian Veterinary Journal 48(3): 235-238.

A disease of chickens characterized by variable haemorrhages in several organs including liver, heart, Spleen, kidney, intestine, and other organs and degenerative changes of cells is reported. The blood clotting mechanism is probably defective.

90. Sivadas, C.G., Nair, M.K., and Gopinath, C. 1962. Bile duct carcinoma in a hen. Indian Veterinary Journal 39:322-324.

A bile duct carcinoma in a Desi hen is reported. Histopathology of the lesions, particularly of those located in the liver, is described.

91. Smith. K.M. 1960. "Disease" of turkey poults (Letter). Veterinary Record 72(32);652.

Observations on Turkey X disease are presented. Outbreaks of "disease" in turkey poults were associated with commercial ration. Mortality of turkey poults often ceased after a change of feed.

92. Stevens, A.J., Sanders, C.N., Spence, J.B., and Newnham, A.G. 1960. Investigations into "disease" of turkey poults (Letter). Veterinary Record 72(31): 627-628.

Preliminary observations on Turkey X disease are presented. The authors encountered 45 outbreaks of "disease" in turkey poults associated with high mortality. Birds died in good condition after a short illness and mortality rates ranged from 10 to 70 %. Affected poults were usually about 4 weeks old, but birds 12 to 15 weeks old were sometimes involved. The consistent post-mortem findings were engorgement and congestion of the kidneys. Other lesions often present included enteritis, distention of the gizzard by coarse material, haemorrhages or necrotic foci in the liver and, less commonly, haemorrhages on the pancreas, white flecks on the air sacs and generalized oedema. More than one commercial ration was involved, but mortality often ceased after a change of feed.

93. Suliman, H.B., Mohamed, A.F., Awadelsied, N.A., and Shommein, A.M. 1987. Acute mycotoxicosis in sheep : field cases. Veterinary and Human Toxicology 29(3): 241-243.

Inappetance, apathy and neurological signs were seen in a flock of sheep near Khartoum, Sudan, fed on groundnut oilmeal contaminated with aflatoxins (750 µg kg^{-1}). The gross and microscopic lesions were confined to the liver. The biochemical analysis of the serum was consistent with the presence of liver damage. The presence of aflatoxins in the feeds and tissues of dead sheep supports the view that the condition was due to aflatoxin poisoning.

94. Swarbrick, O. 1960. Disease of turkey poults (Letter). Veterinary Recced 72(33):671.

Observations on Turkey X disease are presented. The most striking post-mortem lesions were generalised oedema with large quantities of fluid in the peritoneal cavity, and in most of the birds, around the coronary of band of the heart. Extensive swelling of the kidneys, the surfaces of which were covered with petechial

haemorrhages was very evident. Enteritis of various parts of the alimentary canal was also a prominent feature.

95. Upcott, D.H. 1970. Blood coagulation defects in calves fed on toxic groundnut (aflatoxin). Zentralblatt Vet. Med. 17A: 278- 283.

Two calves less than 3 months old at the start of the experiment were on a diet with 20 % non-toxic Indian groundnut meal, and 4 got 20 % toxic Brazilian groundnut meal without or with 150,000 IU vitamin A intramuscularly on days 1, 28, 56, 84, 112 and 134. Blood samples were examined for activity of coagulation factors with particular attention to the prothrombin complex. The first blood sample was taken 14 days before the diets were given and others were taken at weekly intervals. The calves given toxic meal without vitamin A were killed, with extreme signs of poisoning, after 16 and 26 weeks, respectively, and one calf given vitamin A was killed with extreme signs at 12 weeks. The other calves survived without sign of disorder for 61 weeks. Clotting time, one-and two-stage prothrombin time tests and prothrombin consumption index indicated impairment of the activity of prothrombin, factors VII and X, and possibly IX. There was some evidence that vitamin A reduced these effects.

96. Vaid, J., Dawra, R.U., Sharma, O.P., and Negi, S.S. 1981. Chronic aflatoxicosis in cattle. Veterinary and Human Toxicology 23(6): 436-438.

A chrome disease characterized by loss of condition, anorexia, apathy and intermittent diarrhoea with tenesmus in a herd of dairy cattle in India was attributed to prolonged feeding with a diet containing groundnut cake in which aflatoxins B_2, G[and G_2 were detected. Some animals became comatose and died, lactating animals having the highest mortality. Clinical and post-mortem examination revealed liver damage and ulceration of the abomosum.

97. Wannop, C.C. 1961. Turkey "X" disease. Veterinary Record 73: 310-311.

Cases of Turkey "X" disease have been seen in chickens, ducks and turkeys given compound feeds containing no groundnut meal.

98. Yadgiri, B., and Reddy, E.M. 1976. Aflatoxicosis in Poultry. Poultry Advisor 8: 35-40.

Occurrence of aflatoxicosis in poultry in India is reported. Guidelines for usage of contaminated feeds and for avoiding the problem are given.

99. Yadagiri, B., and Tulpule, P.G. 1974. Allatoxin in buffalo milk. Indian Journal of Dairy Science 27(4): 293-297.

Three consignments (CI-3) of groundnut cake (10 samples in all) were screened for aflatoxin using TLC and fed as 30 % of the diet to Murrah buffaloes. Three out of the 10 samples were found to contain 1-3 mg kg^{-1} allatoxin B,. The remainder contained only traces of aflatoxin. Fifty milk samples from these buffaloes were analysed for aflatoxin M, (metabolite of B,) : 10 samples came from C I , and 20 each from C2 and C3. Four of CI showed traces of toxin. 9 C2 and 14 C3 were heavily contaminated, with concentration, ranging from traces to 4.8 ug aflatoxin M, L^{1} milk

100. Younus, M. 1989. Aflatoxicosis in poultry farming. Pashudhan 4(5): 16, 17.

Aflatoxicosis in poultry farming, extraction of aflatoxins from groundnuts, bioconversion of aflatoxins. symptoms and lesions of toxicosis, and prevention and treatment, is discussed.

101. Zimmermann, W., Jost, M, and Wanner, M. 1982 [Effect of groundnut containing allatoxin in the feed on growth, aminopyrine breath test and some clinical chemical indices in pigs.|. Der Einfluss von aflatoxinhaltiger Erdnuss in der Futterration auf Waehstum, Aminopyrin-Atemtest und einige Klinisch-chemische Parameter des Schweiness. Schweizer Archiv fur Tierheilkunde 124(8): 377-387.

The influence of varying amounts of aflatoxin B, in the groundnut feed (0, 4, 98, 491 ug kg^{1}) on health and performance of pigs was studied. Only the highest allatoxin concentration exerted a clear negative influence on weight gain, on the values of the aminopyrine respiratory test, and on some clinico-chemical indices. There were remarkable individual differences with that concentration. It was sometimes possible to find aflatoxin residues in muscles and organs.

1.2.3 Aflatoxicosis in Man

102. Alpert, M.E., Hutt, M.S.R., and Davidson, C.S. 1969. Primary hepatoma in Uganda : A prospective clinical and epidemiological study of forty-six patients. American Journal of Medicine 46: 794-801.

Forty-six patients with primary hepatoma were studied in Mulago Hospital, Uganda, over a nine month period. This represented 2.1 % of all medical admissions. The age peak was 25 to 45 years and the male to female ratio was 2:1. The predominant clinical picture was abdominal pain and hepatomegaly with a hard, frequently tender mass in the right upper quadrant and rapid clinical deterioration and death within several months. Jaundice, fever, hepatic coma and other signs of active hepatocellular disease were infrequent in contrast to observations in the United States and Europe. An unexplained hypercholesterolemia was noted in

approximately one third of the patients. Bloody ascites occurred in 19 % of the patients and was a helpful diagnostic sign. Acute hemoperitoneum occurred in 15 % of cases. The clinical diagnosis of hepatoma, prior to histologic confirmation, was correct in 86 % of the patients. The disease was found more frequently in the poorer immigrants from Rwanda and Burundi than in the local Baganda tribe. Epidemiological study revealed no significant difference in the medical, nutritional or alcoholic history between the patients, the patients' neighbors or a hospital control series. Although hepatomas from different areas appear to be histologically similar, differences in the clinical and epidemiological features suggest that hepatoma in Uganda differs from the disease described from the United States, possibly due to different causative factors.

103. Alpert, M.E., Hutt, M.S.R., Wogan, G.N., and Davidson, C.S. 1971 Association between aflatoxin content of food and hepatoma frequency in Uganda. Cancer Research 28(1): 253-260.

Aflatoxins were estimated in 480 food samples stored for consumption between harvests and collected from different parts of Uganda in 1966-67. Among these samples, 29.6 % contained detectable amounts of aflatoxins and 3.7 % contained more than 1 ug kg^{-1}. The frequency of aflatoxin contamination was particularly high in provinces with a high incidence of hepatoma, or where cultural and economic factors favored the ingestion of moldy foods.

104. Amla, 1., Murthy, V.S., Jayaraj, A.P., and Parpia, H.A.B. 1974. Aflatoxin and Indian childhood cirrhosis - a review. Journal of Tropical Pediatrics and Environmental Child Health 20(1): 28-33.

Aflatoxin B, was detected in the urine of 7 % of 255 cases of childhood cirrhosis during 1964-1969 and in 25 % of breast milk samples from their mothers. The excretion pattern coincided with that of primates injected with C^{14} labelled aflatoxin. Of 25 samples of parboiled rice collected from the homes of patients with cirrhosis, 2 showed both fungal growth and an aflatoxin B, fluorescent spot on chromatographic examination, and all 25 samples of unrefined groundnut oil showed a similar fluorescent spot. Aflatoxin administered to rats caused muscle fragmentation changes, fibrosis and fatty infiltration characteristic of 56 cases of childhood cirrhosis. Growth retardation was noticed both in 24 cirrhotic children and in the litters of lactating rats fed aflatoxin.

105. Amla, 1., Kamala, C.S., Gopalakrishna, G.S., Jayaraj, A.P., Sreenivasamurthy, V., and Parpia, H.A.B. 1971. Cirrhosis in children from peanut meal contaminated by aflatoxin. American Journal of Clinical Nutrition 24: 609-614.

Children suffering from varying degrees of protein-calorie malnutrition had accidentally consumed aflatoxin-contaminated, low fat. commercially produced groundnut protein flour for periods ranging from 5 days to 4 weeks. The hepatic lesions showed a gradual transition from an increase in central and periportal fat to fibrosis and cirrhosis, which does not usually occur in treated Kwashiorkor. The lesions were identical to those of Indian childhood cirrhosis.

106. Amla, **I., Kumari,** S„ Sreenivasamurthy, V., Jayaraj, A.P., and **Parpia,** H.A.B. 1970. Role of aflatoxin in Indian childhood cirrhosis. Indian Pediatrics 7(5): 262-270.

The results of the detection of aflatoxin and its metabolites in the urine and breast milk feeds of both cirrhotic and normal children are presented, and the implication thereof discussed. The possible role of aflatoxin in the aetiology of Indian childhood cirrhosis is further evaluated.

107. Amla, I., Narayan, J.V., Jayaraj, A.P., Sreenivasamurthy, V., and **Parpia,** H.A.B. 1969. Muscle movement in Indian childhood cirrhosis. Indian Pediatrics 6: 305-312.

Ten cases of Indian childhood cirrhosis were studied for changes in the striated muscle. A comparative study of clinical factors, liver and muscle biopsy revealed histopathologic changes in the striated muscles which showed loss of muscle sanation, fatty infiltration and fibrosis. These changes have a direct correlation with the severity of the disease clinically and the histopathologic changes in the liver.

108. Amla, I., Parpia, H.A.B., and Jayaraj, A.P. 1971. Cirrhosis in children after consumption of aflatoxin-contaminated peanut meal. Journal of Pathology 103(2): XIX.

Children suffering from varying degrees of protein calorie malnutrition were accidentally fed with aflatoxin (300 ug kg^{-1}) - contaminated groundnut protein flour for periods of 10 days to 4 weeks. Liver biopsies showed a gradual transition from an increase in central and periportal fat of the liver, to formation of fatty cysts, to fibrosis and cirrhosis.

109. Anonymous. 1966. Alarm about aflatoxin. Nature 212: 1512.

Alarm was expressed at a recent meeting in Rome of the Food and Agriculture Organization of the United Nations about the amount of aflatoxin in groundnuts and other protein supplements. The maximum concentration of aflatoxin which is permitted was laid down in August 1965 by a joint advisory group from the Food and Agriculture Organization and the World Health Organization which took into

account the urgent need to provide extra protein in some parts of the world, and established a level of 30 ug kg' of foodstuff. Clearly the group would have preferred a lower figure, but they concluded that the danger of malnutrition was greater than the danger that allaloxin would produce liver cancer in man.

110. Anonymous. 1984. Aflatoxins and Kwashiorkor. Lancet 2: **1133-1134.**

The possible relationship between aflatoxins and kwashiorkor is **reviewed. It** is concluded that aflatoxins are not primarily responsible for kwashiorkor, but malnourished children may show increased susceptibility to the toxic effects of aflatoxins.

111. Anukarahanonta, T., Chudhabuddhi, C, Temcharoen, P., and Sukroongreung, S. 1984. Pages **339-347 in** Toxigenic fungi - their toxins and health hazard (Kurata. H., and Ueno, Y., eds.).

Data on quality of marketed food in Thailand are reviewed. Groundnut and groundnut products are most frequently contaminated with aflatoxins. A study of chronological and geographical data since 1967 has revealed close correlation between liver cancer incidence and the amount of aflatoxin consumed.

112. Apeagyel, F., Lamplugh, S.M., Hendrickse, R.G., Affram, K., and Lucas, S. 1986. Aflatoxins in the livers of children with Kwashiorkor in Ghana. Tropical and Geographical Medicine 38(3): 273-276.

Aflatoxin **B,** (62 **to 4409 pg g'**) was **detected in** the **livers of 20 children in** Ghana who had died from kwashiorkor. Aflatoxicol (12-99 pg g^{1}) was detected in livers of 2 children.

113. Autrup, H., Seremet, T., Wakhisi, J., and Wasuma, A. 1987 Aflatoxin exposure measured by urinary excretion of aflatoxin-B,-guanine adduct and hepatitis B virus infection in areas with different liver cancer incidence in Kenya. Cancer Research 47: 3430-3433.

Two major etiological agents, hepatitis B virus and aflatoxin B,, are considered to be involved in the induction of liver cancer in Africa. A study was conducted in **various** parts of Kenya with different liver cancer incidence in order to establish the **rate** of exposure to aflatoxin and the prevalence of hepatitis infections. Of all tested individuals, 12.6 % were positive for aflatoxin exposure as indicated by the urinary excretion of aflatoxin B,-guanine. Assuming no annual and seasonal variation, a regional variation in the exposure was observed. The highest rate of aflatoxin exposure was found in Western Highlands and Central Province. The incidence of **hepatitis infection nationwide** as measured **by the** presence of the surface **antigen** was 10.6 %, but a wide regional variation was observed. A multiplicative **and** additive regression analysis to investigate if hepatitis and aflatoxin exposure **had** a synergetic effect in the induction of liver cancer was negative. However, a **moderate** degree of correlation between the exposure to allaloxin and liver cancer was observed when the study was limited to certain ethnic groups. The study gives additional support to the hypothesis that aflatoxin is a human liver carcinogen.

114. Baquetc, E.F., and Freire, M.J. 1989. Present status **and** perspectives **of** aflatoxin research in Mozambique. Pages 93-94 in Aflatoxin contamination of groundnut : proceedings of me International Workshop. 6-9 Oct 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

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 Investigation Veterinaria (INIV) and the Laboratorio Nacional para la Higiene de Agua y Alimentos (LNHAA) are involved in the analysis of food products, for both animals and humans. In 1981, 17 food products, comprising a total of 313 samples were tested and 16 samples were contaminated with aflatoxin B,, 10 with B_2, 4 with G,, and 3 with *G,*. 87-100 % of the groundnut, beer, rice, and maize samples tested were contaminated. The aflatoxin levels in the groundnut samples ranged from 3 to 5500 ug kg', aflatoxin B, being the main contaminant. An analysis program is investigating the possible correlation between consumption of contaminated food and the possible presence of aflatoxin M, in human breast milk. The possibility of further work involving the INIV, LNHAA, and the Faculdade de Agronomia, Universidade Edurado Mondlane, Groundnut Improvement Project is being studied to include an agronomic component and formulate practical recommendations for small farmers and traders.

115. Baxter, C.S., Wey, H.E., and Burg, W.R. 1981. A prospective analysis of the potential risk associated with inhalation of aflatoxin-contaminated grain dusts. Food and Cosmetics Toxicology 19 : 765-769.

In the USA aflatoxin contamination of certain agricultural crops such as groundnut, corn and cottonseed continues to pose a problem, especially in the south-eastern slates where climatic conditions are conducive to growth of aflatoxin-producing fungi. Analysis of airborne dust samples generated from contaminated corn in an agricultural setting has revealed the presence of respirable particles containing aflatoxins. Since aflatoxins are among the most potent of carcinogens, exposure by any route poses a potential hazard. Unfortunately, an adequate assessment of the health hazard resulting from inhalation exposure to aflaloxin- contaminated grain **dust is currently impossible due to the almost total lack of data regarding this route**

of exposure. However, a prospective analysis of the potential risk has been performed using information concerning (1) the absorption and carcinogenic effects of another potent carcinogen, benzo|aJpyrene, when absorbed onto respirable particles, (2) the metabolic capability of cultured human bronchus towards aflatoxins, (3) the irritant nature of respired organic dusts and (4) potential modifying factors known to influence carcinogenic outcome. Suggestions are given regarding the types of information that arc needed in order to adequately assess the risk associated with inhalation of aflatoxin-contaminated grain dust.

116. Beasley, R.P. 1982. Hepatitis B virus as the etiologic agent in hepatocellular carcinoma - Epidemiologic considerations. Hepatology 2(2): 21S-26S.

The epidemiologic evidence suggesting that hepatitis B virus (HBV) is an aetiologic agent for primary hepatocellular carcinoma (PHC) is very strong. It consists of : (i) a strong geographic correlation between HBsAg prevalence and PHC incidence; (ii) multiple case-control studies showing excess HBsAg positivity in PHC patients; (iii) excess HBsAg positivity among mothers compared with fathers of PHC patients, and (iv) a relative risk of 390 among HBsAg carriers in a large general population prospective study in Taiwan. The risk of HBV resulting in the HBsAg carrier state is inversely related to age. Thus, most PHC cases in Taiwan arise from HBV infections which occurred in infancy and childhood.

117. Beasley, R.P., Hwang, L.Y., Lin, C-C, and Chien, C.S. 1981. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22,707 men in Taiwan. Lancet 2: 1129-1133.

A prospective general population study of 22707 Chinese men in Taiwan showed that the incidence of primary hepatocellular carcinoma (PHC) among carriers of hepatitis B surface antigen (HBsAG) was much higher than among non-carriers (1158/100 000 vs 5/100 000 during 75000 man-years of follow-up). The relative risk was 223. PHC and cirrhosis accounted for 54.3 % of the 105 deaths among HBsAg carriers but accounted for only 1.5 % of the 202 deaths among non-carriers. These findings support the hypothesis that hepatitis B virus has a primary role in the aetiology of PHC.

118. Becroft, D.M.O., **and** Webster, D.R. 1972 Aflatoxins and Reyes disease. British Medical Journal 4: 117.

The possible involvement of aflatoxins in the syndrome of encephalopathy and fatty degeneration of the viscera was investigated. Liver tissues from two children with Reye's syndrome were analysed for aflatoxins. All liver extracts examined contained aflatoxin B,. These results suggest a further search for aflatoxin-like compounds in the syndrome of encephalopathy and fatty degeneration of the viscera.

119. Bessard, G.M., **Soubeyrand, J., Chauvier, C, and Pollet, A.** 1990. Alimentary contamination and hepatic presence of aflatoxins in an individual with first-stage liver cancer in Ivory Coast. Journal of Toxicologic Clinique et Experimentale 10(1): 41-44.

Of 78 groundnut samples analyzed in Ivory Coast, 22 contained > 20 ug $kg^{'}$ aflatoxin B, and 27 contained > 200 ug kg^{1} aflatoxins $B_{„}$ B_2, G, or G_2. At post-mortem, aflatoxins were detected in liver samples from a patient with liver cancer : B, 11.8 ug $kg^{'}$; B, 1.9 u*g* $kg^{"1}$; G_2 0.1 ug $kg^{"1}$.

120. Bhat, R.V. 1989. Risk to human health associated with consumption of groundnuts contaminated with aflatoxins. Pages 19-29 in Aflatoxin contamination of groundnut: proceedings of the International Workshop, 6-9 Oct 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

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. Studies carried out in Thailand, the Philippines, and the USA have indicated that the dietary intake of aflatoxins from groundnuts is lower than that from maize. The aflatoxin regulatory actions taken by the European Economic Community (EEC), Japan, and other developed countries on importing groundnuts and groundnut products have resulted in safeguarding exports rather than minimizing health hazards in the developing countries.

121. Bhat, R.V., Nagarajan, V., and Tulpule, P.G. 1978. Health hazards of mycotoxins in India. New Delhi, India : Indian Council of Medical Research. 58 pp.

This reviews discusses the major mycotoxins found in food and feeds, approaches towards prevention and control of mycotoxins, and mycotoxicoses in animals and humans in India. Regulations for mycotoxins are also described.

122. Brudzynski, A., Pee, W.van, and Kornaszewski, W. 1977. The occurrence of aflatoxin B, in peanuts, corn and dried cassava sold at the local market in

Kinshasa, Zaire; its coincidence with high hepatoma morbidity among **the** population. Zeszyty Problemowe Postepow Nauk Rolniczych 189:113-115.

Of 12 maize samples analyzed, 1 had aflatoxin B, at over 200 ug kg^{-1}, and 33 % of dried cassava samples were contaminated with aflatoxin. Select and low quality groundnuts had 80 and 39 % of samples not contaminated. Of the low quality samples 33 % contained more than 250 ug kg^{-1}. Results are related to local incidence of hepatoma and to the high humidity of the climate.

123. Bulatao-Jayme, J., Almero, E.M., Castro, C.A., Jardeleza, T.R., and Salamat, L.A. 1982. A case-control dietary study of primary liver cancer risk from aflatoxin exposure. International Journal of Epidemiology 11(2): 112-119.

The dietary intakes of 90 confirmed primary liver cancer patients were confirmed against those of 90 age-sex matched controls. The frequency and amounts of food items consumed were calculated into units of aflatoxin load per day using a Philippine table of aflatoxin values of these items. Of the total subjects' aflatoxin load, 51.2 % came from cassava, 20.3 % from maize, 6.8 % from groundnuts and 5.8 % from sweet potato. The mean aflatoxin load per day of patients was found to be 44 % that of the controls. The relative risk of developing primary liver cancer was statistically significant in the following order of rank : cassava, groundnuts, sweet potato, maize, and alcohol. The study demonstrates a strong positive association between the ingestion of increasing levels of aflatoxin and the rising risk of developing primary liver cancer. This effect is synergistically aggravated by alcohol consumption.

124. Bulatao-Jayme, J., Almero, E.M., and Salamat, L. 1976. Epidemiology of primary liver cancer in the Philippines with special consideration of a possible aflatoxin factor. Journal of Philippine Medical Association 52(5/6): 129-150.

For many Filipinos maize forms a large part of the daily diet although regional intake varies widely. Correlations between the amount of aflatoxin in the diet and **the** incidence of primary liver carcinoma (PLC) were highly positive. PLC occurred more frequently among younger groups which suggested that a more intensive and frequent toxic insult reduced induction time. Malnutrition was thought to be a contributing factor in PLC but in this study there was no linear correlation between the intake *of* protein and energy and the occurrence of PLC. Regional intakes of **rice,** beans, cassava, groundnuts and maize were estimated; intakes of beans and cassava were not correlated with regional PLC while rice and groundnuts had an inverse correlation. The regional maize intake was the only food which was positively correlated with regional PLC. Regional intake was related to climate, practices in harvesting, storage and eating habits. In 1973. when there was a shortage of rice, more maize was eaten and there was an increase in PLC mortality in all regions. Residents of the Bocol region had a high intake of coconut oil and a relatively low PLC incidence inspite of a moderate intake of highly contaminated maize, suggesting that some component in coconut oil altered the metabolism of aflatoxin in the liver to make it less carcinogenic.

125. Campbell, T.C., Caedo, J.P.Jr., Bulatao-Jayme, J., Salamat, L., and Engel, R.W. 1970. Aflatoxin M, in human urine. Nature (London) 227: 403-404.

Analyses of 500 samples of food products during 1967-1969 showed that manufactured peanut butter samples were highly contaminated with aflatoxin (mean 500 ug kg^{-1}). The authors then examined urine samples from subjects known to have consumed peanut butler. Urine samples were collected before and during consumption of peanut butter; collections were made for 0 to 24 h before and up to 72 h after consumption began. The minimum daily consumption of aflatoxin B, required to produce detectable levels of M, was 15 ug day^{-1}. Of 35 analytical 24 h periods (after consumption began) for the 14 subjects consuming at least the daily minimum, thirty periods were positive for M, and five were equivocal positive because of uncertain interpretations of streaked chromatographic developments. Milk samples collected from 11 mothers known to have consumed peanut butter (3 consumed 4.2 - 8.4 ug day^{-1} and 8 consumed unknown amounts of aflatoxin) were negative for either B, or M_1. However, 3 samples of milk from these mothers contained aflatoxin M_1. Three of eight urine samples from children who had consumed 11.2 - 15.0 ug peanut butter per day contained aflatoxin. It is suggested that determination of aflatoxin M, in human urine provides a simple method of evaluating aflatoxin ingestion.

126. Campbell, T.C., and Salamat, L. 1971. Aflatoxin ingestion and excretion by humans. Pages 271-280 **in** Mycotoxins in Human Health (Purchase, I.F.H., ed.). London : Macmillan Press: London, pp. 306.

Data are presented on the relationship of aflatoxin contamination of food samples (particularly groundnuts and peanut butter) and its ingestion by man. **The** consumption of peanut butter and maize is possibly linked to high rates of liver cancer in some regions of the Philippines. The presence of aflatoxin M, and the presence of aflatoxin B, in the urine, together with the absence of either B, or M, in faeces in known cases of B, ingestion, indicated considerable metabolism of **the** ingested B_1. These results indicated extensive metabolism by humans and a **lower** order of toxicity of aflatoxin for man.

127. Campbell, T.C., and Stoloff, L. 1974. Implications of mycotoxins for human **health.** Journal of Agriculture and Food Chemistry 22(6): 1006-1015.

The veterinary problem, Turkey X **disease, led to the discovery of aflatoxins and to**

studies which demonstrated that toxins in the absence of visible molds could produce effects (carcinogenesis and orange damage) far removed in time from the cause. Only a few cases of acute mycotoxicoses in humans have been recorded. To shed some light on the risk to man from ingestion of aflatoxins in his food supply, published data on comparative metabolism, primate studies, inadvertant human incidents, and epidemiology are analyzed and presented in common terms to facilitate intercomparison. Although the data raise some questions, they presently provide the best available basis for estimating risk. The greatest attention has been given to aflatoxins, but other mycotoxins potentially capable of causing damage on chronic ingestion have also been considered. Among them are patulin, penicillic acid, trichothecenes. luteoskyrin, and citrinin.

128. Carlborg, F.W. 1979. Cancer mathematical models and aflatoxin. Food and Cosmetics Toxicology 17(2): 159-166.

When cancer is caused in laboratory animals by a socially-valuable chemical or an unavoidable environmental contaminant, natural or otherwise, the risks to man from exposure to the chemical at very much lower doses must be estimated. The choice of a mathematical model is an important step in this assessment. Several available models are evaluated with respect to aflatoxin, one of the few agents with usable results both from experiments with animals and from surveys in man.

129. Caster, W.O., Burton, T.A., Irvin, T.R., and Tanner, M.A. 1986. Dietary aflatoxins, intelligence and school performance in southern Georgia. International Journal for Vitamin and Nutrition Research 56 (3): 291-295.

In a region of southern Georgia known for poor school performance, the mothers of the mentally retarded children had diets that differed from the average in terms of foods, but did not differ in terms of critical nutrients. The intake of large amounts of maize, rice, groundnuts and milk (foods potentially high in aflatoxins) were significantly related to mental retardation of children in one county where there were large amounts of aflatoxins in the food supply. No such relation was found in a county with trivial amounts of aflatoxins in the diet.

130. Chen, J.C., Campbell, T.C., Lee, J., and Peto, R. 1990. A preliminary study of dietary, life style, and mortality characteristics of 65 rural populations in the People's Republic of China. Oxford University Press, New York.

No association between primary liver cancer mortality rates among Chinese populations and aflatoxin exposure was found. The authors concluded that there was a strong association of primary liver cancer mortality with hepatitis B virus infection.

131. Constant, J.L., KochelefT, P., Carteron, B., Perrin, J., Bedere, C, and Kabondo, P. 1984. [Geographical distribution of aflatoxins in human food in Burundi.]. Distribution geographique des aflatoxines duns l'alimentation humaine au Burundi. Science des Aliments 4(2): 305-315.

Aflatoxin contamination of foods was greater in areas of low altitude. Food products most frequently and heavily contaminated included groundnuts (maximum aflatoxin 425 ug kg"'), cassava (325 ug kg') and maize (148 ug kg'). The highest incidence of hepatoma in Burundi coincides with the areas of highest aflatoxin contamination.

132. Coulter, J.B.S., Hendrickse, R.G., Lamplugh, S.M., Macfarlane, S.BJ., Moody, J.B., Omer, M.I.A., Suliman, G.I., and Williams, T.E. 1986. Aflatoxins and kwashiorkor : clinical studies in Sudanese children. Transactions of the Royal Society of Tropical Medicine and Hygiene 80(6): 946-951.

Aflatoxin analysis of blood and urine in 584 Sudanese children is reported. The results in 404 malnourished children comprising 34 kwashiorkor, 111 marasmic kwashiorkor and 152 with marasmus were compared with 180 age-matched controls and correlated with clinical findings. Aflatoxin detection rate and mean cocentration were higher in sera of children with kwashiorkor than the other groups. The difference between the detection rate in kwashiorkor and controls was significant ($p < 0.05$). The aflatoxin detection rate in urine was highest in the marasmic kwashiorkor group and the mean concentration was higher in the marasmic kwashiorkor and marasmic groups than in the kwashiorkor and control groups. There were important differences in the detection of certain aflatoxins between the groups. Aflatoxin was detected in the sera of 16 (11.6 %) kwashiorkor, in 6 (6.1 %) marasmic kwashiorkor, but in none of the controls and only one in marasmus. These differences were highly significant ($p < 0.0001$). The ratio of aflatoxin B, to M, was higher in the sera and urines of kwashiorkors than in controls, suggesting that the normal transformation of aflatoxin B, to M, may be impaired in kwashiorkor with consequent increase in transformation of aflatoxin B, to aflatoxicol. It is concluded that the study presents evidence of differences in the metabolism of aflatoxins in children with kwashiorkor compared with children with other kind of malnutrition and normally nourished children and confirms the association between aflatoxins and kwashiorkor.

133. Coulter, J.B.S., Lamplugh, S.M., Suliman, G.I., Omer, M.I.A., and Hendrickse, R.G. 1984. Aflatoxins in human breast milk. Annals of Tropical Paediatrics 4: 61-66.

Aflatoxins were detected in breast milk samples from mothers in the Sudan.

134. Coulter, J.B.S., Suliman, G.I., Lamplugh, S.M., Mukhtar, B.I., and

Hendrickse, R.G. 1986. Aflatoxins in liver biopsies from Sudanese children. American Journal of Tropical Medicine and Hygiene 35(2): 360-365.

Aflatoxin analysis of 40 percutaneous needle liver biopsies **of 27 children** with protein-energy malnutrition and 13 children with miscellaneous liver disease was performed. Aflatoxins B_1 B_2 and aflatoxicol were detected in 5 of the 16 biopsies from kwashiorkor but in none of 11 biopsies from marasmus or marasmic kwashiorkor. Aflatoxins G_1, G_2 and M_2 were detected in 5 of 12 children with chronic liver disease. A very high concentration of aflatoxicol was found in a breast-fed infant with neonatal hepatitis of unknown aetiology.

135. Dvorackova, I. 1976. Aflatoxin inhalation and alveolar cell carcinoma. British Medical Journal 1: 691.

A chemical **engineer aged 68 worked** for three months on a method of sterilising Brazilian groundnut meal which was contaminated by the mold *Aspergillus flavus.* Three months after finishing this work he became ill with high fever and began to expectorate thick, white sputum. X-ray examination showed cavitation in the **left** lower lobe of the lung. At first the process was considered to be due to tuberculosis, and later to mycotic disease. After 2 months further lesions developed in both **lungs.** The condition of the patient became worse and he died 11 months after the onset of his illness. Necropsy showed enlarged, heavy lungs diffusely infiltrated with firm yellow-white or reddish lesions. Histological examination showed bands of fibrous tissue in the parenchyma. The alveoli were lined with high cylindrical or cubital epithelium with giant multinucleated cells, some containing mucous vacoules in their plasma. The interaveolar septa were well preserved. Mitotic figures were rare. The picture was that of pulmonary adenomatosis. No metastases or tumors in other organs were found. Bacteriological examination was negative. A sample of lung tissue was taken for chemical investigation. Thin-layer chromatography of the extract showed the presence of aflatoxin B_1. A colleague of this patient who had been doing the same work had died three years before of pulmonary adenomatosis, but no chemical investigations were done in his case. It seems that men who inhale toxic mold during their work might **well** be at risk

136. Dvorackova, I., Kusak, V., Vesely, D., Vesela, J., and Nesnidal, P. 1977. Aflatoxin and encephalopathy with fatty degeneration of viscera (Reye). Annales de la Nutrition el de L'Alimentation 31: 977-990.

In the past 5 years the authors observed 27 children (3 days to 8 years) who died on the encephalitic syndrome with fatty degeneration of the viscera (Reye). According to the morphological changes in the liver and to the clinical course, the cases were divided into 3 groups. In the first group there were 20 children who died **within 2-10** days after the first symptoms of the disease appeared. In their livers, diffuse fatty degeneration was found. **In the** second **group there were 3 children who** died within 1-2 months after the acute onset of the disease. In their livers, fibrosis with bile duct proliferation and steatosis were found. In the third group, there **were** 4 children who died within 2-4 months after the first symptoms. Their livers showed cirrhosis. Aflatoxin B, was found in the liver specimens of the children in all 3 groups. The source of intoxication in 5 cases was aflatoxin-contaminated **milk** food. The authors suggest that aflatoxin represents an important factor in **the** aetiology of this syndrome and a high risk for human health.

137. Dvorackova, I., and Pichova, V. 1986. Pulmonary interstitial fibrosis **with** evidence of aflatoxin BI in lung tissue. Journal of Toxicology and Environmental Health 18: 153- 157.

Three cases of pulmonary interstitial fibrosis, two in agricultural workers and **one** in a textile worker, are reported. In lung samples of all three patients the presence of aflatoxin BI was demonstrated by radioimmunoassay. A possible occupational **risk of aflatoxin** exposure via the respiratory tract is suggested.

138. Dvorackova, I., Stora, C, and Ayrand, N. 1981. Evidence for aflatoxin B, in **two** cases of lung cancer in man. Journal of Cancer Clin. Oncol. 100: 221-224.

This paper is a report upon two cases of lung cancer in which evidence for presence of aflatoxin in the cancerous tissue is given.

139. Dvorackova, V., Kusak, D., Vesely, J., and Vesela, P. 1976. Aflatoxin and encephalopathy with fatly degeneration of the viscera (Reye). Page 15 in Abstracts of the Third International I.U.P.A.C. Sponsored Symposium on Mycotoxins in Foodstuffs, 16-18 September 1976. Paris, France.

During five years the authors observed 27 children (aged from 3 days to 8 years) who died with symptoms of the encephalitic syndrome with fatty degeneration of the viscera (Reye). According to the morphological changes in the liver and to the clinical course they divided the cases into three groups. In the first group there were 20 children who died within 2-7 days alter the first symptoms of the disease. In their livers diffuse fatty degeneration was found. In the second group there were three children who died within 1-2 months alter the acute onset of the disease. In their livers, fibrosis with bile duct proliferation and steatosis were found. In the third group there were 4 children who died within 2-4 months after the first symptoms of the disease. Their livers showed cirrhosis. Aflatoxin B, was found in the livers of children in all groups. The source of the intoxification in 3 cases was aflatoxin contaminated milk food.

140. Emerole, CO., Uwaifo, A.O., Thabrew, M.I., and Bababunmi, E.A. 1982.

The presence of aflatoxin and some polycyclic aromatic hydrocarbons in human foods. Cancer Letters 15(2): 123-129.

Total aflatoxin content, measured spectrophotometrically, of crops and spices grown locally was 1600 + 500 ug kg' for manihot flour, 400+100 for yam flour, 700 + 150 for red pepper, 1400 + 400 for millet, 400 + 100 for soybeans. 1120 + 500 for maize. 500 + 150 for black eye beans. 40 ± to for rice and 1700 + 400 for groundnuts. Results are discussed in relation to the relatively high incidence of cancer in Tropical Africa.

141. Enwonwu, CO. 1984. The role of dietary aflatoxin in the genesis of hepatocellular cancer in developing countries. Lancet ii(8409): 956-958.

Impaired activity of the liver microsomal mixed-function oxidase (MFO) system is characteristic of protein malnutrition. It explains the accumulation of aflatoxin B, in livers of kwashiorkor victims, whose staple foods are usually contaminated with the toxin. Dietary rehabilitation of such children with high-protein foods not only increases the activity of the liver MFO system but also stimulates DNA replication.

142. Foy, H., Oilman, T., Kondi, A., and Preston, J.K. 1966. Hepatic injuries in riboflavin and pyridoxine deficient baboons - possible relations to aflatoxin induced hepatic cirrhosis and carcinoma in Africans. Nature 212: 150-153.

The livers of the pyroxine-deprived baboons showed striking changes including the presence of intracellular fat globules, interstitial fibrosis and marked variations in size and number of liver cell nuclei. The similarity of these changes to those seen in the livers of rats given aflatoxin and in non-malignant tissue from the livers of Africans with primary liver cancer carcinoma is pointed out. and their possible significance in relation to the pathogenesis of cirrhosis and primary liver carcinoma in Africans is discussed.

143. Groopman, J.D., Zabra, A., Sheabar, F., Wogan, G.N., Montesano, R., and Wild, C.P. 1990. Molecular dosimetry of aflatoxin B, exposures in a human population with high hepatitis B virus infection. Proceedings of the Annual Meeting of the American Association for Cancer Research 31: 230.

A study in the Gambia was performed to investigate two important risk factors for hepatocellular carcinoma, hepatitis B virus (HBV) and aflatoxin B, (AFB). Dietary intake of aflatoxin B, in 20 individuals was determined during a consecutive 8 day period and excretion of AFB-N7-guanine was measured on days 4-8. In addition, on days 1 and 8, blood samples were collected to measure aflatoxin in serum albumin adducts. The individuals chosen were both chronic HBV carriers and non-carriers. In food samples, the primary source of aflatoxin contamination was groundnut sauce with levels of 2-333 ug kg '. Albumin adducts were determined by two different immunoassays, an EL1SA and an RIA, these values ranged from 1.9-399 fmol mg' protein and 400-1922 fmol mg' protein, respectively. It is suggested that the disparity may be due to different specificities of the antibodies used.

144. Hayes, R.B., Nieuwenhuize, J.P.Van, Raatgever, J.W., and Tenkate, F.J.W. 1984. Aflatoxin exposures in the industrial setting : An epidemiological study of mortality. Food and Chemical Toxicology 22(1): 39-43.

Mortality occurring between 1963 and 1980 in a small cohort (N=71) of Dutch oilpress workers exposed between 1961 and 1969 to aflatoxins primarily via the respiratory route was assessed and compared to that of a similar group of unexposed workers (N=67). For the entire period of study, the observed mortalities of total-cancer and respiratory cancer were higher than expected in the aflatoxin-exposed group. Mortality observed in the comparison group was within the expected range. While two deaths in the exposed group were attributed to non-malignant liver disease, no primary liver tumors were observed. The greatest difference between observed and expected mortality was in the period between 1963 and 1968.

145. Hendrickse, R.G. 1984. The influence of aflatoxins on child health in the tropics with particular reference to kwashiorkor. Transactions of the Royal Society of Tropical Medicine and Hygiene 78 (4): 427-435.

The possible relationship between aflatoxins and kwashiorkor is reviewed. Results of recent work are summarized. Aflatoxins were delected frequently in a variety of commonly eaten foods obtained from local markets in the Sudan. Sixty-eight of 85 samples of raw foods obtained from homes contained aflatoxins. Twenty-six of 57 cooked meals from homes had aflatoxins. Analysis of breast milk samples from 99 Sudanese women showed variable amounts of aflatoxins M, and M_2 in about one third of the samples. In a survey of 469 sera and 468 urine samples from children in the Sudan, aflatoxins were detected more often and at higher concentration in sera from children with kwashiorkor than in other malnourished and other control groups. Aflatoxicol was detected in kwashiorkor (12 %) and marasmic-kwashiorkor (6 %) but not in controls and only once in marasmus. Aflatoxins and aflatoxicol were detected in urine of children in all groups. Aflatoxin B, or aflatoxicol was present in all livers from cases of kwashiorkor in a survey of autopsy liver specimens from children in South Africa, Nigeria and Liberia. Aflatoxicol or aflatoxin M, was found in 5 of 6 cases of marasmic kwashiorkor and no aflatoxin was detected in 3 cases of marasmus.

146. Hendrickse, R.G. 1985. Kwashiorkor : 50 years of myth and mystery. Do aflatoxins provide a clue ? Acta Leidensia 53: 11-30.

The relationship between aflatoxin contamination of foods and kwashiorkor is discussed in this review of the disease.

147. Hendrickse, R.G., Coulter, J.B.S., Lamplugh, S.M., Macfarlanc, S.B.J., Williams, T.E., Omer, M.I.M., and Suliman, G.I. 1982. Anatoxins and Kwashiokor : a study in Sudanese children. British Medical Journal 285(6345): 843-846.

Blood and urine samples from 252 children were investigated for their aflatoxin content by high-performance liquid chromatography (HPLC). Aflatoxins were detected more often and at higher concentrations in sera from children with kwashiorkor than in other malnourished and control groups. Aflatoxicol was detected in the sera of children with kwashiorkor and marasmic kwashiorkor but not in the controls and only once in a marasmic child. These differences were significant. Urinary aflatoxin was most often detected in children with kwashiorkor but the mean concentration was lower than in the other groups. Aflatoxicol was not detected in urine in any group. It is suggested either that children with kwashiorkor have greater exposure to aflatoxins or that their ability to transport or excrete aflatoxins is impaired by the metabolic derangements associated with kwashiorkor. Aflatoxins were detected by HPLC in groundnuts (B, 59666 pg g ', B_2 370, G_2 23), limed groundnuts (B, 3517, G, 2816. G_2 6). chickpeas (B, 876 pg g'), dried okra (G_2 12675 pg g') and peanut butter (B, 26300, B_2 9720, G, 84500 pg g '), obtained from local markets.

148. Hermana. 1973. Studies on aflatoxin in Indonesia : Nutrition Research Institute. : 9 pp.

This report reviews aflatoxin contamination problem in groundnuts and groundnut products in Indonesia. Possible relationship between aflatoxin ingestion and hepatocellular carcinoma in humans is also discussed.

149. Husaini, Pang, R.T.L., Tarwotjo, **I., and** Karyadi, D. 1974. Dietary aflatoxin contents, improving agricultural practices and its possible relation to human hepatocellular carcinoma in Indonesia : Nutrition Research Institute. : 14 pp.

Aflatoxin contents of some Indonesian foods are reviewed. Allatoxin contamination problems in groundnuts and groundnut products are discussed. Possible relationship between dietary aflatoxin and hepatocellular carcinoma in humans is also discussed.

150. Keen, P., and Martin, P. 1971. Is aflatoxin carcinogenic in man ? Tropical Geographical Medicine 23(1): **44-53.**

An attempt was made to correlate the presence of aflatoxin in groundnuts with **the** prevalence of primary liver cancer in Swaziland. Primary liver cancer, which is the commonest malignancy in Swazi males, has an unexpected geographical distribution, mainly in the middleveld and lowveld. The Swazis living in the Southern half of the highveld produced fewer primary liver cancers than the equivalent areas in the northern half. The immigrant Shangaans living in Swaziland have a greater tendency to produce primary liver cancer than have Swazis living in the same environments. Infestation of groundnuts by *A. flavus* is present in all parts of Swaziland but is proportionately more prevalent in the middleveld and lowveld. These areas have higher average temperatures and lower rainfall averages than the highveld. Aflatoxin was found in 40 % of the samples tested, and most of the positive samples came from the middleveld and lowveld. Analysis of the eating habits with regard to groundnuts and of methods of harvesting and storage has shown that in the areas with a higher relative incidence of primary liver cancer the opportunities of ingesting aflatoxin are greater. The circumstantial evidence presented suggests that aflatoxin is probably one of the carcinogenic factors in the causation of primary liver cancer in Swaziland, though this cannot explain the global pattern of primary liver cancer.

151. Korobkin, M, and Williams, E.H. 1968. Hepatoma and groundnuts in **the** West Nile districts of Uganda. Yale Journal of Biology and Medicine 41: 69-78.

Hepatoma seen in 22 men and 3 women at a hospital in the West Nile district of Uganda during 15 years is described. The locations of the patients' villages were plotted on a detailed map, accompanied by maps showing the distribution of groundnut cultivation, population and tribal distribution, and the village distribution of patients with other tumors seen during the same period. The difference in distribution of hepatoma patients compared with all other tumor patients is significant. A statistically significant similarity exists between the distribution of groundnut cultivation and the location of the occurrence of hepatoma, but the exact meaning is obscured by the small number of cases and the population distribution of the district. There is also a lack of fungal cultures.

152. Krishnamachari, K.A.V.R., Bhat, R.V., Nagarajan, V., and Tilak, T.B.G. 1975a. Hepatitis due to aflatoxicosis - An outbreak in Western India. Lancet i: 1061-1063.

Parts of Western India have experienced outbreaks of hepatitis affecting man and dogs and characterized by jaundice, rapidly developing ascites, portal hypertension, and a high mortality- rate. The disease was associated with the consumption of maize contaminated heavily with *Aspergillus flavus.* Analysis of contaminated samples showed that affected people might have consumed between 2000 and 6000 u*g* kg' aflatoxin daily over a period of one month. A specimen of liver obtained at

necropsy showed bileduct proliferation and giant cells. The disease appears to be a result of aflatoxicosis.

153. Krishnamachari, k.A.V.K., Bhat, R.V., Nagarajan, V., and Tilak, T.B.G. 1975b. Investigations into an outbreak of hepatitis in parts of Western India. Indian Journal **of** Medical Research 63: 1036-1049.

An outbreak of a disease characterized by jaundice, rapidly developing ascites and portal hypertension, sometimes ending fatally, occurred in over 200 villages of Banswada and Panchamahals districts of Rajasthan and Gujarat. From the epidemiological characters it appeared to be due to a food toxin. Man and dog were afflicted simultaneously. Chemical and biological tests confirmed the presence of high levels of aflatoxin in maize locally grown and consumed by the afflicted tribal population. An estimated intake of about 2000-6000 ug kg' of the toxin daily for several weeks appeared to have caused the outbreak. Bile duct proliferation, one of the characteristic features of aflatoxin liver injury was found in the autopsy of liver studied. Unseasonal rains and faulty method of storage of maize seem to have been responsible for the outbreak.

154. Krishnamachari, K.A.V.R., Bhat, R.V., Nagarajan, V., and Tilak, T.B.G. 1975c. Aflatoxicosis in humans. Proceedings of the Nutrition Society of India 19: 18-22.

The outbreak of aflatoxicosis in rural populations of many villages in Western States (Rajasthan and Gujarat) of India is reported. The disease was characterized by jaundice, rapidly developing ascites, oedema of the lower limbs, and in a few cases by the development of signs of portal hypertension associated with a high rate of mortality. The disease occurred only among maize eating populations. The disease was ascribed to the consumption of aflatoxin-contaminated maize. This provides direct evidence for incriminating aflatoxin in **the causation** of **acute** liver disease with a high mortality rate.

155. Kshirsagar, V.H., Mansukhani, S.H., and Gadgil, R.K. 1968. Primary carcinoma of the liver. Journal of Pathology and Bacteriology 11: 112-120.

Twenty-four hepatomas were encountered in 6356 autopsies over a period of 17 years. The findings are compared with those cited in the Indian and international literature. Twenty-two (91.83 %) of these hepatomas were associated with cirrhosis. It appeared that more carcinomas of the liver were being encountered in the autopsy room than before.

156. Kuiper-Goodman, T., Kirkpatrick, D.C., and Krewski, D. 1979. The aflatoxin intake of various age groups of Canadians. Toxicology and Applied Pharmacology 48(1 **part 2):** A17.

Between 1974 and **1977 the average level of aflatoxins in peanut** butter was 4 ng g '. The average intake **of** these **foods was** 0.4 **g** kg"' body weight for children up to 10 years and declined progressively thereafter to < 0.1 g kg ' bodyweight.

157. Lamplugh, S.M., and Hendrickse, R.G. 1982. Aflatoxins in the livers of children with kwashiorkor. Annals of Tropical Paediatrics 2: 101-104.

The authors consider possible linkages between aflatoxin ingestion and the occurrence of kwashiorkor in children. Aflatoxins have been identified in livers of children suffering from kwashiorkor. Although suggestive, the data are by no means conclusive.

158. Lamplugh, S.M., and Hendrickse, R.G. 1988 Aflatoxins in breast milk, neonatal cord blood, and serum of pregnant women. British Medical Journal 296: 968.

Aflatoxins were **detected in** 90 **(34** %) of 264 **breast milk samples collected** from Accra, Ghana. Aflatoxins were also detected in 63 (34%) of the 188 Ghanaian cord blood samples. Blood samples from Nigeria showed aflatoxins in 16 (21 %) of 77 maternal samples and 9(12 %) in 78 cord blood samples (including a set of twins). These findings confirm that newborn infants in Africa are frequently exposed to aflatoxins in breast milk.

159. Legator, M.S., **and Withrow,** A. 1964. Aflatoxin : Effect on mitotic division in cultured embryonic lung cells. Journal of the Association of Official Analytical Chemists 47: 1007-1009.

Crude **aflatoxin mixtures** as well as crystallized aflatoxin B, suppressed mitotic division in heteroploid and diploid human embryonic lung cells. This inhibition occurred 4 h after exposure of the cells to the toxin and reached a maximum in 8-12 h. A concentration of 0.01 (ig of the toxin could be detected by this method. A sample of 2 ug of TLC pure aflatoxin B, derived from contaminated groundnuts was submitted for assay. A concentration of 0.03 ug produced 51 % reduction in mitosis.

160. Legator, M.S., Zuffante, S.M., and Harp, A.R. 1965. Aflatoxin : Effect on **cultured** heteroploid human embryonic lung cells. Nature 208: 345-347.

The effect of aflatoxin on cell growth, cell morphology, and the synthesis of DNA was investigated. A heteroploid human embryonic lung cell line, L-132, was used. The cells were cultured in monolayer, using basal medium (Eagle) with Earle's

'BSS' and 10 % calf serum. Aflatoxin was added to the growth medium. Up to the 48th hour after the addition of the toxin, cells exposed to 0.05, 0.1, 0.5 and 1.0 ppm increased in number; the 0.05 ppm concentration differed very little from the control. There was little increase in cell numbers between 48 and 93 h at 0.05, 0.1 and 0.5 ppm. At 1.0 ppm there was slight decrease in cell number. Negligible growth occurred at 5.0 ppm. No differences were found in viability of the cells between control and the cells exposed to the toxin except at the concentration of 5 **ppm.** The cells exhibited many abnormal morphological patterns including vacuolization and an accumulation of cellular debris. A 40 % inhibition of DNA synthesis was produced by 0.1 ppm of aflatoxin and more than 40 % by 1 ppm. The suppression of mitosis, inhibition of DNA synthesis, and formation of giant cells, and the previously reported carcinogenic action of aflatoxin, may indicate that aflatoxin effects biological systems as do known alkylating agents.

161. Linsell, A. 1982. Carcinogenicity of mycotoxins. Pages 3-14 in Environmental Carcinogens, Selected Methods of Analysis. Vol. 5. Some Mycotoxins (Egan, H.. Stoloff, L., Scott, P.. Castegnaro, M, Oneill, I.K., and Bartsch, H., eds.). International Agency for Research on Cancer, Lyon.

Possible carcinogenic effects of mycotoxins in animals and man are reviewed. Aflatoxins are carcinogenic in various animals, producing mainly cancer of the liver, colon and kidney. Epidemiological studies have shown a positive correlation between the average dietary concentrations of aflatoxins in human populations and the incidence of primary liver cancer, but it is far from proven that aflatoxins play a part in the etiology of primary liver cancer. Sterigmatocystin is also carcinogenic in rats. Ochratoxin A has nephrotoxic properties in animals, but these mycotoxins are unlikely to be involved in human cancer.

162. Linsell, C.A. 1976. The mycotoxins and the human health aspect. Page 14 in Abstracts of the Third International I.U.P.A.C. Sponsored Symposium on Mycotoxins in Foodstuffs, 16-18 September, Paris, France.

The role of mycotoxins in human disease is briefly reviewed. The acute effects of **the** ingestion of aflatoxins and the role that long term exposure may play in human disease are examined in detail. The necessity for cooperation between chemists, veterinarians, physicians and mycologists in the elucidation of the role of the mycotoxins in human disease is stressed.

163. Lohiya, G., Nichols, L., Hsieh, D., Lohiya, S., **and Nguyen, H.** 1987. Aflatoxin content of foods served to a population with a high incidence of hepatocellular carcinoma. Hepatology, Baltimore 7(4): 750-752.

A total of 36 samples of foods collected during August 1985, December 1985 and March 1986 and served to mentally retarded clients with a high incidence of hepatocellular carcinoma, were analyzed for aflatoxin. Aflatoxin was not detected (< 5 ug kg^{1}) by thin-layer chromatography in 35 food samples containing groundnuts, maize, wheat or milk. One peanut butter sample contained 20 ug kg' aflatoxin. Aflatoxin content of these foods was at or below the level permitted by the Food and Drug Administration. It is concluded that aflatoxin is probably not responsible for liver disease in this population.

164. Nagindu, A., **Johnson, B.K., Kenya, P.R., Ngira, J.A., Ocheng, D.M.,** Nandwa, **H., Omondi, T.N., Jansen, A.J., Ngare, W., Kaviti, J.N., Gatei, D., and** Siongok, T.A. 1982. Outbreak of acute hepatitis caused by aflatoxin poisoning in Kenya. Lancet 1: 1346-1348.

Between March and early June 1981, 20 patients with hepatitis, 12 of whom **died,** were admitted to three hospitals in the Machakos district of Kenya. Two families, from which 8 of 12 sick members died, were eating maize which contained as much as 12000 ug kg' aflatoxin BI. Liver tissue at necropsy contained up to 89 (ig kg ^{1}. aflatoxin. Probably most or all of the hepatitis cases were caused by acute aflatoxin poisoning.

165. Newberne, P.M. 1973. Chronic aflatoxicosis. Journal of the American Veterinary and Medical Association 163: 1262-1267.

Chronic aflatoxicosis is described and reviewed in depth.

166. Newberne, P.M., and Gross, R.L. 1977. The role of nutrition in aflatoxin injury. Pages 51-65 in Mycotoxins in Human and Animal Health (Rodricks, J.V., Hesseltine, C.W., and Mehlman, M.A., eds). Pathotox Publishers, Inc., Illinois : USA, pp. 807.

This paper comprehensively reviews nutritional factors that influence responses of animals and humans to aflatoxin. The effects of nutrients on the metabolism and disposition of aflatoxins are also discussed.

167. Nizami, F., Nizami, H.M., **and** Ahmad, M. 1986. Urinary excretion of aflatoxin and liver cancer in Karachi. Journal of the Pakistan Medical Association 36(5): 112-114.

Two hundred urine samples from apparently healthy subjects were examined for aflatoxins. Aflatoxin was detected in the urine of 9.5 % of subjects from the lower and 5 % from the upper socioeconomic groups. Liver cancer is also more common in less privileged individuals. Aflatoxin excretion **had** no relationship with the age or sex of the subjects studied.

168. Nizami, H.M., and Zuberi, SJ. 1977. Aflatoxin and liver cancer in Karachi, a preliminary survey. Journal of the Pakistan Medical Association 27(6): 351-352.

Twelve of 28 food samples were contaminated with aflatoxin, viz. rice, broken rice, raw grams, almonds, groundnuts, peanut butter, brown beans, white beans, cardamom black, pistachio, maize flour and raw groundnuts. The consumption of foods containing aflatoxins may account for the incidence of liver cancer in Karachi, Pakistan.

169. Nwokolo, C, **and Okonkwo, P.** 1978. Aflatoxin load of common food in savanna and forest regions in Nigeria. Transactions of the Royal Society of Tropical Medicine and Hygiene 72(4): 329-332.

Aflatoxin was present at a high level in most common foods stored poorly for long periods in Nigeria (viz. groundnuts, dried fish, groundnut oil, guinea corn (sorghum), palm oil, rice, maize, beans, yams, acha. cassava and garri). It may work synergistically with other carcinogens to produce the high incidence of primary liver cancer seen in men under 40 years.

170. Oettle, A.G. 1965. The aetiology of primary carcinoma of the liver in Africa : A critical appraisal of previous ideas with an outline of the mycotoxin hypothesis. South African Medical Journal 39: 817-825.

Earlier hypotheses regarding the cause of liver cancer in Africa fail to explain the epidemiologic pattern of this disease, notably its rarity in Egypt. The hypothesis of mycotoxicosis resulting from spoilage of food by toxic molds fits the distribution better, in that it accounts for the rarity of liver cancer in dry areas, where mold Spoilage is minimal, or in populations that consume a predominantly fresh diet.

171. Parpia, H.A.B., and Sreenivasamurthy, V. 1969. Report on recent studies on aflatoxin. Document 2.17/25. P.A.G. (FAO/WHO/UNICEF), September 1969 Meeting - Geneva.

Detoxification of groundnut flour with hydrogen-peroxide is feasible and effective in destroying aflatoxin. This treatment is now used commercially, particularly in the preparation of milk substitutes containing groundnut protein. A mixture of phosphine and ammonia can be used as a fungicide for treatment of groundnuts pods immediately after harvest for controlling growth of *Aspergillus flavus.* Aqueous ethanol can be used effectively to extract about 90 % of ailatoxin from split groundnuts without removing any significant amounts of fat. A study of excretory metabolites of aflatoxin in rats, guinea-pigs and monkeys showed considerable differences in the excretion of aflatoxin M and B between these species. In an exploratory study of the possible role of aflatoxin in infantile liver cirrhosis about 8 % of the urine samples examined contained 10-50 ugof aflatoxin B in a 24 h sample. The pattern of muscular damage in cirrhotic children was similar to the muscular damage caused by ailatoxin fed to albino rats.

172. Parpia, H.A.B., Sreenivasamurthy, V., Shantha, T., Shankaramurti, A., Srikantia, S., and Amla, I. 1972. Urinary ailatoxin B and effects of preservatives on its detection. American Journal of Clinical Nutrition 25: 13-15.

Suitability of hydrochloric acid, chloroform, methanol, and formalin as urine preservatives for aflatoxin analysis was examined. Both hydrochloric acid and formalin were unsuitable as preservatives. Seven per cent of the urine samples from children contained aflatoxin.

173. Payet, M., Cros, J., Quenum, (., Sankale, M., and Moulanier, M. 1966. |Two observations on children who ate for long period of time flour contaminated with *Aspergillus flavus.].* Deux observations d'enfants ayant consomme de facon prolongee des farines souillees par "Aspergillus flavus". Presse Medicale 74: 649-651.

During the period 1958-1960, the acceptability of various groundnut protein concentrates was studied in several MCH centers of Dakar, Senegal. Among the subjects receiving these groundnut protein concentrates, four infants (aged less than one year) were given groundnut protein concentrates at levels ranging from 70 g to 140 g day ' for ten months. Samples of these concentrates which had been saved for storage studies, were analyzed for their aflatoxin content. A 1958 groundnut protein concentrate contained 500-1000 ug kg^{1} aflatoxin; samples of a 1960 concentrate contained 0 to 100 |ig kg' aflatoxin. Two of these children returned to the hospital for follow-up studies in 1963 and 1965. Clinical and laboratory examinations showed that these children were comparable to other children living in the same environment and who had not been given groundnut protein concentrates. Liver biopsis performed in 1963 and 1965 showed in one case lesions of fibrosis; no signs of primary carcinoma of the liver were found or for that matter no indication of carcinogenic degeneration was present.

174. Pearson, C.A. 1990. Aflatoxins and kwashiorkor. Africa Health 12(3): 23-24.

The relationship between aflatoxin contamination of foods and kwasiorkor is discussed in this brief review of the disease in Africa.

175. Peers, F.G., Bosch, X., Kaldor, J., Linsell, C.A., and Pluumen, M. 1987. Aflatoxin exposure, hepatitis B virus infection and liver cancer in Swaziland. International Journal of Cancer 39: 545-553.

A study was carried out in Swaziland to assess the relationship between aflatoxin exposure, hepatitis B virus (HBV) infection, and the incidence of liver-cell carcinoma, which is the most commonly occurring malignancy among males in Swaziland. Levels of aflatoxin intake were evaluated in dietary samples from households across **the** country, and crop samples taken from representative farms. Prevalence of hepatitis B markers was estimated from the serum of blood donors, **and** liver caner incidence was recorded Ior the years 1979-1983 through a national system of cancer registration. Across 4 broad geographic regions, there was a more than 5-fold variation in the estimated daily intake of aflatoxin ranging from 3.1 to 17.5 ug. The proportion of HBV-exposed individuals was very high (86 % in men), **but** varied relatively little by geographic region; the prevalence of carriers of the surface antigen was 23 % in men, and varied from 21 to 28 %. Liver cancer incidence varied over a 5-fold range, and was strongly associated with estimated levels of aflatoxin. In an analysis involving 10 smaller subregions, aflatoxin exposure emerged as a more important determinant of the variation in liver cancer incidence than the prevalence of hepatitis infection. Aflatoxin estimates from crop samples appeared to be a reasonable surrogate for dietary arrangements. A comparison with dietary aflatoxin levels measured in an earlier survey in Swaziland suggested that programs aimed at reducing contamination levels had had some success.

176. Peers, F.G., Oilman, G.A., and Linsell, C.A. 1976. Dietary aflatoxins and human liver cancer : A study in Swaziland. International Journal of Cancer 17(2): 167-176.

A **study in Swaziland to assess the possible relationship** of aflatoxin contamination and the incidence of primary liver cancer is reported. Aflatoxin ingestion levels were determined in food from plate samples collected over a one year period. A significant correlation between the calculated ingested daily dose and the adult male incidence of primary liver cancer in different parts of Swaziland was established. Samples of foodstuffs other than plate samples also reflected the correlation of aflatoxin contamination and liver cancer. This study extends and amplifies the findings of an earlier study in the Murang's district in Kenya and supports the hypothesis that aflatoxin ingestion is a factor in the genesis of primary liver cancer in Africa.

177. Peers, F.G., and Linsell, C.A. 1973. Dietary aflatoxins and liver cancer - A population based study in Kenya. British Journal of Cancer 27: 473-484.

An association between aflatoxin ingested levels and the liver cancer cases in the Murang'a district of the central province of Kenya is reported.

178. Reddy, D.J., and Rao, S.K. 1962. Primary carcinoma of liver among South Indians. Journal of Indian Medical Association 39: 1-6.

The autopsy incidence of **primary carcinoma of the liver found in the two neighbouring provinces of South India, Andhra Pradesh and Madras** was 1.4 and **1**.6 % respectively. The incidence of cirrhosis of the liver was also high, forming 6.1 % of the necropsy examinations. This observation, further strengthened by **the** coexistence of cirrhosis in nearly 80 % of livers with primary cancer, has naturally suggested a relationship between the two conditions, but a careful histological examination of tissues taken from different parts of the cirrhotic livers failed to show a transition of hyperplastic regenerating cells into malignant ones. These findings, along with the occurrence of carcinomatous growth in non-cirrhotic livers fail to establish a direct relationship between the two conditions. It is postulated that there is yet an unidentified factor which is responsible for initiating malignancy and while it can act as a carcinogen on normal cells occasionally, actively regenerating cells in cirrhotic livers are more prone to malignant transformation. Some aspects of the histological patterns of the tumors arc dealt with and this shows a high percentage of cholangio hepatomas. Further analysis of **the** case records revealed that 80 % of the subjects were Andhras who consume **hot curries.**

179. Robinson, P. 1967. **Infantile** cirrhosis of **the liver in India.** With special **reference to probable aflatoxin etiology. Clinical Pediatrics 6(1):** 57-62.

The possibility of **aflatoxin** ingestion being **involved** in cases of infantile cirrhosis of liver in **India** is considered.

180. Rodricks, J.V. 1979. Aflatoxin and the public health. Peanut Journal of Nut World 58(8): 10-13.

This paper provides a popular review of the aflatoxin problem in relation to public health.

181. Rogan, W.J., Yang, G.C., and Kimbrough, R.D. 1985 Aflatoxin and Reye's syndrome : a study of livers from deceased cases. Archives of Environmental Health 40(2): 91-95.

Aflatoxins were detected in only 1 of the liver specimens of 12 children **who** presumably died of Reye's syndrome. It is concluded that aflatoxin is not regularly recoverable from cases of Reye's syndrome at a high rate and the proposed aetiological relationship is questioned.

182. Shank, R.C., Bhamarapravati, N., Gordon, J.E., and Wogan, G.N. 1972. Dietary aflatoxins and human liver cancer IV. Incidence of primary liver cancer in

two municipal populations of Thailand. Food and Cosmetics Toxicology 10: 171-179.

The incidence of primary liver cancer was determined in two selected populations in Thailand by direct field investigation. Liver viscerotomy specimens were obtained from 21 % of the persons aged 15 years or more who lived and died in the municipal areas of Ratburi, Potharam, Baan Pong and Nakorn Pathom (combined population 99,537) in south-western Thailand, and from 33 % of comparable persons in the municipal areas of Songkhla and Haad Yai (combined populations 97,867) in southernmost Thailand. Incidence was six new cases 100,000 people/year in the south-west area and two new cases/ 100,000/year in the Songhla-Haad Yai area. The available evidence supports the hypothesis that ailatoxin consumption is related to primary liver cancer in Thailand.

183. Shank, R.C., Bourgeois, C.H., Keschamras, N., and Chandavimol, P. 1971. Aflatoxins in autopsy specimens from Thai children with an acute disease of unknown aetiology. Food and Cosmetics Toxicology 9: 501-507.

Chemical analyses for aflatoxins were performed on autopsy specimens from 23 Thai children who died with acute encephalopathy and tatty dengeneration of the viscera (EFDV) and from 15 children and adolescents who died from unrelated causes. The highest levels detected were 93 µg aflatoxin B_1 kg^{-1} in a liver specimen, 123 µg kg^{-1} in stool, 127 pg kg^{-1} in stomach and intestinal contents and 8 pg mL^{-1} in bile. Trace amounts were detected also in brain, kidney and urine. Ailatoxin B_2 was indicated, but not confirmed, in 12 specimens of brain, liver, kidney, stool and intestinal contents. A blue fluorescent spot with the chromatographic properties of aflatoxin M_1 was found in trace amounts in two urine specimens, and a spot similar to that of ailatoxin B_1 was found in another. None of the urine specimens from healthy control children contained any of the anatoxins, but very small amounts of aflatoxin B_1 (1-4 pg kg^{-1} tissue) were demonstrated in some autopsy specimens from 11 of the 15 control subjects.

184. Shank, R.C., Gordon, J.E., Wogan, G.N., Nondasuta, A., and Subhamani, B. 1972. Dietary aflatoxins and human liver cancer. 111. Field survey of rural Thai families for ingested aflatoxins. Food and Cosmetics Toxicology 10: 71-84.

Aflatoxin consumption through cooked foods was determined by three 2-day surveys over a period of 1 year for 144 randomly selected households in nine villages within three areas of Thailand. The Singburi and Ratburi areas had the highest levels of aflatoxin ingestion, with respective annual means ranging from 73 to 81 and from 45 to 77 ng total aflatoxins kg^{-1} bodyweight day^{-1} on a family basis. Intake as high as 1072 ng total aflatoxins kg^{-1} bodyweight day^{-1} were recorded for individuals. A verge annual ailatoxin consumption in Songkhla was 10-14 times less than in Singburi. The Singburi dietary load of aflatoxin represents 20-30 % of intake values that induce a liver-tumor incidence of nearly 100 % in rats following continous exposure.

185. Shank, R.C., Wogan, G.N., Gibson, J.B., and Nondasuta, A. 1972. Dietary aflatoxin and human liver cancer. II. Aflatoxins in market foods and foodstuffs of Thailand and Hongkong. Food and Cosmetics Toxicology 10: 61-69.

Among more than 2000 samples of market foods and foodstuffs collected in Thailand, representing some 170 different human foods, groundnut products were the most frequently and most highly contaminated with aflatoxins. Dried com, millet, wheat, barley. Job's tear seeds and dried chili peppers also were frequently contaminated, although concentrations were low. The frequency and extent of contamination of Thai foodstuffs showed geographical distributions and followed seasonal trends. Beans of various strains were the chief source of aflatoxin in the Hongkong foodstuffs examined. In general, aflatoxin contamination in Honkong was less frequent and at lower levels than that in Thailand. Rice, the staple food in both areas, was seldom contaminated and (hen only at low levels. The results of this study provided the basis upon which Thai populations were selected to measure directly the amounts of aflatoxins ingested in food.

186. Stoloff, L. 1983. Aflatoxin as a cause of primary liver-cell cancer in the United States : A probability study. Nutrition and Cancer 5: 165-186.

Primary liver-cell cancer (PLC) mortality ratios, computed from deaths certificate records compiled by the National Center for Health Statistics, for the periods 1968-1971 and 1973-1976 were sorted by race, sex, urbanization, and region. From this sort, rural white males from the Southeast and the "North and West" regions were selected for comparison of mortality ratios and past dietary exposure to aflatoxin. Based on projections of recent aflatoxin contamination information back to the 1910-1960 period, and estimates of corn and groundnut usage from household food consumption surveys relating to that period, the expected average daily ingestion of aflatoxin B1 or each group was calculated (Southeast 13-197 ng kg^{-1} bodyweight; North and West 0.2-0.3 ng kg^{-1} bodyweight). An age-adjusted excess PLC mortality ratio was observed for the Southeast population when compared with the "North and West"- 10 % excess PLC deaths at all ages, and 6 % excess PLC deaths for the 30-49 year age-group-but although the difference was in the expected direction in ration to projected past exposure to afltoxin, it was far from the manyfold difference that would have been anticipated from experiments with rats and from prior epidemiological studies in Africa and Asia. The remaining major portion of the PLC mortality in the Southeast may be attributed to many unidentified causes for which the two populations that were compared were not controlled, leaving in doubt the validity of any attribution for the excess PLC mortality to

ailatoxin ingestion. A considerable excess over average US PLC mortality ratios was seen for all Orientals resident in the US and for urban black males. Occurrence of PLC in Orientals has been related to the presence of markers for hepatitis B virus in the blood serum of affected individuals.

187. Stoloff, L. 1989. Aflatoxin is not a probable human carcinogen : The published evidence is sufficient. Regulatory Toxicology and Pharmacology 10: 272-283.

Since the early 1960s, when aflatoxin, the mold-produced contaminant of a number of important food commodities, was found to be a potent hepatocarcinogen for laboratory rats, there has been a sustained search for evidence to support the regulatory presumption that aflatoxin is a probable human carcinogen. The developing laboratory evidence of differences between species in metabolism of aflatoxin and susceptibility to its oncogenic effects indicated that humans were probably refractory to aflatoxin carcinogenesis, but the early epidemiological evidence indicated otherwise. That epidemiological evidence, however, contained flaws so that Working Groups of the International Agency for Research on Cancer (IARC) meeting in 1970, 1976, and 1982, although ignoring the biochemial evidence, did consider the available epidemiological evidence insufficient for a conclusion of human carcinogenicity. During the 1970s and 1980s, studies on the connection between chronic infection with hepatitis B virus (HBV) and primary liver cell cancer (PLC), the expected lesion from aflatoxin exposure, had established a very strong etiological relationship between HBV and PLC. Since all the epidemiological studies of aflatoxin and PLC conducted prior to 1982 had been of populations with endemic HBV infection, and, in addition to other flaws, had not been controlled for this confounding factor, there was a solid basis for their rejection. Most epidemiological studies in the 1980s of aflatoxin and PLC were either in the United Suites, where HBV-infected groups could be excluded from the study, or, when in areas of chronic HBV infection, attempts were made to include that factor. The study of U.S. populations showed no difference in mortality rates from PLC that could be attributed to aflatoxin exposure. The studies of populations with endemic HBV infection produced no convincing evidence to support a primary role for aflatoxin in the induction of human PLC, although an accessory role to HBV infection for aflatoxin could not be ruled out. However, the epidemiological studies of the HBV/PLC relation indicate that an accessory factor is not an essential condition, a conclusion supported by animal models and a laboratory study that specifically found no interaction between ailatoxin and a hepatitis virus in the duck, a species in which liver cancer can be induced by either agent. It was surprising that an IARC Working Group meeting in 1987 concluded, on the basis of much of this evidence that was available at that time, and citing other studies that appear to be irrelevant to the issue that there was sufficient evidence to consider aflatoxin a probable human carcinogen.

188. Tulpule, P.G. 1969. Aflatoxicosis. Indian Journal of Medical Research (Supplement) 57(8): 102-114.

Recent work on mycotoxin contamination of animal and human foods is reviewed and some experimental work on agricultural and biological aspects is reported. Irrespective of growing and harvesting conditions, length of storage affected degree of aflatoxin contamination. In tests with 60 different kinds of groundnuts, including the variety U.S. 26, immune to aflatoxin development but not yet grown in India, the unique resistance of this variety was confirmed. Extensive field experiments showed that it was suitable for cultivation in India. Laboratory tests indicated that Zn, Cu, Co and Cd were required for effective growth and sporulation of the fungus and production of aflatoxin. Feeding experiments with young monekys and with rats confirmed that the level of dietary protein modified the extent of aflatoxin liver damage. Investigations of 16 cases of childhood cirrhosis, however, failed to show conclusively that aflatoxin was responsible.

189. Van Nieu Wenhuize, J.P., Herber, R.F.M., DeBruin, A., Meyer, I.P.B., and Diba, W.C. 1973. Aflatoxins : epidemiological study on the carcinogenicity of prolonged exposure to low levels among the workers of a plant. Tijadschrift voor Diergeneeskunde 51: 754.

Of 55 workers in a Dutch groundnut oil factory who had been exposed to an estimated 0.039-22.5 µg aflatoxin week^{1}, 11 developed various forms of cancer, mainly of the respiratory tract.

190. Van Rensberg, S.J., Van der Watt, J.J., Purchase, I.F.H., Coutinho, L.P., and Markham, R. 1974. Primary liver cancer rate and aflatoxin intake in a high cancer area. South Afrian Medical Journal 48(60): 2508a-2508d.

Data on the age and sex distribution of primary liver cancer rates in goldminers from the Inhambane district of Mozambique were obtained from a hospital registration program. Annual values for 1964-68 and 1969-71 were 35.5 and 25.4/100000. Occurrence was twice as frequent in males. Aflatoxin was found in 9.3 % of 880 random foods, equivalent to a mean consumption of 222.4 ng kg^{-1} bodyweight. Comparisons with similar studies in areas of lower incidence showed a significant correlation between aflatoxin consumption and liver cancer rates.

191. Van Rensburg, S.J., Cook-Mozaffari, P., van Schalkwkyk, D.J., van der Watt, J.J., Vincent, T.J., and Purchase, I.F.H. 1985. Hepatocellular carcinoma and dietary aflatoxin in Mozambique and Transkei. British Journal of Cancer 51 (5): 713-726.

Estimations of the incidence of hepatocellular carcinoma during 1968-1974 in the

Province of Inhambanc, Mozambique, were calculated and together with rates observed in South Africa among mineworkers from the same Province indicated very high incidence in certain districts of Inhambane. Exceptionally high incidences in adolescents and young adults were not sustained at older ages and suggested the existence of a group of highly susceptible persons. A sharp decline in incidence occurred during the period of study. Concurrently with the studies of incidence, 2183 samples of prepared food were randomly collected from 6 districts of Inhambane and from Tahica-Magude, a region of lower hepatocellular carcinoma incidence to the south. A further 623 samples were taken during 1976-1977 in Transkei, much further south, where an even lower incidence had been recorded. The mean aflatoxin dietary intake values for the regions were significantly related to hepatocellular carcinoma rates. Information on aflatoxin B, contamination of prepared food from 5 different countries showed overall a highly significant relation with crude hepatocellular carcinoma rates. In view of the evidence that chronic hepatitis B virus (HBV) infection may be a prerequisite for the development of almost all cases of hepatocellular carcinoma and given the merely moderate prevalence of carrier status which was observed in some high-incidence regions, it is likely that an interaction between HBV and aflatoxin is responsible for the exceptionally high rates in Africa and Asia. Indications from Mozambique suggest that aflatoxin may have a late-stage effect on the development of hepatocellular carcinoma.

192. Van Rensburg, S.J., Kirsipuu, A., Coutinho, L.P., and Van der Watt, J.J. 1975. Circumstances associated with the contamination of food by aflatoxin in a high primary liver cancer area. South African Medical Journal 49(22): 877-883.

In Mozambique, methods of food production, harvesting, storage and preparation were studied to detect points of aflatoxin contamination. Groundnuts, the main dietary source of protein provided the most aflatoxin contamination, traditional agricultural methods promoting fungal infection and growth. Aflatoxin production also occurred in the main carbohydrate sources, cassava and maize, during storage. Western-type foods had particularly low aflatoxin contents. A simple educational program could reduce the incidence of primary liver cancer associated with aflatoxin poisoning; westernisation of some living habits could also reduce the incidence of the disease.

193. Van Rensburg, SJ., Vander Watt, J J., Purchase, I.A.H., Coutinho, L.P., and Markham, R. 1974. Primary liver cancer rate and aflatoxin intake in a high cancer area. South African Medical Journal 48: 2508a-2508d.

Possibility of aflatoxin ingestion being related to primary liver cancer incidence is considered for a high cancer area of South Africa.

194. Vries, H.R.De., Lamplugh, S.M., and Hendrickse, R.G. 1987. Anatoxins and kwashiorkor in Kenya : a hospital based study in a rural area of Kenya. Annals of Tropical Paediatrics 7(4): 249-251.

Aflatoxin analyses were made on serum and urine samples from 41 children admitted to a rural hospital in Kenya with kwashiorkor, marasmus, marasmic kwashiorkor or normal nutrition. Aflatoxins were detected most frequently and at highest concentration in the sera of children with kwashiorkor who, conversely, showed aflatoxins least frequently in their urine and in concentrations that were disproportionately low compared with serum/urine aflatoxin levels in other groups. These findings indicate altered aflatoxin metabolism in kwashiorkor and support the hypothesis that there is a special relationship between aflatoxins and kwashiorkor.

195. Willis, R.M., Mulvihill, J.J., and Hoofnagle, J.H. 1980. Attempted suicide with purified aflatoxin. Lancet 1: 1198-1199.

In 1966, while working in a U.S. Department of Agriculture research laboratory, a 25-year-old woman attempted suicide twice by ingesting mixtures of aflatoxin containing 15-45 % of aflatoxin B_1. She consumed 5.5 mg of aflatoxin over 2 days and, 6 months later, 35 mg more over 2 weeks. After the first episode, she was admitted to hospital with a transient, non-pruritic, macular rash, nausea, and headache; on the second occasion, she reported nausea only. Both times, physical, radiological, and laboratory examination were normal, except for sulphobromophthalein retentions at 45 min of 9 % and 7 %. Percutaneous liver biopsy on each occasion was normal by light microscopy. On follow-up examination after 14 years, she was found normal.

196. Wogan, G.N. 1968. Aflatoxin risks and control measures. Federation Proceedings 27(3): 932-938.

This paper reviews research on toxicity and carcinogenicity of aflatoxins to animals, and summarizes control measures applied or proposed for protecting human food supplies from aflatoxin contamination. Control measures include use of effective crop handling, processing and storage procedures, segregation of contaminated produce by various sorting and diversion systems, and detoxification.

197. Yadgiri, B., Reddy, V., Tulpule, P.G., Srikantia, S.G., and Gopalan, C. 1970. Aflatoxin and Indian childhood cirrhosis. American Journal of Clinical Nutrition 23: 94-98.

Of 16 Indian children from 3 months to 2.5 years old with a diagnosis of Indian childhood cirrhosis confirmed histologically, 10 had a compound in their urine which gave a fluorescent spot. The ultraviolet and infrared spectra of aflatoxin B_1

and the fluorescent spot in the urine were quite dissimilar. The unknown fluorescent compound was highly soluble in ethyl ether whereas aflatoxin B_1 was insoluble.

198. Yaobin, W., Lizun, L., Benfa, Y., Yaochu, X., Yunyuan, L., and Wenguang, L. 1983. Relation between distribution of liver cancer and climate - Aflatoxin B_1 in China. Sci. Sin. Ser. B 26: 1166-1175.

The geographical distribution of primary liver cancer in China is related to contamination of foodstuffs (including groundnuts) with aflatoxins. The warm, humid climate is conducive to contamination of foodstuffs with aflatoxin.

199. Yeh, F-S., Mo., C-C., and Yen, R-C. 1985. Risk factors for hepatocellular carcinoma in Guangxi, People's Republic of China. Natl. Cancer Inst. Monogr. 69: 47-48.

The major risk factor for primary liver cancer was identified as hepatitis virus B infection in certain populations in southeastern China.

200. Yeh, F-S., Yu, M.C., Mo, C-C, Luo, S., long, M.J., and Henderson, B.E. 1989. Hepatitis B virus, aflatoxins, and hepatocellular carcinoma in southern Guangxi, China. Cancer Research 49: 2506-2509.

Investigations were made into the roles of hepatitis B virus and alflatoxin B_1 in the development of primary hepatocellular carcinoma (PHC) in a cohort of 7917 men aged 25 to 64 yr old in southern Guangxi, China, where the incidence of PHC is among the highest in the world. After accumlating 30188 man-yr of observation, 149 deaths were observed, 76 (51 %) of which were due to PHC. Ninety-one % (69 of 76) of PHC deaths were hepatitis B surface antigen (HBsAg) positive at enrollment into the study in contrast to 23 % of all members of the cohort (RR=38.6). Three of the four patients who died of liver cirrhosis also were HBsAG positive at enrollment. There was no association between HBsAG positivity state and other causes of deaths. Within the cohort, there was a 3.5-fold difference in PHC mortality by place of residence. When estimated aflatoxin B_1 levels in the subpopulations were plotted against the corresponding mortality rates of PHC, a positive and almost perfectly linear relationship was observed. On the other hand, no significant association was observed when the prevalence of HBsAG positivity in the subpopulations was compared with their corresponding rates of PHC mortality.

201. Zhu, J.Q., Zhang, L.S., Hu, X., Xiao, Y., Chen, J.S., Xu, Y.C., Fremy, J., and Chu, F.S. 1987. Correlation of dietary aflatoxin B_1 levels with excretion of aflatoxin M_1 in human urine. Cancer Research 47(7): 1848-1852.

Maize and groundnut oil (253) samples were collected from 32 households in Fushui County of the Guangxi autonomous region of the People's Republic of China, where high liver cancer incidence has been reported, every day over a period of 1 week and analyzed for aflatoxin B_1. A total of 252 urine samples were collected simultaneously from the residents in the households which had consumed aflatoxin and were analyzed for allaioxin M_1. A good correlation between dietary aflatoxin B_1 intake and total aflatoxin M_1 excretion in human urine was observed during a 3-day study. Between 1.23 and 2.18 % of dietary aflatoxin B_1 was present as aflatoxin M_1 in human urine. A good correlation was also found between aflatoxin B_1 concentration in maize and aflatoxin M_1 concentration in human urine. It is suggested that analysis of aflatoxin M_1 in urine by ELISA could be used as an index for human exposure of aflatoxin B_1 in an expensive epidemiological study.

202. Zuckerman, A.J. 1975. Peanuts and cancer. Nature 256: 540.

Liver cell cancer is prevalent in many regions of Africa and South-east Asia. The author comments on the possible connection between liver cell cancer and aflatoxins.

1.2.4 Laboratory Studies on Toxicity and Carcinogenicity of Aflatoxin

203. Adamson, R.H., Correa, P., and Dalgard, D.W. 1973. Occurrence of a primary liver carcinoma in a rhesus monkeys fed aflatoxin B_1. Journal of National Cancer Institute 50: 549-551.

A primary liver carcinoma occurred in a rhesus monkey given aflatoxin B_1 orally over a period of 6 years. The fact that this tumor rarely arises spontaneously in monkeys, and pathologic changes seen in the livers of other monkeys given aflatoxin B_1 suggested that the neoplasm was induced by this compound.

204. Adamson, R.H., Correa, P., Sieber, S.M., McIntire, K.R., and Dalgard, D.VV. 1976. Carcinogenicity of aflatoxin B_1 in rhesus monkeys. Two additional cases of primary liver cancer. Journal of National Cancer Institute 57(1): 67-78.

Three of 42 monkeys, 20 *Macaco mulatta,* 20 *Macaca jascicularis* and 2 *Cercopithecus aethiops,* given aflatoxin B_1 (AFB_1) by mouth, nasogastric tube or intraperitoneally for longer than 2 years developed primary malignant neoplasm of the liver. Liver biopsies made at intervals indicated that neoplasia was preceded by pathological lesions of the liver, including toxic hepatitis, proliferation of pseudotubules and hyperplastic nodules. Serum α-fetoprotein, estimated in one of

the monkeys by radioimmunoassay, paralleled tumor growth and recurrence of the hepatocellular carcinoma. Normal serum a-fetoprotein was present in a monkey with haemangioendothelial sarcoma. The results implicate AFB_1 as a liver carcinogen in monkeys, and add support to the hypothesis that human populations exposed to this substance may be at risk of developing liver cancer.

205. Allcroft, R. 1965. Aspects of aflatoxicosis in farm animals. Pages 153-162 in Mycotoxins in Foodstuffs (Wogan, G.N., ed.). Cambridge : Massachusetts Institute of Technology Press. 291 pp.

The susceptibility, symptoms, and pathological changes in cattle, sheep, pigs, and poultry caused by feeding groundnut meal containing aflatoxin are discussed. Some functional changes observed in experimental aflatoxicosis in calves, pigs, and chickens are reported, and the excretion of aflatoxin or a metabolite in faeces and urine from sheep, which are resistant to the toxin, has been compared with excretion in cattle which are susceptible.

206. Annau, E., Corner, A.H., Magwood, S.E., and Jericho, K. 1964. Electrophoretic and chemical studies on sera of swine following the feeding of toxic groundnut meal. Canadian Journal of Comp. Medical and Veterinary Science 28: 264-269.

Diets with different proportions of a toxic groundnut meal, containing aflatoxin B_1 (5000 µg kg^{-1}) and aflatoxin G_1 (1000 µg kg^{-1}), or a non-toxic soybean oil meal, were given to 42 pigs of about 12 weeks of age. Diets with 10% or more groundnut meal were unpalatable but 2.5 and 5 % were eaten readily. Growth rate and efficiency of feed utilization were progressively reduced by feeding more than 2.5 % groundnut meal. There was also a progressive degeneration of the liver with pronounced pericellular fibrosis and an increase of bile duct epithelium. The electrophoretic serum patterns were grossly affected by feeding groundnut meal. With large proportions there was a relative decrease in the albumin, α1-, α2- and ß-globulins and an increase in Y-globulins as measured by starch gel electrophoresis.

207. Armbrecht, B.H., Hodges, F.A., Smith, H.R., and Nelson, A.A. 1963. Mycotoxins. I. Studies on aflatoxin derived from contaminated peanut meal and certain strains of *Aspergillus flavus.* Journal of the Association of Official Agricultural Chemists 46(5):805-817.

Aspergillus flavus strains were obtained from British and domestic sources, and were cultured on natural substrates. Aflatoxin was extracted from groundnut meal and from wheat. The amounts of toxic substances present were estimated by paper chromatography. Concentrates were obtained by precipitation. One concentrate was reduced. Toxicity tests on ducklings confirmed British findings.

208. Ashley, L.M., Halver, J.E., Gardner, W.K.Jr., and Wogan, G.N. 1965. Crystalline aflatoxins cause trout hepatoma. Federation Proceedings 24: 627.

Trout fed Bloor-La Roche extracted cottonseed meal fats developed classical trabecular liver cell neoplasms in five months. One of five cottonseed meals with known agricultural history assayed 300 µg kg^{-1} allatoxin B,, and trout fed this fat showed a high incidence of hepatoma. Trout fed crystalline aflatoxin B_1 and G_1 confirmed previous experiments with these toxins; trabecular hepatoma developed within six months. Small fish-force fed 1, 3, or 5 mg of crude allatoxin kg^{-1} bodyweight had from slight focal to extensive liver cell necrosis with copious haemorrhage in severe cases. Extent of these lesions varied directly with the amount of toxin ingested. Prenecrotic or concomitant pathology included generalized cytoplasmic and nuclear vaculation, nuclear pleomorphisrn and occasional cholangiolar proliferation. Other trout loaded with crystalline aflatoxin B_1 at 0.1,0.5, 1.0 or 3.0 mg kg^{-1} bodyweight showed a range of variation in the extent of necrosis and haemorrhage together with accompanying pathology essentially similar to fish fed the crude allatoxin. Allatoxin B_1 appeared to be from five to ten times more toxic to trout than the crude aflatoxin.

209. Ashley, L.M., Halver, J.E., and Wogan, G.N. 1964. Hepatoma and aflatoxicosis in trout. Federation Proceedings 23: 105.

In rainbow trout fed wheat aflatoxins for 6-9 months (80 µg kg^{-1} crude aflatoxin), 60 % had classical multinodular hepatoma. Fish force-fed high levels (30-300 µg kg^{-1} day^{-1}) of thin-layer chromatogram-purified B_1 and G_1 compounds for 5 days died with acute aflatoxicosis at 10-15 days. Tentative LD_{50} total dose was 1500 µg kg^{-1}.

210. Barnes, J.M., and Butler, W.H. 1964. Carcinogenic activity of aflatoxin to rats. Nature 202: 1016.

Rats received in a powdered cube diet 1750 µg kg^{-1} aflatoxin from toxic groundnut meal. Three rats given aflatoxin for 89 days ultimately developed liver carcinoma after being returned for 300 or more days to stock diet. The carcinogenic dose was not greater than 2500 µg kg^{-1} per rat. The three rats had multiple trabecular hepatocarcinoma with adenomatous areas. The possible hazard to human health was indicated.

211. Bourgeois, C.H., Shank, R.C., Grossman, R.A., Johnsen, D.O., Wooding,

V.L., and Chandavimol, P. 1971. Acute aflatoxin B_1 toxicity in the macaque and s similarities to Reye's syndrome. Laboratory Investigation 24: 206-216.

ighteen monkeys were given various doses of chromatographically pure aflatoxin ,. Death occurred in one animal receiving 4500 μg kg^{-1} and in all animals receiving 13500 µg kg^{-1} or more. Cough, vomiting, diarrhoea and coma were characteristic clinical findings. Changes in the serum included hypoglycaemia, increased nonesterified fatty acids and transaminases and decreased phospholipids, crebral oedema with neuronal degeneration, bile duct hyperplasia, hepatic cell ecrosis, lymphocytosis and marked fatty degeneration of the liver, heart and sidneys were found post-mortem.

12. Brown, J.M.M. 1965. Biochemical changes in the livers of domestic birds pisoned with aflatoxin. South African Medical Journal 39: 778.

iochemical studies were made on liver tissue and blood from ducklings and various seeds of chickens maintained on standard rations containing 500 μg kg^{-1} aflatoxin $_1$. The results obtained from these birds were compared with those from birds of e same age maintained on groundnut-free mashes. Of the various breeds of lickens studied only New Hampshires were susceptible to the effects of this level i allatoxin. In the livers of affected chickens or ducklings, a marked decrease in e activity of certain mitochondrial dehydrogenase and enzymes of the electron ansfer chains or oxidative phosphorylation mechanisms was apparent. Mild to oderate anaemia, severe hypoproteinaemia and grossly abnormal plasma protein ectrophoretograms were presented by affected birds. Elevations in the activity of "/lain plasma enzymes, notably those of lactic dehydrogenase, aldolase and utamic-oxalacetic or glutamic-pyruvic transaminase, were also noted in these birds, the ability of affected birds maintained on rations containing 500 µg kg^{-1} aflatoxin recover rapidly when placed on toxin-free rations was noted.

13. Bryden, W.L., Cumming, R.B., and Balanave, D. 1979. The influence of tamin A status on the response of chickens to aflatoxin B_1 and changes in liver did metabolism associated with aflatoxicosis. British Journal of Nutrition 41(3): 29-540.

flatoxin B_1 levels greater than 1 mg kg^{-1} diet had a deterimental effect on /eability, bodyweight gain, food intake and food conversion efficiency. When fed r more than 2 weeks, aflatoxin increased relative liver weight and liver lipid concentration. The effects were less pronounced with vitaminotic A chickens. A nergistic effect on hydropericardium development was observed between aflatoxin and retinol. This effect was not observed when the dietary level of α-tocopherol as increased ten-fold. Liver fatty acid composition was significantly influenced by aflatoxin B_1. The extent of these changes was reduced by the inclusion of additional dietary biotin.

214. Butler, W.H. 1964a. Acute liver injury in ducklings as a result of aflatoxin poisoning. Journal of Pathology and Bacteriology 88: 189-196.

This paper describes the histological changes in the liver produced by a single dose of aflatoxin and contrasts them with the lesions produced in day-old ducklings by other well-studied liver poisons. Day-old Khaki Campbell ducklings given 15 µg aflatoxin by mouth developed an extensive biliary proliferation in the liver with fatty degeneration of the peripheral parenchyma cells. This lesion reached its maximum in 3 days, and then regressed with repair of the liver parenchyma. A comparable lesion was seen in ducklings given single doses of dimethylnitrosamine and cycasin, but not with other hepatotoxic agents such as carbon tetrachloride, ethionine and thioacetamide. The lesions varied greatly at the same dose level, and it was not possible to estimate the amount of aflatoxin by histological examination of the livers of poisoned birds. Evidence of possible direct action upon the biliary epithelium is given, and it is suggested that aflatoxin may be an alkylating agent.

215. Butler, W.H. 1964b. Acute toxicity of aflatoxin B_1 in rats. British Journal of Cancer 18(4): 756-762.

Acute toxicity of aflatoxin B_1 in rats is reported. The LD_{50} of aflatoxin B, to male rats was estimated as 7.2 mg kg^{-1} per os and 6 mg kg^{-1} inuaperiioneal and female rats 16 mg kg^{-1} per os. A periportal zone of liver necrosis developed in 3-4 days. Following this there was a slow recovery so that by 1 month mere was a persistant biliary proliferation and the presence of large hyperchromatic parenchymal cells. Lesions in other organs are described.

216. Bulter, W.H. 1966a. Early hepatic parenchymal changes induced in the rat by allatoxin B_1. American Journal of Pathology 49: 113-119.

Sequential ultrastructural changes were induced in rat liver parenchymal cells by the oral administration of aflatoxin B_1. These were described at intervals of 1, 3 and 6 hours. At 1 h some dilatation of the RER cisternae and dislocation of ribosomes appeared in periportal cells. By 3 hours disruption of the RER was more evident; cenfrilobular cells remained normal. At 6 hours when the periportal cells exhibited further disruption with alteration in mitochondria, centrilobular parenchymal cells revealed dilatation of RER cisternae and di.slocation of ribosomes. At 3 and 6 hours the formation of nucleolar caps was prominent. There appears to be a correlation between inhibition of the protein synthetic mechanism and the disruption of the RER but this does not appear to be related to the nucleolar alterations.

217. Butter, W.H. 1966b. Acute toxicity of aflatoxin B_1 in guinea-pigs. Journal of Pathology and Bacteriology 91: 277-280.

The effects of a single dose of aflatoxin B_1 on guinea-pigs were compared with the effects of feeding groundnut meal containing aflatoxin. The LD_{50} of aflatoxin B_1 in male and female guinea-pigs was estimated as 1.4 mg kg^{1} body weight by interaperitoneal injection. The main hepatic lesion was a centrilobular zone of necrosis developing with biliary proliferation over 2 days. The lesion was healed over a few days and the biliary proliferation rapidly regressed. The pathological changes in other organs are described.

218. Butler, W.H. 1966c. Carcinoma of the grandular stomach in rats given diets containing aflatoxin. Nature (London) 209: 90.

Of six one-year-old rats receiving a diet containing 3000-4000 μg kg^{-1} aflatoxin. five survived longer than 39 weeks and of these, three showed anaplastic hepatocellular carcinomas. Adenocarcinoma of the rectum or stomach also occurred in two of these three rats. One case each of hepatic carcinoma and carcinosarcoma of the stomach occurred among six young rats which received the diet for three weeks and then returned to a normal diet. Aflatoxin may therefore be a factor in the aetiology of carcinomas in organs other than the liver.

219. Butler, W.H. 1969. Aflatoxicosis in laboratory animals. Pages 223-236 in Aflatoxin : Scientific background, control and implications (Goldblatt, L.A., ed.). New York, USA : Academic Press. 472 pp.

The acute toxicity and carcinogenic activity of aflatoxin and the pathological changes which this toxin induces in laboratory animals are described. The organ primarily affected is the liver but changes are seen in most other organs. The carcinogenicity of aflatoxins has been demonstrated in rats, ducks, and trout. In sheep, mice and guinea-pigs, there is some evidence of carcinogenicity. Guinea-pigs are extremely susceptible to aflatoxin.

220. Butler, W.H., and Barnes, J.M. 1963. Toxic effects of groundnut meal containing aflatoxin to rats and guineapigs. British Journal of Cancer 17: 699-710.

Trials were conducted with rats of both sexes of about 100 g bodyweight given diets With from 3500 to 4000 µg kg^{-1} aflatoxin B_1 supplied by up to 50 % groundnut meal, and with guinea pigs given up to 1600 µg kg^{-1}. With the largest amount of toxic meal rats grew normally for 3 weeks, then growth declined. When the diet was changed to exclude the toxic meal, after 16 weeks growth improved. Other diets were given continously or intermittently for different periods. There was evidence that some irreversible damage was done to the liver by 12 weeks on the diet with 1400 ug kg^{-1} aflatoxin. Damage to the liver in different conditions is described and illustrated. The malignant tumors of the liver are compared with those produced by other known carcinogens. Guinea-pigs given 1400 µg kg^{-1} aflatoxin died, usually within 14 to 28 days. As the dose was reduced the development of clinical and pathological signs was correspondingly retarded. The histological picture was of acute poisoning, and malignant tumors did not develop, probably because the animals died too soon.

221. Butler, W.H., and Wigglesworth, J.S. 1966. The effects of aflatoxin B_1 on the pregnant rat. British Journal of Experimental Pathology 47: 242-247.

Aflatoxin in one quarter of the lethal dose for the non-pregnant female rat was given by mouth to rat at various stages of pregnancy. Animals dosed in early pregnancy were no more severely affected than non-pregnant rats, and there was no evidence of toxic effects on the foetus or placenta other than slight reduction in placental weight at term. Rats dosed on the 16th day, however, had more severe liver injury than non-pregnant rats with increased fatty infiltration and retardation of foetal growth. It is concluded that foetal growth retardation in the latter group of animals occurs secondarily to the toxic effects in the mother and may be due to the maternal liver damage.

222. Carnaghan, R.B.A. 1965. Hepatic tumours in ducks fed on low level of toxic groundnut meal. Nature (London) 208: 308.

Sixteen 7-day-old Khaki Campbell ducklings were fed on a commercial poultry feed free of aflatoxin, and 37 had 0.5 % toxic Brazilian groundnut meal included in the feed. The groundnut meal contained 7000 µg kg^{-1} aflatoxin B_1,. The regression of growth up to 11 weeks did not differ significantly between the groups but those given groundnut meal were significantly lighter at 3, 4 and 5 weeks, and the difference was maintained up to the end of the trial after 14 months. During the first 4 weeks 19, and in the next 7 months 7 ducks in the group given toxic groundnut meal died; all had damage to liver typical of poisoning by aflatoxin. After 14 months tumours were found in livers of 8 of the 11 survivors of the group. There was no tumor in the 10 survivors of the group given no groundnut meal.

223. Carnaghan, R.B.A. 1967. Hepatic tumours and other chronic liver changes in rats following a single oral administration of aflatoxin. British Journal of Cancer 21: 811-814.

Two groups of weanling female rats were each given a single sublethal dose of aflatoxin by mouth. Almost half the animals in each group developed hepatic tumors. Other chronic changes were found in the liver of a high proportion of the remainder. The mean time of tumor formation was 26 months.

224. Carnaghan, R.B.A., Hartley, R.D., and O'Kelly, J. 1963. Toxicity and fluorescence properties of the aflatoxins. Nature (London) 200: 1101.

Relative intensities of fluorescence of the four major components of aflatoxin (aflatoxins B_1, B_2, G_1 and G_2) and their acute toxicities to day-old ducklings are presented. The LD_{50} value for each compound was calculated on the basis of 50 g bodyweight of day-old ducklings. The LD_{50} for B_1 was 18.2 µg, for B_2 84.8 µg, for G_1 39.2 µg, and for G_2 172.5 µg.

225. Carnaghan, R.B.A., Lewis, G., Patterson, D.S.P., and Allcroft, R. 1966. Biochemical and pathological aspects of groundnut poisoning in chickens. Pathol. Veterinaria 3(6):601-615.

Rhode Island Red chicks were fed from hatching to 8 weeks of age a commercial ration to which was added a highly toxic groundnut meal; groups were killed at regular intervals for biochemical and histological examinations. Growth was severely retarded and the liver/bodyweight ratio was increased compared with control chicks. Increased hepatic fat, reduced vitamin A storage, and fluctuation in the RNA/DNA ratio were also observed. These biochemical features of groundnut toxicity are discussed in relation to pathological changes.

226. Carnaghan, R.B.A., and Sargeant, K. 1961. The toxicity of certain groundnut meals to poultry. Veterinary Record 73: 726-727.

Day-old ducklings in groups of 6 were given 2 turkey diets which had been associated with outbreaks of Turkey "X" disease. The diets had about 6 % Indian groundnut meal. Other groups were given similar amounts of Indian groundnut meal known to be non-toxic. Those given the toxic meal did not grow well and 5 in each group died within 5 weeks. Gross and microscopical lesions, similar to those produced by toxic Brazilian and Last African groundnut meals, were found. Extracts of the Indian meals in amounts equivalent to 100, 200 and 750 g in 5, 5 and 11 days did not kill day-old ducklings, but liver lesions were found.

227. Clifford, J.I., and Rees, K.R. 1967. The **a**ction of aflatoxin B_1 on the rat liver. Biochemical Journal 102: 65-75.

The administration of a single dose of aflatoxin B_1 to the rat (7000 µg kg^{-1} bodyweight) resulted in the slow development of a periportal necrosis. Hepatic enzymes were released into the serum in the second 24 h of the poisoning, closely preceding the onset of the necrosis, which was followed by a rise in serum alkaline-phosphatase activity and bilirubin concentration. Aflatoxin B_1 was detected **in** the nucleus of the poisoned liver cell and in vitro it was shown to interact with DNA. The toxin inhibits the production of nuclear RNA, probably by preventing the transcription of DNA by the RNA polymerase. It is proposed that the interaction of the toxin with DNA gives rise to its inhibitory action on mitosis and its necrogenic action.

228. Clifford, J.I., Rees, K.R., and Stevens, M.E.M. 1967. The effect of the anatoxins B_1, G_1 and G_2 on protein and nucleic acid synthe**sis in rat liver.** Biochemical Journal 103: 258-261.

A comparison was made of the different spectra obtained by causing various aflatoxins (B_1, G_1 and G_2) to interact with calf-thymus DNA. The effect of these toxins on RNA and protein synthesis by rat-liver slices was measured. The extent of their inhibitory action on the synthetic reactions was proportional to the degree of spectral shift obtained with their interaction with DNA. It is proposed that their toxicity depends upon this interaction. It was demonstrated that RNA polymerase of nucleoli isolated from the livers of aflatoxin B_1-poisoned rats was inhibited. This finding is in agreement with the proposed mechanism for the hepatotoxic action of aflatoxin.

229. Cuthbertson, W.F.J., Laursen, A.C., and Pratt, D.A.H. 1967. Effect of groundnut meal containing aflatoxin on Cynomolgus monkeys. British Journal of Nutrition 21: 893-908.

Both male and female Cynomolgus (*Macaca irus)* monkeys survived for 3 years without apparent ill health when fed on diets containing groundnut meal to provide up to 0.36 µg aflatoxin B_1 g^{-1} diet, and thus supplying a mean daily consumption of up to 2 µg aflatoxin B_1 kg^{-1} bodyweight. No histological changes attributable to aflatoxicosis were discovered in any of the organs from the monkeys receiving these quantities of aflatoxin. Few monkeys survived for more than a month or two when given a diet containing 1.8 µg aflatoxin B^1 g^{-1}, which provided about 50 (ig aflatoxin B_1 kg^{-1} bodyweight day^{-1}. No depression in growth rate nor effect on health was noted in these monkeys which survived on diets containing 1.8 (ig aflatoxin B_1, nor in any of the other monkeys. Histological changes were observed in the livers of all monkeys receiving the diet containing 1.8 (ig aflatoxin B_1 g^{-1} for more than a month or two. but no abnormalities related to aflatoxicosis could be detected in any of the other organs, except for minor changes in the kidneys of two monkeys. No tumors were seen in any of the monkeys, even in those surviving for 3 years on diets providing 1.8 (ig aflatoxin B_1 g^{-1}. Thus, no conclusions can be drawn from this work as to the carcinogenicity of aflatoxin in monkeys (still less in man), because the animals were young and little is known of the duration of exposure required to demonstrate carcinogenicity in this species.

230. Datta, P.R., and Gajan, R.J. 1965. Plasma protein index of aflatoxin-fed ducklings. Federation Proceedings 24: 392.

The protein index method, based on Brdicka's Cobalt catalysed polarographic waves, is the most sensitive measure of pathological changes in blood proteins. This technique was applied to plasma from ducklings fed various levels of aflatoxin. The protein indexes were determined and the statistical parameters of the data were calculated. The protein index of the ducklings fed 7 µg of crystalline aflatoxin B, was twice the index value obtained from control birds. The protein index of ducklings fed aflatoxin mold culture, estimated to contain 20 µg aflatoxin B_1 chemically, was 5 to 6 times the control index. These results correlate with the histological liver data and reflect structural changes in the liver.

231. Deo, M.G., Dayal, Y., and Ramalingaswami, V. 1970. Aflatoxins and liver injury in the rhesus monkeys. Journal of Pathology 101: 47-56.

The effects on the liver of oral administration of a range of doses of a partially purified mixture of aflatoxins B_1 and G_1 were studied in a group of 64 young male rhesus monkeys. The dosage levels of the toxin used were : 1 mg kg^{-1} bodyweight daily (group 1); 0.25 mg kg^{-1} bodyweight twice a week (group II); and 62 *µg* once a week (group III). The toxin was administered continuously according to this schedule. The animals in groups I and II were further subdivided in order to study the effect of a diet poor in protein on the liver injury induced by aflatoxin. All animals in group I died before the end of the 3rd week of aflatoxin administration; extensive haemorrhagic necrosis of the liver was a characteristic finding. Animals in group II were studied at intervals up to nearly 5 months of aflatoxin administration. Large bizarre hyperchromatic liver cells and bile-duct proliferation appeared around the middle of this period and thereafter progressed in severity. Animals in group III showed comparable changes in a much milder form, which appeared much later and did not then progress significantly up to the end of the 2 year period of observation. No tumors were seen in any of the groups. Protein deficiency did not exert any clear-cut deleterious influence on the liver injury induced by aflatoxin. The investigation emphasises the importance of long-term studies in non-human primates, using low doses of aflatoxins, to elucidate their role in the causation of liver disease (including liver cancer) in man.

232. Dickens, F., and Jones, H.E.H. 1963. The carcinogenic action of aflatoxin after its subcutaneous injection in the rat. British Journal of Cancer 17: 691-698.

Sarcoma or fibrosarcoma developed in 11 rats at the site of subcutaneous injection of repeated doses of aflatoxin. A preparation containing 3.8 % aflatoxin B and 56 % aflatoxin G was injected twice a week in doses of 50 µg for 50 weeks or 500 µg for 8 weeks.

233. Diener, U.L., Davis, N.D., Salmon, W.D., and Prickett, C.O. 1963. Toxin producing Aspergillus isolated from domestic peanuts. Science 142: 1491-1492.

Nine species of fungi isolated from stored domestic groundnuts were grown on sterilized groundnuts and were incorporated into diets fed to ducklings. Symptoms of acute toxicity resulted only after consumption of one of the diets and this one contained material incubated with the fungus *Aspergillus flavus*.

234. Duthie, I.F., Lancaster, M.C, Taylor, J., Thomas, D.C, Shacklady, C.A., Attfield, P.H., and Fuller-Lewis, E. 1966. Toxic groundnut meal in feeds for pigs. I. A trial made at two laboratories with pigs from about 40 to 200 lb live-weight fed to a restricted scale. Veterinary Record 79: 621-625.

In an experiment with pigs from 40 to 200 lb live-weight, the effect was studied of inclusion in the diet of groundnut meal at levels of 0, 2.5, 5 and 7.5 % giving concentrations of 2, 140, 280 and 410 *µg* kg^{-1} aflatoxin B_1, respectively. Depression of growth rate and feed conversion efficiency occurred at the two highest levels of inclusion at both centres where the experiment was replicated. These effects were detected mainly between 40 and 140 lb live-weight. A significant linear relationship could be represented by regression equations based on aflatoxin B, content of feeds for the live-weight ranges of 40 to 140 lb and 40 to 200 lb. The results for both centres could be combined because there was little variation between them. The combined results could be represented also by regression equations. It is suggested that the sets of equations may be of some value in predicting levels at which groundnut meal of defined toxicity may be used for pigs. No clinical signs could be attributed to the levels of toxic groundnut meal used. Carcass abnormalities were not reported and no macroscopic differences between livers were found. Microscopic liver lesions were minimal, except in pigs on diets containing 410 *µg* kg^{-1} aflatoxin B_1.

235. Ferrando, R., N'Diaye, L., Gautier, F., and Henry, N. 1974. [Effects on ducklings of diets based on groundnut cake containing aflatoxins with or without DDT or phenobarbital: comparison with a soya-based diet.]. Effets, chez le caneton, de regimes renfermant du tourteau d'arachide contenant des aflatoxines en association ou non avec du DDT ou du phenobarbital : comparaison avec un regime a base de soja. Recueil de Medecine Veterinaire 150(7): 601-606.

Day-old Muscovy ducklings of mixed sex were divided into 6 groups of 34 or 35 and given for 3 weeks diets in which the control group had 25 % soya oilmeal and the test groups groundnut oilmeal containing 1000 (ig aflatoxin B_1 kg^{-1}. For 4 weeks of the test groups the diet contained also DDT 50 or 150 or phenobarbital 100 or 200 mg kg^{-1}. The diets had 23 % crude protein. Cumulated mortality in 3 weeks was for the controls 6.6 % and for groups with groundnut meal alone or the additives in order as above 45.7, 41.1, 20.5, 14.7 and 14.7 %. Final weights of survivors were 484, 291, 338, 366, 448 and 433 g and weight of liver as proportion of bodyweight was 5.03, 5.97, 6.58 (only 1 value for DDT), 7.68 and 8.58 %. The mean liver

reserved for vitamin A were 174, 58, 101, 59 and 60 IU g^{-1}. It was concluded that DDT and phenobarbital, especially in the larger dose of the first and the smaller of the second, tended to offset the toxic effects of aflatoxin; their effects were highly significant. The effects on vitamin A reserved might be attributable to the role of vitamin A in enzyme induction.

236. Friedman, M.A., and Wogan, G.N. 1966. Effects of aflatoxin B_1 on enzyme induction and nuclear RNA metabolism in rat liver. Federation Proceedings 25: 662.

The induction of tryptophan pyrrolase and tyrosine transaminase in rat liver by hydrocortisone (50 mg kg^{-1}) was significantly inhibited by simultaneous administration of aflatoxin B_1 (1 mg kg^{-1}) in adrenalectomized or intact rats. Inhibition also occurred when the toxin was administered 1 hour after hydrocortisone. However, the induction became intensive to aflatoxin B_1 3 hours or longer after hydrocortisone administration. In comparative experiments, these effects were shown to be unlike those caused by puromycin but analagous to those of actinomycin D. The latter suggestion was corroborated by the findings that aflatoxin B_1 at an IP dose of 5 mg kg^{-1} (LD_{50}) resulted in 62 % inhibition and $I I^3$ - cytidine incorporation into rat liver nuclear RNA within 30 minutes after administration. The depression of incorporation was accompanied by a 23 % reduction in the nuclear RNA/DNA ratio. In animals killed 90 rnin. following aflatoxin B_1 administration, the incorporation of a 30 min. H^3 - cytidine pulse was inhibited by 73 % with a concomitant reduction of 28 % in the nuclear RNA/DNA rates. The observed effects on nuclear RNA metabolism may be a primary determinant of the toxicity of aflatoxin B_1

237. Friedman, M.A., and Wogan, G.N. 1967. Effects of aflatoxin B_1 on RNA polymerase activity and incorporation of cystine into RNA of rat liver nuclei. Federation Proceedings 26: 358.

RNA polymerase activity, incorporation of tritiated cytidine into RNA, and RNA/DNA ratios were determined in liver cell nuclei isolated from rats killed at intervals following a single sublethal dose (3 mg kg^{-1}) of aflatoxin B_1. Twelve hours after dosing, cytidine incorporation was decreased to 7 % of control values and me RNA/DNA ratio was suppressed by 29 %. At 24 hours, incorporation was 6.5 % of control, and RNA/DNA ratios had risen to 85 % of control values. By 5 days after dosing, the RNA/DNA ratios had returned to prc-treatment levels, but cytidine incorporation was still suppressed by 63 %. The latter inhibition persisted for prolonged periods beyond 5 days. RNA polymerase activity of nuclei isolated from treated animals was inhibited by 32 % at 5 minutes, 75 % at 30 minutes and 78 % at 48 hours.

238. Gardiner, E.E. 1962. A comparison of me toxicity to poults and chicks of a certain peanut oil meal. Poultry Science 41: 1348-1350.

Effects of various levels of toxic Brazilian groundnut meal on growth and mortality of poults and chicks were investigated. The growth rate of poults led each level of the meal was markedly reduced and was inversely related to the level of the meal in the birds' diets. Mortality of the poults fed the two higher levels (37 and 55 %) of meal occurred early and by 21st day of the experiment all were dead. Mortality of the poults receving meal at me lowest level (18 %) reached 100 % by me 28th day. Gross observations of the livers from the sacrificed poults (at 15th day) indicated severe lesions of the livers from those poults receiving groundnut meal. There were no marked differences in the percent mortality of the various groups of chicks. The 4-week weights of the chicks receiving the two higher levels of groundnut meal were significanlty ($P < 0.01$) lower man the other two groups and significantly different from each other. The livers of chicks fed the meal were yellow throughout (on all levels of the meal) while those from controls appeared normal.

239. Gopalan, C., Tulpule, P.G., and Krishnamurthi, D. 1972. Induction of hepatic carcinoma with aflatoxin in the rhesus monkey. Food and Cosmetics Toxicology 10: 519-521.

The carcinogenic effect of aflatoxin was investigated in the rhesus monkey The toxin was administered to one male and one female monkey in a sub-acute dose for a period of 5.5 years. In a male monkey, a rapidly growing hepatocellular carcinoma of a giant-cell type developed 2.5 years after the toxin administration had been discontinued. The histopathology of the tumor is described. Observation of the female is continuing.

240. Halver, J.E. 1965. Aflatoxicosis and rainbow trout hepatoma. Pages 209-234 in Mycotoxins in Foodstuffs (Wogan, G.N., ed.). Cambridge, Massachussetts : Mass. Inst. Technol. Press. 291 pp.

Rainbow trout fed appropriate levels of 11 chemical carcinogens incorporated into a negative control test ration developed typical adenomatous, uabecular, or mixed-pattern hepatoma. Occasionally a cholangioma was also observed. Fat carefully extracted from the commercial ration and fed as part of the diet to young trout for 1 year also appeared to be a vector in hepatomagenesis. High load tests with crude aflatoxin concenuates showed massive liver damage and multiple haemorrhagic areas throughout the liver, caeca, and other viscera of small trout in a single-dose, 10-day test. Considerable typical liver damage could be observed when only 0.2 mg of the crystalline aflatoxins kg^{-1} body weight were administered. In a long-term feeding study crude and crystalline aflaloxins B_1 and G_1 extracted,

separated, and purified from contaminated groundnuts or cultured on wheat showed a very high incidence of primary liver cell carcinoma in trout fed only low levels of aflatoxins for 6 months to 1 year. Further current investigations in many laboratories should extend and define the effects of aflatoxicosis in rainbow trout hepatomagenesis.

241. Halver, J.E., Ashley, L.M., Smith, R.R., and Wogan, G.N. 1968. Age and sensitivity of trout to aflatoxin B_1. Federation Proceedings 27:552.

Young rainbow trout fed 20 µg kg^{-1} aflatoxin B_1 diet alter 4. 8. 12, and 16 or 20 weeks on control diet developed degrees of hepatomas directly related to size before insult; whereas, control fish fed CTD without toxin failed to exhibit nodules after 20 months on test. Numbers of gross tumors per group varied inversely with age before fish received toxin. Total dose was near constant at 9-10 µg B_1 $fish^{-1}$. Another study showed total dose at early age spread over 2-20 weeks yielded nearly the same number of tumors at termination. More aflatoxin B_1 increased incidence slightly but when 0.4 µg was ingested during the first four weeks, growth was impaired and acute liver damage was detected. Survivors developed classical hepatoma at 12 or 20 months. In contrast, fish fed 20 µg kg^{-1} of tetrahydrodesoxy aflatoxin or Wogan's compound 11 had only one or two with tumors alter 20 months. Total dose of each exceeded 10 *µg* $fish^{-1}$. Positive controls showed over 90 % with hepatoma at 20 months of 20 µg kg^{-1} B_1 diet insult and some fish had metastasis to other organs. Coho Salmon fed the same levels of aflatoxin B_1 as positive trout controls failed to develop gross hepatoma.

242. Harding, J.D.J., Done, J.I., Lewis, G., and Allcroft, R. 1963. Experimental groundnut poisoning in pigs. Res. Vet. Sci. 4: 217-229.

Landrace x Wessex pigs given feed from a farm where there had been outbreaks of groundnut poisoning developed typical signs, and 20 % toxic groundnut meal in the feed produced the expected lesions. The progress of changes in liver is described. There were sometimes high serum glutamic oxalacetic transaminase and alkaline phosphatase, in liver concentration of fat was increased with vitamin A was less. and ratio of weight of thyroid to bodyweight was low.

243. Hertrampf, J. 1978. [Groundnut meal and its problems.]. Erdnussschrot und seine probleme. Muhle + Mischfuttertechnik 115(3):36.

Problems arising from the contamination of groundnut meal with aflatoxin are reviewed. Progress in the detection of related compounds and knowledge of their toxic properties are summarized, with details of acute toxicity on monkeys, and indications of the chronic effects of very small doses on liver and kidneys of children. Transmission of the toxic constituents from animal feeds to cow's milk and pig liver and kidneys is also reported, almough there are no apparent indications of progressive accumulation. Aflatoxin in imported groundnut meal is discussed and the limits imposed by the EEC on feeds of different kinds are tabulated. The practical impliations of stringent limits on aflatoxin contents of meal imported into European and other countries are discussed, with particular reference to the danger that more heavily contaminated batches may be retained in the developing countries where the population relies heavily on groundnut meal as a source of protein. The possibility of detoxification with ammonia is briefly discussed, and it is noted that tests with rats indicate that the protein quality if thereby decreased by about 10%.

244. Hsieh, D.P.H., Wong, Z.A., Wong, J.J., Michas, C, and Ruebner, B.H. 1977. Comparative metabolism of aflatoxin. Pages 37-50 in Mycotoxins in Human and Animal Health. (Rodricks, J.V., Hesseltine, C.W., and Mehlman. M.A., eds.). Pathotox Publishers, Park Forest South. Illinois, USA. 807 pp.

This paper gives a comparative profile of the in vitro hepatic metabolism of aflatoxin B_1 by rat, mouse, duck, monkey, hamster, and human, and introduces certain correlations for predicting human and animal susceptibilities to acute and chronic aflatoxicosis comparing the relative activities of several in vitro metabolic pathways.

245. Koppang, N., and Helgebostad, A. 1972. [Aflatoxin poisoning in milk.J. Aflatoxinforgiftning hos mink. Vara Palsdjur 43(9): 262-265.

Groups of 3 mink were given a basal feed with 2, 1.5 or 1 % groundnut meal containing 4500 µg kg^{-1} aflatoxin. Two other groups of 3 were given 0.5 % and one group received no groundnut meal. The mink given 1 % groundnut meal or more died after 24 to 42 days with nonspecific inflammation of the stomach and intestines, degeneration and fatty infiltration of the liver, and extensive liver cell changes. Of one group given 0.5 % groundnut meal, 1 mink died after 48 days and the others were killed for examination; all had only slight liver changes. Of the other group, 1 died after 371 days and the others were killed; they had widespread tumors in the liver as well as fatty infiltration.

246. Kulczycki, J., Lesiak, M., and Polkowski, K. 1985. Effect of subtoxic doses of aflatoxin fed to cows on serum and milk composition. Pages 561-579 in Proceedings of V International Symposium on Mastitis Control (Wisniowski, J., ed.).

Experimental feed containing groundnut oilcake contaminated with aflatoxins B_1, B_2 and G_1 was fed once daily to 16 lactating cows to provide daily intakes of 1000, 2000 or 3000 µg aflatoxin. There was no correlation between aflatoxin intake and milk yield or incidence of udder disorders (including mastitis), but contents of

aflatoxin M_1 in milk were proportional to intake of aflatoxin B_1, irrespective of milk yield.

247. Lafont, P., Sarfati, J., Jacquet, J., Gaillardin, M., and Lafont, J. 1983. [Effect of pathological and nutritional factors on the mammary excretion of aflatoxin in cows.]. Influences de facteurs patholgiques et nutritionneles sur l'elimination de l'aflatoxine par la mamelle chez la vache. Microbiologic - Aliments - Nutrition 1(3): 292-300.

A field study involved 139 dairy cows in 7 herds, all receiving naturally contaminated groundnut meal at 1.5-4 kg day^{-1}. The concentration of aflatoxin M_1 in milk from hind quarters of 34 cows ranged from 290 to 4040 ng L^{-1}, increasing as milk somatic cell count (SCC) increased from 50000 to 1 million mL^{-1} and dose of aflatoxin B_1 increased from 1.35 to 2.75 mg day^{-1}. The average percentage of the aflatoxin B_1 dose which was excreted in milk increased from 0.74 ±0.11 to 3.22 ± 0.41 as SCC increased. Milk from 2 cows with Ketosis, and from 1 other cow, contained aflatoxin B, in concentrations similar to those of aflatoxin M_1 found in milk from other cows. Changes to the roughages part of the diet in 3 of the herds did not significantly alter the excretion of aflatoxin M_1 in milk, but in 2 herds aflatoxin M_1 was found in milk after the diet change.

248. Lancaster, M.C., Jenkins, F.P., and Philp, J. 1961. Toxicity associated with certain samples of groundnuts. Nature 192: 1095-1096.

Rats (4 weeks old) in 3 groups of 9 males and 7 females were fed for 30 weeks on a purified diet based on casein with 0.75 % L-cystine added, groundnut oil, starch and cellulose, or that diet with 20 % either Brazilian or Indian groundnut meal to replace the same percentage of starch. In each group, 2 of each sex were killed after 9 weeks. Up to 9 weeks, growth rate, intake of feed and efficiency of feed utilisation were similar on the purified diet and that with the Indian meal. Growth on Brazilian meal was less, significantly so in males, and intake and efficiency also were reduced. On exmination post-mortem at 9 weeks macroscopic lesions were seen in livers of rats given the Brazilian meal, but not in other groups. After 30 weeks there were macroscopic lesions in livers and lungs. Findings are described in detail. They indicate that the diet with Brazilian groundnut meal is carcinogenic.

249. Lewis, G., Markson, L., and Allcroft, R. 1967. The effect of feeding toxic groundnut meal to sheep over a period of five years. Veterinary Record 80: 312-314.

A group of 5 Kerry crossbred lambs 3 months old at the start were fed for 5 years on hay and a concentrate with 20 % highly toxic groundnut meal, containing 1750 µg kg^{-1} aflatoxin during the first 3-5 years, and 1000 µg kg^{-1} since then. Lambs born were added to the experimental group. A control group, for the first 3 years, got concentrate with fishmeal and maize gluten supplying protein. In the group given aflatoxin, fertility was lower and growth during the first 18 months was also retarded. One lamb was killed after 5 months and others died during the experiment. Damage to the liver was found in only one, and there were no typical signs of groundnut poisoning. In 2 sheep there were nasal chondromata; the rarity of this disorder and the possibility of its being related to the feed are discussed.

250. Loosmore, R.M., and Harding, J.D.J. 1961. A toxic factor in Brazilian groundnut causing liver damage in pigs. Veterinary Record 73: 1362-1364.

High mortality is reported in young pigs in a herd given Brazilian groundnut meal (17.5 % in the starter pellets and 8.75 % in the sow and weaner meal). Pigs of the same herd transferred to another farm were not affected. Sows lost appetite and tended to subsist on grass. Liver lesions in acute, subacute and chronic types of poisoning are described. There were some similarities to chronic copper poisoning. There was no difference in toxicity between solvent-and expeller-processed meals. Experimental poisoning was also studied. The suspected diet was given to 2 pigs for 4 weeks and 5 others got a meal with 20 % Brazilian groundnut meal. All developed signs of poisoning, with typical acute or subacute liver damage. Pigs given another commercial meal or 20 % Nigerian groundnut meal were not poisoned.

251. Madejski, Z. 1978. [Investigation of the toxic effects on rats and ducks of diets supplemented with rape seed oil meal and groundnut meal, containing some natural goitrogens (VTO, ITC0 and aflatoxins.]. Badanie toksycznosci pasz z dodatkiem naturalne goitrogeny (VTO, ITC) i aflatoksyny dla szczurow i Kaczek. Annales Universitatis Mariae Curie-Sklodowska Sectio DD 32/33: 95-116.

252. Madhavan, T.V. 1967. Effect of prednisolone on liver damage in rat induced by aflatoxin. Journal of Pathology and Bacteriology 93: 443-447.

The effect of prednisolone on aflatoxin liver injury was studied in two experiments on weanling rats kept on diets containing 20 % and 5 % protein. Rats on the high protein diet were apparently healthy and showed only mild histological changes in the liver when they were given 70 µg of the toxin per day for 20 days. The administration of 1000 µg or 200 µg of prednisolone daily did not alter the picture in any way. Rats on the low protein diet readily developed typical lesions in less than 20 days on the same dose of the toxin. In the presence of the higher dose of prednisolone there was marked inhibition of bile-duct proliferation and fat accumulation. This inhibitory effect was less marked when the smaller dose was given. The clinical signs and mortality due to the toxin did not appear to be influenced by the steroid.

253. Madhavan, T.V., and Gopalan, C. 1965. Effect of dietary protein on aflatoxin liver injury in weanling rats. Arch. Path. 80: 123-126.

Weanling male rats fed low protein diets (4 % casein) and given 50 ug of aflatoxin daily, developed severe liver lesions characteristic of aflatoxin injury within 20 days. Those fed 20 % casein in the diet had only mild changes in the liver, including vacuolation and typical, possibily precancerous, cells. These were not encountered in the protein-deficient groups.

254. Madhavan, T.V., and Gopalan, C. 1968. The effect of dietary protein on carcinogenesis of aflatoxin. Arch. Pathol. 85: 133- 137.

Three experiments were conducted in which different daily oral doses of aflatoxin were administered to weanling rats for varying periods. In each experiment, the animals were divided into two groups, one (HP) receiving 20 % casein and the other (LP) a 5 % casein in the diet, the former being pair fed to the latter. In all, 30 rats on the HP diet and 12 on the LP diet survived for more than a year. Of the former. 15 (50 %) developed hepatomas or tumors in other organs while the other 15 showed only precancerous lesions in the liver. In contrast, none of the 12 rats on the low-protein diet showed tumors or precancerous lesions of the liver while only one developed a kidney tumor. It is concluded that a low level of dietary protein has an inhibitory effect on aflatoxin carcinogenesis in the rat.

255. Madhavan, T.V., and Rao, K.S. 1966. Hepatic infarction in ducklings in aflatoxin poisoning. Arch. Pathol. 81: 520-524.

The results of feeding different daily doses of pure aflatoxin to day-old ducklings up to a maximum period of eight weeks are reported. Ducklings given 40 ug to 10 µg of aflatoxin per day died within five days. In addition to the characteristics lesions, a number of them had hepatic infarcts, with or without arterial occlusion. Severe congestion was present in all. Lower doses of the toxin caused milk chronic lesions which were regressive.

256. Madhavan, T.V., and Rao, K.S. 1967. Tubular epithelial reflux in the kidney in aflatoxin poisoning. Journal of Pathology and Bacteriology 93: 329-331.

Aflatoxin produces liver lesions in different species. Renal changes have been non-specific, such as parenchymal haemorrhages in ducklings given large doses of the toxin, and lipid accumulation in the tubular epithelium along with exudates in Bowman's spaces in monkeys. In this paper the authors describe tubular epithelial refluc resulting from acute aflatoxin poisoning in guinea-pigs.

257. Madhavan, T.V., Rao, K.S., and Tulpule, P.G. 1965. Effect of dietary protein level on susceptibility of monkeys to aflatoxin liver injury. Indian Journal of Medical Research 53: 984-989.

Studies were undertaken to examine the effect of dietary protein deficiency on the susceptibility of monkeys to aflatoxin injury. Young rhesus monkeys were kept on low and high protein diets and given aflatoxin in two doses in different groups. All monkeys in both dietary groups receiving 500 ug a day of aflatoxin developed fatty liver and biliary fibrosis in 16 to 30 days. Monkeys receiving 100 ug a day on low protein diets behaved similarly, whereas the corresponding high protein group was apparently healthy, It is concluded that dietary protein deficiency markedly enhances the susceptibility of primates to aflatoxin. The implications for the human situation are discussed.

258. Madhavan, T.V., Tulpule, P.G., and Gopalan, C. 1965. Aflatoxin induced hepatic fibrosis in rhesus monkeys. Arch. Pathol. 79: 466-469.

Daily oral administration of high doses of aflatoxin to young monkeys fed a stock diet produced fatty livers and biliary fibrosis in four weeks. The pathological features are described in detail and the implications in experimental and human liver injury discussed.

259. Magwood, S.E., Annau, E., and Corner, A.H. 1966. Induced tolerance in turkeys to aflatoxin poisoning. Canadian Journal of Comp. Medical and Veterinary Science 30: 17-25.

The consumption of moderately poisonous levels of toxic groundnut meal by turkey poults induced a degree of tolerance to aflatoxin. Poults thus "conditioned" gained more than "unconditioned" birds or successively higher levels of aflatoxin, and survived and gained on rations which killed mature turkeys. Ingestion of aflatoxin led to changes in the serum electrophoretic patterns followed in a few weeks by a return to nearly normal patterns. Subsequent increases in aflatoxin intake induced only minimal changes in the serum pattern but the tolerance appeared unrelated to the hepatotoxic effect because extensive pathological changes developed in the liver.

260. Maryamma, K.I., and Sivadas, C.G. 1975. Aflatoxicosis in goat - an experimental study. Indian Veterinary Journal 52: 385-392.

Pathological changes in experimental aflatoxicosis in goat are described. Pathological changes included renal and hepatic degeneration and necrosis. These changes were observed in the pituitary, thyroid and adrenal glands in the aflatoxin fed goats. Considerable reduction in serum vitamin A level was also noted.

261. Murthy, T.R.K., Jemmali, M., Henry, Y., and Frayssient, C. 1975.

Aflatoxin residues in tissues of growing swine : effect of separate and mixed feeding of protein and protein-free portions of the diet. Journal of Animal Science 41(5): 1339-1347.

In diet of growing pigs, half the protein was from groundnut meal contaminated with aflatoxin. Pigs were given complete diet freely, or the protein portion in the amount eaten by the first group, with the non-protein portion given freely. With separate protein, pigs had aflatoxin B_1, B_2 and M in liver, gall bladder, heart, muscle and kidney, and developed signs of poisoning. With complete diet there was no sign of poisoning, and no aflatoxin was delected in organs of the one pig killed of that group.

262. Nabney, J., Burbage, M.B., Allcroft, R., and Lewis, G. 1967. Metabolism of aflatoxin in sheep : Excretion pattern in the lactating ewe. Food and Cosmetics Toxicology 5: 11-17.

The excretion *of* aflatoxin components was detemiined in milk, urine and faeces of a lactating ewe after oral administration of a single dose (1 mg kg^{-1}) of a mixture of anatoxins (B_1 36 %; G_1 52 %; B_2 3 %; G_2 2 %). About 90 % of the total anatoxin excretion in the milk and urine occurred in the first 48 h. No aflatoxin was detected in milk after 6 days, and none in urine and faeces after 8 and 9 days respectively. Only 8.1 % of the total dose was recovered in an identifiable form, the

milk containing 0.1 %, the urine 6.4 % and the faeces 1.6 %. Some unchanged anatoxin B_1 was present in the faeces but only trace amounts were found in milk and urine. Aflatoxin M, was excreted in relatively large amounts by all three routes, but mainly in the urine, and was the major form of aflatoxin in milk and faeces. Aflatoxin G, was excreted chiefly in the urine and faeces with only traces in the milk.

263. Newberne, P.M., Carlton, W.W., and Wogan, G.N. 1964. Hepatomas in rats and hepatorenal injury induckIings fed peanut meal or *Aspergillus flavus.* Pathol. Vet. 1: 105-132.

When rats were fed groundnut meal (made from groundnuts grown in the USA) for 10-12 months, a high incidence of hepatomas and renal damage was observed. Direct extension of hepatomas to the mesentery and metastasis to lungs occurred in several animals. Ducks fed the toxic meal (which was later found to contain between 100 and 3500 µg kg^{-1} aflatoxin) for a month developed liver cell damage and bile duct hyperplasia. Lesions induced in ducklings by administering into the stomach by tube extracts of *A. flavus* cultures daily for five days were comparable to those induced with toxic groundnut meal.

264. Newberne, P.M., and Gross, R.L. 1977. The role of nutrition in aflatoxin injury. Pages 51-65 *in* Mycotoxins in Human and Animal Health (Rodricks, J.V., Hesseltine, C.W., and Mehlman, M.A., eds.).

This paper comprehensively reviews nutritional factors that influence responses of animals and humans to aflatoxin. The effects of nutrients on the metabolism and disposition of aflatoxins are also discussed.

265. Newberne, P.M., Harrington, D.H., and Wogan, G.N. 1966. Effects of cirrhosis and other liver insults on induction of liver tumors by aflatoxin in rats. Laboratory Investigation 15: 962-969.

Choline deficiency, partial hepatectomy, repeated biopsy, and iron injections, alone or in combination, did not result in liver tumor induction unless aflatoxin was superimposed at some point during the experiment. Serum protein levels were elevated in rats with tumors induced by aflatoxin-contaminated groundnut meal. It was concluded that cirrhosis was the only form of liver injury among those used in these experiments that influenced neoplasia.

266. Newberne, P.M., Hunt, C.E., and Wogan, G.N. 1967. Neoplasmas in the rat associated with administration of urethan and aflatoxin. Experimental Molecular Pathology 6: 285-289.

Groundnut meal was extracted with methanol to remove over half the aflatoxin. or again with chloroform to remove all that could be detected, or it was not extracted. Groups of 10, 20 or 25 male Charles-River caesarean-derived rats aged 3 weeks were given freely (until they died) one of 4 diets with casein 7 %, groundnut meal 34 % and sucrose 32 to 40 %, or one of 2 diets with casein 18 %, no groundnut, and sucrose about 55 %, all with minerals and vitamins. Lard, 12 or 20 %, was added to give a total lipid level of 20 %. From one diet with the twice-extracted meal and one with casein 18 % were omitted the choline chloride that was in the other diets 0.2 % for the first week, 0.1 % for the second, and the vitamin B_12 50 mg per 100 g. Aflatoxin extracted chemically and biologically was for diet with whole meal 1500, with meal extracted once 400, and for other diets under 5 μg kg^{-1}. To some of those diets was added urethane (ethyl carbamate) 0.1, 0.2, 0.4 or 0.6 %. Liver carcinoma as described in the literature was found in 13 of 20 mice given 400 µg kg^{-1}, and in 9 of 10 mice given 1500 µg kg^{-1} aflatoxin. Lifespan ranged between groups from 219 to 568 days. The shortest was that of the group given 1500 μg kg^{-1} aflatoxin and 0.6 % urethane; the lower though still significant incidence of carcinoma in that group might depend on the short lifespan, but in other groups a lower incidence appeared to depend on the giving of both additives together. The effect of aflatoxin and counter effect of urethane were both related to dose. The less

potent carcinogen, urethane, caused malignant lymphoma and vascular lesions of liver and lungs. Choline deficiency caused nodular cirrhosis but no tumors.

267. Newberne, P.M., Russo, R., and Wogan, G.N. 1966. Acute toxicity of aflatoxin B_1 in the dog. Pathol. Veterinaria 3(4):331-340.

This work is a study of the acute effects of aflatoxin B_1 in dogs. The dog has a remarkable susceptibility to aflatoxin B_1 by oral and intraperitoneal routes. Intraperitoneal administration resulted in the shortest survival time and the most profound pathological changes. However, massive single oral doses produced lesions of similar nature and intensity. Oral administration of aflatoxin in small, divided doses, over extended periods proved to be less toxic. The experimentally induced disease resembled hepatitis X in many aspects.

268. Newberne, P.M., Wogan, G.N., Carlton, W.W., and Abdel-Kader, M.M. 1964. Histopathologic lesions in ducklings caused by *Aspergillus flavus* cultures, culture extracts and crystalline aflatoxins. Toxicology and Applied Pharmacology 6: 542-546.

Male White Peking ducklings were given from 32 h old a purified diet with aflatoxin fractions B_1, B_2 or G_1, or were given extracts from infected groundnuts or wheat or from liquid cultures of *Aspergillus flavus.* Histopathological lesions produced in liver, necrosis of parenchyma and proliferation of bile ductule cells, are described and illustrated. Variations were only in degree and intensity. Each of the crystalline aflatoxins could cause lesions in liver identical with those produced by extracts and cultural extracts containing the toxins; aflatoxin B_1 had the greatest potency. Ducklings given infected groundnuts or wheat as 10 % of the diet, Otherwise of a chick starter mash, had similar lesions but of greater severity.

269. Newberne, P.M., Wogan, G.N., and Hall, A. 1966. Effects of dietary modifications on response of the ducklings to aflatoxin. Journal of Nutrition 90: 123-130.

The short-term effects of dietary modifications on the response of ducklings to aflatoxin were determined. In the presence of aflatoxin, dietary supplements of 4 % of methionine, 1 % of arginine or 0.8 % of lysine, as individual additions, depressed weight gain and decreased mortality. The addition to the diet of 1 % arginine and 0.8 % lysine with, but not without, aflatoxin sharply decreased weight gain and increased mortality. The addition of glutathione or cysteine to the diet as sources of sulfhydral groups had no effect on toxicity. Autoclaving aflatoxin-contaminated groundnut meal decreased toxicity and markedly increased weight gains of ducklings over a 9-day period.

270. Panda, P.C., Murti, S.A., Sreenivasamurthy, V., Murti, I.A.S., and Amla, I. 1975. Effect of aflatoxin on the haematological picture of albino rats and guineapigs. Indian Journal of Experimental Biology 13: 569-570.

Studies on the effect of aflatoxin B_1 on the blood picture of albino rats and guineapigs revealed a depression in the total RBC and WBC counts including haemoglobin content of blood. Toxin fed animals exhibited anisocytosis and poikilocytosis. The blood clotting time was prolonged. The implication of these findings with respect to anaemic symdrome in rats and guineapigs is discussed.

271. Panda, P.C., Sreenivasamurthy, V., and Parpia, H.A.B. 1970. Effect of aflatoxin on reproduction in rats. Journal of Food Science and Technology 7: 20-22.

The effects of feeding aflatoxin B at a level of 50 µg day^{-1} $animal^{-1}$ on the reproductive performance of albino rats was studied. No effect on the oestrus cycle of the adult was observed. The litter size and the birth weight of the young rats was unaffected. However, significant increase in intrauterine foetal resorption in the pregnant rat and significant decrease in the weaning weight of the young rats were observed in the group fed with aflatoxin.

272. Parpia, H.A.B., and Sreenivasamurthy, V. 1969. Report on recent studies on aflatoxin. Document 2.17/25. P.A.G. (FAO/WHO/UNICEF), September 1969 Meeting - Geneva.

Detoxification of groundnut flour with hydrogen-peroxide is feasible and effective in destroying aflatoxin. This treatment is now used commercially, particularly in the preparation of milk substitutes containing groundnut protein. A mixture of phosphine and ammonia can be used as a fungicide for treatment of groundnuts pods immediately after harvest for controlling growth of *Aspergillus flavus.* Aqueous ethanol can be used effectively to extract about 90 % of aflatoxin from split groundnuts without removing any significant amounts of fat. A study of excretory metabolites of aflatoxin in rats, guinea-pigs and monkeys showed considerable differences in the excretion of aflatoxin M and B between these species. In an exploratory study of the possible role of aflatoxin in infantile liver cirrhosis about 8 % of the urine samples examined contained 10-50 µg of aflatoxin B in a 24 h sample. The pattern of muscular damage in cirrhotic children was similar to the muscular damage caused by aflatoxin fed to albino rats.

273. Paterson, J.S., Crook, J.C., Shand, A., Lewis, G., and Aileron, R. 1962. Groundnut toxicity as the cause of exudative hepatitis (oedema disease) of guinea-pigs. Veterinary Record 74: 639-640.

Diets with 15 % toxic groundnut meal were made up and tested at 2 centres. All guinea-pigs given the diets died within 55 days. Histological changes in the liver were typical of groundnut poisoning. A diet used in 1957, containing 15 % toxic-groundnut meal, had caused oedema and deaths in guinea-pigs, and when tested it twice as toxic as the newly formulated feed. In breeding guinea-pigs there was no ill effect when they were given the new diet with hay to appetite. When the hay was restricted to 10 g the stock deteriorated and all guinea-pigs died of oedema disease.

274. Patterson, D.S.P., Shreeve, B.J., Roberts, B.A., Berrett, S., Brush, P.J., Glancy, E.M., and Krogh, P. 1981. Effect on calves of barley naturally contaminated with ochratoxin A and groundnut meal contaminated with low concentration of aflatoxin B,. Research in Veterinary Science 31(2):213-218.

Four groups of six 12-week-old male Friesian calves were fed diets containing 390 to 540 µg kg^{-1} ochratoxin A, 320 to 500 µg ochratoxin A plus 12 to 13 µg kg^{-1} aflatoxin B_1, 10 to 13 µg kg^{-1} anatoxin B,, or a control diet containing neither toxin. At the end of the 87-day experiment there was no evidence of kidney or liver damage in any group of animals as judged by serial plasma and urine enzyme assays, haematological examination including the assay of blood coagulation factors, gross appearance at autopsy or histopathological assessment at the light microscopic level. Nor was there any significant alteration in serum IgA, IGM, IgG, and IgG, levels or in litres to environmental bacterial and viral antigens. Trace amounts of ochratoxin A were detected in kidneys of five of the 12 calves exposed to this toxin, but all 12 kidneys contained residues of the metabolite ochratoxin alpha (less than 5 to 10 µg kg^{-1}). Traces of aflatoxins B_1 and M_1 were detected in the liver of one

of the 12 calves exposed to aflatoxin B_1, but nine kidneys contained the metabolite aflatoxin M_1 (less than 0.01 to 0.03 µg kg^{-1}). There was no evidence of interaction between the two toxins.

275. Platonow, N. 1964. Effect of prolonged feeding of toxic groundnut meal in mice. Veterinary Record 76: 589-590.

Groups of 12 male A.D.R.I. mice of 20 to 25 g bodyweight were fed for at least 3 months on diets with no toxic groundnut meal or 15, 30 or 80 %. The meal had aflatoxins B_1 and G_1 (each 4500 ug kg^{-1}), and aflatoxins B_1 and G_2 (each 600 µg kg^{-1}). There was no ill effect on appetite or growth, and no gross pathological signs of poisoning were seen in carcases at the end of the experiment.

276. Platonow, N. 1965a. The effect of feeding toxic groundnut meal to chickens on oxidized pyridine nucleotides levels of liver and serum. Canadian Journal Comp. Med. 29: 23-27.

Oxidised pyridine nucleotides were estimated by measuring the extinction coefficient at 260 mµ (F260) of the acid extracts of liver homogenates and serum of chickens during 6 weeks on a diet with 30 % groundnut meal contaminated with aflatoxins. The chickens were White Leghorns of 510 g liveweight at the start in 5 groups each of 6 males and 6 females; groups were killed after 1, 2, 4 and 6 weeks. Serum was tested by iodine agglutination. After 2 weeks on the toxic diet, liver extracts showed a significant depression of F260. The value returned to within the normal range after 4 weeks on the diet and remained constant until the end of the experiment. In serum values were also reduced after 2 weeks and remained low. The iodine agglutination test was negative after 1 and 2 weeks, showed a weak reaction after 4 weeks and a positive reaction after 6 weeks.

277. Platonow, N. 1965b. Detection of urocanase in the blood of chickens chronically poisoned with toxic groundnut meal. Canadian Journal Comp. Medical and Veterinary Science 29: 94-96.

Male White Leghorn chickens (4 weeks old) were divided into 3 groups of at least 8 each. Half of each group got a diet wtih 30 % of toxic groundnut meal for 2, 4 or 6 weeks. The diet contained 3100 µg kg^{-1} aflatoxin. The other half of each group were given a balanced commercial feed. Urocanase activity was detected in the blood serum of 3 of 9 chickens with signs of poisoning at 2 weeks. By the sixth week 5 of 8 poisoned chickens had urocanase in serum. In liver urocanase per g tissue was uniform throughout the experiment but in total liver its activity increased toward the end of the experiment.

278. Purchase, I.F.H. 1967. Acute toxicity of aflatoxins M_1 and M_2 in one-day-old ducklings. Food and Cosmetics Toxicology 5: 339-342.

The acute toxicity of aflatoxins B_1, M_1, and M_2 in one-day-old Pekin ducklings was estimated to be 12, 16 and 61.4 µg $duckling^{-1}$. Single doses of each of the three aflatoxins produced similar liver lesions. Whereas a high dose produced extensive haemorrhagic necrosis with little or no bile-duct proliferation, an intermediate dose induced slight bile-duct proliferation and varying degrees of hepatocellular necrosis and a low dose caused considerable bile-duct proliferation and extensive degenerative changes in liver cells. Renal congestion at low doses of M_1 and M_2 was accompanied by degenerative changes (vacuolation) of the convoluted tubules but frank necrosis was only seen with M_1. Aflatoxin B_1, even in a high dose, induced only slight degenerative changes of the tubules. None of the aflatoxins induced glomerular damage.

279. Rajan, A., Nair, K.M., and Sivadas, C.G. 1973. Comparative study of experimental aflatoxicosis in cross-bred Jersey and buffalo calves. Kerala Journal of Veterinary Science 4: 109-113.

A comparative study of aflatoxicosis in crossbred Jersey calves and buffalo calves showed the greater susceptibility of buffalo calves. Clinically, icterus was present in both species. Other features of aflatoxicosis included weight loss, weakness, failure to thrive, and ascites. Considerable reduction in erythrocyte sedimentation rate was observed in buffalo calves. Liver was pale yellow, enlarged and fixable with round border.

280. Reddy, G.S., Tilak, T.B.G., and Krishnamurthi, D. 1973. Susceptibility of vitamin A - deficient rats to aflatoxin Food and Cosmetics Toxicology 11: 467-470.

Weanling albino rats were divided into groups of six males and six females. They were maintained on a vitamin A-free diet, one group being fed ad lib. and the second being pair-fed with the first. Rats in the second group were given an oral supplement of 100 IU vitamin A animal^{-1} day^{-1}. At the end of a feeding period of 9 weeks, a crystalline preparation of aflatoxin was administered in a single dose of 3.5 mg kg^{-1} bodyweight. Male rats in the first group showed an increased susceptibility to the toxin as evidenced by rapid mortality and severe liver damage, midzonal in distribution. Their hepatic stores of vitamin A were severely depleted. Vitamin A-supplemented male rats showed only mild hepatic damage to the periportal parenchyma. Liver damage was minimal in female rats of both groups and was unaffected by the amount of vitamin A stored in the liver.

281. Richir, C., Martineaud, M., Toury, J., and Dupin, H. 1964. [Carcinogenic effect of diets with contaminated groundnuts. |. Sur les effets cancerigenes de regimes contenant des arachides contaminees. C.R. Soc. Biol. 158: 1375-1379.

Groups of 9 W.A.G. rats of each sex and of average weight of 168 g were given for 200 days diets with 20, 40 or 80 % crushed groundnuts. Chemical and biological tests with ducklings showed that the nuts had 1000 µg kg^{-1} aflatoxin. Ten male rats got a control diet with 20 % groundnut oilmeal. All rats that ingested groundnuts became ill and many died. Hepatomata were found in some rats in all groups, or there were signs of a precancerous state in the liver. Other lesions were in the bronchi, and in the testes of males which became impotent. Fertility of females was unimpaired. Intake of aflatoxin by the first 2 rats with tumors was 2350 and 1310 *µg* in 122 and 134 days. The lethal dose of aflatoxin for rats is considered to be 10000 µg per kg bodyweight.

282. Richir, C., Toury, J., Giorgi, R., and Dupin, H. 1964. [The liver lesions in ducks treated with extracts of groundnuts contaminated with *Aspergillus flavus*.]. Analyse des lesions hepatiques du caneton traite par des extraits d'arachides contaminees par *Aspergillus flavus*. Pathol. Biol. 12:980-987.

The method of estimating aflatoxin by fluorescence and its extraction by chloroform from samples from Senegal of groundnuts contaminated with *Aspergillus flavus* are described. The extract was given to groups of Pekin Khaki ducklings (2 days old) to supply different amounts of the toxic substances for up to 7 days. Damage caused in the liver of the ducklings by aflatoxin is described. Most deaths of the birds occurred within 2 days. In the ducks dying early, there was generally necrosis of the liver. In those which died later there were sometimes signs of necrosis, but mainly there was some proliferation of cells. In survivors there was regeneration after initial necrosis. It is suggested that the toxin may contain different substances, one causing necrosis and the other proliferation. Relative toxicity estimated from fluorescence, number of deaths and types and degree of lesions found were not closely related.

283. Rogers, A.E., and Newbcrne, P.M. 1969. Aflatoxin B_1 in carcinogenesis in lipotrope-deficient rats. Cancer Research 29: 1965-1972.

Male Fischer rats were fed either a diet severely deficient in lipotropic agents or control diet which induced a borderline lipotrope deficiency. They were given a carcinogenic dose of aflatoxin B_1 over a 3-week period and were scarified at intervals of 1 day to 1 year later. A localized hyperplastic response of hepatocytes, measured by thymidine-^{3} H labeling in autoradiographs and by mitotic counts, was found as early as 1 day alter administration of the full dose of aflatoxin in the rats fed the control diet. This response persisted up to the time of carcinoma development 6 months to 1 year later and was accompanied by histologic and histochemical abnormalities of the hyperplastic cells. The early hyperplastic response was not found in the livers of the severely deficient rats, but a few hyperplastic nodules developed after 6 months.

284. Salmon, W.D., and Newherne, P.M. 1963. Occurrence of hepatomas in rats fed diets containing commercial peanut meal as a major source of protein. Cancer Research 23: 571-575.

Charles River CD rats (4 to 5 weeks old) were given diets in which the chief source of protein was dried lean beef or extracted or commercial groundnut meal, the last from 4 lots of high-quality solvent-process meal. In the first trial. 13 of 59 rats given diets wim 6 or 7.9 % dried beef and 33.3 or 25 % methanol-extracted groundnut meal and 2 of 10 given 34 % groundnut meal alone developed hepatomata, but none of the 50 rats given beef as sole protein. In subsequent work. 64 of 73 rats given commercial meal developed hepatomata; that was not affected by substitution of 7.9 % casein for the beef or of lard for beef fat. In addition, 16 of those 73 rats developed adenomata of kidney tubules, not found in rats on the extracted groundnut meal. The hepatomata was of a type histologically different from that produced by choline deficiency and was not affected by choline intake.

The groundnut meal showed no sign of mold but the possibility of some infection by molds before the meal was prepared was not eliminated. The relatively low incidence of tumors in the first compared with the later trials suggested that the carcinogenic agent was decreased by extraction with hot methanol.

285. Sargeant, K., Allcroft, R., and Carnaghan, R.B.A. 1961. Groundnut toxicity. Veterinary Record 73: 865.

Samples of grounndut meals and decorticated nuts from Nigeria, French West Africa, and the Gambia were examined. Many were previously found toxic in trials with ducklings. Some of the meals were already suspect, being associated with the deaths of calves and sheep, others were not, and among those several were not toxic. Some extracts from decorticated nuts were also toxic. It was suggested that all consignments of groundnuts should be tested before inclusion in livestock feeds.

286. Sargeant, K., O'Kelly, J., Carnaghan, R.B.A., and Allcroft, R. 1961. The assay of a toxic principle in certain groundnut meals. Veterinary Record 73: 1219-1223.

The toxic principle in certain samples of Brazilian groundnut meal was extracted and concentrated 250 times on a weight basis. This extract produced mortality in ducklings and turkey poults and histological liver lesions identical to those seen in field outbreaks of so-called Turkey "X" disease. The dosing of concentrated extracts to young ducklings gives a rapid and sensitive method for testing the toxicity or otherwise of groundnut meals. The toxic principle is neither a pyrrolizidine alkaloid nor the N-oxide of such an alkaloid. It is suggested that the toxic principle is derived from a microorganism, possibly a fungus. A similar toxic principle was found in some groundnut meals from India, Uganda and Tanganyika, French West Africa, Nigeria, Gambia and Ghana.

287. Sargeant, K., O'Kelly, J., Carnaghan, R.B.A., and Allcroft, R. 1961. The assay of a toxic principle in certain groundnut meals. Veterinary Record 73: 1215-1219.

The toxic substance present in some Brazilian and African groundnut meals which causes Turkey "X" disease was shown to be even more toxic to ducklings. The signs included loss of appetite, poor growth rate, a tendency to down or feather pulling, liver damage and sometime purple discoloration of the legs and feet in white-skinned birds over 3 weeks old. occasionally accompanied by lameness. Not all groundnut meals tested were toxic. Toxic groundnut meal as 10 % of the diet caused death within 6 to 20 days. This interval and the severity of the gross lesions depended on the toxicity of the sample, the amount eaten and the age of the ducklings ; younger birds were more susceptible. With chicks, 10 % groundnut meal reduced growth rate but did not cause death ; with chickens 45 days old 15 % groundnut meal caused 3 deaths in the first 3 weeks but no more in the rest of the feeding period lasting 9 months.

288. Schoental, R. 1961. Liver changes and primary liver tumours in rats given toxic guinea pig diet (M.R.C. diet 18). British Journal of Cancer 15: 812-815.

A batch of guinea pig Diet 18 containing groundnut meal was suspected to be toxic; when fed to rats it induced liver tumours.

289. Sen, S. 1972. Aflatoxin hepatic carcinogeneis : An experimental comparative study. Journal of Medical Research 60: 1261-1266.

Toxic groundnut meals containing aflatoxin when fed to albino rats produced hepatic carcinoma. Morphological alterations in the liver induced by aflatoxin were studied in great detail. The biological picture of primary hepatic carcinoma in rats has been analysed. Probable mode of aflatoxin hepatic carcinogenesis is discussed. Attempts have been made to compare these experimental observations to those seen in naturally occurring primary hepatic carcinoma in man.

290. Shank, R.C., and Wogan, G.N. 1966. Acute effects of aflatoxin B_1 on liver composition and metabolism in the rat and duckling. Toxicok gy and Applied Pharmacology 9: 468-476.

In the weanling male rat. a single oral $LD_{2()}$ of aflatoxin B_1 caused a transient inhibition of leucine incorporation into liver proteins. The brief (6-h) suppression was followed by a prolonged period of stimulation of heaptic protein synmesis which persisted for 3-6 days alter dosing. Liver deoxyribonucleic acid metabolism was not altered during this period. Five consecutive daily administrations of sublethal doses of the toxin, each equivalent to 10 % of the $LD_{5()}$, caused significant suppression of growth and liver weight in the duckling. Liver glycogen content and glycogenesis were also decreased, whereas lipid content was elevated. Treatment of weanling male rats according to the same dosing schedule resulted in suppresion of growth and lowering of food intake, efficiency of food utilization, and liver weight; but liver composition was not significantly altered in this species.

291. Shankaran, P., Shankaran, R., Raj, H.G., and Venkitasubramanian. 1970. Biochemical changes in liver due to aflatoxin. British Journal of Experimental Pathology 51(5): 487-491.

The effect of a single dose of aflatoxin on me content of glycogen, lipid and protein of mouse liver was studied 2 h after injection of me toxin. No significant changes in these compounds were observed. Enzymes in livers of mice were assayed 2 h and 8 h alter aflatoxin treatment. In general, the enzymes of particulate fraction and

isocitrate dehydrogenase in the supernatant showed a significant increase; the remaining enzymes of the supernatant fraction decreased at both time intervals. The response of the activities of aconitase, fumerase and ATP-ase 2 h after injection were the opposite of those at 8 h. The results are explicable in terms of mitochondrial injury of the aflatoxin- treated animals.

292. Shankaran, K., Raj, H.G., and Venkitasubramanian, T.A. 1970. Effect of aflatoxin on carbohydrate metabolism in chick liver. Enzymologia Acta Biocatalytica 39: 371-378.

The activities of UDP glucose-glycogen transglucosylase, glycogen-phosphorylase, phosphoglucomutase, glucose-6- phosphatase, and combined HMP dehydrogenases were assayed and the incorporation of glucosc-U-^{14}C into liver glycogen in vivo studied in aflatoxin B_1-treated and control chicks. A significant decrease in the activities of the first four enzymes and an increase in that of the last one were noted. The decrease in UDP glucose-glycogen transglucosylase activity was of significant magnitude to account for the depletion of hepatic glycogen, while the catabolic phase of glycogen metabolism was almost unaffected in aflatoxin B_1 administered chicks. The increase in combined HMP dehydrogenases may probably be due to an enhanced HMP shunt pathway.

293. Siller, W.G., and Ostler, D.C. 1961. Letters to Editor. Veterinary Record 73: 134-138.

Diagnostic symptoms of Turkey X disease are described. These are based on the presence of histopathological liver lesions.

294. Solomon, G., Jensen, R., and Tanner, H. 1965. Hepatic changes in rainbow trout *(Salmo gairdneri)* fed diets containing peanut, cottonseed, and soybean meals. American Journal of Veterinary Research 26: 764-770.

An outbreak of hepatoma in hatchery rainbow trout led to investigation of the effects of some feedingstuffs on the liver. The control diet was the Colorado diet, of spray-dried skimmed milk 5, delactosed whey 5, white-fish meal 16, solvent-extracted soya meal 16, wheatings 20, dried brewer's yeast 10, fermentation solubles 10, unextracted liver meal 15, vitamins A and D in oil 2 and salt 1 % by dry weight, with vitamin premixes. In one test diet a solvent-extracted degossypolished cottonseed meal with 41 % protein replaced the soya meal, and to another was added crude toxic groundnut meal with 10000 µg kg^{-1} aflatoxin. The diets were given for 12, 10 and 9 months, respectively, to replicate groups of 300 fish, and some of each group were killed after 6, 9 and 12 months. With the control diet the livers showed no significant pathological change. With the cottonseed meal diet some hyperplasia of bile duct epithelium and cholangitis and hyperplasia of bile duct epithelium in fish 6 months old which had had it for 3 1/2 months, progressing in the next 6 months until cytoplasmic degeneration, cholangioma, hepatoma, hyperchromarism and hypertrophic hepatocytes were common. Mixed cholangioma-hepatoma was rare.

295. Svobodu, D., Grady, H., and Higginson, J. 1966. Aflatoxin B_1 injury in rat and monkey liver. American Journal of Pathology 49(6): 1023-1051.

Acute studies of ultrastructural and biochemical abnormalities induced by aflatoxin B_1 in the liver of rats and monkeys revealed abnormalities in nuclear fine structure. These were accompanied by decreased cytoplasmic RNA and protein content as well as a fall in nuclear protein levels. The early abnormalities in nuclear ultrastructure resembled those due to actinomycin and other agents which cause nuclear "capping". The biochemical lesion primarily related to the nucleolar responses, however, requires further elucidation. Mitochondrial function, as determined by P.O ratios, was abnormal in early stages following aflatoxin administration, but it appeared that respiratory activity recovered a simultaneous and complete degree of phosphorylative capacity. The acute hepatic lesions in monkeys, by light microscopy, bore some resemblance to the changes in human liver in acute viral hepatitis. In chronic experiments, nucleolar changes such as those present in acute experiments were not observed. On the other hand, well differentiated hepatocellular carcinomas appeared in a significant number of animals whose livers otherwise showed little architectural abnormality.

296. Svoboda, P.J., and Higginson, J. 1966. Ultrastructure lesions in rat and monkey liver due to aflatoxin. Federation Proceedings 25: 622.

Acute ultrastructural and metabolic changes were studied in the liver of rats and monkeys after one dose of aflatoxin. By 1 h, the nucleolus showed formation of nucleolar "caps" similar to those seen with actinomycin and lasiocarpine and followed by dispersion of nucleolar contents. Simultaneously there was proliferation of SER. Lesions became more severe at 48 h when fat droplets were present and accompanied by mitrochondrial degeneration. In non-necrotic cells, these changes reversed by 72 h. A corresponding decrease occurred in the RNA/DNA ratios in the homogenate and in the nuclear fraction. In monekys, while the cytoplasmic lesions were similar, the nucleolus showed unmasking of an additional granular component and other previously undescribed aggregates. In chronic experiments, rats were fed aflatoxin (1000 to 2000 µg kg^{-1}) for 30 weeks and studied serially. In contrast to the acute studies, changes were minimal and consisted of proliferation of SER; nuclear chnages were not observed. Tumors occurred at 26 weeks in isolated foci. The acute results suggest a similarity in action to lasiocarpine and certain natural carcinogens.

297. Theron, J.J. 1965. Acute liver injury in ducklings as a result of aflatoxin poisoning. Laboratory Investigation 14: 1586-1603.

Crystalline aflatoxin B_1, 100 µg in wheat germ oil, was given to 30 Peking ducklings 1 day-old, and 10 similar ducklings got only the wheat germ oil. One bird from each group was killed 1, 2, 4, 8, 16 and 24 h later and the rest after 72 h. Aflatoxin B_1 caused necrosis of the parenchymal cells of liver with focal haemorrhages. There was progressive decrease in activities of succinic dehydrogenase, alkaline phosphatase, adenosine triphosphatase, inosine diphosphatase and thiamine pyrophosphatase during development of the lesions, but an increase in acid phosphatase. Ultrastructure changes in the parenchyma are described. Mode of action of the poison is discussed.

298. Tilak, T.B.G. 1975. Induction of cholangiocarcinoma following treatment of a rhesus monkey with aflatoxin. Food and Cosmetics Toxicology 13: 247-249.

A female rhesus monkey, which had received aflatoxin for a period of over 5 years, partly by intramuscular injection but principally by oral intubation, developed a metastasizing intrahepatic bile-duct carcinoma, which was detected at autopsy 5.25 years after toxin administration was discontinued.

299. Tilak, T.B.G., Nagarajan, V., and Tulpule, P.G. 1975. Microsomal metabolism as a determinant of aflatoxin toxicity. Experientia 31: 953.

The evidence to incriminate the possible role of microsomal drug metabolism system in aflatoxin toxicity in rat is presented. It is suggested that an effective drug metabolism results in decreased acute toxicity and the products of metabolism are more carcinogenic than the native toxin to the rat liver.

300. Tulpule, P.G., Madhavan, T.V., and Gopalan, C. 1964. Effect of feeding aflatoxin to young monkeys. Lancet i: 962-963.

Young rhesus monkeys of 1.5-2.0 kg bodyweight were fed daily 1 mg of aflatoxin (containing 60 % aflatoxin B_1 and nearly 40 % aflatoxin G_1). They appeared unaffected at the end of the second week, when liver biopsies showed only slight changes. Between the 2nd and 4th weeks, all animals developed anorexia and apathy and within two days died in a coma. The liver lesions were similar to those described in ducklings.

301. Wogan, G.N. 1965. Experimental toxicity and carcinogenicity of the aflatoxins. Pages 163-173 *in* "Mycotoxins in Foodstuffs" (Wogan, G.N., ed.). M.I.T. Press : Cambridge, Massachusetts, USA.

Toxicity and carcinogenicity of aflatoxins in various laboratory animals are reviewed. Results of experiments on effects of pure aflatoxins on ducklings and rats are presented. Aflatoxin- contaminated diets are carcinogenic to rats, and aflatoxins in partially purified form or pure aflatoxin B, induced hepatomas in rats.

302. Wogan, G.N. 1966. Chemical nature and biological effects of the aflatoxins. Bacteriological Review 30(2): 460-470.

The information reviewed here emphasized the importance of the discovery of aflatoxins. This discovery, arising from astute observations in a seemingly obscure toxicity syndrome in poultry flocks, has led to increasing general attention to the possible significance of toxic mold metabolites as contaminants of foodstuffs and therefore as potential biological agents in problems of animal and human health. In addition, the potency of these compounds as toxic agents and as carcinogens should make them useful and powerful tools in investigations into the mechanism of toxicity and chemical carcinogenesis.

303. Wogan, G.N., and Friedman, M.A. 1965. Effects of aflatoxin B_1 on tryptophan pyrrolasc induction in rat liver. Federation Proceedings 24: 627.

The influence of single oral doses of aflatoxin B, on the increase of liver tryptophan pyrrolase six hour following IP injection of 150 mg kg^{-1} hydrocortisone or 600 mg kg^{-1} tryptophan was studied in weaning male rats. Animals treated with 1 mg kg^{-1} toxin and hormone showed no increase in enzyme activity 6 and 12 hours after toxin administration compared to approximately four- fold increase in those receiving hormone only. Although the effect at this dose level was not apparent 5 days later, larger amounts (3 or 5 mg kg^{-1}) of toxin caused inhibition of enzyme induction which persisted for at least 10 days. Animals treated with 5 mg kg^{-1} toxin and tryptophan showed significant but incomplete inhibition of induction which was apparent after 24 hours and up to 10 days after treatment. These results indicate that the biochemical effects of aflatoxin B_1 include significant alterations in the ability of the liver to respond to hormonal regulation of enzyme activity.

3C 4. Wogan, G.N., and Newberne, P.M. 1967. Dose-response characteristics of aflatoxin B_1 carcinogenesis in the rat. Cancer Research 27: 2370-2376.

Highly purified aflatoxin B_1 administered to male and female Fischer rats by intubation, or by feeding, induced hepatocellular carcinoma, and other liver lesions. Lesions of other tissues were also observed. Male rats surviving a single LD_{50} dose (5000 µg kg^{-1}) of the compound developed persistent liver lesions, but no hepatocellular carcinoma, within 69 weeks alter dosing. In males dosed with 400 µg administered in 10 equal daily doses, 4/24 animals developed liver cell carcinoma 35-82 weeks after treatment. A similar dose was ineffective in inducing carcinomas in females within an 82-week period, although preneoplastic liver lesions occurred at high incidence. When aflatoxin B, was fed continuously at a level of 1000 (ig in the diet, hepatocellular carcinomas were induced in 18/22 males within 35-41 weeks, but did not appear in females until 64 weeks. At a dietary level of 15 µg kg^{-1}, carcinomas were induced in 12/12 males alter 68 weeks and in 13/13 females killed after 80 weeks. Feeding of the 1000 µg diet for only the 14 initial days of the

experiment resulted in minimal carcinoma incidence in both sexes 80 weeks after withdrawal.

305. Wogan, G.N.. Paglialunga, S., and Newberne, P.M. 1974. Carcinogenic effects of low dietary levels of aflatoxin B_1 in rats. Food and Cosmetics Toxicology 12: 681-685.

Aflatoxin B_1 was added to a semi-synthetic diet at levels of 1. 5, 15, 50 and 100 µg kg^{-1} These diets were fed to male Fischer rats, beginning when their body weights were 80 g and continuing for up to 105 wk. A control group received the same diet without aflatoxin. Hepatocellular carcinomas were induced in a high incidence by levels of 50 and 100 |ig kg^{-1}, and in a lower incidence by the other dietary levels. Tumors were induced in two of 22 animals fed 1 µg kg^{-1} and pathological lesions were present in the livers of seven of the remaining survivors. These results suggest that, under these experimental conditions, the sensitivity of the Fischer rat to aflatoxin carcinogensis is approximately the same as that previously reported for the rainbow trout.

306. Yadgiri, B., and Tulpule, P.G. 1975. Metabolism of aflatoxin "in vitro" in the liver of farm animals. Indian Journal of Dairy Science 28(2):108-112.

The hepatic metabolism of aflatoxin in different species of farm animals, namely, cow, buffalo, sheep and goat was studied in vitro. A known level of aflatoxin B_1 was incubated with liver slices and concentration of unmetabolised toxin and its metabolite (aflatoxin M_1) were determined. The sheep and goat appeared to metabolise the toxin faster man the cow or the buffalo. These metabolic studies could be correlated with the relative species susceptibility to the toxin.

307. Zuckerman, A.J., Rees, K.R., Inman, D., and Petts, V. 1967. Site of action of aflatoxin on human liver cells in culture. Nature (London) 214: 814-815.

Human liver tissue culture preparations were exposed to different doses of aflatoxins B_1, G_1 and G_2. The LD_{50} for aflatoxin B_1 was 1 mg kg^{-1} 5 mg kg^{-1} for aflatoxin G_1 and 16 mg kg^{-1} for aflatoxin G_2. The autoradiography investigations revealed that human embryo liver cells in culture incorporated uridine and thymidine, indicating the ability of the hepatic cells to synthesize both RNA and DNA. The addition of aflatoxin B_1 to the cell culture in low concentrations resulted in an inhibition of both nuclear RNA synthesis and nuclear DNA synthesis by the parenchymal cells. It is concluded that the action of the toxin on the human liver cell is similar to that occurs in the rat liver cell.

308. Zuckerman, A.J., Tsiquaye, K.N., and Fulton, F. 1966. Tissue culture of human embryo liver cells and the cytotoxicity of aflatoxin B_1. British Journal of Experimental Pathology 48: 20-27.

Monolayers of differentiated parenchymal human embryo liver cells were maintained in culture for periods varying from 10-16 days after implantation on polythene. Cell disaggregation by trypsinisation gave the best results. Survival of the liver cells was found to be most consistent in growth medium containing 10 % foetal calf serum. Preparations were examined at intervals by fluorescence microscopy after staining with 1/1000 acridine orange. Complete recovery of the cells after implantation occurred rapidly, and the normal appearance of hepatic tissue was assumed after overnight incubation. After four to eight days many granules of neutral fat accumulated in the cytoplasm of the hepatic cells. When hepatic cell degeneration and death occurred this tended to be rapid and fairly uniform throughout the implanted tissue. Fibroblasts increased in number after about 10 days in culture and on occasions sheets of fibroplasts overgrew the hepatic tissue. The effect of purified aflatoxin B_1 on the liver cells was investigated. Marked changes were observed after 16 h exposure of the cells to 10 µg mL^{-1} of aflatoxin B_1. The overall dimensions of the hepatic cells were reduced. There was complete loss of orange (RNA) fluorscence from the cytoplasm and the cytoplasm became opaque and fluoresced deep green. The nucleus also showed marked changes and death of the cells followed.

2. *ASPERGILLUS FLAWS* INFECTION AND AFLATOXIN CONTAMINATION IN GROUNDNUTS

2.1 REVIEW

The groundnut is unusual because flowers are formed and fertilized above the soil with subsequent fruit development in the soil. The subterranean pod is associated with the soil microflora over an extended period of time and thereby facilitating its invasion by fungi and other organisms. In several groundnut-producing countries extensive research has been done on various aspects of the geocarposphere fungal ecology. This researche was accelerated by the discovery in 1960 that the fungus *Aspergillus flavus* could produce a toxic substance (aflatoxin) when growing upon groundnuts. Research institutions in a number of groundnut-producing countries placed a high priority on determining the stage or stages in crop production at which groundnuts are most likely to be invaded by the aflatoxin-producing fungus and became contaminated with aflatoxins.

The literature relevant to the invasion of groundnuts by *A.flavus* and subsequent aflatoxin contamination before harvest, during postharvest field drying, and during storage is critically reviewed in this section.

2.1.1 Preharvest

Several researchers in the USA and Nigeria studied fungal infection of groundnut fruits from the early stages of fruit development until harvest (Garren 1966, McDonald and Harkness 1964, McDonald 1970a, b). Garren (1966) reported a fairly well defined endogeocarpic myeoflora within the fruit as it developed in the soil, and suggested that a normal succession of soil fungi led to predominantly healthy pods with quiescent fungi. However, Garren concluded that a disturbance of the succession could lead to rotting of fruits. The balance could be disturbed by low calcium content or high organic matter content of the soil with the possibility of subsequent rotting of pods. Garren (1966) regarded *A.flavus* as a persistent species in the pod shell but of little quantitative importance in undamaged pods. The presence of a 'natural barrier' to invasion of undamaged pods was considered by Garren. He suggested possible antagonism and competition by *Trichoderma viride* and *Penicillium* spp. within the endogeocarpic community. Investigations by Porter and Garren (1968) showed *Penicillium* spp. were predominant in the shell myeoflora. *Trichoderma* spp. and *Fusarium* spp. were dominant groups and *A. flavus* was a subdominant fungus. Jackson (1968), in Georgia, USA, investigated the geocarpic myeoflora of undamaged pods and he obtained similar results. In both Virginia and Georgia, USA, the *penicillin* were an important group while the *aspergilli* were of little quantitative importance. However, the aspergilli were dominant in Mokwa, Nigeria (McDonald 1970a, b). These differences are probably attributable to environmental variation rather than qualitative differences in the soil mycofloras because most of the shell fungi are commonly present in groundnut soils in widely distributed groundnut production areas of the world (Borut and Joffe 1966, Joffe 1969. McDonald 1970b). These studies and several other investigations conclusively showed that while shells of immature and mature fruits were commonly infected by fungi including *A. flavus,* very little seed infection occurred at this stage (Jackson 1965, 1968, Ashworth et al. 1965, McDonald and Harkness 1964, Lyle 1966, Austwick and Ayerst 1963, Barnes 1971). Diener et al. (1965) conducted trials in Alabama, Georgia and North Carolina in the USA to determine the extent of shell and seed invasion at harvest in respect of *A. flavus* and other fungi. In 1963 in Alabama the percentage of seeds and shells invaded by *A.flavus* increased with time and maturity of the groundnut. In 1964, only low levels of *A.flavus* were found in seeds and shells in all three states, and low levels were attributed to abundant and well distributed rainfall and moderate temperatures. However. *A. flavus* was frequently isolated from seed and pods from 10 to 12 fields of different soil types in Israel (Joffe and Borut 1966). In the Gambia (Gilman 1969). *A.flavus* made up 14% of the fungi infecting seed of mature intact pods at lifting.

After visiting three groundnut-producing countries (The Gambia, Nigeria and Uganda) in Africa, Bampton (1963) reviewed the agricultural aspects of the problem of fungal infection of groundnuts in the field and subsequent aflatoxin production by *A.flavus.* He recommended procedures that might be used to determine when *A. flavus* infection of the kernels occurs. In the Gambia it was noticed that no sample taken from me soil showed evidence of fungal infection, with the exception of some plants which were suffering from rosette disease. Some aflatoxin contamination was found in kernels from the diseased plants. In Nigeria, *A. flavus* infection and aflatoxin contamination were detected in kernels from crops that had been left in the ground for approximately four weeks beyond maturity. Fungus and toxin were also detected in samples from a late-sown experimental crop that had been maintained for the normal length of time. Bampton reported analytical data from only a few of the samples collected in these countries, but complete results were given in confidential reports to the countries concerned. He concluded that *A.flavus* infection in the soil was unlikely to be of any significance.

McDonald and his coworkers made comprehensive investigations of the field occurrence of *A. flavus* infection and aflatoxin contamination in several groundnut varieties in Nigeria (McDonald and Harkness 1965, 1967). They conducted several serial harvesting trials at Mokwa and Kano Agricultural Research Stations, Northern Nigeria, in 1962, 1963 and 1964 to investigate the occurrence of aflatoxin in the groundnut crop at harvest, when lilted before, at, and after normal maturity. Crops

harvested at or earlier than the normal time were free from aflatoxin, but late harvesting usually resulted in aflatoxin contamination. The weather, as well as crop age, affected fungal infection and aflatoxin contamination. In the Kano trials there was an increase in the toxicity of the crop at harvest with increase in age. It appeared that the time of appearance of aflatoxin in the Kano crop depended on the occurrence of late-season drought stress (McDonald and Harkness 1967). At Mokwa the long wet season apparently delayed the appearance of aflatoxin in the crop. At Mokwa groundnut is normally harvested before the end of the rains, while in Kano it is harvested either at the end of the rains or later.

Data from Alabama, USA also demonstrated that a much higher percentage of *A. flavus* invasion occurred in overmature seed and pods than in immature and mature seed and pods from the same plants at harvest (Diener et al. 1965). In India, Mehan et al. (1986) showed that levels of *A. flavus* and aflatoxin B_1 were much higher in seeds from overmature pods of several groundnut genotypes than in seeds from immature and mature pods, especially under drought stress conditions. Seeds become susceptible to *A. flavus* invasion when seed moisture content is below 30% (Dickens and Pattee 1966, McDonald and Harkness 1967). Drought stress, lowered seed moisture content, over-maturity, and decreased plant vigour in groundnuts are interrelated and moisture related, and these factors contribute to increased susceptibility to *A. flavus* invasion and aflatoxin contamination (Bampton 1963, McDonald and Harkness 1967, Mehan 1987).

Many investigators have concluded that pods with shells that were damaged while the crop was in the soil were more likely to contain toxic kernels than were pods with undamaged shells (Bampton 1963, Diener et al. 1965, Ashworth and Langley 1964, McDonald and Harkness 1967. Schroeder and Ashworth 1965).

A number of soil-inhabiting pests including pod borers, millipedes, mites, white grubs, termites and nematodes have been implicated in *A. flavus* infection of groundnuts before harvest. The lesser cornstalk borer *(Elasmopalus lignosellus* Teller), a common pest of groundnuts in the USA, predisposes groundnut fruit to *A. flavus* infection (Dickens 1977, Wilson and Lynch 1984). *A. flavus* propagules may be carried by the insect to ideal infection sites where the kernels are damaged. Kernels from damaged pods often contain very high levels of aflatoxins. Another serious pest of groundnut in the USA, the southern corn rootworm *(Diabrotica undecimpunctaia howardi* Barber), has been associated with increased fungal invasion of groundnut fruit (Porter and Smith 1974). The insect feeding sites on the pod provide portals of entry for *A. flavus* and other fungi.

Aspergillus flavus invasion and aflatoxin contamination in groundnuts have been widely associated with pod damage in the ground by termites in South Africa (Sellschop 1965), and in Nigeria (McDonald and ABrook 1963, McDonald et al. 1964, Johnson and Gumel 1981). Pod scarification by termites *(Microtermes* spp. and *Odontotermes* spp.) is particularly important in facilitating invasion *by A. flavus*. Additional research is needed to determine if there is a quantifiable relationship between the degree of pod scarification and infection of the kernels by *A. flavus.*

Several species of mites penetrate groundnut pods, feed on the kernels, and disseminate spores of *A. flavus* (Aucamp 1969). Several studies have been conducted on the potential involvement of nematodes in the aflatoxin problem in groundnuts in Georgia, USA (Bell et al. 1971. Jackson and Minton 1968, Minton and Doupnik 1969, Minton and Jackson 1967). However, no conclusive relationship between nematode infestation and *A. flavus* invasion or aflatoxin contamination was established.

Premature death of plants, particularly during pod development and maturity, from root and stem infections by pathogens such as *Rhizoctonia solani, Sclerotium rolfsii,* and *Fusarium* spp. increase the chance of seed contamination with aflatoxins (Ashworth and Langley 1964, McDonald 1970, Ahmed et al. 1989). Lesions produced by these pathogenic fungi facilitate invasion of seeds by *A. flavus* (Ashworth and Langley 1964, Schroeder and Ashworth 1965). Some virus diseases such as groundnut rosette and bud necrosis may also predispose groundnuts to invasion by *A. flavus* (Bampton 1963).

Rapid invasion of pods by *A. flavus* in the soil has been widely associated with mechanical damage to pods during field cultivations and at the time of harvest (Ashworth and Langley 1964, Bampton 1963, McDonald and Harkness 1967, Schroeder and Ashworth 1965). Kernels from broken pods had extensive fungal invasion and aflatoxin contamination (McDonald and Harkness 1963, 1964). Schroeder and Ashworth (1965), in the USA, found that kernels from pods with mechanical damage and growth cracks had higher levels of aflatoxin than those with rot and insect injury. Damage to shell or kernel provides an increased probability of rapid and direct invasion of the kernel by *A. flavus,* which in turn increases the possibility of aflatoxin formation. Damage also increases nutrient availability for rapid growth of *A. flavus.*

Mechanical injury, growth cracks, and pod damage by soil- inhabiting pests have received considerable attention in research on aflatoxin contamination, but less is known about associations between diseases of groundnut and preharvest aflatoxin contamination of seeds. Information on the incidence of *A. flavus* and level of aflatoxins in seeds from plants attacked by various pathogens in different groundnut-producing regions is needed.

Preharvest infection of groundnut seeds by *A. flavus* was previously attributed to depend largely on physical and biological damage to pods (Ashworth and

Langley 1964, McDonald and Harkness 1964, Schroeder and Ashworth 1965). However, undamaged pods can also be invaded by *A. flavus* and contaminated with aflatoxin before harvest (Butler et al. 1973, Sanders et al. 1981, Hill et al. 1981). Drought stress during late stages of pod development favors invasion of groundnuts by *A. flavus* and subsequent aflatoxin production (Hill et al. 1981, Cole et al. 1982, Sanders et al. 1981).

Late-season drought stress, particularly in the semi-arid tropics, is a major factor associated with aflatoxin contamination (Blaney 1985, Mehan 1987). An association between late season drought stress and increased *A. flavus* invasion and aflatoxin contamination in groundnuts was documented as early as 1965 in South Africa (Sellschop 1965) and has been confirmed by researchers in Nigeria (McDonald and Harkness 1967), in the USA (Dickens et al. 1973, Pettit et al. 1971. Sanders et al. 1981), and in India (Mehan et al. 1986, Mehan et al. 1988). In studies in Texas, USA, the levels of *A. flavus* infection and aflatoxin in groundnuts (cv. Starr) harvested from rainfed and irrigated plots in 1967 and 1969 revealed that drought had a significant influence on preharvest fungal infection and aflatoxin contamination (Pettit et al. 1971). The incidence of *A. flavus* was highest in kernels from rainfed plots in South Texas in 1967 and 1969, years during which moderate and severe droughts occurred. Aflatoxin levels in kernels harvested from rainfed plots 120 and 130 days after sowing averaged from 694 to 10,240 µg kg^{-1} aflatoxin while either zero or trace amounts of aflatoxin were detected in kernels from irrigated plots. Davidson ct al. (1983), in Georgia, USA, highlighted the role of drought stress in accelerating *A. flavus* infection and aflatoxin production in sound mature kernels. Aflatoxin contamination in kernels from three growers' fields which had no, moderate, and severe drought stress averaged 6, 73, and 444 µg kg^{-1}, respectively (Davidson et al. 1983). However, some studies revealed that drought Stress alone was not responsible for aflatoxin contamination since drought-stressed groundnuts were not always contaminated with aflatoxins (Hill et al. 1983, Wilson and Stansell 1983).

Recent reports from extensive research conducted at the USDA controlled environment plots near Dawson, Georgia, USA, provide additional information about the influence of soil temperature and moisture levels on the extent of *A. flavus* infection and aflatoxin contamination in groundnuts (Blankenship ct al. 1980, Blankenship et al. 1983, 1984, Blankenship et al. 1989. Cole et al. 1984, Cole et al. 1985, Cole ct al. 1989, Sanders et al. 1985). Cole et al. (1985) determined the optimum mean pod-zone soil temperature range to be 28-30.5°C for aflatoxin production in drought conditions during the last 30-50 days of the growing season. Cole and his coworkers reported no aflatoxin contamination in kernels of undamaged pods in plots with adequate irrigation (irrespective of pod-zone soil temperature), or from drought-stressed crops when the mean pod-zone soil temperature during the last 30-50 days before harvest was < 25°C or > 32°C (Sanders et al. 1983, Cole et al. 1985). Their results suggest that groundnuts subjected to drought stress may not be contaminated with aflatoxins unless drought is accompanied by mean pod-zone soil temperatures of 25-31°C during late stages of pod development. liven a small mean temperature change may significantly influence aflatoxin production in drought-stressed groundnuts. These researchers reported high levels of *A. flavus* infection (25-70 %) in different sizes of kernels, even in those from irrigated plots. The high levels of the fungus in kernels of undamaged pods from a well irrigated plot were not explained.

Sanders et al. (1985) conducted studies to determine the duration of end-of-season drought stress necessary for prehavest *A. flavus* invasion and aflatoxin production in groundnuts when mean pod-zone soil temperatures were in the optimum range (28-30.5"C) for aflatoxin development. Their results indicate that more than 20 days, but probably less man 30 days, of drought stress at soil temperatures optimum for aflatoxin development are required for preharvest aflatoxin contamination. Increased duration of drought and temperature stress generally resulted in increased percentages of kernels infected *by A. flavus* (Sanders et al. 1985).

Larger, more mature groundnut kernels require considerably longer drought stress periods to become invaded by *A. flavus* than do smaller, immature kernels (Sanders et al. 1985, Cole et al. 1989).

In some studies, lack of aflatoxin contamination in heavily *A. flavus-infecled* kernels from heated irrigated plots (mean geocarposhpere temperature of 34.5"C) has been interpreted to mean that adequate irrigation prevents aflatoxin production (Hill et al. 1983). This may be due to the effect of the high or low mean temperature on fungus metabolism rather than adequate irrigation. This is supported by the observations of Cole et al. (1985) and Sanders et al. (1983) who demonstrated that a drought stressed soil temperature mean of 31.3°C was apparently too high for aflatoxin production even with a high incidence of kernel invasion (> 50 %) by *A. flavus.*

Recent studies in the USA have demonstrated that drought and temperature stress conditions in the pod zone and not in the root zone predispose groundnuts to contamination with aflatoxin (Blankenship et al. 1989).

Reduced metabolic activity associated with decreased pod moisture content under drought stress seems to increase susceptibility of groundnuts to *A. flavus* infection. Another possible role of drought stress in preharvest fungal infection could involve supression of microbial competitors of the aflatoxin-producing fungus by elevating the soil temperature in the pod zone.

Although results of various studies emphasize the importance of late-season drought stress in *A. flavus* infection and aflatoxin contamination, very little is known of the effects of early, mid season or multiple drought stress periods.

Pod splitting is another factor contributing to aflatoxin contamination. Pods maturing under fluctuating soil moisture conditions during seasons of inadequate or irregular rainfall, are prone to pod splitting. Seed in split pods are frequently invaded by *A. flavus* and subsequently become contaminated with aflatoxins (Graham 1982).'

Some research has been done on possible effects of calcium content of pods and seeds on preharvest aflatoxin contamination, but no definite relationship has been established (Cole et al. 1985, Wilson et al. 1985). If a relationship exists it could be a complex one because there is an interaction between drought and calcium deficiency.

Limited research has shown that the incidence of A *flavus* infection and aflatoxin contamination is likely to be much higher in groundnuts planted on light sandy and red sandy loam soils than in groundnuts planted on Vertisols (Graham 1982, Mehan et al. unpublished). This appears to be related mainly to the water potential and aeration in the soils; light sandy and red sandy loam soils have lower water-holding capacity and groundnuts grown on these soils are more prone to drought stress than those grown on Vertisols that have higher water-holding capacity. Light sandy and red sandy loam soils appeal to favor rapid proliferation of the aflatoxigenic fungus, especially under conditions of low water potential al which the activity of other microrganisms is minimal. More intensive studies are needed to determine interactions between moisture stress and *A. flavus* invasion of groundnuts in Vertisols, and to determine the effects of other soil types on preharvest aflatoxin contamination of groundnuts.

It is well established that *A. flavus* invasion can occur in soil during pod development and maturation; the fungus directly penetrates the pod wall or enters passages created by pod pests and disease/lesions. However, the exact mode of infection of groundnut fruit has not been fully elucidated. Some researchers in the USA (Lindsey 1970, Wells et al. 1972, Styler et al. 1983) have suggested that *A. flavus* may invade the flowers, travel down the pegs and become established in the developing seed. However, recent studies in Australia (Pitt 1989) have failed to establish a definite link between flower and peg invasion, and between peg and fruit invasion. In comparative studies of the invasion of flowers, aerial pegs, and kernels by wild-type and mutant strains of *A.flavus* or *A. parasiticus,* Cole et al. (1986) in the USA have conclusively proven that preharvest *A. flavus* infection and subsequent aflatoxin contamination originates mainly from the soil. They presented the following evidence in support of soil invasion as opposed to aerial invasion : (i) a greater percentage invasion of kernels rather than flowers or aerial pegs by either wild-type *A.flavus* or mutants; (ii) significant invasion by an *A. parasiticus* color mutant occurred only in groundnut from soil supplemented with the mutant, whereas adjacent plants in close proximity but in nontreated soil were invaded only by endemic strains of *A. flavus* or *A. parasiticus;* (iii) aflatoxin data from drought stressed, visibly intact groundnut kernels showed that samples from soil not supplemented with a mutant strain contained a preponderance of aflatoxins Bs (from wild-type *A. flavus)* whereas adjacent samples from mutant-supplemented soil contained a preponderance of aflatoxins Bs plus Gs (from wild-type and mutant *A. parasiticus);* and (iv) data from two air samplings showed no propagules of *A. flavus* or *A. parasiticus* in the air near the experimental site.

More research is needed to answer the important question "can flower and aerial peg invasion lead to significant invasion of groundnut fruit by *A.flavus'?";* and, "can this occur under both normal and drought stress situations?" If would be interesting to determine if the fungus could become systemic in the groundnut plant.

2.1.2 During Postharvest Drying

At harvest, mature pods contain a complex of microorganisms, the endogeocarpic microflora, which includes *A. flavus* (Garren et al. 1969). When these infested groundnut pods are lifted from the soil to be cured and dried, they are subjected to rapidly changing environmental conditions which cause shifts in the dominant and sub-dominant fungal species on and within the pods. The terms curing and drying have been defined as two distinct phases of change in groundnut composition following lifting (Blatchford and Hall 1963a). Curing of groundnuts is generally considered to occur after lifting during the period when the groundnuts are still attached to the haulms. It has been hypothesized that during curing several chemical and physical changes occur which influence kernel quality. The hypothesis is based on reported differences in seed germination, nutritional and taste qualities which develop during curing. Pods dried off the haulms are generally of reduced quality. The term "curing" thus relates to those as yet unidentified processes which terminate when the plants become dry or the groundnut pods are removed from partially dried haulms. The term "drying" is used to describe all phases of moisture removal from groundnuts, including the moisture lost during curing and after removal from the haulms. At lifting time, moisture levels in pods and kernels from living plants range from as high as 50 % to approximately 20%, depending upon soil conditions. The most important environmental factor which influences the endogeocarpic microflora during curing and drying is pod and kernel moisture. When high moisture groundnuts are lifted and cured/dried in windrows there may be considerable invasion of seeds by *A. flavus* and other fungi already established in the shell (McDonald and Harkness 1963), McDonald and A' Brook 1963, Burrell et al. 1964, Bampton 1963, Jackson 1965, Gilman 1969, Troeger et al. 1970). This is

encouraged if drying is slow and seeds are in the very susceptible range of 12-30 % moisture content for extended periods. In warm, wet weather the drying time is extended and the risk of aflatoxin contamination is increased (Jackson 1967b). A rain shortly after lifting is not particularly harmful, but a rain alter the groundnuts are partially dried, followed by poor drying, is likely to result in aflatoxin contamination (Troeger et al. 1970). Rains in the evening may keep the groundnuts wet all night, thus providing fungi with the needed moisture. Rains early in the morning are less likely to slow down drying and accelerate mold growth, because of effective daytime drying.

Several investigations of sun-drying methods in relation to moisture content of groundnuts have been done in two areas of Nigeria (Burrell et al. 1964, McDonald et al. 1964, McDonald and Harkness 1965). Groundnuts were harvested during the wet season in one area and during the dry season at the other location. In the wet area, where large differences occurred in the rate of drying between the various treatments, it was found that the slower rate of drying was associated with a higher incidence of *A. flavus* and other fungi and a higher incidence of toxic samples. When the pods were separated from the haulms shortly after lilting and then sun-dried in thin layers on matting with protection from rain, the drying rate was fast and pods were free of aflatoxin. Treatments in which the pods were field dried on the haulms did not give a toxin-free crop and the majority of the produce was of very low quality. In the area where the crop was lifted under dry conditions, no effect of drying method on toxicity or fungal infection of kernels was observed. In areas where rains continue after harvest, field drying of groundnuts can present problems and serious aflatoxin contamination is likely to occur.

The use of inverted windrows compared to random windrows has been shown to speed the curing and drying process (Dickens and Khalsa 1967, Porter and Garren 1970, Petlil el al. 1971). Groundnut pods positioned at the top of windrows (inverted windrows) reside where air currents move more rapidly and where the atmospheric relative humidity (RH) is low as compared with positions closer to the soil surface (random windrows). When the soil is wet from recent rains, the RH near the soil surface exceeds 90 %, especially on nights when there is little air movement. Pods near the wet soil surface dry much more slowly than those in inverted windrows. Lower levels of *A.flavus* infection and aflatoxin contamination have been reported in groundnuts dried in inverted windrows than in random windrows (Porter and Garren 1970, Dickens and Khalsa 1967). Dickens and Khalsa (1967) observed that average moisture content of groundnuts from inverted windrows was 8 % lower than the moisture content of groundnuts from random windrows. They also examined the influence on drying rates of using air at 85% or 50% R.H. The drying rate was slow when 85 % RH air was used and 20 to 51 % of the kernel samples from this treatment contained aflatoxin. In comparison, when the RH of the drying air was 50 %, only 1 % of the kernel samples contained aflatoxin. Inverted windrows shorten the time required to cure groundnuts in field and help to reduce the number of kernels invaded by *A. flavus* and other fungi. However, to avoid infection and aflatoxin contamination because of prolonged rainy periods the groundnuts should be threshed as soon as possible with final drying achieved under controlled conditions.

McDonald and A 'Brook (1963). in Nigeria, examined the toxicity of artificially dried groundnuts and sun-dried in windrows. Aflatoxin was present in the sun-dried material, whereas material artificially dried shortly after harvest was free of toxin.

Windrowing of lifted plants is the standard procedure for groundnuts produced in the United States (Dickens 1977). A digger-shaker inverter implement is used to dig the plants, shake soil from the roots and pods, and invert the plants. After several days, the partially dried groundnuts are removed from the haulms with a combine. They are then piled about 1.5 meters deep in dryer wagons with perforated floors and dried, by forcing heated air up through the pods, to achieve an average moisture content of less than 10% (wet basis). If the combine damages the pods, the groundnuts become more susceptible to subsequent mold damage than groundnuts in sound pods (Dickens and Khalsa 1967). Precautions should be taken to protect groundnuts from rain while they are in combine baskets, dryer wagons, or other containers in the field, and during transport to the dryer. A layer or batch of wet groundnuts in a dryer may mold before drying.

When drying capacity is inadequate, groundnuts should be left in the windrows rather than combined and held for drying. Even during periods of rain, the risk of aflatoxin contamination is probably less for groundnuts in inverted windrows than for those held in dryers without proper ventilation (Dickens 1977). Adequate procedures have been developed to prevent molding of sound intact groundnuts during bulk drying (Dickens and Pattee 1966).

2.1.3 During Storage

The number of ecological studies of storage fungi involving quantitative mold counts of populations is limited (Diener 1960. Schroeder and Boiler 1971, Borut and Joffe 1966, Joffe 1969, Moubasheret al. 1980). In Alabama, USA, quantitative data on the mycoflora of seed from farmers' slock groundnuts stored in 26 farm-size bins were related to initial moisture, seed damage, type of bin, and length of storage. Species of the *A. glaucus* group, *A.flavus* group, and *P. ciirinum* were dominant in the mycoflora of the seed of farmers' stock groundnuts stored for 8-56 months. The number and kind of fungi associated with seed stored for 1-6 months were determined (Welty and Cooper 1969). Initially, *A. repens* was isolated from 54% of the seed, *Penicillium* spp. from 80% and *A.flavus* from 7%. After 2-3 months of storage, the percentage of seed with *A. repens* and *PeniciUium* spp. dropped to about

10% and then gradually increased to 40% after 6 months. *A.flavus* remained at 7% for the first 3 months, but after 4-6 months it was isolated from 18% of the seed.

In Israel, *A. niger* was the most common species in 114 stored groundnut samples from the 1963 and 1964 crops (Borut and Joffe 1966). *A. niger* occurred in 97.4% of the samples, while *A.flavus* was detected in 78.4% of the 1963 samples and in 63.5% of the 1964 samples. However, *A. niger* occurred in large numbers (40% of the total colonies) in 114 samples, whereas *A. flavus* made up only 5.7% of the mycoflora of stored seed. *A. niger* was the dominant species in the mycoflora of 419 samples of stored seed examined over a 5-year period in Israel (Joffe 1969), and *A.flavus* was present in relatively small quantities in comparison with *A. niger.* The high incidence of *A. niger* in stored seed that were disinfected with mercuric chloride was considered to be attributable to selective removal of antagonists (Joffe 1968).

In Egypt, groundnut seed were adjusted to 8.5, 13.5, 17.5, and 21% moisture levels and stored for 6 months at 5, 15, 28, and 45°C (Moubasher et al. 1980). *A. fumigatus* was the dominant fungus followed by *A.flavus, A. niger, A. terreus,* and *P. funiculosum.*

The main factors influencing the growth of *A.flavus* and otlier storage fungi in groundnuts are moisture (relative humidity), temperature, time, and gaseous composition of the atmosphere. High mycofloral counts have been associated more often with high initial moisture contents of groundnuts going into storage than with any otlier factor (Diener 1960). Austwick and Ayerst (1963) studied the growth of *A.flavus* and *A. chevalieri* of the *A. glaucus* group at different relative humidities and temperatures; they were unable to grow at less than 80% RH equilibrium. The literature on the influence of moisture, temperature, and other factors on the growth of *A.flavus* in groundnuts has been comprehensively reviewed by Diener and Davis (1977).

A. flavus infection and aflatoxin contamination may increase in groundnuts during storage until their moisture content drops below 9%. Increases in moisture from rewetting in storage or exposure to high humidity for extended periods of time result in rapid invasion by the aflatoxigenic *A. flavus* with consequent aflatoxin contamination. Natural accumulation of carbon dioxide (CO_2) and decreased levels of oxygen (O_2) in closed storage reduce mycofloral development (Landers et al. 1967, Jackson and Press 1967). Low temperatures and uniform moisture distribution reduce mold growth and insect activity. Aeration is necessary to reduce aflatoxin contamination during storage. High relative humidity and temperatures, rain water leakage, condensation, and insect infestation are all important factors that contribute to aflatoxin contamination of groundnuts in storage (Diener and Davis 1977).

2.1.4 Aflatoxin-Producing Potential of *Aspergillus flavus* Isolates from Groundnuts

Various investigators have determined the aflatoxin-producing potential in groundnuts and nutrient media of isolates of *A. flavus* obtained from groundnuts grown in various geographical areas. A number of researchers have qualitatively determined aflatoxin production on natural and nutrient media (Wallbridge 1963, Austwick and Ayerst 1963, Sreenivasamurthy et al. 1965, Rao et al. 1965, Borut and Joffe 1965. 1966). Austwick and Ayrest (1963) found that 52% of a selection of *A. flavus* isolates from groundnuts from several African countries were toxin producers. Investigators in India (Rao et al. 1965, Sreenivasamurthy et al. 1965) reported that less than 6% of 179 isolates formed aflatoxin. In Israel, Borut and Joffe (1965, 1966) reported that 71.2% of 330 *A.flavus* isolates from groundnut soils and kernels produced aflatoxin. In Egypt, El-Khadem et al. (1975) found over 50% of *A.flavus* isolates from groundnut shells and seeds to be aflatoxin producers.

Many investigators have made quantitative determinations of aflatoxin producing ability of *A.flavus* isolates. Diener and Davis (1966) reported aflatoxin production on groundnuts and in a nutrient solution from about 86% of a number of *A.flavus* strains isolated from several natural substrates. Taber and Schroeder (1967) assayed 78 samples of Spanish groundnuts from nine geographical areas in Texas, USA, for aflatoxin and isolated 213 *A.flavus* isolates, 107 of which were toxin producers. In Israel, Joffe (1970) reported that 89.6% of 1626 *A.flavus* isolates from groundnut kernels (1084), rhizosphere (30) and geocarposphere (11), and from groundnut soils (501) were aflatoxigenic. These data indicated a remarkably uniform and high frequency of toxicity among such isolates, ranging in soils from 81.6% to 90%, in fresh kernels from 84.6% to 100%. and in stored kernels from 78.2% to 97.7%. A summary of data from investigations in the U.K., Holland, India, South Africa, and the USA indicated that 58% of 1390 isolates of the *A.flavus* group were aflatoxin producers (Diener and Davis 1969). This included 330 isolates from Israel (Borut and Joffe 1965), 235 (71.2%) of which produced some aflatoxin. Some other workers have reported a very high frequency of aflatoxigenic isolates from groundnut kernels (Chen et al. 1988, Hasegawa et al. 1987, Sripathomswat and Thasnakorn 1981). Hasegawa et al. (1987) reported that 91.5% of 47 isolates of A. *flavus* (from groundnuts imported into Japan) were aflatoxin producers. Chen et al. (1988) found that all *A. flavus* and *A. parasiticus* isolates from raw groundnut kernels from several areas of Taiwan were aflatoxigenic.

Isolates of *A. flavus* and *A. parasiticus* vary widely in the amount of aflatoxin produced on groundnuts, and in their capacity to produce different aflatoxins. Some isolates produce no aflatoxins (Codner et al. 1963, Diener and Davis 1966, Taber and Schroeder 1967). In the UK, Codner et al. (1963) tested 5 isolates of *A.flavus*

and one isolate of *A, parasiticus* for ailatoxin-producing potential on groundnuts. The *A. parasiticus* isolate produced 265 µg g^{-1} of a mixture of all four anatoxins, while *A. flavus* isolates produced from 14-162 µg g^{-1}. In Texas, aflatoxin B_1 production by 213 *Aflavus-oryzae* isolates from Spanish groundnuts ranged from 0 to 349 µg g^{-1} on a groundnut substrate (Taber and Schroeder 1967). In general, some isolates produced 8 to 10 times more aflatoxin B_1 than B_2; no isolate producing aflatoxins G_1 or G_2 was found. Isolates collected from groundnuts in seven southern states varied in aflatoxin B_1 production from 0.07 to 17 µg g^{-1} in groundnuts (Diener and Davis 1966). Of 1626 isolates of *A. flavus* tested in Israel, 1.7% produced only aflatoxin B_1, 95% produced both aflatoxins B_1 and B_2, 8.4% produced aflatoxins B_1, B_2, G_1, and G_2, and 10.4% produced no aflatoxins (Joffe 1969). This indicated that 8.4% isolates were probably *A. parasiticus,* which commonly produces all four aflatoxins (Diener and Davis 1977, Ogundero 1987). About 7% of the isolates produced less than 1 µg g^{-1}, 70% produced from 1 to 100-125 µg g^{-1}, and 23% produced more than 100-125 µg g^{-1}. Doupnik (1969) screened 244 A. flavus isolates from three varieties of groundnut, and 66% produced aflatoxin. About 18.6% produced only aflatoxin B_1, 31% produced aflatoxins B_1 and B_2, and 50.3% produced both aflatoxins B_1 and G_1 and in addition some produced aflatoxin B_2 and/or G_2. This indicated that about 50% of the Georgia isolates were *A. parasiticus.* About 10% of the isolates produced less than 1 µg g^{-1}, 40% produced from 1 to 100- 125 µg g^{-1}, and 50% produced more than 100-125 µg g^{-1} (Doupnik 1969). Table 2 summarizes data on aflatoxigenic isolates of *A. flavus* reported by various investigators.

Some workers reported that repeated culture of *A. flavus* isolates on artificial media can lead to partial or total loss of aflatoxin producing ability, whereas culture on natural substrates can lead to an increase in production of aflatoxins (Armbrecht et al. 1963, Diener and Davis 1969). However, some specific strains e.g., NRRL 2999, Ala-6, and Ala-1 did not lose aflatoxin-producing ability when grown continuously on a series of natural or synthetic substrates (Diener and Davis 1969).

Table 2. Incidence of aflatoxigenic isolates of *Aspergillus flavus* reported by various investigators

Source of isolates	Country	Investigators	Substrate	No. of isolates		%Aflatoxigenic isolates
				Tested	Aflatoxigenic	
Groundnuts	UK	Austwick and Ayerst (1963)		59	11	18.6
Groundnuts	UK	Codner et al. (1963)	Groundnut	6	6	100
Groundnuts, *C.C.*	UK	Wallbridge (1963)	NM	43	32	74.4
Groundnuts, C.C.[b]	USA	Armbrecht et al. (1963)	NM	10	7	70.0
Groundnuts	USA	Diener and Davis (1966)	Groundnut	26	25	96.1
Groundnuts	USA	Taber and Schroeder (1967)	Groundnut, rice	213	107	50.2
Groundnuts	USA	Doupnik (1969)	NM	244	161	66.0
Groundnuts, soils	Israel	Borut and Joffe (1965)	Groundnut	330	235	71.2
Groundnuts, soils	Israel	Joffe (1969)	NM	1626	1463	90.0
Groundnuts, groundnut meal	India	Sreenivasamurthy et al. (1965)	Groundnut	150	4	2.6
Groundnuts	India	Rao et al. (1965)	Groundnut	29	6	20.7
Groundnuts	India	Kang (1970)	NM	21	18	85.7
Groundnuts	India	Subrahmanyam and Rao (1974)	NM	240	72	30.0
Groundnut seedlings[c]	India	Mehan (1979 unpublished data)	NM	17	16	94.1
Groundnuts	Japan	Haseaawa et al. (1987)	Groundnut meal	47	43	91.5
Groundnuts	France	Lafont and Lafont (1971)	Groundnut	26	26	100
Groundnuts	Nigeria	Ogundero (1987)	Groundnut	10[4]	6	60

a NM = Nutrient medium
[b] C.C.= Culture collection [c] From infected cotyledons of 'aflaroot' affected seedlings
[d] Tour isolates of *A. parasiticus* and 6 *of A. flavus;* all 4 isolates *of A. parasiticus* produced aflatoxins

2.2 BIBLIOGRAPHY

2.2.1 *ASPERGILLUS FLAWS* INFECTION AND AFLATOXIN CONTAMINATION OF GROUNDNUTS

309. Dickens, J.W. 1977. Aflatoxin occurrence and control during growth, harvest and storage of peanuts. Pages 99-105 in Mycotoxins in human and aninmal health. (Rodricks. J.V., Hessekine, C.W., and Mehlman, M.A., eds.). Illinois, USA : Pathotox Publishers, Inc.

This paper discusses causes of aflatoxin contamination and methods of prevention and segregation to control the aflatoxin problem in farmers' stock groundnuts from the time the groundnuts are growing in the field until they are marketed, stored, and shelled.

310. Diener, U.L. 1973. Deterioration of peanut quality caused by fungi. Pages 523-557 in Peanuts : Culture and Uses. American Peanut Research & Education Association, Stillwater, OK, USA.

This paper reviews seed infection by *Aspergillus flavus* and other fungi, and aflatoxin contamination of groundnuts from the time the groundnuts are growing in the field until they arc marketed, stored and shelled. Causes of aflatoxin contamination and methods to control the aflatoxin problem are discussed.

311. Diener, U.L., Pettit, R.E., and Cole, R.J. 1982. Aflatoxins and other mycotoxins in peanuts. Pages 486-519 *in* Peanut Science and Technology (Pattee, H.E., and Young, C.T., eds.). American Peanut Research and Education Society, Inc., Yoakum, Texas 77995, USA. 825 pp.

Of the mycotoxins that have played important roles in human and animal health in the last century, aflatoxin is the most significant threat in modern agriculture. The aflatoxin-producing fungi, *Aspergillus flavus* and *A. parasiticus,* are omnipresent in soils and air throughout the world. Groundnuts, corn, and cottonseed and their products are constantly monitored for aflatoxin contamination. When environmental conditions are favorable for growth of the fungus, *A. flavus* may rapidly invade and contaminate groundnuts with aflatoxin in the field before harvest and after harvest during curing/drying in the windrow. Drought stress has been the factor most frequently correlated with aflatoxin contamination in groundnuts before harvest. Control of aflatoxin in groundnuts in the field can probably be accomplished most successfully with well-managed irrigation practices that prevent drought stress. The use of rotations, fungicides, and soil insecticides has not been demonstrated to control or greatly reduce pod invasion by *A. flavus* ano/or aflatoxin contamination in the field or windrow. Breeding for resistance to *A. flavus* invasion and/or aflatoxin formation is a sound but long-term approach to control. Inversion of groundnut pods in the windrows reduces the probability of aflatoxin contamination during curing. However, groundnuts contaminated before harvest must be diverted from the human food chain by inspection or eliminated by sorting and other techniques during processing. Aflatoxin removal or inactivation by chemical treatments is feasible, but the product may be suitable for animal feeds and not for human consumption. Aflatoxin appears to be the only mycotoxin problem in groundnuts of any consequence at this time.

312. Elamin, N.H.H., Abdel-Rahim, A.M., and Khalid, A.E. 1988. Aflatoxin contamination of groundnuts in Sudan. Mycopathologia 104(1): 25-31.

Groundnut samples, collected soon after harvest, from different districts in the irrigated region (Central Sudan) were free from aflatoxins. Samples collected from the rainfed region (Western Sudan) showed incidence of aflatoxin contamination ranging from 100 % samples in El Hamdi to only 10 % samples in Casgeal. Damaged pods were highly contaminated with *Aspergillus flavus* and aflatoxins. Sound intact pods had lower fungal contamination and were almost free of aflatoxins. Groundnut products (groundnut paste, red and grey roasted pods) collected from Khartoum North (Bahri) had higher levels of aflatoxin than those collected from Khartoum and Umdorman. Grey and red roasted pods showed higher levels of aflatoxins than the groundnut paste. None of the three varieties (MH383, Barberton, Ashford) of groundnut tested was completely resistant to *A. flavus* contamination and aflatoxin production. A temperature of 30"C and 86.3 % relative humidity were the optimum conditions for both *A. flavus* growth and aflatoxin production in groundnuts.

313. Fonseca, H. 1976a. [Study of aflatoxin in groundnut from harvest to processing in the Monte Alto region, Sao Paulo.]. Estudos da aflatoxina no amendoim, da colheita a industrializacao. na regiao de Monte Alto, S.P. Anais da Escola Superior de Agricultura "Luiz de Quciroz" 33: 375-384.

The presence of aflatoxin in groundnuts was studied at the time of sale by the grower, during storage, and after oil extraction. Of 40 samples of groundnut and 20 of groundnut flour, 90 % contained aflatoxin. Aflatoxin B_1 at >1000 |ig kg^{-1} was found in 90 % of the samples, the level rising from the time of sale to milling, then falling after oil extraction.

314. Fonseca, H, 1976b. [Study of aflatoxin in groundnut from harvest to processing in the Santa Adelia region, Sao Paulo.]. Estudo da aflatoxina no

amendoim. da colheita a industrializacao, na regiao de Santa Adelia. A.P. Anais da Escola Superior de Agricultura "Luiz de Queiroz" 33: 385-393.

The presence of aflatoxin in groundnuts was examined at the time of sale by the grower, during storage, and after oil extraction. Of 40 samples of groundnut and 20 of groundnut flour, all but one contained aflatoxin. Aflatoxin B_1 levels were very high in 31.6 %, and high in 55 % of the samples. Aflatoxin levels fell from the time of sale to milling. Five samples had >10000 µg kg^{-1}.

315. Fonseca, H. 1976c. [Study of aflatoxin in groundnut from harvest to processing in the Fernandopolis region, Sao Paulo.]. Estudo da aflatoxina no amendoim. da colheita a industrializacao, na regiao de Fernandopolis, S.P. Anais da Escola Superior de Agricultura "Luiz de Queiroz" 33: 395-405.

The presence of aflatoxin in groundnuts was examined at the time of sale by the grower, during storage, and after oil extraction. Of 40 samples of groundnut and 20 of groundnut flour, all but one contained aflatoxin. Aflatoxin levels were very high in 38.3 %, and high in 51.7 % of the samples. Aflatoxin levels rose from the time of sale till milling, then fell. Five samples had > 10000 µg kg^{-1} aflatoxin.

316. Fonseca, H. 1976d. Study of aflatoxin in groundnuts from harvest to processing in the region of Matao, S.P. Anais aa Rscola Superior de Agricultura Luiz de Queiroz (Brazil) 33: 365-374.

In the present work the occurrence of aflatoxin in groundnut in the region of Matao, Sao Paulo, was investigated at three stages from harvest to processing : (a) at the time the grower sells it to the oil mill; (b) during its storage prior to milling; and (c) after oil extraction (groundnut flour). At each stage two collections of 10 samples each were made. The conclusions were : (1) the majority of the samples (85 %) were toxic; (2) the toxicity level of samples, in terms of aflatoxin B_1, grew from stage (a) to stage (c) giving average values of 60 pg kg^{-1} at the first and 1220 pg kg^{-1} at the second stage. The mean value was 440 pg kg^{-1}; (3) good drying practices and proper storage can minimize aflatoxin levels and even prevent its occurrence; and (4) aflatoxin is already present when groundnuts are delivered to the mill.

317. Fonseca, H., Nogueira, J.N., Graner, M., Oliveira, A.J., Caruso, J.G.B., Boralli, C, Calori, M.A., and Khatounian, C.A. 1983. Natural occurrence of mycotoxins in some Brazilian foods. II. Pages 53-54 in. Proceedings of the Sixth International Congress of Food Science and Technology. Vol. 3 (McLoughlin, J.V., and McKenna, B.M., eds.). Boole Press Ltd., Dublin : Irish Republic.

Surveys were carried out for two years to determine the natural occurrence of mycotoxins (aflatoxin, ochratoxin and zearalenone) in some common Brazilian foods including raw and salted roasted groundnuts, pacoca (a very popular groundnut candy), peanut butter, maize, soybean, cured cheese, salami and copa (cured and dried pork). Eight characteristic regions, Vale do Paraiba, Mogiana, Paulista Velha, Araraquarense, Noroeste, Paulista Nova. Sorocabana and Vale do Ribcira/Litoral Sul, were surveyed, and 742 samples were analyzed. Estimation of toxins was made by thin- layer chromatography (TLC) by comparison with standards. Six samples of corn (4.7 %) were contaminated with aflatoxin, ranging from 41 to 2000 µg kg^{-1} of aflatoxin B_1; 81 samples of pacoca (63.3 %), ranging from < 20 to 1187 µg kg^{-1}; 80 samples of peanut butter (62.5 %), from < 20 to 275 pg kg^{-1}; 17 samples of raw groundnuts (17.7 %), from 19 to 3125 µg kg^{-1}; and 9 samples of roasted salted groundnuts (9.4 %) from < 30 to 4250 µg kg^{-1}. From the first to the second year, the percentage of contaminated samples increased slightly for raw groundnuts (from 15.6 to 18.8 %) and decreased for pacoca (67.2 to 59.4 %) for roasted salted groundnuts (12.5 to 7.8 %), corn (6.2 to 3.1 %), and for peanut butter (84.4 to 40.6 %). The average content of aflatoxin of the contaminated samples increased for raw groundnuts (from 166.7 to 641.7 pg kg^{-1}) and roasted salted groundnuts (410 to 980 pg kg^{-1}) and decreased for pacoca (165.6 to 108.8 pg kg^{-1}), peanut butter (91.3 to 50.4 µg kg^{-1}), and corn (853.3 to 700 µg kg^{-1}).

318. Martin, P.M.D., and Oilman, G.A. 1976. A consideration of the mycotoxin hypothesis with special reference to the mycoflora or maize, sorghum, wheat and groundnuts. Tropical Products Institute Report; Series G (UK) no. 105, 119 pp.

This review traces the connection between the mycology of foodstuffs and the onset of disease due to the toxins that various fungi produce within those foodstuffs. Particular emphasis is placed on the practical aspects, especially with reference to the physiological interactions among fungi, the incidence of fungi and fungal toxins in various substrates, the ecology of mycotoxin formation, and the acute and chronic effects of mycotoxicosis.

319. McDonald, D. 1966. Research on the aflatoxin problem in groundnuts in Northern Nigeria 1961-1965. Samaru Miscellaneous Paper 14, 34 pp.

A chronological review is presented of research into the toxicity of groundnuts in Northern Nigeria caused by aflatoxin. Most results obtained up to 1963 have already been published. Investigations in 1964-65 included varietal resistance trials, studies of the mycoflora of groundnut roots and fruits, and trials on times and methods of lifting, methods of crop drying, and decortication in connection with the moisture content of the crop. Though it seems possible to develop varieties resistant to *Aspergillus flavus,* the standard varieties form the most resistant material at present available. An appendix by D. Halliday summarizes research on aflatoxin carried out by the Nigerian Stored Products Research Institute; 2 further appendices contain

recommendations to farmers on the avoidance of aflatoxin contamination, on the basis of conclusions drawn from the experimental work.

320. McDonald, D. 1969. *Aspergillus flavus* on groundnut *(Arachis hypogaea* L.) and its control in Nigeria. Journal of Stored Products Research 5: 275-280.

Groundnuts are produced in two distinct zones in Nigeria; the dry northern zone where 95 % of the crop is grown, and the wetter riverain zone. In the northern zone the crop is normally harvested after the rains have ended, but in the riverain zone harvesting takes place during the rains. Investigations on groundnuts from the 1961 crop showed that *A. flavus* infection of kernels and aflatoxin production occurred in both zones. The condition of the shell had a marked influence on the fungal infection and toxicity of the kernels. Kernels from undamaged pods were rarely infected by *A. flavus,* whereas kernels from perforated pods had a high degree of infection with this and other fungi. Kernels from termite-scarified pods were intermediate in this respect. Kernels from all pod grades showed higher fungal infection in material from the riverain zone than in that from the northern zone, indicating that poor drying conditions could be important. Trials at Mokwa in the riverain zone and at Kano in the northern zone in which various methods of drying were tested demonstrated that rapid drying gave kernels with low fungal infection and little or no toxicity. Slow drying with kernel moisture contents above 20 % for extended periods resulted in heavily infected, toxic kernels. Kernels from undamaged, mature pods were found to be free from fungal infection at lifting, infection by *A. flavus* not normally occurring until 4-6 days after harvest. Over-mature pods and pods from plants that had wilted and died before harvest had kernels infected by fungi at lifting in the northern zone. On the basis of these findings recommendations were provided for management of *A. flavus* in groundnut.

321. McDonald, D. 1976. Aflatoxins: Poisonous substances that can be present in Nigerian groundnuts. Samaru Miscellaneous Paper 53, Institute for Agricultural Research, Samaru, Ahmadu Bello University, Zaria, Nigeria, 14 pp.

This paper outlines events leading up to the discovery of aflatoxin, describes briefly research done on the groundnut aflatoxin problem in the northern states of Nigeria, considers the implications of aflatoxin in relation to animal and human health, and discusses measures for elimination of aflatoxin from Nigerian groundnuts, or to at least greatly reduce incidence of aflatoxin.

322. Mehan, V.K. 1987. The aflatoxin contamination problem in groundnut-Control with emphasis on host plant resistance. Pages 63-92 in Proceedings of the first Regional Groundnut Plant Protection Group Meeting and Tour, 15-21 February 1987, Harare, Zimbabwe.

The status of the global aflatoxin problem is reviewed with special reference to African groundnut producing countries, and research needs are highlighted. Possible practical control measures are discussed with emphasis on use of host plant resistance to the aflatoxin-producing fungus *Aspergillus flavus.*

323. Mixon, A.C. 1980. Potential for aflatoxin contamination in peanuts *{Arachis hypogaea* L.) before and soon after harvest - a review. Journal of Environmental Quality 9(3):344-349.

Cultural and preharvest conditions and early postharvest conditions which influence the vulnerability of groundnuts to seed invasion by *Aspergillus flavus* and to aflatoxin contamination are discussed. Management practices, including chemical control and genetic resistance are considered.

324. Peers, F.G. 1967. Aflatoxin - A summary of recent work. Tropical Science IX(4):186-203.

The important published information on aflatoxin during the period January 1964 to March 1967 is summarized. The topics covered include (a) assay of aflatoxins, (b) toxicology of aflatoxins, (c) biosynthesis of aflatoxins. (d) biochemical and intercellular effects of aflatoxins, (e) metabolism of aflatoxins in animals, (0 incidence, control and detoxification, and (g) implication in man.

325. Sarnaik, S., Godbole, S.H., and Pradnya Kanekar. 1988. Incidence of toxigenic *Aspergillus flavus* in marketed edible vegetable oils. Current Science, India 57(24):1336-1337.

Twenty-six groundnut oil samples (12 unrefined, 7 refined and 7 from ration shops) were collected from the local market and assessed for *Aspergillus flavus* contamination. Three unrefined and three ration oil samples contained the fungus. Five of these six isolates produced aflatoxins in a nutrient medium. One isolate from unrefined groundnut oil was not toxigenic. When the ability of all isolates to produce aflatoxin in sterile groundnut oil was tested, the isolates did not produce any toxin during a 3-month period.

326. Sargeant, K., Sheridan, A., O'Kelly, J., and Carnaghan, R.B.A. 1961. Toxicity associated with certain samples of groundnuts. Nature 192: 1096-1097.

The toxic extract of Brazilian groundnut meal was further purified and a fluorescent method of identification after chromatographic separation was devised. The toxic substance was isolated from a fungus, *Aspergillus flavus.* When the fungus was grown on sterilized groundnuts and fed to ducklings, it resulted in typical liver lesions in ducklings.

327. Stoloff, L. 1980. Aflatoxin control : past and present. Journal of the Association of Official Analytical Chemists 63(5):1067-1073.

History of control of aflatoxin levels in foods, especially groundnut products, in the USA is surveyed, and data presented show changes in aflatoxin contamination from 1967 to 1978 for raw shelled groundnuts, consumer groundnut products, almonds, pecans and walnuts, and imported Brazil and pistachio nuts. The effect of establishment of an FDA action level of 0.5 ng aflatoxin MI mL^{-1} milk on aflatoxin concentration in market milk is discussed. Aflatoxin contamination of food is effectively controlled in the USA, with the possible exception of some cornmeal and corn grits products locally made and consumed in rural South Eastern USA.

2.2.2 Preharvest

328. Ahmed, N.E., Younis, Y.M.E., and Malik, K.M. 1989. *Aspergillus flavus* colonization and aflatoxin contamination of groundnut in Sudan. Pages 255-261 in Aflatoxin Contamination of groundnut: proceedings of the International Workshop, 6-9 Oct 1987, ICRISAT Center. India. Palancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

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-wcek intervals and harvested at the normal time, and at 1 week before, and 1 week after, were free from *A. flavus* infection and aflatoxin contamination. *Aspergillus flavus* infected a low percentage (2.7 - 7 %) of groundnuts left in the soil for 6 weeks alter harvest. However, no aflatoxin contamination was delected. Wilt diseases and insect damage, mainly by white grubs and termites, predisposed seeds to prehavest *A. flavus* infection (56.4-69.8 %) and aflatoxin contamination (18-21 µg kg^{-1}). Groundnuts stored for 3 months in a well-ventilated room with an average temperature of 15°C were infected by *A. flavus* at a low level, but had no aflatoxin contamination. Infection increased with time in storage. Groundnuts harvested 1 week before maturity were not affected by *A. flavus* infection of seeds in storage, and there were no differences among genotypes. Groundnuts stacked in sacks at shelling sites were sampled; 4 % were contaminated with aflatoxin, the average level being 11 µg kg^{-1}. Fifteen per cent of the samples from oil mill sites were contaminated, the average aflatoxin content being 20 (ig 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 contaminated samples, with an average aflatoxin content of 10 µg kg^{-1}. This produce is usually allocated for local processing.

329. Ashworth, L.J.Jr., and Langley, B.C. 1964. The relationship of pod damage to kernel damage by molds in Spanish peanuts. Plant Disease Reporter 48(11): 875-878.

Results of 10 field experiments indicated that *Rhizoetonia solani,* alone and in combination with insect larvae, caused 87 *%,* and *Seterotium rolfsii* and unidentified agents 13 %. of the preharvest pod damage in Spanish groundnuts in Texas, USA. Kernel damage and the entry of fungi such as *Aspergillus niger* and *A. flavus* into the kernels was associated with the breakdown in pod structure, primarily by *R. solani.*

330. Ashworth, L.J.Jr., Schroeder, H.W., and Langley, B.C. 1965. Anatoxins : Environmental factors governing occurrence in Spanish peanuts. Science 148: 1228-1229.

Aflatoxins were absent from freshly harvested groundnuts although *Aspergillus flavus* infected most of the kernels from pods with visible openings. Microbial competition, governed by kernel moisture, limited aflatoxin content of kernels. The toxins were subjected to microbial breakdown, but the amount of toxin destruction was governed by initial aflatoxin concentration.

331. Aucamp, T.L. 1969. The role of mite vectors in the development of aflatoxin in groundnuts. Journal of Stored Products Research 5: 245-249.

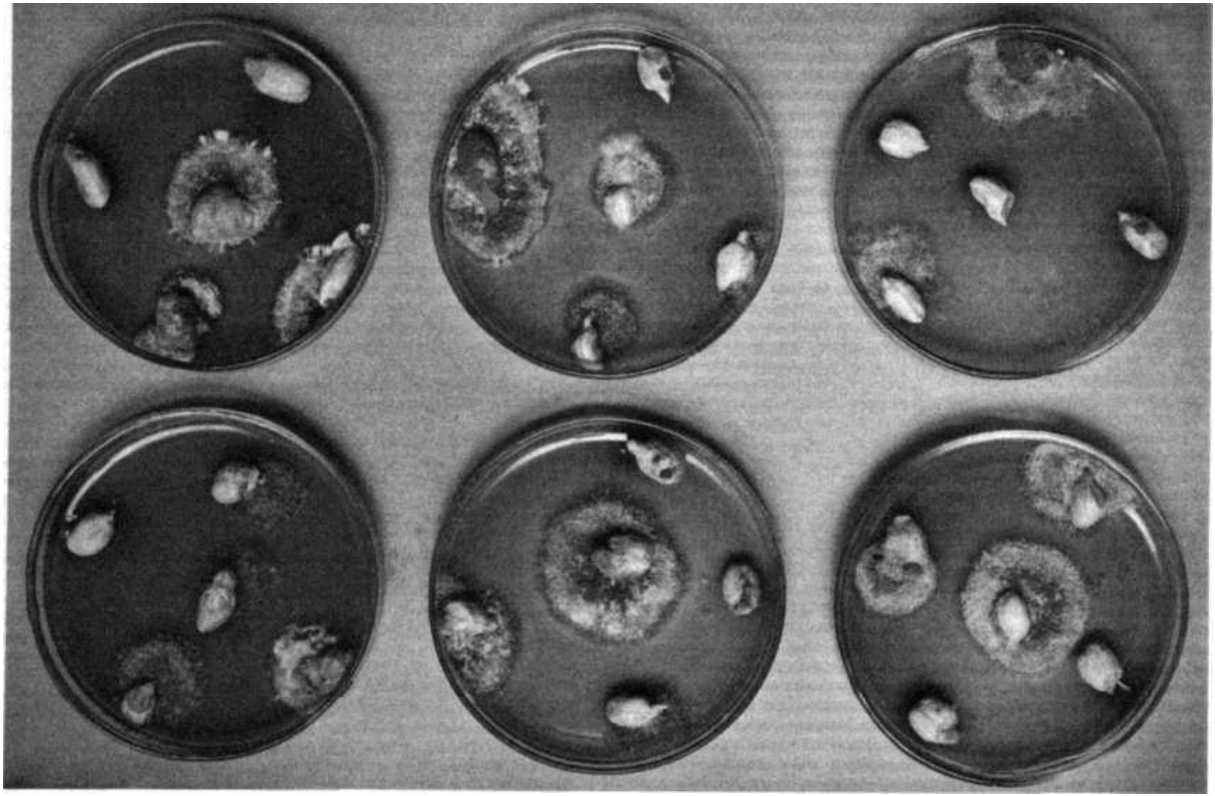
Field surveys in northern Transvaal, the Republic of South Africa, showed large numbers of Astigmatid mites associated with the subterranean parts of the groundnut plants. Mites were usually heavily contaminated with spores of species of the fungus *Aspergillus.* Under suitable conditions, contaminated mites may enter the groundnut kernels, causing proliferation of the fungus and production of aflatoxin.

332. Bampton, S.S. 1963. Growth *of Aspergillus flavus* and production of aflatoxin in groundnuts - Part 1. Tropical Science V(2): 74-81.

This article was written as an introduction to a series of research papers on the agricultural aspects of the problem of infection of groundnuts and subsequent aflatoxin production by *Aspergillus flavus.* It briefly reviews the problem in the widest sense, and then deals with the preliminary steps required to determine the point of entry of the fungus into the groundnut and subsequent development of toxicity.

333. Barnes, G.L. 1971. Mycoflora of developing peanut pods in Oklahoma. Mycopathologia et Mycologia Applicata 45(2):85-92.

Starr and Argentine groundnut cultivars were grown in soil fungicide-treated



Illustrations to show preharvest seed infection by *Aspergillus flavus*.

irrigated and non-irrigated plots in a 2 year study designed to determine possible-effects of management practices on invasion of developing pods and seeds by *Aspergillus flavus* and other soil fungi. Many genera and species of fungi were isolated from whole pods and half shells throughout each season, fungi were seldom isolated from kernels. No significant differences in populations between fungicide treatments, irrigation, or cultivars were found. Significant shifts in frequency of isolation of dominant genera *{Fusarium* and *Penicillium)* occurred during 1965 and 1966. *Trichoderma viride,* a minor component of the population at the first sampling in 1966, beacme a sub-dominant member by the last sampling. The reverse was true with *A. niger.* Several species of fungi, not previously isolated from groundnut pods, were found in pods in this study : *Actinomucor elegans, A. sclerotiorum, Sordid fumicola, S. humana,* and *Sporormia australis.*

334. Baur, F.J., and Parker, W.A. 1984. The aflatoxin problem : industry-FDA-USDA cooperation. Journal of the Association of Official Analytical Chemists 67(1): 3-7.

The role of groundnut growers, shellers and manufacturers of consumer groundnut products, together with USDA and FDA, played in forming a joint task force to develop a program of research into incidence, causes and control of aflatoxin contamination of groundnuts and groundnut products is discussed.

335. Bell, D.K., Minton, N.A., and Doupnik, B.Jr. 1970. Infection of peanut pods by *Aspergillus flavus* as affected by *Meloidogyne arenaria* and length of curing time. Phytopathology 60: 1284.

Studies were conducted to determine the effects *of Meloidogyne arenaria* and curing time on pod infection by *Aspergillus flavus.* Groundnuts of the cultivar Argentine were grown in field microplots containing methyl bromide-treated soil inoculated with either *A. flavus, M. arenaria,* or *A. flavus* + *M. arenaria.* Noninoculated microplots served as controls. F,ach treatment was replicated six times. At maturity the plants were dug, the pods rated for root knot galling, and one-third of the pods harvested. The remaining pods were left attached to the plants and placed on a wire-mesh greenhouse bench to cure. Additional samples were made after 5 or 12 days of curing. After each sampling, 25 two- seeded pods from each replicate were assayed for fungi. The remaining pods were dried for subsequent aflatoxin analyses. Pods from *M.* arenaria-inoculated plants were heavily galled, but the incidence of *A. flavus* and total fungi was not affected. The length of the curing time did not affect the incidence of *A. flavus* or total fungi. Only in pods from *A.* flavus-inoculated plants was the incidence of *A. flavus* increased. Aflatoxin contamimuion was not affected by any of the treatments. *M. arenaria* damage to groundnut pods did not affect *A. flavus* infection.

336. Bell, D.K., Minton, N.A., and Doupnik, B.Jr. 1971. Effects *of Meloidogyne arenaria, Aspergillus flavus,* and curing time on infection of peanut pods by *Aspergillus flavus.* Phytopathology 61: 1038-1039.

The effects of *Meloidogyne arenaria, Aspergillus flavus,* and curing time on infection of groundnut pods by *A., flavus* were studied. Plants were grown in field microplots of fumigated soil inoculated with either *A. flavus, M. arenaria,* or *A. flavus* + *M.* arenaria. Pods from *M. arenaria* inoculated plants were heavily galled, but the incidence of *A. flavus* and total fungi was not affected. Length of curing time also did not affect the incidence of *A. flavus* or total fungi. Only in pods from *A. flavus*-inoculated plants was the incidence of *A. flavus* increased. Aflatoxin contamination was not affected by any of the treatments. It was concluded that *M. arenaria* damage to groundnut pods did not affect *A. flavus* infection.

337. Blaney, B.J. 1985. Mycotoxins in crops grown in different climatic regions of Queensland. Pages 97-108 in Trichothecenes and other mycotoxins (Lacey, J., ed.). John Wiley & Sons Ltd. : UK.

This paper considers climatic conditions in relation to mycotoxin contamination in different crops in Queensland, Australia. Queensland has a history of recurring drought, and drought stress is an important factor in preharvest aflatoxin contamination of the groundnut and maize crop, particularly in the drier Burnett region. Conversely, while drought depresses yields, dry conditions usually limit the extent of fungal damage to wheat, barley and sorghum. However, the 1983 season was exceptional because persistent heavy rain resulted in extensive mold damage to all crops. Mycotoxins produced by *Fusarium* spp. had not been implicated in widespread mycotoxicosis in Queensland, although they may contaminate maize and sorghum in regions with high rainfall during the growing season, e.g., the tablelands in Far North Queensland. Mycotoxicosis of man seems extremely unlikely to occur in Queensland, but mycotoxicosis of livestock occurs occasionally. It is usually associated with moldy feed that is stored in moist conditions. In addition, cases are likely to occur in drought stress seasons when feeds are scarce, and of low quality.

338. Blankenship, P.D., Cole, R.J., and Sanders, T.H. 1980. Rainfall control plot facility at National Peanut Research Laboratory. Proceedings of the American Peanut Research and Education Association 12:46.

Six 18 ft x 40 ft plots with automatic mechanized roof systems for rainfall control were constructed. The 6-ft-deep artificial soil profiles in the plots were positively drained and protected from lateral soil moisture movement. Soil physical property data were measured and collected automatically. The facility was designed to provide absolute moisture control so that factors and relationships affecting *Aspergillus flavus* invasion of groundnuts in the field could be studied.

339. Blankenship, P.D., Cole, R.J., Sanders, T.H., and Hill, R.A. 1983. [Environmental control plot facility with manipulate soil temperature.]. Parcelles a environnement controle avec temperature du sol reglablc. Oleagineux 38(11): 615-620.

Six 66.92 sq m environmental control plots with automatic, movable shelters were designed and built at the National Peanut Research Laboratory, Dawson, Georgia, for the study of *Aspergillus flavus* invasion of groundnuts during drought stress. The shelters move over the plots at the onset of rainfall and off the plots when rainfall ceases. Plots were fitted with apparatus which allows soil temperature manipulation in the fruiting zone of groundnuts. Each plot has a separate shelter-mounted irrigation system. During operation, soil temperature and moisture data of the plots are collected automatically with a microprocessor-based, digital data-acquisition system.

340. Blankenship, P.D., Cole, R.J., Sanders, T.H., and Hill, R.A. 1984. Effect of geocarposphere temperature on pre-harvest colonization of drought-stressed peanuts by *Aspergillus flavus* and subsequent aflatoxin contamination. Mycopathologia 85: 69- 74.

Florunner groundnuts in research plots were subjected to 5 soil temperature and moisture treatment regimes resulting in *A. flavus* infection and subsequent aflatoxin contamination in drought- stressed groundnuts. Treatments imposed beginning 85 days after planting were drought, drought with heated soil, and 3 drought treatments with cooled soil. The incidence of *A. flavus* in drought-stressed, unshelled, sound mature kernels (SMK) decreased with a decrease in the mean 5 cm deep soil temperature. The incidence of *A. flavus* was greater in inedible categories and in damaged kernels than in SMK. The mean, threshold, geocarposphere temperature required for aflatoxin development during the latter part of the peanut growth cycle was between 25.7°C and 27°C.

341. Blankenship, P.D., Cole, R.J., and Sanders, T.H. 1985. Comparative susceptibility of four experimental peanut lines and the cultivar Florunner to pTeharvest aflatoxin contamination. Peanut Science 12: 70-72.

Four peanut genotypes, selected as resistant to invasion by *Aspergillus flavus* in laboratory screening with rehydrated, stored seed and the cultivar Florunner were subjected to preharvest drought and temperature conditions conducive to *A. flavus* invasion and aflatoxin contamination. Preharvest aflatoxin contamination of peanuts has been previously correlated with geocarposphere temperature and moisture conditions during drought. AII genotypes were highly contaminated with aflatoxin. This study indicates that a critical assessment should be made *of* the value of using the current laboratory method to select germplasm for resistance to *A. flavus* invasion and assuming resistance to aflatoxin contamination under field conditions.

342. Blankenship, P.D., Sanders, T.H., Dorner, J.W., Cole, R.J., and Mitchell, B.W. 1989. Engineering aspects of aflatoxin research in groundnuts : evolution of an environmental control plot facility. Pages 269-278 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 Oct 1987, ICRISAT Center, India. Patancheru 502 324, A.P., India : International Crops Research Institute for the Semi-Arid Tropics.

In 1980, an environmental control plot facility was designed and built at the National Peanut Research Laboratory, Dawson, Georgia, 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 x 5.5-m wide x 1.8 m deep, isolated plots with electric-motor-powered roofs for rainfall exclusion as required. Geocarposphere temperature manipulation was accomplished with thermostatically 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 micro-computer-based temperature control/alarm system was designed and installed to replace manual controls for soil temperature manipulation. The functional performance of the facility was adequate to provide a wide variety of required environmental conditions for research.

343. Bushnell, D.G. 1965. The incidence of aflatoxin in the Rhodesian groundnut crop. Rhodesia Agricultural Journal, Bulletin no. 2287. 62 : 94-96.

There was a low incidence of aflatoxin contamination in the 1962/63 Southern Rhodesian groundnut crop (50-250 µg kg^{-1} aflatoxin B_1), whereas with the 1963/64 crop, an appreciable number of samples were contaminated with more than 250 µg kg^{-1} aflatoxin B_1. This incidence occurred before marketing and was not aggravated by storage conditions used in Rhodesia. Although fungal infection might occur before harvesting, the major portion of aflatoxin contamination occurred in the period between lifting of the crop and delivery of the shelled groundnuts to the Grain Marketing Board. There was no evidence that the distribution of contamination was dependant upon the rainfall in the harvesting period. There appeared to be a higher level of aflatoxin B_1 contamination in areas with high air temperatures (65°F and above) in the harvesting period. The aflatoxin research program for the 1965 crop is described.

344. Butler, J.L., Cole, R.J., Holaday, C.E., Williams, E.J., Samples, L.E., McGill, J.F.. Blankenship, P.D., and Redlinger, L.M. 1973. Conditions related to aflatoxin contamination in the field. Proceedings of the American Peanut Research and Education Association 5: 195-196.

Samples of groundnuts were collected in the field prior to harvest, immediately after harvest, and from fanners' stock storage warehouses at widely separated points in Southwest Georgia, Alabama and North Florida. Samples were analyzed for aflatoxin contamination. Some groundnuts showing no visible hull damage (mechanical or insect damage) were analyzed just as pods came from the ground, and the kernels contained high levels of allatoxin, but no mold was apparent, even when examined under a microscope. Extremely dry weather during the latter part of the growing season allowed the groundnuts to dry to the moisture level which was conducive for aflatoxin production in the field. Some groundnuts were dug, inverted and sprayed immediately with fungicides. Low levels of aflatoxin were present at digging and aflatoxin increased with exposure in the windrow even though they were sprayed with fungicides.

345. Cole, R.J. 1989. Preharvest aflatoxin in peanuts. International Biodcterioration 25(4): 253-257.

The allatoxin problem in groundnuts was first associated with poor windrow drying conditions and, to a lesser extent, improper storage due to condensation and/or roof leakage. Recent research has resulted in a re-evaluation of the etiology of the allatoxin contamination of groundnuts. Preharvest contamination is thought to be the most significant source of contamination with storage contamination being a less significant problem. The probable sequence of events leading up to preharvest contamination of groundnuts is outlined. This is based on detailed studies carried out over a number of years.

346. Cole, R.J., Blankenship, P.D., Hill, R.A., and Sanders, T.H. 1984. Effect of geocarpospherc temperature on preharvest colonization of drought stressed peanuts by *Aspergillus flavus* and subsequent aflatoxin contamination. Pages 44-51 in Toxigenic Fungi - Their Toxins and Health Hazard (Kurata. H., and Ueno, Y., eds.). Kodansha Ltd. : Tokyo. Japan ; Elsevier : Amsterdam. Netherlands.

Groundnuts (cv. Morunner) grown three consecutive years in environmental control plots were subjected to various soil temperature and moisture treatment regimes resulting in *Aspergillus flavus* infection and subsequent aflatoxin contamination in some of the drought-stressed groundnuts. Treatments imposed beginning 85-100 days after sowing were drought, drought with heated soil, and drought with cooled soil treatments. The incidence of *A. flavus* in drought-stressed, unshelled, sound mature kernels (SMK) decreased with decreases in the mean 5 cm deep soil temperature. The incidence of *A. flavus* was greater in inedible categories and in damaged kernels than in sound mature kernels. The mean, threshold, geocarposphere temperature required for allatoxin development during the latter part of the groundnut growth cycle was between 26.3°C and 29.6°C with a mean geocarposphere temperature of 31.3°C, i.e.,too high for aflatoxin contamination.

347. Cole, R.J., Blankenship, P.D., Sanders, T.H., and Cole, R.A. 1984. Relation of preharvest aflatoxin contamination to duration of environmental stress. Journal of American Peanut Research and Education Society 16(1): 45.

Previous experiments established the optimum conditions of temperature and moisture for preharvest aflatoxin contamination of groundnuts. The optimum conditions are a mean temperature in the geocarpospherc of 29.5-30°C with a moisture level of between 40-60 bars. Visibly-undamaged groundnuts subjected to these stress conditions during the last 45-50 days of the growing season were highly contaminated with aflatoxin at harvest. The objective for CY 1983 studies was to determine the length of stress period required for preharvest contamination of groundnuts. Stress conditions were imposed 20, 30, 40 and 50 days before harvest. A stress period of 20 days before harvest was not sufficient to cause contamination. Groundnuts subjected to stress conditions for 30, 40 and 50 days were contaminated, therefore, a threshold stress period for preharvest aflatoxin contamination of groundnuts was 20-30 days before harvest.

348. Cole, R.J., Csinos, A.S., Blankenship, P.D., Sanders, T.H., Gaines, T.P., and Davidson, J.I. 1985. Evaluation of soil calcium as methods of prevention of preharvesi aflatoxin contamination of peanuts. Proceedings of the American Peanut Research and Education Society 17: 71.

In 1984, studies were conducted to evaluate the role of calcium in controlling preharvest aflatoxin contamination of groundnuts using the environmental control plots at Dawson, Georgia, USA. Florunner groundnuts were grown under soil environmental stress conditions optimum for preharvest allatoxin contamination. Calcium treatments were 0, 58 and 230 lbs $acre^{-1}$ calcium as $CaSO_4$ added to pretreatment levels. The levels of calcium in me kernels from the 0 and 230 lbs treatment levels were significantly different, reflecting a response to added calcium in spite of relatively high pretreaunent levels of soil calcium. There were no significant differences in calcium levels of kernels between the 0 and 58 lbs calcium treatments. No significant relationship between aflatoxin contamination and kernel calcium levels was found. The authors concluded that application and uptake of calcium by the groundnut seed was not a viable method to prevent preharvest aflatoxin contamination.

349. Cole, R.J., Hill, R.A., Blankenship, P.D., Sanders, T.H., and Garren, K.H. 1982. Influence of irrigation and drought stress on invasion by *Aspergillus flavus*

of corn kernels and peanut pods. Developments in Industrial Microbiology 23: 229-236.

Invasion of groundnuts and corn by *Aspergillus flavus* and *A. parasiticus* and contamination with aflatoxin occur primarily under drought-stress conditions. However, the elevated soil (groundnut) and ambient temperatures (corn) are extremely important factors. In the case of groundnuts, irrigation may provide effective management by reducing soil temperatures in the geocarposphere. This same protection apparently is not available for corn since irrigation may not be adequate to sufficiently alter ambient temperatures sufficiently.

350. Cole, RJ., Hill, R.A., Blankenship, P.D., and Sanders, T.H. 1986. Color mutants of *Aspergillus flavus* and *Aspergillus parasiticus* in a study of preharvest invasion of peanuts. Applied and Environmental Microbiology 52:1128-1131.

A comparison of the invasion of flowers, aerial pegs, and groundnut kernels by wild-type and mutant strains of *Aspergillus flavus* and *A. parasiticus* along with aflatoxin analyses of kernels from different drought treatments have supported the hypothesis that preharvest contamination with aflatoxin originates mainly from the soil. Evidence in support of soil invasion as opposed to aerial invasion was the following : (i) A greater percentage of invasion of kernels rather than flower or aerial pegs by either wild-type *A. flavus* or mutants, (ii) Significant invasion by an *A. parasiticus* color mutant occurred only in groundnuts from soil supplemented with the mutant, whereas adjacent plants in close proximity but in untreated soil were only invaded by wild-type *A. flavus* or *A. parasiticus,* (iii) Aflatoxin data from drought-stressed, visibly undamaged groundnut kernels showed that samples from soil not supplemented with a mutant strain contained a preponderance of aflatoxin Bs (from wild-type *A. flavus)* whereas adjacent samples from mutant- supplemented soil contained a preponderance of aflatoxins Bs plus Gs (from wild-type and mutant *A. parasiticus),* (iv) Preliminary data from two air samplings showed an absence of propagules *of A. flavus* or *A. parasiticus* in air around the experimental site.

351. Cole, D.L., and Masuka, AJ. 1989. Evaluation of new rapid methods for aflatoxin detection in groundnuts in Zimbabwe. Pages 185-189 in Proceedings of the third Regional Groundnut Workshop for Southern Africa, Lilongwe, Malawi, 13-18 March 1988, Patancheru 502 324. A.P., India : International Crops Research Institute for the Semi-Arid Tropics.

Two new rapid methods that use monoclonal antibodies to detect aflatoxins in groundnuts were tested. The Quantitox is an enzyme immunoassay using microtitre wells and the Aflatest is based on monoclonal antibodies absorbed onto an affinity column. It was confirmed that aflatoxin contamination occurs preharvest and that storage under low-moisture conditions prevents additional toxin development. There were differences in susceptibility of local cultivars to aflatoxin development. The Aflatest procedure was useful to rapidly screen groundnut samples for aflatoxin contamination, but there were problems with the Quantitox method. Aflatoxin contamination occurred in the field prior to harvest and was not aggravated by storage conditions at Cleveland Depot during 1987. There was a 19.23 % incidence of aflatoxin contamination in the samples taken in early 1987, but it was not related to the farm management system (communal, small-scale commercial, and large-scale commercial) or any particular pest or disease.

352. Cole, R.J., Sanders, T.H., Dorner, J.W., and Blankenship, P.D. 1989. Environmental conditions required to induce preharvest aflatoxin contamination of groundnuts : summary of six years' research. Pages 279-287 in. Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 Oct 1987, ICRISAT Center. India. Patacheru 502 324, A.P.. India : International Crops Research institute tor the Semi-Arid Tropics.

Environmental conditions necessary for preharvest aflatoxin contamination oi visibly sound groundnuts are reviewed on the basis of studies conducted at Dawson, Georgia. USA during six 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 infection 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, immature kernels. Phytoalexin-based resistance can readily explain the resistance in immature kernels, but it does not explain the broader-based resistance observed in 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.

353. Cole, R.J., Sanders, T.H., Hill, R.A., and Blankenship, P.D. 1985. Mean geocarposphere temperatures that induce preharvest aflatoxin contamination of peanuts under drought stress. Mycopathologia 91: 41-46.

Apparently undamaged groundnuts grown under environmental stress in the form of drought and heat become contaminated with *Aspergillus flavus* and aflatoxin in the soil prior to harvest. The upper mean temperature limit for aflatoxin contamination in undamaged groundnut kernels grown under drought stress during the latter 4-6 weeks of the growing season was between 29.6 - 31.3°C. The lower limit was between 25.7 - 26.3°C. Groundnuts grown under drought stress with a mean geocarposphere temperature of 29.6°C were highly contaminated while those at 31.3°C were not contaminated. Likewise, those grown under drought stress with

a mean geocarposphere temperature of 25.7°C were not contaminated while those subjected to a mean geocarposphere temperature of 26"C resulted in some categories becoming contaminated. Increasing the mean temperature to 29.6°C increased the amount of contamination.

354. Davidson, J.I.,Jr., Hill, R.A., Cole, R.J., Mixon, A.C., and Hcnning, R.J. 1982. Field performance of two peanut cultivars relative to resistance to invasion by *Aspergillus flavus* and subsequent aflatoxin contamination. Proceedings of the American Peanut Research and Education Society 14(1):74.

Two runner type groundnut cultivars. Sunbelt Runner and Florunner, identified by a laboratory method as having large differences in seed resistance to colonization by *Aspergillus flavus,* were evaluated for resistance to natural seed infection by *A. flavus* and subsequent aflatoxin contamination. Groundnuts were grown on three nonirrigated farms during 1980 using two planting dates and three harvest dates for each cultivar. Groundnuts grown on two farms experienced moderate to severe drought stress and both cultivars contained high levels of aflatoxin. Groundnuts on the third farm had adequate rainfall and contained only very low levels of aflatoxin. Sunbelt Runner (reported to be resistant to *A flavus* colonization of seeds) had no advantage over Florunner (reported to have only moderate resistance to seed colonization) in respect of levels of *A. flavus* and subsequent aflatoxin contamination under field conditions. Levels *of A. flavus* infection and aflatoxin contamination were related primarily to environmental conditions, especially drought stress, during pod maturation. Genetic resistance to invasion by *A. flavus* and subsequent aflatoxin production must be verified in field environments or under conditions simulating those environments.

355. Davidson, J.I.Jr., Hill, R.A., Cole, R.J., Mixon, A.C., and Henning, R.J. 1983. Field performance of two peanut cultivars relative to aflatoxin contamination. Peanut Science 10(1): 43- 47.

Two runner type groundnut cultivars, "Sunbelt Runner" and "Florunner", were compared under differing field conditions for natural seed infection by *Aspergillus flavus* and aflatoxin contamination. Laboratory tests had shown marked differences in seed resistance to colonization by *A. flavus.* Groundnuts were grown on three nonirrigated farms during 1980 using two planting dates and three harvesting dates for each cultivar. Groundnuts grown on two farms experienced moderate to severe drought stress and both cultivars contained high levels of aflatoxin. Groundnuts on the third farm received adequate rainfall and contained only very low levels of aflatoxin. Sunbelt Runner (reported to be resistant to *A. flavus* colonization of seeds) had no advantage over Florunner (reported to have moderate resistance to seed colonization) in respect of levels of *A. flavus* infection and subsequent aflatoxin contamination under field conditions. Levels of *A. flavus* infection and aflatoxin contamination were related primarily to environmental conditions, especially drought stress, during pod maturation. These results show that the current laboratory assay method for selecting resistant lines should be carefully reassessed.

356. Dickens, J.W. 1977. Aflatoxin occurrence and control during growth, harvest and storage of peanuts. Pages 99-105 in Mycotoxins in human and aninmal health. (Rodricks, J.V., Hesseltine, C.W., and Mchlman, M.A., eds.). Illinois, USA : Pathotox Publishers, Inc. 807 pp.

This paper discusses causes of aflatoxin contamination and methods of prevention and segregation to control the aflatoxin problem in fanners' stock groundnuts from the time the groundnuts are growing in the field until they are marketed, stored, and shelled.

357. Dickens, J.W., Satlerwhite, J.B., and Sneed, R.E. 1973. aflatoxin-contaminated peanuts produced on North Carolina farms in 1968. Proceedings of the American Peanut Research and Education Society 5(1): 48-58.

The geographical distribution of rainfall and of farms which produced segregation-3 groundnuts in North Carolina suggest that drought after groundnuts are formed but prior to harvest is conducive to infection by *Aspergillus flavus* before harvest. Damage from the lesser cornstalk borer (LCB) may also favor infection. However, many drought-area fields infested with LCB did not produce segregation-3 groundnuts. *Aspergillus flavus* growth and aflatoxin contamination probably occurred before harvest. Some groundnuts which contained visible *A, flavus* growth and high concentrations of aflatoxin were found on freshly-dug plants in two fields. Typical LCB damage was found on some pods in 96 % of the samples taken from lots of segregation-3 groundnuts. The incidence of kernels with visible *A. flavus* growth (AFK) appeared to be related to pod condition. There were 9.4, 7.6, 2.5, 0.0 and 0.8 AFK kg^{-1} of kernels from LSK, insect- damaged pods, mechanically-damaged pods, sound-mature pods and other pods, respectively. Kernels from LCB-damaged pods apparently had a much higher incidence of AFK than did kernels from pods with any other type of damage. Kernels with no visible damage that passed over a 15/64 inch slotted screen (R 15) contained an average of 149 µg kg^{-1} aflatoxin. The portion that consisted of all damaged kernels in the samples and all kernels that passed through the screen contained an average of 10,018 µg kg^{-1} aflatoxin. Even the R15 kernels from only the sound pods contained an average of 29 µg kg^{-1} aflatoxin. Kernels from pods not damaged by LCB often contained aflatoxin.

358. Diener, U.L. 1965. Relation *of Aspergillus flavus* invasion to maturity of peanuts at harvest. Journal of the Alabama Academy of Siences 36: 21.

Groundnuts were hand-picked from plants or ground immediately after digging and separated into immature, mature, and overmature-damaged categories Groundnuts within each category were separated on the basis of pod color, appearance, and

location on the plant. Microfloral analysis showed that *Aspergillus flavus* was present only in the overmature-damaged groundnuts (100 spores g^{-1} of kernels). Two per cent of the mature and 8 % of the overmature-damaged kernels were infected with *A. flavus*.

359. Diener, U.L., Cole, R.J., Sanders, T.H., Payne, G.A., Lee, L.S., and Klich, M.A. 1987. Epidemiology of aflatoxin formation by *Aspergillus flavus*. Annual Review of Phytopathology 25: 249- 270.

The epidemiology of aflatoxin contamination in grounduts, maize and cottonseed, the life cycle of the aflatoxin-producing fungus, *Aspergillus flavus,* from sources of primary and secondary inoculum, the mode of entry of *A .flavus* into the plant, seed infection, and aflatoxin formation in the seed is reviewed.

360. Diener, U.L., and Davis, N.D. 1977. Aflatoxin formation in peanuts by *Aspergillus flavus*. Bulletin of the Alabama Agricultural Experimental Station (No. 493), 49 pp. Auburn, Alabama, USA : Auburn University.

This review discusses factors influencing production of aflatoxin in groundnuts including: the aflatoxin-producing fungus *Aspergillus flavus,* the substrate, relative humidity, moisture, temperature and time, aeration, the pod, and damage to the kernel.

361. Diener, U.L., Jackson, C.R., Cooper, W.E., Stipes, R.J., and Davis, N.D. 1965. Invasion of peanut pods in the soil by *Aspergillus flavus.* Plant Disease Reporter 49(11): 931-935.

The extent of pod (shell) and kernel invasion by *Aspergillus flavus* and other fungi by digging time was determined in groundnut fields in Alabama, Georgia, and North Carolina. Groundnut fruits were classified into maturity, damage, and discoloration categories. Pods were surface-sterilized, shells and kernels plated on Czapek-Dox agar medium containing 20 % sucrose, and incubated at 26-30°C for 4 to 7 days. Data from Alabama in 1963 showed that the percentage of kernels and shells invaded by *A. flavus* increased with time and with maturity of the groundnuts. In 1964, low levels of *A. flavus* were found in kernels and shells in all three States. Low infections were attributed to abundant and well-distributed rainfall and moderate temperatures.

362. Dorner, J.W., Cole, R.J., Blankenship, P.D., and Sanders, T.H. 1988. Pattern of preharvest aflatoxin contamination of peanuts based on single kernel analysis. Proceedings of the American Peanut Research and Education Society 20(1):24.

Cultivar Florunner groundnuts were grown in an environmental control plot at the National Peanut Research Laboratory, Dawson, USA. Plants were subjected to late-season drought stress under conditions favoring preharvest aflatoxin contamination. Individual plants were harvested during the drought period, and single-kernel analyses were performed for aflatoxin contamination. An association between aflatoxin-contaminated kernels and certain individual plants was observed. Of the 1340 kernels analyzed from 44 plants, only 3.5 % had > 10 μg kg^{-1} aflatoxin, but 87.2 % of those kernels came from 9.1 % of the plants. Over half of the contaminated kernels (> 10 µg kg^{-1}) came from one plant. Of the kernels that contained > 10 µg kg^{-1} aflatoxin, 42.6 % were from yellow 2 hull-scrape maturity stage pods, and 78.8 % were confined to the immature yellow 1, yellow 2, and orange A stages combined. Only 17 % were from the mature brown and black maturity stages. There was not a strong relationship between aflatoxin contamination and the location of the kernel in the pod. Both kernels were contaminated in 45.5 % of the pods containing kernels with > 10 µg kg^{-1} aflatoxin. In 21.1 % only me apical kernel was contaminated and in 15.2 % contamination was confined to the basal kernel. One-kemel pods accounted for 18.2 % of the contaminated kernels.

363. du Toit, A.A. 1977. Aflatoxin research in Rhodesia. Rhodesia Agricultural Journal, Research Report no. 1. 33 pp.

Since 1962, a research team in Rhodesia has studied the factors that may affect the incidence and level of aflatoxin contamination of groundnuts and ways of dealing with the aflatoxin problem. This report gives a brief account of the research projects and discusses pertinent results. Among these is the possibility of predicting levels of aflatoxin contamination of the crop resulting from different meteorological, seasonal and cultural factors.

364. El Nur, E., and Ibrahim, G. 1970. *Aspergillus flavus* and aflatoxin production. 1.- Kernel moisture content and kernel contamination. Sudan Agriculture Journal 5(1):5-15.

In the Gezira, the Sudan, field experiments showed that *Aspergillus flavus* and other fungi such as *Penicillium* spp. and *Fusarium* spp., were always present in kernels from broken groundnut pods. On watering before harvest, the kernel moisture content immediately after lifting was 58.7 % for the cultivar Ashford, and 46 % for the cultivar Barberton, but after 7 days in the windrow the moisture content dropped to 4.7 and 3.8 %, respectively. Groundnuts dried immediately after harvest for at least 7 days were considered to be free from aflatoxin.

365. Elamin, N.H.H., Abdel-Rahim, A.M., and Khalid, A.E. 1988. Aflatoxin contamination of groundnuts in Sudan. Mycopathologia 104(1):25-31.

Groundnut samples, collected soon after harvest, from different districts in the

irrigated region (Central Sudan) were free from aflatoxins. Samples collected from the rainfed region (Western Sudan) showed incidence of aflatoxin contamination ranging from 100 % samples in El Hamdi to only 10 % samples in Casgeal. Damaged pods were highly contaminated with *Aspergillus flavus* and aflatoxins. Sound intact pods had lower fungal contamination and were almost free of aflatoxins. Groundnut products (groundnut paste, red and grey roasted pods) collected from Khartoum North (Bahri) had higher levels of aflatoxin than those collected from Khartoum and Umdorman. Grey and red roasted pods showed higher levels of aflatoxins than the groundnut paste. None of the three varieties (MH383, Barberton, Ashford) of groundnut tested was completely resistant to *A. flavus* contamination and aflatoxin production. A temperature of 30°C and 86.3 % relative humidity were the optimum conditions for both *A. flavus* growth and aflatoxin production in groundnuts.

366. Garren, K.H. 1966. Peanut (Groundnut) microfloras and pathogenesis in peanut pod rot. Phytopathology Zeitschrift 55(4): 359-367.

In Virginia (USA), *Trichoderma viride* appears to be dominant, and *Penicillium* spp. sub-dominants, in the climax endogeocarpic community of sound and rotting pods, *Aspergillus flavus* and *A. niger* being minor but persistent and potentially dangerous. Pathogenicity tests suggest a stage in fruit growth when factors as yet unkown weaken the natural resistance of the pod to invasion by rot fungi so that it becomes susceptible to several efficient saprophytes from the geocarposphere. *Pythium myriotylum* was indicated as the prime pod-rot pathogen and *Rhizoctonia solani* as sporadically important.

367. Garren, K.H. 1981. Drought, irrigation, and field infection of peanuts and corn by *Aspergillus flavus* in Virginia in 1980. Proceedings of the American Peanut Research and Education Society 13(1): 60.

Field infections of groundnuts and corn by the toxigenic *Aspergillus flavus* were studied in plots of an irrigation study. April-September rainfall in the study area was 40 % of normal. Prior to the 1980 harvests the hidden ("unseen") *A. flavus* infections in freshly dug groundnuts - i.e. pockets of infection from which the mold proliferates when groundnuts are not handled properly - varied from none to 1.5 %. On 10/2/80 this hidden infection was 0.75 % in irrigated groundnuts and 3.5 % in non-irrigated groundnuts. Visible infections (the hallmark of "Seg.3") had never been reported at digging in Virginia before 1980, but in 1980 visible *A. flavus* infections were found on many windrowed groundnuts (pods) within a few hours after digging in the non-irrigated plots. Corn taken directly from the field to the laboratory had much more *A. flavus* infection if it came from non-irrigated plots, but careful handling of the irrigated com was indicated. Hot, dry weather induced *A. flavus* infection in the irrigated corn. The 1980 droughts' effects on aflatoxin formation in com and on aflatoxin potentials in groundnuts increased the area's 1980 economic woes.

368. Garren, K.H., Christensen. C.M., and Porter, D.M. 1969. The mycotoxin potential of peanuts (Groundnuts) : The U.S.A. viewpoint. Journal of Stored Products Research 5: 265-273.

The mycotoxin potential of a plant product can be guaged by seeking answers to two or three questions basic to the mycotoxin problem, namely, 'Which of the fungi invading the product can be toxigenic ?', and 'How can they become established in the product?' Both questions relate to the quite new science of microbial synecology. In contrast, the third basic question ('What factors are necessary for toxin production by a given toxigenic fungus?') relates to the much older but more complex science of microbial autecologv. Fruits of groundnuts are completely hypogeic in their development, and there seems to be a successional invasion of them by soil-borne or 'field' fungi with the result that sound mature fruits have a dormant or quiescent endogeocarpic microflora community. Results of co-operative research in six groundnut producing states (U.S.A.) and at the University of Minnesota, interpreted in the light of present-day groundnut harvesting practices in the U.S.A., justify the following conclusions : (1) *Aspergillus flavus* can be toxigenic under field conditions and is a serious problem. (2) Many other fungi from the groundnut endogeocarpic community are toxigenic under laboratory conditions. (3) Until the conditions under which these fungi may become toxigenic are better known, their presence in lifted groundnuts constitutes a real mycotoxin potential. (4) The genera posing the greatest threat arc, in estimated order of importance, *Penicillium, Aspergillus* (primarily because of *A. flavus). Alternaria,* and *Fusarium.* (5) Much care must be exercised with groundnuts after lifting, to minimise opportunity for proliferation of such fungi. This care must be exercised at least until we determine the extent to which such fungi might be expected to produce toxins in groundnuts on the farm and during subsequent handling.

369. Gilman, G.A. 1969. An examination of fungi associated with groundnut pods. Tropical Science 11(1):38-48.

Studies in the Gambia showed that mature kernels inside visibly intact pods of the groundnut varieties 'Senegal' and 'G.O.M.B.' were frequently associated with the fungus *Macrophomina phaseolina* before and directly after harvest. Some mature pods were occasionally contaminated with the fungus *Aspergillus flavus* at lifting. The degree of invasion by these fungi was influenced, among other things, by soil moisture, shell and kernel maturity, correct lifting techniques, and the intensity of sun drying. Postharvest invasion increased with higher rainfall intensities. In general, invasion patterns were independent of varieties.

370. Goarin, P., and Goarin, S. 1970. (Contribution to the knowledge of

groundnut aflatoxin in Senegal.]. Contribution a la connaissance de l'aflatoxine de l'arachide au Senegal. L'Agronomie Tropicale 25(3):277-293.

Research conducted mainly in Senegal on the significance of aflatoxin contamination of groundnut, methods of analysis, and control measures are reviewed. Contrary to the general assumption that high aflatoxin contents arc associated with wetting of crop produce, in northern Senegal it was found that aflatoxin contamination was attributed to failure of crops to attain physiological maturity during dry years. Less arid production area, where infection is less, improved crop drying and possibly grading of produce should allow the production of acceptable edible or confectionery groundnuts and even groundnut meals. In general, the use of varieties better adapted to the environmental conditions, and careful harvesting, will keep the aflatoxin contents of groundnut and oil-cakes at an acceptable level.

371. Graham, J, 1982. Aflatoxins in peanuts: occurrence and control. Queensland Agricultural Journal 108(3):119-122.

The problem of aflatoxin contamination, which was serious in the Queensland groundnut industry in drought years, is reviewed with special reference to factors influencing aflatoxin formation including moisture stress, insect damage, and cultivar resistance. Control measures included crop hygiene, resistant varieties, avoiding moisture stress, harvesting the crop at optimum maturity, avoiding pest and mechanical injury, drying rapidly and segregating contaminated batches of groundnuts.

372. Griffin, G.J. 1972. Conidial germination and population *oi Aspergillus flavus* in the geocarposphere of peanut. Phytopathology 62:1387-1391.

Under greenhouse conditions, pegs of Viriginia Bunch 46-2 groundnut plants were introduced into small pots containing a nonsterile loamy line sand artificially infested with washed conidia of a clone of *Aspergillus flavus* isolated from groundnut fruit. Dilution plate analysis indicated the inner 0.5 mm layer of geocarposphere soil of pegs and mature fruits had *A. flavus* populations slightly different from nongeocarposphere soils. In contrast, the population of total fungi was greater, and the populations of bacteria and actinomycetes were much greater, in geocarposphere soils. Microscopic observation of the inner 0.5 mm soil layers indicated that no germination of *A. flavus* conidia occurred in peg geocarposphere soil, and trace germination occurred in geocarposphere soil for plants maintained in a growth chamber at 30°C. But *A. flavus* conidia germinated readily in soil adjacent to pods after 16 h at 30°C and 35°C when a 4- to 6- sq mm area of pod surface was superficially injured and inoculated with infested soil. Dry conidia applied to aerial portions of pegs in the greenhouse germinated at a low percentage. These results are discussed in relation to the exogenous carbon and nitrogen requirements for spore germination.

373. Griffin, G J., and Garren, K.H. 1976. Colonization of aerial peanut pegs by *Aspergillus flavus* and *A. niger-group* fungi under field conditions. Phytopathology 66(10):1161-1162.

Selective media were used to isolate *Aspergillus flavus* and fungi of the *A. niger* group from aerial groundnut pegs in the field during 1973,1974, and 1975 and from groundnut flowers in 1974 and 1975. *Aspergillus flavus* was isolated from about 7 % of washed groundnut flowers in both years, but isolation frequency from terminal portions of washed aerial pegs and surface-sterilized aerial pegs over the same period was lower (1.5 % and 0.3 %, respectively). In 1973, *A. flavus* was isolated from 0.2 % of surface-sterilized aerial pegs. Isolation frequency of antagonists of *A. flavus* in the *A. niger* group was lower than *A. flavus* on flowers and similar on aerial pegs. Low levels of colonization of groundnut fruits by *A. flavus* via flower and aerial peg colonization appears possible under Virginia field conditions.

374. Habish, H.A., Abdulla, M.H., and Broadbent, J.H. 1971. The incidence of aflatoxin in Sudanese groundnuts. Tropical Science XIII(4):279-287.

A preliminary survey of the incidence of aflatoxin in the main groundnut production areas of the Sudan is described, correlating the extent of infection with *Aspergillus flavus* and other kernel fungi with the aflatoxin content of the kernels. In general, the rainfed samples had a higher incidence of kernel contamination than those from the irrigated areas. The majority of the groundnut samples from the irrigated areas were aflatoxin-free, whereas in those from the rainfed areas none was free, and 48 % were classified low-medium (<50 to 50-250 μg kg^{-1} aflatoxin), while 52 % were in the high to very high category (250-1000 μg kg^{-1} to > 1000 μg kg^{-1} aflatoxin).

375. Hanafi, M.O., and Hassan, S.H. 1982. Prospects for improved control of aflatoxin in Sudanese groundnuts through traditional marketing systems. Pages 417-420 in Proceedings of the International Symposium in Africa on Production, World Oilseeds Market and Intra-African Trade in Groundnuts and Products, 7-11 June 1982, Banjul, The Gambia : African Groundnut Council, Lagos, Nigeria.

Groundnut is an important crop in the Sudan, both in rainfed areas where end-of-season droughts are common, and under irrigation in the Gezira. Quality factors arc important in determining acceptability and market price. Aflatoxin contamination is a problem in the rainfed crop. Aflatoxin levels are important in determining quality grade and price. The groundnut marketing system is described. A detoxification plant is under construction and will be used to detoxify groundnut cake and meal.

376. Hanlin, R.T. 1969. Fungi in developing peanut fruits. Mycopathologia ct Mycologia Applicata 38(1-2): 93-100.

Results showed that 84 % of groundnut pegs contained fungi before entering the soil; shell invasion remained high (90-100 %) throughout the season and seed invasion rose to 82 %, declining at harvest. The most common shell fungi were *Penicillium, Fusarium,* and *Phoma* spp., and seed fungi *Penicillium* and *Gliocladium* spp. Groundnut fungi can be ecologically grouped into an aerial flora, terrestial flora, and species colonizing fruits above the soil and in the soil.

377. Hanlin, R.T. 1970. Invasion of peanut fruits by *Aspergillus flavus* and other fungi. Mycopathologia et Mycologia Applicata 40: 341-348.

Intensive studies of the mycoflora of "Dixie Spanish" groundnuts grown under different combinations of tillage, organic matter, and previous crop revealed no consistent reduction in either total fungi, *Aspergillus* spp., *A. niger, A. flavus, Penicillium* spp., or *Fusarium* spp. for any of the 16 combinations studied. The level of fungal invasion in apparently sound groundnut shells and seeds was consistently high, but the species composition of this flora changed as the season progressed. *Penicillium* was the most common genus, being isolated from over half of the shells sampled. The level in seeds was lower, but in both shells and seeds there were more *Penicillium* spp. in fruits in the soil than in fruits from stacks. *Aspergillus* spp. occurred at lower levels than *Penicillium,* but they also decreased in fruits in the stack. Of particular interest is that at all three collecting dates the level of *Aspergillus* isolated from seeds was at least twice that from shells. The lower frequency of certain fungi in fruits from stacks is undoubtedly related to the lower moisture level in the drying fruits. Other fungi, especially *Fusarium* spp., increased sharply in the dried tissues so that the overall level of invasion remained high. This may be of considerable practical importance since certain species of *Fusarium* produce toxins.

378. Hanlin, R.T. 1985. Ecology of mycotoxigenic fungi in nut crops. Pages 175-184 in Filamentous microorganisms. Biomedical aspects (Arai, T. ed.). Japan Scientific Societies Press, Tokyo : Japan.

Current information is reviewed, with special reference to work on the mycoflora of groundnuts and pecans and factors which favor invasion of seeds by *Aspergillus flavus* and the production of aflatoxin.

379. Hill, R.A., Blankenship, P.D., Cole, R.J., Sanders, T.H., Kirksey, J.W., and Greene, R.L. 1981. Influence of soil temperature and moisture on microflora, aflatoxin concentration, maturity and damage in peanuts. Proceedings of the American Peanut Research and Education Society 13(1):61.

Cultivar Florunner groundnuts were grown for 145 days in experimental plots in 1980. Different treatment regimes, imposed 94 days after sowing were irrigated (I); irrigated with heated soil (III); drought-stressed (D); and drought-stressed with cooled soil (DC). Soil temperature and moisture tension at 2", 12" and 24" below the surface were measured throughout the growing season. At harvest, the incidence of the *Aspergillus flavus* group within kernels and aflatoxin concentration were increased by any kind of damage for all treatments. In sound mature kernels (SMK) colonization by the *A. flavus* group was greatest with treatment D (75 % kernels colonized), least in 1 (7 %) and DC (11 %) and intermediate for III (26 %). Aflatoxin was absent from or negligible (<1 μg kg^{-1}) in SMK with 1, 111 or DC treatments but there were 244 μg kg^{-1} aflatoxin in D treated SMK. The proportion of immature and damaged kernels at harvest was increased by drought-stress and decreased by irrigation but little affected by temperature. More aflatoxin was found in immature than mature sound kernels. Extensive colonization of SMK by the *A. flavus* group, and subsequent aflatoxin production, was favored by hot, dry conditions when most associated microorganisms did not grow. Elevated temperature alone or drought stress alone did not cause aflatoxin contamination in SMK. When the ratio of SMK colonized by *A.flavus* compared to *A. niger* was > 19:1 there was aflatoxin contamination, but no aflatoxin if this ratio was < 9:1. Irrigation is recommended to prevent aflatoxin contamination in groundnuts.

380. Hill, R.A., Blankenship, P.D., Cole, R.J., and Sanders, T.H. 1983. Effects of soil moisture and temperature on preharvest invasion of peanuts by the *Aspergillus flavus* group and subsequent aflatoxin development. Applied and Environmental Microbiology 45(2):628-633.

Four soil temperature and moisture treatment regimes were imposed on cultivar Florunner groundnuts 94 days after sowing in experimental plots in 1980. At harvest (145 days alter sowing), the incidence of the *Aspergillus flavus* group and the aflatoxin concentration were greatest in damaged kernels. Extensive colonization of sound mature kernels (SMK) by the *A.flavus* group occurred with the drought stress treatment (56 % kernels colonized); colonization was less in the irrigated plot (7 %) and the drought stress plot with cooled soil (11 %) and was intermediate in the irrigated plot with heated soil (26 %). Aflatoxin was virtually absent from SMK with the last three treatments, but it occurred at an average concentration of 244 μg kg^{-1} in drought-stressed SMK. Colonization of SMK by the *A. flavus* group and aflatoxin production were greater with hot dry conditions. Neither elevated temperature alone nor drought stress alone caused aflatoxin contamination in SMK. When the ratio of SMK colonized by *A. flavus* compared with *A. niger* was > 19:1, there was aflatoxin contamination, but there was none if this ratio was < 9:1. Irrigation caused a higher incidence of *A. niger* than did drought. This may have prevented the aflatoxin contamination of undamaged groundnuts.

381. Hill, R.A., Wilson, D.M., McMillian, W.W., Widstrom, N.W., Cole, R.J., Sanders, T.H., and Blankenship, P.D. 1985. Ecology of the *Aspergillus flavus* group and aflatoxin formation in maize and groundnut. Pages 79-95 in Trichothecenes and Other Mycotoxins (Lacey, J., ed.). John Wiley & Sons Ltd. : UK.

The effects of environmental factors on preharvest aflatoxin contamination of groundnuts and maize are reported. Results on aflatoxin production by various isolates of *Aspergillus flavus* and *A. parasiticus* from maize and groundnuts are summarized. Temperature, of the air (maize) and in the geocarposphere (groundnut), during seed development was the single most important determinant of colonization by the *Aspergillus flavus* group of fungi and subsequent aflatoxin contamination. Other factors, especially the availability of water to plant and fungus, interactions between temperature and the availability of water, and the effects of these variables on the *A.flavus* group fungi and associated microorganisms were also important. No aflatoxin contamination occurred in undamaged groundnut kernels from crops grown with adequate irrigation (irrespective of geocarposphere temperature) or from drought-stressed crops when the mean geocarposphere temperature during the last 45 days before harvest was < 25°C or > 32°C. There were differences between edible and inedible grades of kernels and within U.S. Commercial edible grades (Jumbo, Medium, No.1 and other edible) both for *A.flavus* infection and aflatoxin contamination. Any kind of kernel damage, irrespective of the crop growth conditions, could enhance aflatoxin contamination. Aflatoxin concentration in edible-grade kernels increased with increased geocarposphere temperature to a maximum of nearly 500 µg kg^{-1} (mean temperature, 30.5°C). Groundnut kernel infection by the *A.flavus* group fungi was also maximal at 30.5 (95.7 % kernels infected at harvest) with a smaller proportion of kernels infected at lower and higher temperatures. In maize, kernel infection by the *A.flavus* group fungi and aflatoxin contamination were both maximal during the growing season when air temperatures were around 30°C. Damaged ears also were more often colonized by the *A.flavus* group fungi and more likely to be contaminated with aflatoxins than undamaged ears. Most *A.flavus* group isolates from maize were identified as *A.flavus* and a smaller proportion (< 10 %) of isolates as *A. parasiticus.* In groundnuts, also, the majority of *A.flavus* group isolates were *A.flavus.*

382. Jackson, C.R. 1965. Peanut pod mycoflora and kernel infection. Plant and Soil 23(2): 203-212.

The mycoflora in soil clinging to dry pods of groundnuts (cv. Argentine) was sampled in two experiments by serially washing pods for increasing periods in changes of sterile water. Of the nine principal fungi, *Aspergillus niger, A.flavus, A. terreus. Rhizopus* spp. and *Sclerotium bataticola* were present initially in relatively small numbers and decreased rapidly in subsequent dilutions. The decrease paralleled a decrease in weight of suspended material and in percentage of soil and organic particles greater than 0.016 mm in size. *Penicilliumfuniculosum, P. rubrum, P. citrinum,* and *Fusarium* spp. occurred in large numbers and either increased or slowly decreased in numbers in subsequent dilutions. In some instances variations in numbers followed trends in percentages of soil and organic particles less than 0.016 mm in size. When dry pods with this known mycoflora were allowed to hydrate over a 6-day period at 26°C, 32°C, or 38"C, there was extensive pod penetration and kernel infection by *A. niger, A.flavus, S. bataticola* and *Rhizopus* spp., but not by other fungi. The degrees of *A. flavus* and *A. niger* infection increased with increasing temperatures.

383. Jackson, C.R. 1968. A field study of fungal associations on peanut fruit. Georgia Agricultural Experiment Station Research Bulletin 26, 29 pp.

The fungal associations that occurred on groundnut fruit of the Argentine, Early Runner, and Florigiant cultivars were studied during a two-year period by sampling pods from three experiments at various intervals before and after maturity. No significant differences were found among cultivars, in estimated numbers of all fungi found on pod surfaces, or in kernels. The dominant fungi were *Penicillium* spp. *(P. funiculosum, P. rubrum,* and *P. citrinum)* and *Aspergillus* spp. *(A. niger, A. terreus, A. flavus). Fusarium* was a sub-dominant genus. A succession of well-defined communities was not recognized, although the densities of the dominant fungi fluctuated and *Rhizopus* became prominent alter maturity. Immature kernels from pods removed from the soil had a variable endocarpic community dominated by *Aspergilli* and *Penicillia.* A post-maturity community succeeded the first community and was evident at 15 days after maturity. It was characterized by the dominance of *Aspergilli, Penicillia, Sclerotium* and *Rhizopus.* The endocarpic community that developed in the windrow was characterized by the same fungi found in the post-maturity community. The windrow community was dominated by *Fusarium, Rhizopus* and *Sclerotium. Rhizoctonia, Trichoderma, Nigrospora,* and *Curvularia* were sub-dominant genera.

384. Jackson, C.R., and Minton, N.A. 1968. Peanut pod invasion by fungi in the presence of lesion nematodes in Georgia. Oleagineux 23(8-9):531-534.

A study conducted in groundnut in the U.S.A. in 1965 indicated a relationship between the presence of lesion nematodes *(Pratylenchus brachyurus)* and the numbers of fungi in the shells. In 1966, however, such a relationship was not ascertained, either for the shells or for the kernels. The results of the 1967 microplot experiment indicated that, although total numbers of all fungi in kernels at maturity were increased by the presence of lesion nematodes, the numbers of *Aspergillus flavus* and *A. niger* in the kernels were not changed.

385. Joffe, A.Z. 1969. The mycoflora of fresh and stored groundnut kernels in Israel. Mycopathologia et Mycologia Applicata 39: 255-264.

The mycoflora of groundnut kernels was studied over a 5 year period in Israel. A total of 132 samples of fresh, and 419 samples of stored kernels were examined. A list of 173 species isolated from these kernels is given, comprising 109 from fresh, and 153 from stored kernels. In three of the four years in which a comparative study of fresh and stored kernels was done, the number of fungal colonies from the fonner exceeded that from the latter by about 25 *%. Aspergillus flavus* usually occurred only in small quantities. *Aspergillus niger* was the most abundant fungus. On stored kernels it was even more predominant than on fresh kernels. *Penicillium funiculosum* and *P. rubrum* also occurred frequently.

386. Joffe, A.Z. 1970. The presence of aflatoxin in kernels from five years groundnut crops and of *Aspergillus flavus* isolates from kernels and soils. Plant and Soil 33: 91-96.

Aflatoxin was found in up to 6.4 % of fresh groundnut kernels and in up to 32 % of stored kernels, although the intensity of toxicity was always very low (up to 125 |ig kg^{-1}). About 90 % of 1626 *Aspergillus flavus* isolates from groundnut kernels, rhizospheres, and geocarpospheres produced aflatoxin in culture. In quantitative tests with 750 isolates, 60 % of the isolates produced > 25 µg g^{-1} aflatoxin.

387. Joffe, A.Z., and Borut, S.Y. 1966. Soil and kernel mycoflora of groundnut fields in Israel. Mycologia 58: 629-640.

Soil mycoflora in 12 groundnut fields of different edaphic and climatic conditions throughout Israel was studied. The greatest number of fungi gm^{-1} of soil was found in the Huleh peat location (217,000), whereas other locations gave lower counts (20,000-80,000). No significant differences occurred on the four collection trips. A list of the 95 species that were isolated from soils is given. Those that occurred in at least 6 fields were : Mucor racemosus, *Rhizopus oryzae, Aspergillus amstelodami, A. flavus, A. nidulans, A. niger, A. sulphureus, A. ustus, A. versicolor, A. wentii, Penicillium funiculosum, P. lilacinum, Cephalosporium curtipes, Fusarium equiseti, F. oxysporum, F. solani, Hormodendrum nigrescens, Myrothecium verrucaria, Paecilomyces flavescens, Trichoderma lignorum* and *Rhizoctonia* spp. The others were found in fewer locations. The mycoflora of freshly collected kernels from the same fields consisted of only 34 species. Those found in at least 6 samples were : *Rhizopus* orzyae, *A.flavus, A. niger, P. funiculosum, P. rubrum, F. oxysporum* and *F. solani.* Species that were common in soils and kernels also occurred on the groundnut shells.

388. Joffe, A.Z., and Lisker, N. 1969. The mycoflora of fresh and subsequently stored groundnut kernels on various soil types. Israel Journal of Botany 18(2): 77-89.

During the first six months of storage *Aspergillus niger* was the dominant fungus in samples of groundnut kernels from the 1965-1966 and 1966-1967 crops on all types of soil. *A. flavus* and *Fusarium* spp. were present in only small amounts, whereas prevalence of *Penicillium* spp. declined in early stages of storage. *Aspergillus niger* appeared to suppress other fungi in the kernel mycoflora. The numbers of A. *niger* colonies in the geocarposphere and within groundnut kernels were positively related, but for other fungi no relationship was observed.

389. Joffe, A.Z., and Lisker, N. 1970. Effects of crop sequence and soil types on the mycoflora of groundnut kernels. Plant and Soil 32: 531-533.

In Israel, the effects of crop sequence and soil types on the mycoflora of groundnut kernels was investigated over 3 years in 81 fields. Some of the fields were previously fallowed, and others were continuously cropped with or without groundnuts. The general mycoflora of kernels was most dominant in fields previously planted to groundnuts. In the cropped fields, whether groundnuts were included in the previous crops or not, *Aspergillus niger* accounted for 50-60 % of the total mycoflora. *Penicillium funiculosum* and *P. rubrum* were considerably more prevalent on previously fallowed fields. Prevalence of *A.flavus* was always low and was not influenced by crop sequences. Total kernel mycofloras were consistantly higher on medium and heavy soil than on other soils.

390. Johnson, R.A., and Gumel, M.H. 1981. Termite damage and crop loss studies in Nigeria - the incidence of termite scarified groundnut pods and resulting kernel contamination in the field and market samples. Tropical Pest Management 27(3):343-350.

The incidence of termite-scarified groundnut pods and resulting kernel contamination were studied in field and market samples in northern Nigeria. Scarification was caused by *Microtermes lepidus* and was restricted to the more mature pods. In the field, scarification was much higher (40.9-87.9 % of the pods) in the dead stands where the tap root had been invaded by *Microtermes* than in healthy stands (7.9-31.6 %). In the market samples, the number of scarified pods rarely exceeded 5 %; 85-91 % of the kernels from scarified pods were infected, the dominant fungi being *Macrophomina phaseolina* (37-61 %) and *Fusarium* spp. (8-26 %). Less than 5 % of the kernels were infected by *Aspergillus* spp. The fungi carried by the termites bore little relation to kernel infecting fungi. Of the kernels from undamaged pods collected from dead stands, 67 % were infected, indicating that, compared with plant death, scarification was of secondary importance in inducing fungal

infection. The quality of infected planting seed was not altered when the seed was dressed with Fernassan D (a mixture of lindane and thiram).

391. Lillehoj, E.B. 1979. Natural occurrence of mycoioxins in feeds : pitfalls in determination. Pages 139-153 in Interactions of mycotoxins in animal production :proceedings of a Symposium, 13 July 1978, Michigan State University, Washington, D C, USA : National Academy of Sciences.

This review emphasized the major pitfalls in the detection of mycotoxins that occur before harvest, with reference to variation in toxins in forages and silages, aflatoxin in groundnuts, cottonseed and maize, and trichothecenes and zearalenone in maize. Variations attributable to interaction with other feed components and detection hazards in presumptive tests are also discussed.

392. Lindsey, D.L. 1970. Effect of *Aspergillus flavus* on peanuts grown under gnotobiotic conditions. Phytopathology 60:208-211.

Two varieties of groundnut (Tennessee Red and Virginia Bunch 46-2) were grown under gnotobiotic conditions, and the pods were inoculated with a conidial suspension of the aflatoxin-producing fungus *Aspergillus flavus.* No evidence of pathogenicity to groundnut plants or pod rot symptoms was observed. *A. flavus* penetrated the shell tissue consistently, but was limited in its seed invasion to the testa. Invasion and colonization of the embryos by *A. flavus* appeared to be limited.

393. Lisker, N., Joffe, A.Z., and Frank, Z.R. 1970. Penetration of *Aspergillus flavus* and some other fungi into pods of various peanut varieties. Oleagineux 25(6):347-348.

In Israel, nonsterilized soil was infested with *Aspergillus flavus* before sowing four varieties of groundnut. Seed infection by this fungus did not increase as compared with the controls (no-infestation). A depressing effect on various other fungi and inverse relationships between fungi in the soil were noted, as well as differences in susceptibility among varieties.

394. Lyle, J.A. 1966. Ecology and aflatoxin-potential of peanut pod fungus flora. Journal of the Alabama Academy of Science 37: 252.

Studies were conducted to determine mycoflora of groundnuts in Alabama, Georgia, North Carolina. Oklahoma, Texas and Virginia states. *Aspergillus flavus* was isolated infrequently from pegs, shells, and kernels. Species of *Fusarium, Penicillium, Rhizopus,* and *Curvularia* were predominant fungi isolated from pegs, while species of *Rhizoctonia, Fusarium, Rhizopus,* and *Penicillium* predominated among those isolated from shells and kernels. Soil type, location, and groundnut cultivar had no apparent effect on frequency and number of fungi isolated. Assays of kernel and shell samples obtained 3 weeks prior to harvest showed consierable levels of aflatoxin. No appreciable differences in aflatoxin occurred in samples taken from the top and bottom of the windrow during the curing process.

395. Lynch, R.E., Ouedraogo, A.P., and Some, S.A. 1990. Effect of harvest date and termite-resistant varieties on termite and millipede damage to groundnut in Burkina Faso. Pages 87-89 in Summary Proceedings of the first 1CR1SAT Regional Groundnut Meeting for West Africa, ICRISAT Sahelian Center, Niamey, Niger, 13-16 Sep 1988. Patancheru, Andhra Pradesh, India : International Crops Research Institute for the Semi-Arid Tropics.

Research carried out at the Gampela research Station in Burkina Faso during 1986 and 1987 indicated that pod scarification and penetration by termites were enhanced by late harvest, pod damage was greater on plants where the tap root had been invaded and the plants killed by termites. Millipede damage was significantly greater in plants harvested at 70 and 90 days than for plants harvested at 125 days. Delayed harvest enhanced *Aspergillus flavus* invasion of groundnut pods and kernels. Insecticide application for the control reduced insect damage and yields were significantly greater and increased with longer growing time. In the evaluation of groundnut cultivars for resistance to millipedes and termites, millipede damage was significantly greater on QH 243. The lowest millipede damage was recorded for NCAc 2240 and NCAc 343. Banga, a local cultivar, showed least termite damage to plants and pods, while maintaining acceptable yields.

396. Lynch, R.E., and Wilson, D.M. 1984. Relation of lesser cornstalk borer damage to peanut pods and the incidence of *Aspergillus flavus.* Proceedings of the American Peanut Research and Education Society 16(1): 35.

During 1983, studies were conducted on the relationship between lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller), damage to groundnut pods in the field, and the incidence of *Aspergillus flavus.* Florunner groundnuts were sown on April 8, April 27, May 18, and June 10. Groundnut pods were sampled on September 1, 8, and 15, and separated into undamaged, externally damaged, and pod penetrated classes according to lesser cornstalk borer feeding damage. The pods and kernels were assayed for *A. flavus* and *A. niger.* The incidence of *A. flavus* on the pods and kernels was significantly greater on pods that were penetrated by lesser cornstalk borers.

397. Manzo, S.K., and Misari, S.M. 1989. Status and management of aflatoxin in groundnuts in Nigeria. Pages 77-90 in Aflatoxin contamination of groundnut : Proceedings of the International Workshop, 6-9 October 1987, ICRISAT Center,

India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

A committee to coordinate action on the problem of aflatoxin contamination in Nigeria was formed in 1961 with representatives from four ministries, i.e., the Institute for Agricultural Research (IAR), Zaria, the Nigerian Stored Products Research Institute (NSPRI), and the Northern Nigerian Marketing Board. This Committee was given the responsibility of assessing the extent of the aflatoxin problem in groundnut in the country and of initiating and coordinating all actions leading towards elimination of aflatoxin. IAR investigated the aflatoxin contamination of the groundnut crop up to the stage where the produce was sold by farmers, while NSPRI studied the problem from the time of storage until produce was exported or consumed. IAR investigated the time of invasion of groundnut kernels by *Aspergillus flavus,* and when, and under what conditions it produced aflatoxin. An interplay of temperature, relative humidity, drought, and erratic rainfall patterns, and maturity of the crop at lifting affected 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, while in the major groundnut growing areas that lie in the drier Northern Guinea and Sudan Savanna the problem is largely 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 groundnuts to either 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 government 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 with an aflatoxin content that does not exceed the maximum permissible limit of 20 µg kg^{-1} set by the liuropean Economic Community. 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.

398. McDonald, D. 1969. *Aspergillus flavus* on groundnut (*Arachis hypogaea L.)* and iLs control in Nigeria. Journal of Stored Products Research 5: 275-280.

Groundnuts are produced in two distinct zones in Nigeria; the dry northern zone where 95 % of the crop is grown, and the wetter riverain zone. In the northern zone the crop is normally harvested after the rains have ended, but in the riverain zone harvesting takes place during the rains. Investigations on groundnuts from the 1961 crop showed that *A. flavus* infection of kernels and aflatoxin production occurred in both zones. The condition of the shell had a marked influence on the fungal infection and toxicity of the kernels. Kernels from undamaged pods were rarely infected by *A. flavus,* whereas kernels from perforated pods had a high degree of infection with this and other fungi. Kernels from termite-scarified pods were intermediate in this respect. Kernels from all pod grades showed higher fungal infection in material from the riverain zone than in that from the northern zone, indicating that poor drying conditions could be important. Trials at Mokwa in the riverain zone and at Kano in the northern zone in which various methods of drying were tested demonstrated that rapid drying gave kernels with low fungal infection and little or no toxicity. Slow drying with kernel moisture contents above 20 % for extended periods resulted in heavily infected, toxic kernels. Kernels from undamaged, mature pods were free from fungal infection at lifting, infection by *A. flavus* not normally occurring until 4-6 days after harvest. Over-mature pods and pods from plants that had wilted and died before harvest had kernels infected by fungi at lifting in the northern zone. On the basis of these findings recommendations were provided for management of *A. flavus* in groundnut.

399. McDonald, D. 1970a. Fungal infection of groundnut fruit before harvest. Transactions of the British Mycological Society 54(3): 453-460.

In trials at Mokwa, Nigeria, fruits of the long-season groundnut variety Samaru 38 were tested at intervals during development for fungal infection of the shell and of the seed. The shells were infected by fungi at an early stage in development, but infection of seeds did not occur until later. The dominant fungi in the endogeocarpic mycoflora were *Fusarium* spp., and *Macrophomina phaseoli*; other common species included *Aspergillus* spp., *Botryodiplodia theobromae, Penicillium* spp. and *Rhizopus* spp. Dominant fungi in seeds were *Fusarium* spp., *Aspergillus* spp. and *Penicillium* spp.

400. McDonald, D. 1970b. Fungal infection of groundnut fruit after maturity and during drying. Transactions of the British Mycological Society 54(3): 461-472.

In trials at Mokwa, Nigeria, shells and seeds of the long-season groundnut variety Samaru 38 were examined for fungal infection. Samples were taken at normal lifting time, during windrow drying, after sun-drying on mats, and after becoming over-mature in the ground. At normal maturity the shells were commonly infected, although very few seeds were invaded. Fruits lifted when mature and dried in windrows showed an increase in invasion of seeds. Seeds from fruits picked shortly after lifting, then sun-dried on mats, showed little increase in fungal infection during drying. When fruits were left in the soil after reaching maturity, there was a gradual increase of fungal invasion of the seeds. *Macrophomina phaseoli* and *Fusarium* spp. were the dominant fungi in shells; they were also dominant in seeds of over-mature and windrowed-dried fruit. *Aspergillus* spp. were not abundant in shells or seeds but were frequently present, the toxigenic *Aspergillus flavus* being the most common.

401. McDonald, D., and Harkness, C. 1963. Growth *of Aspergillus flavus* and production of aflatoxin in groundnuts - Pan II. Tropical Science V(3): 143-154.

With the discovery of aflatoxin in groundnuts from many producing countries preliminary investigations were made on the 1961 crop in Nigeria. Results of the toxin tests showed that none of the varieties sampled was more resistant to toxin formation than another and that toxic samples appeared with equal frequency in both Northern and Riverain Provinces. Within each provincial group individual sites varied considerably for toxicity of samples and this may have been partially associated with different post-harvest drying and storage procedures. The fungal contamination tests showed no evidence of varietal resistance but did indicate considerably more contamination in samples from the Riverain Provinces than in samples from the Northern Provinces. Condition of the shell markedly influenced the degree of contamination of the kernels and, as for toxicity, there were considerable differences between individual sites for contamination with *Aspergillus flavus* and other fungi.

402. McDonald, D., and Harkness, C. 1964. Growth of *Aspergillus flavus* and production of aflatoxin in groundnuts - Part IV. Tropical Science VI(1): 12-27.

Investigations on aflatoxin in groundnuts were carried out during 1962 at Mokwa and five other localities in the Riverain Provinces of Northern Nigeria. The results indicated that : (1) *Aspergillus flavus* grew much more successfully on moribund or dead groundnut tissue than it did on living tissue. (2) There was virtually no toxicity in the crop at harvest, the exceptional cases being from broken pods. (3) Contamination of kernels with *A. flavus* and appearance of the toxin did not occur until at least five days after harvest. (4) Kernels from broken pods were more likely to be contaminated with *A. flavus* and to be toxic than were kernels from undamaged pods. (5) Toxicity of the crop varied from year to year at specific localities.

403. McDonald, D., and Harkness, C. 1965. Growth of *Aspergillus flavus* and production of aflatoxin in groundnuts - Part VIII. Tropical Science VII(3): 122-137.

Trials were conducted at Mokwa Agricultural Research Station, Northern Nigeria, in 1963 to test various natural methods for postharvest drying of groundnuts. Samples of groundnuts were taken from the range of treatments at intervals from harvest until the end of drying, and these were tested for aflatoxin content and kernel contamination with *Aspergillus flavus* and other fungi. Large differences in rates of drying occurred between the various treatments and, in general, the slower the rate of drying the higher the kernel contamination with *A. flavus* and other fungi and the higher the incidence of toxic samples. When pods were hand-picked from the haulms and sun-dried in a layer, with protection from rain, the rate of drying was high and a toxin-free crop resulted. None of the treatments in which the pods were dried on the haulms in the field resulted in a toxin- free crop.

404. McDonald, D., and Harkness, C. 1967. Aflatoxin in the groundnut crop at harvest in Northern Nigeria. Tropical Science IX(3):148-161.

Serial harvesting trials were carried out at Mokwa and Kano Agricultural Research Stations, Northern Nigeria, in 1963 and 1964 to investigate me occurrence of aflatoxin in the groundnut crop at lifting, when harvested before, at, and after the normal time. This work formed part of the programme of work on the toxicity of the groundnut crop in Northern Nigeria. Crops harvested either at or earlier than the normal time were free from aflatoxin, but late harvesting usually resulted in some toxicity. The weather, as well as the age of the crop, had some effect on toxicity, wet weather delayed the appearance of aflatoxin. Pods with shells damaged while the crop was in the ground were more likely to contain toxic kernels than were pods with undamaged shells.

405. McDonald, D., Harkness, C., and Stonebridge, W.C. 1964. Growth of *Aspergillus flavus* and production of aflatoxin in groundnuts - Part VI. Tropical Science VI(3): 131-154.

Trials were carried out at Kano Agricultural Reserch Station, Northern Nigeria in 1963 to test various natural methods for postharves: drying of groundnuts. Samples of groundnuts were taken from the different treatments at intervals from harvest until the end of drying and tested for aflatoxin content and kernel contamination with *A. flavus* and other fungi. There was very little difference in rate of drying between the various treatments, and no effect on toxicity or fungal contamination was observed. Some kernel samples were found to be toxic at harvest and this was associated with both pod damage and kernel moisture content.

406. Mehan, V.K. 1987. The aflatoxin contamination problem in groundnut - Control with emphasis on host plant resistance. Pages 63-92 in Proceedings of the first Regional Groundnut Plant Protection Group Meeting and Tour, 15-21 February 1987, Harare, Zimbabwe.

The status of the global aflatoxin problem is reviewed with special reference to African groundnut producing countries, and research needs are highlighted. Possible practical control measures are discussed with emphasis on use of host plant resistance to the aflatoxin-producing fungus *Aspergillus flavus,*

407. Mehan, V.K., McDonald, D., Ramakrishna, N., and Williams, J.H. 1986. Effects of genotype and date of harvest on infection of peanut seed by *Aspergillus flavus* and subsequent contamination with aflatoxin. Peanut Science 13(2): 46-50.

Several groundnut genotypes reported as resistant, susceptible or highly susceptible to *in vitro* colonization of rehydrated, mature, stored, undamaged seed by *Aspergillus flavus* (IVSCAF) were tested for natural seed infection by *A. flavus* and other fungi in two or more replicated field trials at 1CRISAT Center, Patancheru, India, in 1979-1984. Undamaged pods were sampled before maturity, at optimum maturity (normal harvest) and when over-mature (late harvest) and seed examined for infection by *A. flavus* and other fungi. In the 1983 and 1984 rainy and 1983/84 postrainy seasons, only four genotypes (one resistant and three susceptible) were tested, and seed were also tested for aflatoxin content. In all seasons the genotypes reported as IVSCAF-resistant had significantly lower levels of seed infection with *A. flavus* and other fungi than did genotypes reported as IVSCAF- susceptible. Genotypic differences in levels of seed infection by *A. flavus* were consistent over seasons. The resistant cultivar J 11 had a significantly lower aflatoxin content than the other three IVSCAF-susceptible genotypes tested in the 1983 and 1984 rainy and 1983/84 postrainy seasons. Drought stress in the 1984 season apparently increased susceptibility to seed infection by *A. flavus* and other fungi, and to aflatoxin contamination, in all genotypes. Seed infection by *A. flavus* and other fungi, and aflatoxin contamination increased with increasing maturity of pods, indicating the importance of lifting the groundnut crop at optimum maturity.

408. Mehan, V.K., Rao, R.C.N., McDonald, D., and Williams, J.H. 1988. Management of drought stress to improve field screening of peanuts for resistance to *Aspergillus flavus.* Phytopathology 78(6):659-663.

Drought stress during late stages of pod maturation in an irrigated groundnut crop during the postrainy season significantly increased the level of seed infection by *A. flavus.* A line-source sprinkler irrigation system imposing a drought- stress gradient was used for field screening of groundnut genotypes for resistance to seed infection by *A. flavus.* A significant, positive, linear relationship was found between water deficit (drought intensity) and seed infection in groundnut genotypes. Genotypic differences for seed infection by *A. flavus* were evident at all levels of drought stress, but, under the more severe drought stress conditions, the genotypes resistant to *A. flavus* had low but positive levels of seed infection giving improved statistical precision.

409. Middleton, K.J. 1989. Queensland Department of Primary Industries' involvement with aflatoxin in groundnuts in Australia and Indonesia. Pages 209-213 in. Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 Oct 1987, ICRISAT Center, India. Patancheru 502 324. A.P., India : International Crops Research Institute for the Semi-Arid Tropics.

Rainfed groundnut production in Queensland, Australia, is oten severely affected by aflatoxin contamination. The Queensland Department of Primary Industries (QDP1) provides extension and research services to groundnut producers, and has addressed this problem in a variety of ways since becoming aware of it. Extension activates 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 activites. The Australian Centre for International Agricultural Research (AC1AR) - funded groundnut project in Indonesia conducted in collaboration by scientists from QDPI and the Agency for Agricultural Research and Development (AARD), in future, consider the inclusion of research on production aspects of aflatoxin contamination.

410. Mintnn, N.A., Bell, D.K., and Doupnik, B.Jr. 1969. Peanut pod invasion by *Aspergillus flavus* in the presence of *Meloidogyne hapla.* Journal of Nematology 1(4): 318-320.

'Argentine', 'Early Runner' and 'Florigiant' groundnut cultivars: were grown in methyl bromide-treated soil in field microplots inoculated with : (1) *Aspergillus flavus* or (ii) *A. flavus* + *Meloidogyne hapla.* Nematode infection produced heavy root galling and light pod galling equally on all cultivars. *A. flavus, A. niger, Cephalosporium* spp., *Colletotrichum* spp., *Curvularia* spp., *Fusarium* spp., *Penicillium* spp. and *Trichoderma viride* were isolated from shells and kernels. A significantly greater incidence and density *of A. flavus* was obtained from kernels of plants inoculated with both organisms than from kernels of plants receiving only the fungus. Differences were not significant, however, for incidence and density of *A. flavus* in shells or for the total of all fungal propagules in shells and kernels. Shells of 'Early Runner' contained significantly greater incidence and density of *A. flavus* than the other two cullivars; also, kernels of this cultivar contained more fungal propagules than kernels of 'Argentine'. A significantly larger number of total fungi was isolated from kernels of 'Argentine' than from 'Florigiant'. Anatoxins occurred in only two shell samples and were not detected in kernels.

411. Minton, N.A., and Jackson, C.R. 1967. Invasion of peanut pods by *Aspergillus flavus* and other fungi in the presence of root-knot nematodes. Oleagineux 22:543-546.

Groundnut plants grown in steam-heated soil were inoculated with the root-knot nematode *Meloidogyne arenaria.* Inoculation with the nematode, resulting in severe galling of pods and pegs, increased the incidence of shell infection by *Aspergillus flavus, A. niger,* and *Scleroiium bataticola,* and increased the infection of the kernels by A. *niger.* The random occurrence of trace amounts of aflatoxins among

treatments showed little relation to distribution of nematodes in shells or to that of *A.flavus* in shells or in kernels.

412. Musingo, M.N., Basha, S.M., Sanders, T.H., Cole, R.J., and Blankenship, P.D. 1989. Effect of drought and temperature stress on peanut (*Arachis hypogaca* L.) seed composition. Journal of Plant Physiology 134: 710-715.

Groundnut plants were subjected to drought and temperature stress for various periods and the seeds from these plants were separated into Jumbo, Medium and No. 1 market size categories and analyzed for soluble and total carbohydrates, alpha amino nitrogen, total protein and oil. Results showed that soluble and total carbohydrate content of the seed increased due to drought and temperature stress, with Jumbo and Medium sizes showing the highest increase. It is suggested that drought and temperature stresses caused increased accumulation and/or synthesis of carbohydrates and certain polypeptides and thus may enhance seed invasion by *Aspergillus flavus* and aflatoxin production.

413. Nagaraj, G., and Kumar, K. 1986. Location variations in the aflatoxin content of some Virginia groundnut varieties. Journal of the Oil Technologists' Association of India 18(3):89-91.

Seven Virginia groundnut varieties, viz. S 230, Kadiri I, Kadiri 3, Karad 4-11, M13, GAUG 10 and TMV 10 were grown in four locations (Chintamani, Jalgaon, Khargaon, and Junagadh) in India in the rainy season of 1982. After harvest the pods were received at Junagadh during February-March 1983. Pods were shelled and aflatoxin analyses carried out on seeds with a thin-layer chromatography (TLC) method. Aflatoxin B_1 was found in all the groundnut varieties (0.8 to 65.8 µg kg^{-1}). Aflatoxins B_2 and G_1 were found in a few samples from some locations. Among varieties, S 230 had the highest level of aflatoxins at all the locations (49.8 to 85.9 µg kg^{-1}) followed by GUAG 10 (15 to 88.3 µg kg^{-1}) and Kadiri 3 (17.8 to 106.7 *µg* kg^{-1}). Karad 4-11 had low levels of aflatoxins at all four locations (1.6 to 30.3 µg kg^{-1}) followed by Kadiri 1 (4 to 52 *µg* kg^{-1}). M13 and TMV 10 had moderate levels of aflatoxins. Among the locations, Junagadh and Chintamani samples contained higher levels of aflatoxins. Unfavorable postharvest drying conditions at Junagadh and Chintamani probably resulted in higher levels of aflatoxins at these locations. The varieties with more than 3 % phenols in the seed testa generally contained low levels of aflatoxins. The varieties with protein content higher man 25 % contained more aflatoxins, while those having about 22 % proteins contained less aflatoxins except TMV 10 which also had less total phenols. Sugar and oil content did not appear to have any effect on the aflatoxin levels.

414. Norse, D. 1971. Aflatoxin studies. Pages 239-246 [in] Annual Report of the Department of Agriculture, Malawi, for the year 1967/68, 279 pp.

The incidence of *Aspergillus flavus* and aflatoxin in groundnuts was greater in the hotter, lower altitude areas. *A.flavus* and the toxin were found primarily in broken pods, but they occasionally occurred in apparently undamaged pods. No significant correlation was found between drying treatments and incidence of the fungus in kernels. There was no correlation between superficial shell and kernel infection as long as the shell remained intact.

415. Pettit, R.E., and Chan, A.K. 1980. Detection of mold and mycotoxin damaged peanut kernels with helium-neon laser reflected energies. Proceedings of the American Peanut Research and education Society 12:43.

An improved nondestructive technique for accurately detecting the extent to which kernels are damaged by molds and aflatoxin has been under study. A helium-neon laser emitting light at 6550 A wavelength with horizontal and vertical polarized components was first standarized on a known background target. The laser was then directed on the groundnut kernel surface and the back scattering amplitude of both polarizations recorded along with a computer calculated ration of the amplitudes. Preliminary results indicate that the amplitudes detected for pickout kernels are 30 to 40 % below the amplitude recorded for sound mature healthy kernels. The amplitudes for *Aspergillus flavus* inoculated kernels were 15 to 20 % below those recorded for the sound mature kernels. The polarization ratio for sound mature healthy groundnut kernels deviated only slightly from unity. With mold damaged kernels examined along the long axis the horizontal wave component decreased so that the polarization ratio ranged from 0.60 to 0.80. Evidence indicates that the amplitude of the reflected laser beam correlates with the surface features of the kernels while the polarization ratio provides information on the internal composition of the kernels.

416. Pettit, R.E., and Geiger, R.L. 1981. Dielectric properties of mold and mycotoxin damaged peanuts. Phytopathology 71: 249.

Dielectric characteristics of good sound mature kernels and damaged kernels of groundnut were investigated over a frequency range of 20 KHz to 20 MHz. Groundnuts were placed in a test capacitor and measurements were made on a HP 4342A Q meter. Equivalent moisture contents were maintained using humidity chambers as determined by the oven dry method. Preliminary results indicate that the real part of the dielectric constant (permittivity) is essentially independent of mold contamination level whereas the imaginary pan (alt. loss tangent) is strongly dependent upon contamination level. The simultaneous determination based upon these dielectric characteristics, provides a means of detecting varying degrees of mold and mycotoxin damage.

417. Pettit, R.E., Taber, R.A., Schroeder, H.W., and Harrison, A.L. 1971.

Influence of fungicides and irrigation practice on aflatoxin in peanuts before digging. Applied Microbiology 22(4): 629-634.

Groundnuts (cultivar Starr) grown rainfed under drought stress conditions had markedly higher levels *of Aspergillus flavus* infection and aflatoxin contamination of kernels before digging than had groundnuts grown under irrigation, in field trials at Yoakum and Stephenville, USA. in the 1967. 1968 and 1969 seasons. Levels of seed infection and aflatoxin contamination were considerably higher in 1967 and 1969 than in 1968, *A. flavus* infection and aflatoxin contamination levels were higher in drought-stressed groundnuts produced at Yoakum than in groundnuts produced at Stephenville. In 1969 some samples of freshly-dug groundnuts from irrigated plots had high levels *of A. flavus* infection (16-59 %), but no aflatoxin was detected in these samples. Absence of aflatoxin in these samples is attributed to high seed moisture levels and to low soil temperatures (10 to 21° C). Several fungicides applied to the soil and foliage did not affect fungal infection and aflatoxin contamination of groundnuts

418. Picasso, C. 1986. [Aflatoxin, rosette, and groundnut rust - the climatic environment that promotes their presence and development]. Aflatoxinc. rosette et rouille de l'arachide- environnement climatique propice a leur presence et dcveloppmcnt. Pages 151-161 in Agrometeorology of groundnut : Proceedings of an International Symposium, 21-26 Aug 1985, ICRISAT Sahelian Center, Niamey, Niger. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

This paper deals with three groundnut diseases in West Africa that affect yield and quality of this crop. The infection of pods and seeds by the aflatoxin-producing fungus *Aspergillus flavus* is linked to climatic factors. Field infection is increased by drought at the end of the growing cycle. Rosette is a virus disease transmitted by an aphid. Its development and propagation are directly related to those of the insect, which in turn result from well-defined climatic conditions, notably temperature and humidity. Groundnut rust, a fairly recent but fast-developing disease in West Africa, is caused by the fungus *Puccinia arachidis*. The fungus has, however, a short viability period for its development in tropical climate, the spread of uredospores, their release, and dissemination by the wind and the conditions for infection are presented. A good knowledge of the agroclimatic environment that affects these diseases, well defined for certain parameters, facilitates assessment of risks and of the need to take protective measures. It also cuts down the loss caused by these diseases through appropriate cropping methods, so that the pathogens do not encounter satisfactory conditions for their development on the plants. This is, however, an interim solution that awaits the use of resistant varieties, the only really effective method of control that could be employed by all farmers.

419. Pitt, J.I.1989. Field studies on *Aspergillus flavus* and aflatoxins in Australian groundnuts. Pages 223-235 in Anatoxin contamination of groundnut: proceedings of the International Workshop, 6-9 Oct 1987, ICRISAT Center. India. Patancheru. A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

Anatoxins 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 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 spores g^{-1} to as high as 10^8 spores 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, aflatoxin is not 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 sufficiendy 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.

420. Porter, D.M., and Garren, K.H. 1968. An analysis of the endogeocarpic microflora of peanuts in Virginia. Tropical Science 10 (2): 100-106.

In Virginia. U.S.A., the endogeocarpic microflora of groundnuts was studied over a 3-year period. The density of fungi in the pods decreased and that of bacteria increased as the pods matured. On the basis of the isolation density, the isolated fungi were divided into dominant and sub-dominant groups. Shells were more susceptible to fungal infection than seed. The dominant genera of the isolated fungi were *Penicillium, Trichoderma, Chaetomium,* and *Fusariurn.* The isolation density of *Aspergillus flavus,* a sub-dominant fungus, was only 3 %. The isolation density

of *A. flavus* from shells remained constant during the growing season, but it increased for isolations from seed as the season progressed.

421. Porter, D.M., and Garren, K.H. 1970. Endocarpic microorganisms of two types of windrow-dried peanut fruit *{Arachis hypogaea* L.). Applied Microbiology 20(1):133-138.

The endocarpic microorganisms of groundnut fruit dried in either a random windrow (plants left as they fell from the digger) or an inverted windrow (plants inverted to expose fruit to sunlight) were different from that of freshly dug fruit. *Chactomium, Penicillium, Trichoderma, Rhizoctonia,* and *Fusarium* were dominant fungi associated with shells (pericarp) of freshly dug fruit. The dominant fungi of shells of windrowed fruit included *Chaetomium, Rhizoctonia, Fusarium, Sclerotium,* and *Alternaria.* Seeds of freshly dug fruit were dominated by *Fenicillium* and *Aspergillus.* The only dominant species in seed of windrowed fruit was *Fenicillium.* Microorganisms were isolated from shells and seed of freshly dug fruit at a frequency of 79 % and 52 %, respectively. The percentage of infection was reduced by drying in the field. This was particularly true of the inverted windrows. The proportion of shells and seed infected with a microorganism was reduced 13 % and 36 % respectively, after field drying for 5 to 7 days in random and inverted windrows. Microorganisms were isolated much more frequently from shell pieces (73 *%)* than from seed (36 %).

422. Porter, D.M., and Smith, J.C. 1974. Fungal colonization of peanut fruit as related to southern corn rootworm injury. Phytopathology 64 : 249-251.

Field-grown groundnut fruit injured by the feeding of southern corn rootworm larvae were more susceptible to fungal colonization than noninjured fruit. Seed from injured fruit were colonized by fungi at a much greater frequency than seed from noninjured fruit. However, colonization by the toxigenic fungus *Aspergillus flavus* was not affected. In greenhouse tests, pod breakdown, an important in-soil rot of groundnut fruit caused by *Pythium myriotylum* was greatly enhanced by the presence of rootworm larvae. Under high inoculum densities of P, *myriotylum* and abnormally high rootworm populations the incidence of pod breakdown was almost twice that observed when only the fungus was present. Rootworm population densities influenced the severity of pod breakdown; severity increased as rootworm populations increased. The data suggest that insect feeding sites could provide portals of entrance into the groundnut fruit for many fungi, including *P. myriotylum.*

423. Porter, D.M., Wright, F.S., and Steele, J.L. 1972. Relationship of shell damage to colonization of peanut seed by *Aspergillus flavus.* Proceedings of the American Peanut Reserach and Education Association 4: 207.

Differences in infection of seed by *Aspergillus flavus* were noted when comparisons were made between seed from sound or damaged groundnut fruit with different moisture contents. Seed from pods with visible damage (shell damage detected visually) and invisible damage (shell damage detected by a staining technique) were infected more frequently by *A. flavus* than those from sound fruit (no visible or invisible damage). Seed from invisibly damaged fruit were infected almost as rapidly as seed from visibly damaged fruits. The isolation frequency of *A. flavus* from invisibly and visibly damaged fruit was 23 % and 26 %, respectively. *A. flavus* was isolated at a frequency of 8 % from nondamaged fruit. After incubation at a temperature and relative humidity conducive to the rapid proliferation of *Aspergillus* spp. for a period of 24 to 48 hours, *A. flavus* was isolated just as rapidly from seeds from invisibly damaged fruit as from visibly damaged fruit. Although the isolation frequency of A. *flavus* from seed from sound fruit increased with time, the frequency did not approach that obtained from seed from damaged fruit. Before incubation, the isolation frequency *of A. flavus* from seed from non-inoculated fruit with moisture contents of 10 %, 30 % and 50 % was 4 %, 7 % and 10 %, respectively. Following incubation the isolation frequency of *A. flavus* from seed was greater in partially dried fruit than in fruit with a high moisture content. The isolation frequency *of A. flavus* from seed from partially dried fruit with a moisture content of 10 % and 30 *%* was 20 % and 25 %, respectively, following incubation. *A. flavus* was isolated less frequently (12 %) from seed from fruit with a 50 % moisture content, probably due to competition with other fungi during incubation.

424. Porter, D.M., Wright, F.S., and Steele, J.L. 1984. |Relationship of microscopic shell damage to colonization of peanut by *Aspergillus flavus.].* Rapport entre les deteriorations microscopiques de la coque et la colonisation des graines d'arachide par *Aspergillus flavus.* Oleagineux 41(1): 23-30.

Seeds from groundnut pods exhibiting microscopic shell damage, only detected with a staining technique, were as susceptible to colonization by *Aspergillus flavus* as seed from pods with visible damage. The purpose of mis study was to determine the relationship between incubation time, pod moisture, shell damage and groundnut seed colonization by *A. flavus* and to develop a mathematical expression to represent this relationship. It appeared that pod resistance to fungal colonization was gready reduced when protective physical barriers were breached. Therefore, practices that minimize microscopic damage should be developed to aid in minimizing seed colonization by undesirable fungi.

425. Quitco, R., Bautista, L., and Bautista, C. 1989. Aflatoxin contamination of groundnuts at the post-production level of operation in the Philippines. Pages 101-110 in Aflatoxin contamination of groundnut: proceedings of the International Workshop. 6-9 October 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

The results of surveys in the Philippines have shown that farm level aflatoxin

significantly increased from harvest to farm storage during the main cropping season. At harvest, groundnuts contained, on average, 3.16 μg kg^{-1} aflatoxin. During windrowing. aflatoxin levels increased at the rate of 1.5 µg kg^{-1} day^{-1}. In farm storage aflatoxin contamination continued to increase at the rate of 1 µg kg^{-1} day^{-1}. Aflatoxin contamination was signifiantly higher during the main cropping season than the second cropping season. At the traders' level, groundnut samples taken from various middlemen contained 35 µg kg^{-1} aflatoxin. On the other hand, samples taken from the wholesalers' newly procured groundnuts contained 188 μg kg^{-1} aflatoxin. Groundnuts that had been in wholesalers' warehouse for more than 3 months contained 275 µg kg^{-1} aflatoxin. At the processors' level, raw groundnuts for confectionery use (roasted and fried) contained 7.73 ug aflatoxin kg^{-1}, grounduts intended for peanut butter contained 17.13 µg kg^{-1}, and rejected groundnuts had 120.6 |ig kg^{-1}. Aflatoxin contamination could start during harvest. Aflatoxin content increased to a significantly high level during trade and processing. This continued increase was attributed to insufficient drying of groundnuts after harvest.

426. Saito, S., Kawasugi, S., Tsuruta, O., and Manabe, M. 1985. *Aspergillus flavus* infection of peanuts in Thailand. Proceedings of the Japanese Association of Mycotoxicology No. 21: 34-35.

Groundnut samples were collected from test fields and fanners' fields at harvest during the rainy season and from kernels sold in a market. *Aspergillus flavus* infection was only detected in the samples collected from the market. It is suggested that infection of groundnuts occurs during postharvest drying.

427. Sanders, T.H., Blankenship, P.D., Cole, R.J., and Hill, R.A. 1984. Effect of soil temperature and drought on peanut pod and stem temperatures relative to *Aspergillus flavus* invasion and aflatoxin contamination. Mycopathologia 86: 51-54.

Groundnut stem and pod temperatures of plants growing in irrigated, drought, drought-heated soil, and drought-cooled soil treatments were determined near the end of the growing season. Mean soil temperatures of the treatments during this period were 21.5, 25.5, 30 and 20°C, respectively. Groundnut stem temperatures in all drought treatments reached a maximum of ca. 40°C and for 6-7 h each day were as much as 10°C warmer than irrigated groundnut stems. Pod temperatures in drought-heated soil and drought treatments were ca. 34°C and 30°C, respectively, for several hours each day. As pod temperatures approached the optimum for *A. flavus* growth (ca. 35°C), the proportion of kernels infected and aflatoxin concentration increased. Increased plant temperature without accompanying pod temperature increases (drought-cooled soil) resulted in infection percentages and aflatoxin concentrations only slightly higher than those of the irrigated groundnuts.

428. Sanders, T.H., Blankenship, P.D., Cole, R.J., Ashley, K.H., and Hill, R.A. 1985. Conclusive test for aflatoxin resistance in peanuts. Journal of the American Oil Chemists' Society 62(4):654-655.

Data compiled over 4 years showed that 5 cm of soil at a mean of 29.4°C and severe drought 40-50 days before harvest resulted in *Aspergillus flavus* infection and aflatoxin contamination in preharvest groundnuts. Four genotypes, which were rated as resistant to seed colonization by *A, flavus* in a laboratory screening assay, and 2 other genotypes were grown in plots and subjected to the above stress conditions 98 days alter sowing. Tests of dried groundnuts harvested 143 days after sowing showed that nuts of all the genotypes contained aflatoxin and were extensively infected by *A. flavus.*

429. Sanders, T.H., Blankenship, P.D., Cole, R.J., and Smith, J.S. 1986. Role of agrometeorological factors in postharvest quality of groundnut. Pages 185-192 in Agrometeorology of groundnut: proceedings of an International Symposium, ICRISAT Sahelian Centre, Niamey, Niger, Patancheru, India : International Crops Research Institute for the Semi-Arid Tropics.

Posmarvest quality of groundnut is influenced by the particular set of environmental and cultural practices that influence physiology and maturation. Groundnut composition, although related to environment, changes dramatically as groundnuts mature. There is biochemical basis for inferior quality in immature groundnut. Drought stress and soil temperature influence maturation rate and thus have an indirect effect on postharvest quality. *Aspergillus flavus* invasion and aflatoxin contamination in groundnuts are related to drought stress, soil temperature, and maturity. Small, immature seed are more likely to be contaminated with *A. flavus* than larger, mature seed. The biochemical composition, fungal contamination, and the tendency toward higher moisture content complicate storage of immature seed. Each of these factors predisposes immature seed to rapid quality deterioration in storage. Agrometeorological studies must include an awareness of the interrelationships of environment, maturity, and postharvest quality.

430. Sanders, T.H., Cole, R.J., Blankenship, P.D., and Hill, R.A. 1983. Drought soil temperature range for aflatoxin production in preharvest peanuts. Proceedings of the American Peanut Research and Education Society 15(1): 90.

In 1982 Florunner groundnuts were grown in experimental plots to evaluate the effect of various drought soil temperatures on aflatoxin production in preharvest groundnuts. Drought and soil temperature regulation were initiated 90 days after sowing. Mean soil temperatures under the groundnut rows in the various treatments were 31.7°C. 29.9°C, 27.7°C, 26.3°C. 24.7°C and 25.6°C (irrigated control). At harvest, no aflatoxin was found in groundnuts from the control or 31.7°C plot and concentrations of aflatoxin decreased with decreasing temperature for other drought

treatments. At 24.7°C only the other edible size category contained aflatoxin (20 µg kg^{-1}). *Aspergillus flavus* group fungi were found in groundnuts from each plot.

431. Sanders, T.H., Cole, R.J., Blankenship, P.D., and Hill, R.A. 1985. Relation of environmental stress duration to *Aspergillus flavus* invasion and aflatoxin production in preharvest peanuts. Peanut Science 12(2):90-93.

Previous experiments have established that late-season water deficit conditons and 28-30.5°C in the geocarposphere are optimum for preharvest aflatoxin contamination of Horunner groundnuts. Visibly-undamaged groundnuts from plants exposed to these stress conditions during the last 40-45 days before harvest were highly contaminated with aflatoxin at harvest. The objective of this study was to determine the duration of water and soil temperature stress required for extensive preharvest invasion by *Aspergillus flavus* and contamination of groundnuts with aflatoxin. Stress conditions were imposed 20, 30. 40 and 50 days before harvest. Incidence of *A. flavus* was greatest in edible groundnuts from 30. 40, and 50 day stress treatments. A stress period of 20 days before harvest did not result in aflatoxin contamination of edible groundnuts by *A. flavus.* Groundnuts subjected to defined temperature and water deficit stress conditions for 30, 40, and 50 days became contaminated. Therefore, a threshold stress period for preharvest aflatoxin contamination of groundnuts by *A. flavus* was more than 20, and possibly less than 30 days before harvest.

432. Sanders, T.H., Hill, R.A., Cole, R.J., and Blankenship, P.D. 1981. Effect of drought on occurrence of *Aspergillus flavus* in maturing peanuts. Journal of the American Oil Chemists' Society 58(12): 966A-970A.

Cultivar Florunner groundnuts were grown in experimental plots with soil moisture and soil temperature modified during the last third of the growing period to produce drought, drought with cooled soil, irrigated and irrigated with heated soil treatments. Twice each week, beginning 97 days after sowing, random samples were harvested and maturities of individual pods were determined without destroying pod integrity. The nature and quantity of the microflora associated with the pods and kernels were subsequently assessed. Drought and lower soil temperature resulted in maturity distributions containing higher proponions of immature pods. On groundnuts with no visible damage to the pod or kernel, colonization by *Aspergillus flavus* was more frequent in immature than mature kernels. Drought stress increased the incidence of *A. flavus* and irrigation decreased it, except when soil temperatures were modified. *A. flavus* infection was greatly increased at all maturity levels by pod damage.

433. Schroeder, H.W., and Ashworth, L.J.Jr. 1965. Aflatoxins in Spanish peanuts in relation to pod and kernel condition. Phytopathology 55: 464-465.

Nondamaged and damaged groundnut kernels from broken and perforated pods were analyzed to determine their relative importance as sources of aflatoxins. Apparently damage-free kernels (accounting for 48.5 % of the total kernels) contained between 500 and 1000 µg kg^{-1} aflatoxins. Damaged kernels, on the other hand, contained 2000 µg kg^{-1} or more aflatoxins. Subsequently, kernels from mechanically broken, rot- and insect-perforated, and growth-cracked pods were assayed separately for aflatoxin content. Results indicated that kernels from pods with growth cracks are more subject than kernels from pods with other types of damage to aflatoxin development.

434. Schroeder, H.W., and Boiler, R.A. 1973. Aflatoxin production of species and strains of the *Aspergillus flavus* group isolated from field crops. Applied Microbiology 25(6):885-889.

Groundnuts, cottonseed, rice and sorghum from Texas, USA, were sampled over a 3-year period. Aflatoxins were present each year in groundnut and cottonseed and in 2 of 3 years in rice and sorghum. The *Aspergillus flavus* group fungi were much more prevalent in groundnut and rice than in cottonseed and sorghum. Of the isolates of the A. flavus group, 96 % from groundnut, 79 % from cottonseed. 49 % from sorghum and 35 % from rice produced aflatoxins. The average toxin production of isolates was much less for rice than for the others. More than 90 % of all isolates of the *A. flavus* group were identified as the species *A. flavus. A. parasiticus* was isolated from all crops. Only *A. parasiticus* isolates produced aflatoxin G as well as B.

435. Sellschop, J.P.F. 1965. Held observations on conditions conducive to the contamination of groundnuts with the mould *Aspergillus flavus* Link ex Fr. Pages 47-52 in Symposium on Mycotoxins in Foodstuffs, Agricultural Aspects (Abrams, L., Sellschop, J.P.F., and Rabie, C.J., eds.). Dept. of Agriculture, Technical Services, Pretoria, South Africa.

Environmental and biological factors responsible for aflatoxin contamination of the South African groundnuts are discussed. During the 1963/64 growing season, maturing groundnuts in the North-Western Transvaal areas were damaged by certain species of termites; this predisposed groundnuts to seed invasion by *Aspergillus flavus.* Protracted droughts followed by the late rains in these areas were also conducive to infection of groundnuts by the aflatoxigenic fungus. Most of the infection of groundnuts by the fungus was believed to occur in the groundnut before harvest.

436. Styer, C.H., Cole, R.J., and Hill, R.A. 1983. Inoculation and infection of peanut flowers by *Aspergillus flavus.* Proceedings of the American Peanut Research and Education Society 15: 91.

Flowering groundnuts (cv. Florunner) in pots were placed in a growth chamber with

14 hours of light, 80-90 % humidity, and a temperature of 29°C. Freshly opened flowers were inoculated by dusting the stamens and style with a camel's hair brush carrying spores of *A. flavus* cultures isolated from groundnuts. Flowers were collected in 70 % ethanol immediately before and after inoculation and after periods of one to eight days. The styles were removed from the flowers and placed in a mixture of malachite green, acid fuchsin, lactophenol and glycerol. No spores were observed on styles of uninoculated flowers, whereas styles from inoculated flowers were covered with large numbers of spores. Some germination had taken place 24 hours post inoculation (PI) but by 48 hours PI, many spores had germinated and hyphae were observed growing over the surface of the stigma and pollen grains. Some of the hyphae entered the style tlirough the stigma and ramified in the stylar tissue proximal to the stigma, until some hyphae had grown down to the top of the ovary. In some flowers, after as little as two days PI, conidiophores bearing spores were observed on the anthers and distal portions of the filaments, thus providing a secondary source of inoculum. Research is being undertaken to determine if the ovary and later, the fruit harbor the fungus.

437. Subrahmanyam, P., and Rao, A.S. 1974. Occurrence of aflatoxins and citrinin in groundnut *(Arachis hypogaea* L.) at harvest in relation to pod condition and kernel moisture content. Current Science 43(22): 707-710.

Groundnut pods were collected from fields on the day of harvest in November 1972, graded into undamaged and damaged pods and seed moisture contents determined. High levels of aflatoxin were found in seeds with low moisture (< 30 %), which occurred under rainfed conditions and low levels or no toxins in those with high moisture content (under irrigation). In all instances damaged seeds were found to contain the toxins. Seed moisture content and pod damage appeared to be the major governing factors for fungal infection and toxin accumulation before harvest. The accumulation of a yellow pigment in some seeds, especially in damaged pods, was noticed and it was identified as citrinin. Only *Aspergillus flavus* isolates were found to produce aflatoxins while isolates of *Penicillium citrinum, P. jenseni* and *A. terreus* produced citrinin.

438. Subrahmanyam, P., and Rao, A.S. 1977. Fungal infection of groundnut pods and aflatoxin accumulation before harvest. Proceedings of the Indian Academy of Sciences 85(6):432-443.

Shells and kernels of groundnut were examined in plants from 60 days old to harvest in the rainy (Kharif) and postrainy (Rabi) seasons. Most were free of fungal infection at first but nearly all shells were infected by harvest time. There was no aflatoxin in kernels from undamaged pods from Rabi season, but aflatoxins (118-128 µg kg^{-1}) were found in samples from rainfed plots in Kharif season. In kernels from damaged pods, higher levels of aflatoxin were detected in samples from rainfed plots (780-6700 µg kg^{-1}) than from irrigated plots (820-1840 µg kg^{-1}) in Kharif season. Low levels of aflatoxins (82-180 µg kg^{-1}) were found in damaged pods from Rabi season.

439. Vidal (Jaona, G., and Zenteno Zevada, M. 1982. The mycoflora of groundnuts (*Arachis hypogaea* L.) destined for human consumption. Anales del Instituto de Biologia Universidad Nacional Autonoma de Mexico, Botanica 47-53: 229-238.

Groundnut samples from Mexico FD, and several localities in Jalisco, Oaxaca and Guerrero were tested using malt salt agar (6 % NaCl. MSA) and mall acar (MA) media for *Aspergillus flavus.* MSA was more favorable for the development of mycoflora than MA. Groundnuts from Mexico FD showed both high and low numbers of fungi and those retaining the testa were more susceptible to fungal colonization. Since *A. glaucus* was present in all the tests and *A. flavus* in few samples and in low quantity, the relative humidity (RH) equilibrium of the fruits was deduced to be less than 75 *%.*

440. Waliyar, F., and Roquebert, M.F. 1979. [Mycoflora of groundnut pods and seeds in Senegal.]. Mycoflore des gousses et des graines d'arachide au Senegal. Revue de Mycologie 43: 169-186.

Fungi isolated from groundnut pods and seeds are listed. *Aspergillus,* particularly *A. flavus, Penicillium* and *Fusarium* spp. occurred most commonly.

441. Waliyar, F., and Zambettakis, Ch. 1979. [Study of the mycoflora of groundnut pods and seeds in Senegal.]. Etude de al mycoflore des gousses et des graines d'arachide au Senegal. Oldagineux 34(4):191-198.

About 50 species of fungi were isolated from groundnut pods and seeds at harvest. *Aspergillus, Penicillium, Fusarium* spp., and *Mucorales* occurred most commonly; *A. flavus* was predominant. There were no significant differences in the mycoflora over the two successive years of experimentation. A few pathogenic species *(Fusarium solani* and *Rhizotonia solani)* were found only rarely and did not appear to present a threat to groundnuts in Senegal.

442. Wells, T.R., Kreutzer, W.A., and Lindsey, D.L. 1972. Colonization of gnotobiotically grown peanuts by *Aspergillus flavus* and selected interacting fungi. Phytopathology 62:1238- 1242.

Groundnut plants (cv. Tennessee Red) were grown under gnotobiotic conditions in the presence of *Trichoderma viride, Penicillium funiculosum,* and the aflatoxin-producing *Aspergillus flavus.* These fungi singly or in combinations colonized aerial and subterranean parts of groundnut plants; however, colonization by *T. viride* was restricted primarily to subterranean tissues. Immature pods, mature

pericarp, and testae, to a lesser extent, were susceptible. Embryos showed limited invasion. Colonization of immature and mature pericarps by *A.flavus* was reduced in the presence of *T. viride. Penicillium funiculosum* not only nullified this antagonistic effect, but also appeared to stimulate colonization of mature groundnut pericarps and testae by *A.flavus*.

443. Wilson, D.M., and Flowers, R.A. 1974. Low aflatoxin levels in windrowed peanuts and population changes of the *Aspergillus flavus* group in soil, pods and kernels before and after harvest. Proceedings of the American Peanut Research and Education Association 6: 52-53.

Soil populations of the *Aspergillus flavus* group fungi gradually increased in two groundnut fields during the 1973 growing season. In one field, populations increased from approximately 50 propagules g^{-1} of dry soil in June to about 225 propagules g^{-1} in October. In another field the increase was from 10 in June to 25 propagules g^{-1} in October. Less than 0.1 % of groundnut kernels in windrows had *A. flavus* infection when collected daily from digging to combining (0-7 days). However, the day after combining and drying to 10-12 % moisture *A. flavus* was isolated from 15-25 % of the kernels. Recovery of *A.flavus* from pods by washing with sterile water demonstrated that there were enough propagules on the pod surface to allow this infection. The number of propagules on the surface did not increase from digging to combining in either field. Even though there was a low *A.flavus* kernel infection at harvest, 2 to 15 µg kg^{-1} aflatoxins were found in 21 of the 37 samples collected from digging to combining (0-7 days). The aflatoxins must have been produced by early infections by the *A. flavus* group fungi that did not persist or were not isolated by the technique used.

444. Wilson, D.M., and Flowers, R.A. 1978. Unavoidable low level aflatoxin contamination of peanuts. Journal of the American Oil Chemists' Society 55: 111A-112A.

In order to determine whether there is a detectable background level of aflatoxins in sound mature groundnuts at the time they are dug, samples were collected from each of the 4 replications in 1973, 1974, 1975 and 1976, in the USA. Each year 5 lb samples of sound groundnuts from each plot were shelled, hand-sorted to remove damaged and immature kernels, ground, and samples analysed for aflatoxin using the AOAC method. Level of *Aspergillus flavus* infection was : 1973, 0.9 % of 8000 seeds ; 1974. 1.4 % of 3000 seeds ; and 1975 and 1976, 0.6 % of 2000 seeds. Quality control measures have been instituted in the US to ensure that unavoidable aflatoxins in consumer groundnuts and groundnut products do not exceed 20 µg kg^{-1}. However, it was found that aflatoxin contamination at a low level (trace to 50 (ig kg^{-1}) can occur before the groundnuts arc dug. The data indicate that low level contamination is not related to high level of *A. flavus* infection or to current production practices. Low level of aflatoxin contamination of groundnuts may be endemic and current sorting procedures may not be effective in removing unblemished contaminated groundnuts.

445. Wilson, D.M., and Lynch, R.E. 1984. Effect of lesser cornstalk borer peanut damage on colonization by a mutant of *Aspergillus parasiticus.* Proceedings of the American Peanut Research and Education Society 16(1):47.

Lesser cornstalk borer (LCB) larvae were fed groundnut pods, maturity stage 2-6, as described by Lynch (1984). In one half of the laboratory test the LCB larvae were infested with a color mutant of *Aspergillus parasiticus;* the remaining larvae were not infested. After 10 days the groundnuts were sorted according to damage category and the surviving larvae were recovered. Groundnut hulls, kernels and larvae were placed on 10 % malt salt medium and incubated at 30° C for 6 days before observation. Infestation of the larvae with A. *parasiticus* decreased LCB damage but did not influence larval survival. LCB damage was stage related. Kernels from penetrated pods contained more green *A. flavus* group and *A. parasiticus* than kernels from pods with no damage or external damage. Damage by LCB did not affect the incidence of fungi recovered from hulls. *Aspergillus flavus* was recovered from uninfested more often than infested treatments. *Aspergillus parasiticus* was recovered more often from kernels in stage 3 than those in 2, 4, and 5. Stage 6 kernels had the least *A. parasiticus.* These results show that LCB larvae can be vectors of *A. parasiticus* and that kernels in penetrated pods are often colonized.

446. Wilson, D.M, and Stansell, J.R. 1981. Effects of irrigation on aflatoxin contamination of peanuts. Proceedings of the American Peanut Research and Education Society 13(1):60.

Florunner and Florigiant groundnuts were grown in 1974, 1975, 1976 and 1977 and inoculated with *Aspergillus parasiticus* 30 days after sowing. Four replicates were grown in plots for 140 to 145 days under rainfall controlled shelters with six irrigation treatments: (1) wet from 0-140, (2) dry from day 36-70, (3) dry from day 71-105, (4) dry from day 106-140, (5) dry from day 36- 105, (6) dry from day 71-140. Aflatoxin concentrations from Florunner groundnuts showed significant differences among treatments (P=0.01) in 1974 and 1976 but not in 1975 or 1977. In 1974 and 1976, Florunner sound mature kernels had significantly more aflatoxin in treatments 4 and 6 than in other treatments. Aflatoxin concentrations from Florigiant treatments 4 and 6 were significantly greater (P=0.01) than other treatments in 1974 and 1975, but not in 1977. No data were taken in 1976 for Florigiant groundnuts. Water stress during the last 35 or 70 days of the season affected aflatoxin contamination of sound mature kernels in three of the four years on one or both cultivars. Because of year to year variation, drought stress alone will not account for high levels of field aflatoxin contamination. However, in all

treatments with irrigation during the last 35 days of the season no significantly high levels of aflatoxin contamination occurred in any year or cultivar.

447. Wilson, D.M., and Stansell, J.R. 1983. Effect of irrigation regimes on aflatoxin contamination of peanut pods. Peanut Science 10: 54-56.

Effects of irrigation and drought stress treatments on preharvest aflatoxin contamination of groundnuts were investigated in the 1974, 1975, 1976 and 1977 seasons. Two groundnut cultivars, Florunner and Florigiant, were grown under rainfall controlled shelters with six irrigation treatments : (1) full irrigation throughout the growing season, (2) no irrigation from 36 to 70 days after sowing (DAS), (3) no irrigation from 71 to 105 DAS, (4) no irrigation from 106 to 145 DAS, (5) no irrigation from 36 to 105 DAS, and (6) no irrigation from 71 to 145 DAS. Groundnut plants in each replicated plot were inoculated by sprinkling them with a spore suspension of an aflatoxigcnic isolate (NRRL 2999) of *Aspergillus parasiticus.* Significant differences in aflatoxin contents of Florunner groundnuts were attribuatable to the irrigation treatments in 1974 and 1976 but not in 1975 and 1977. In 1974 and 1976, sound mature kernels of Florunner from the treatments 4 and 6 had significantly higher levels of aflatoxins than had the kernels from other treatments. Aflatoxin contamination levels in sound mature kernels of cultivar Florigiant from treatments 4 and 6 in 1975 and from treatment 6 in 1974 were significantly greater than in sound mature kernels from other treatments. Such differences in aflatoxin contamination were not evident in 1977. Drought stress during the last 40 to 75 days of the season favored aflatoxin contamination in three of the four seasons in one or both cultivars. Because of season to season variation, drought stress was not alone responsible for preharvest aflatoxin contamination. In some seasons, other environmental factors interacted with drought stress to either favor or inhibit aflatoxin contamination. No aflatoxin was detected in seeds of the test cultivars from treatments where irrigation was applied during the last 40 days of the season.

448. Wilson, D.M., Walker, M.E., Gaines, T.P., Csinos, A.S., Win, T., and Mullinix, B.G.Jr. 1985. Effects of *Aspergillus parasiticus* inoculation, calcium rates and irrigation on peanuts. Proceedings of the American Peanut Research and Education Society 17: 72.

The effects of gypsum, irrigation and inoculation with *Aspergillus parasiticus* (NRRL 2999) on mycoflora and aflatoxin contamination of groundnuts were examined. Two rows of groundnut plants in each plot were inoculated by sprinkling them with a spore suspension of the *A. parasiticus* isolate. Two irrigation regimes and four gypsum rates were tested. Soil samples were collected three times during the season and at harvest to monitor populations of *A. flavus* group fungi (*A. parasiticus* and *A.flavus).* Groundnut pods were collected at harvest for P, K, Ca, Mg, aflatoxin and mycoflora analyses. No aflatoxins were found in groundnuts from any treatment. Populations of *A.flavus* group fungi were significantly higher in soil from inoculated plots for the first two sampling dates only; *A. parasiticus* apparently did not persist in the soil throughout the growing season. More kernels were infected with the *A. flavus* group fungi and other fungi in plots with no gypsum treatments than in plots with gypsum treatments. The relationship between calcium nutrition and *A. flavus* infection in groundnuts may be important in preharvest aflatoxin contamination.

2.2.3 During Postharvest Field Drying

449. Bampton, S.S. 1963. Growth of *Aspergillus flavus* and production of aflatoxin in groundnuts - Part I. Tropical Science V(2): 74-81.

This article was written as an introduction to a series of research papers on the agricultural aspects of the problem of infection of groundnuts and subsequent aflatoxin production by *Aspergillus flavus.* It briefly reviews the problem in the widest sense, and then deals with the preliminary steps required to determine the point of entry of the fungus into the groundnut and subsequent development of toxicity.

450. Barnes, G.L., Nelson, G.L., Clary, B.L., Moseley, Y.C., and Manbeck, H.B. 1970. Effects of drying, storage gases and temperature on development of mycoflora and aflatoxin in stored high-moisture peanuts. Proceedings of the American Peanut Research and Education Association 2: 42-46.

This paper reports results of research at Oklahoma State University directed toward control of mold development on high moisture groundnut pods by storage in anaerobic and fungitoxic gases at two temperatures during 1968 and 1969. Storage of pods in N_2 or CO_2 to prevent mold growth and aflatoxin production was tested. Nondried (freshly harvested) and partially dried pods were inoculated with an aflatox in-producing strain of *Aspergillus flavus* and stored in chambers held at 3 and 24°C. During 1968 and 1969, undiluted N_2 and CO_2 were used as lest anaerobic storage gases. During 1969, a mixture of 5 % SG_2 and 95 % N_2 by volume was also tested. Compressed air was used as a check gas each year. The gases were fed through the test chambers for 32 days. Spaced samples were assayed for quality, moisture, mycoflora, and aflatoxins. All air-treated pods at 24"C soon became covered with mycelium of species of *Fusarium, Rhizopus,* and *Mucor,* but the moldy, partially dried pods soon became overgrown with *A.flavus.* In both years, mold development was greatly delayed by CO_2 or N_2. CO_2 prevented mold development for over 2 weeks at 24°C while pods held 36 days at 3°C in CO_2 had their original mold-free appearance and a near-normal odour. While 5 % SO_2 in N_2 prevented mold development and aflatoxin contamination, the treatment caused serious off flavors in both raw and roasted kernels from the treated pods. Oil

extracted from SO_2-treated kernels was very dark and had an odour distinctly different from normal. Moldy pods had a fermentation odour. High levels of aflatoxins occurred in kernels from pods stored in air at ambient room temperature; levels increased with time. Higher levels of aflatoxins occurred in high moisture kernels than in those from partially dried pods. All CO_2, N_2 and SO_2 treatments produced aflatoxin-free kernels. Molds were isolated from all visibly mold-free CO_2 and N_2 treated pods.

451. Barnes, G.L., Nelson, G.L., and Manbeck, H.B. 1970. Effects of drying, storage gases, and temperature on development of mycoflora and aflatoxins in stored high moisture peanuts. Phytopathology 60: 581.

Molds develop on high-moisture groundnuts when these are improperly dried or held in bulk. Storage of pods in N_2 or CO_2 to prevent mold growth and atlatoxin production was tested. Nondried and partially dried pods were inoculated with an aflatoxin- producing strain *of Aspergillus flavus* and stored in chambers held at 3 and 24°C. Air, N_2 and CO_2 were metered through replicated chambers. Spaced samples were assayed for quality, moisture, mycoflora, and aflatoxins. All air-treated pods at 24°C soon became covered with mycelium of species of *Fusurium, Rhizopus,* and *Mucor,* but the moldy, partially dried pods soon became overgrown with *A. flatus.* Mold development was gready delayed by CO_2 or N_2. CO_3 prevented mold development for over 2 weeks at 24°C while pods held 36 days at 3°C in CO_2 had their original mold-free appearance and a near-normal odour. Moldy pods had a fermentation odour. High levels of aflatoxins occurred in kernels from pods stored in air at 24°C; levels increased with time. Higher levels of aflatoxin occurred in nondried pods than in partially dried pods. CO_2 and N_2 treatments produced aflatoxin-free kernels. Molds were isolated from all pod samples regardless of treatment. CO^2 and N_2 were fungistatic.

452. Barnes, G.L., and Young, H.C.Jr. 1971. Relationship of harvesting methods and laboratory drying procedures to fungal populations and aflatoxins in peanuts in Oklahoma. Phytopathology 61: 1180-1184.

Fusarium spp., *Penicillium* spp., and *Alternaria tenuis* were the dominant fungi in harvested groundnut pods in 1965 in Oklahoma, USA. *Fusarium* spp., *Trichoderma viride,* and mucoraceous species (*Rhizopus* sp. and *Mucor* sp.) were dominant in 1966. *Aspergillus flavus* was rarely isolated either year. Populations of the fungi from machine-combined pods were not significantly different from those of hand-picked pods except for mucoraceous species, which were greater in combine-harvested pods. Aflatoxins were found in all samples from the 1965 season, but only in a few in the 1966 season. Aflatoxins were not correlated with presence of *A. flavus,* but were correlated with isolations of *A. tenuis.*

453. Bell, D.K., Minton, N.A., and Doupnik, B Jr. 1970. Infection of peanut pods by *Aspergillus flavus* as affected by *Meloidogyne arenaria* and length of curing time. Phytopathology 60: 1284.

Studies were conducted to determine the effects of *Meloidogyne arenaria* and curing time on pod infection by *Aspergillus flavus.* Groundnuts of the cultivar Argentine were grown in field microplots containing methyl bromide-treated soil inoculated with either *A. flavus, M. arenaria,* or *A. flavus* + *M. arenaria.* Noninoculated microplots served as controls. Each treatment was replicated six times. At maturity the plants were dug, the pods rated for root knot galling, and one-third of the pods harvested. The remaining pods were left attached to the plants and placed on a wire-mesh greenhouse bench to cure. Additional samples were made after 5 or 12 days of curing. After each sampling, 25 two-seeded pods from each replicate were assayed for fungi. The remaining pods were dried for subsequent aflatoxin analyses. Pods from *M. arenaria-inoculated* plants were heavily galled, but the incidence of *A. flavus* and total fungi was not affected. The length of the curing time did not affect the incidence of *A. flavus* or total fungi. Only in pods from *A.* flavas-inoculated plants was the incidence of *A. flavus* increased. Aflatoxin contamination was not affected by any of the treatments. *M. arenaria* damage to groundnut pods did not affect *A. flavus* infection.

454. Bell, D.K., Minton, N.A., and Doupnik, B.Jr. 1971. Effects of *Meloidogyne arenaria, Aspergillus flavus,* and curing time on infection of peanut pods by *Aspergillus flavus.* Phytopathology 61: 1038-1039.

The effects of *Meloidogyne arenaria, Aspergillus flavus,* and curing time on infection of groundnut pods by *A. flavus* were studied. Plants were grown in field microplots of fumigated soil inoculated with either *A. flavus, M. arenaria,* or *A. flavus* + *M. arenaria.* Pods from *M. arenaria* inoculated plants were heavily galled, but the incidence of *A. flavus* and total fungi was not affected. Length of curing time also did not affect the incidence of *A. flavus* or total fungi. Only in pods from *A.* flavus-inoculated plants was the incidence of *A. flavus* increased. Aflatoxin contamination was not affected by any of the treatments. It was concluded that *M. arenaria* damage to groundnut pods did not affect *A. flavus* infection.

455. Blatchford, S.M., and Hall, D.W. 1963a. Methods of drying groundnuts : I. Natural methods (Literature survey). Tropical Science 5(1):6-33.

Natural groundnut drying methods used by farmers in several countries are described. The necessity of adequate drying to prevent the development of the aflatoxin-producing fungus *Aspergillus flavus* in seeds is emphasized.

456. Blatchford, S.M., and Hall, D.W. 1963b. Methods of drying groundnuts : II Artificial methods. Tropical Science 5(2):82-98.

Information on the artificial drying of groundnuts, which is of special importance in preventing the development of the fungus *Aspergillus flavus* is summarized. It is recommended that, before drying, the nuts should be cured on the plant in windrows and should then be threshed rather than remain on the haulms. Reference is made to the extensive experimental work carried out in the USA. various types of dryers used there and in Australia, Israel, Nigeria and Tanzania are described with special reference to batch dryers. In the tropics, where ambient temperatures can be above 29°C, the use of forced air without additional heat may be sufficient for drying groundnuts.

457. Burrell, N.J., Grundey, J.K., and Harkness, C. 1964. Growth of *Aspergillus flavus* and production of aflatoxin in groundnuts - Part V. Tropical Science VI(2): 74-90.

Investigations of sun-drying methods in relation to moisture content of groundnuts were carried out in two areas in Northern Nigeria. Groundnuts arc harvested during the wet season in one area and during the dry season in the other. In the wet area, of the several sun-drying methods tried, the only one which was satisfactory was to pick the nuts from the haulms as soon as practicable alter lifting and to expose them to the sun on materials, such as matting, which could be carried under cover at night and during rain. In the dry area, a variety of sun-drying methods was used and in all cases the groundnuts dried rapidly. Those picked and placed on materials which were protected under cover during showers and at night dried the most rapidly and were consequently less susceptible to the production of aflatoxin.

458. Bushnell, D.G. 1965. The incidence of aflatoxin in the Rhodesian groundnut crop. Rhodesia Agricultural Journal, Bulletin no. 2287. 62 : 94-96.

There was a low incidence of aflatoxin contamination in the 1962/63 Southern Rhodesian groundnut crop (50-250 µg kg^{-1} aflatoxin B_1), whereas with the 1963/64 crop, an appreciable number of samples were contaminated with more than 250 µg kg^{-1} aflatoxin B_1. This incidence occurred before marketing and was not aggravated by storage conditions used in Rhodesia. Although fungal infection might occur before harvesting, the major portion of the build-up of aflatoxin contamination occurred in the period between lifting of the crop and delivery of the shelled groundnuts to the Grain Marketing Board. There was no evidence that the distribution of contamination was dependant upon the rainfall in the harvesting period. There appeared to be a higher level of aflatoxin B_1 contamination in areas with high air temperatures (65°F and above) in the harvesting period. The aflatoxin research program for the 1965 crop is described.

459. Butler, J.L., Cole, R.J., Holaday, C.E., Williams, E.J., Samples, L.E., McGill, J.F., Blankenship, P.D., and Kedlingcr, L.M. 1973. Conditions related to aflatoxin contamination in the field. Proceedings of the American Peanut Research and Education Association 5: 195-196.

Samples of groundnuts were collected in the field prior to harvest, immediately after harvest and from farmers stock storage warehouses at widely separated points in Southwest Georgia, Alabama and North Florida. Samples were analyzed for aflatoxin contamination. Some groundnuts showing no visible hull damage (mechanical or insect damage) were analyzed just as pods came from the ground, and the kernels contained high levels of aflatoxin, but no mold was apparent, even when examined under the microscope. Extremely dry weather during the latter pan of the growing season allowed the groundnuts to dry to the moisture level which was conducive for aflatoxin production in the field. Some groundnuts were dug. inverted and sprayed immediately with fungicides. Low levels of aflatoxin were present at digging and aflatoxin increased with exposure in the windrow even though they were sprayed with fungicides.

460. Dickens, J.W., and Khalsa, J.S. 1967. Windrow orientation and harvesting damage to peanuts. Oleagineux 22: 741-746.

Studies on the effects of plant orientation in windrows were conducted in North Carolina, USA. Groundnuts on inverted plants dried much more rapidly than those on plants in random windrows. Both plant orientation and moisture content at time of combining affected pod damage during combining, the amount of kernel damage caused by subsequent shelling operations and seed germination. Apical kernels were more subject to mechanical damage during combining than basal kernels. In groundnuts inoculated with *Aspergillus flavus,* kernels in broken pods and shelled kernels were more often contaminated with aflatoxin during bulk curing than were groundnuts in sound pods.

461. Dickens, J.W., and Pattee, H.E. 1966. The effects of time, temperature and moisture on aflatoxin production in peanuts inoculated with a toxic strain of *Aspergillus flavus.* Tropical Science 8(1):11-22.

Effects of time, temperature and moisture on the production of aflatoxin in groundnuts inoculated with an aflatoxin-producing strain of *Aspergillus flavus* were studied. Moisture contents between 15 and 30% were conducive to aflatoxin production. At least 48 and over 100 hours from the time of inoculation were required for aflatoxin to develop in kernels at 32°C and 21°C, respectively, at all moisture levels. Groundnut curing usually involves partial drying in windrows and subsequent drying in bulk curing bins. Unless considerable mold growth occurs before the start of bulk curing, aflatoxin is not likely to develop during the curing process if recommended practices are followed. In cool weather, even prolonged

periods in the windrows at high moisture contents did not cause aflatoxin development in groundnuts previously inoculated with *A. flavus* isolates.

462. Diener, U.L., and Davis, N.D. 1967. Relation of environment to allatoxin production by *Aspergillus flavus* in freshly dug peanuts. Phytopathology 57: 458.

Freshly dug Early Runner and Florigiant groundnuts were surface- disinfected, inoculated with an aflatoxin-producing isolate of *Aspergillus flavus,* and incubated for 3 weeks in eight 10 ft^1 environmental chambers at 10-45 + 0.5°C and relative humidities (RH) of 70-98 ± 1 %. Groundnuts were sampled after 7 and 21 days of incubation and determinations of kernel moisture, aflatoxin, and free fatty acid content were made. In 1965 at 30°C, aflatoxin formed in Early Runner kernels in 21 days at 92 and 98 % RH. whereas in Florigiant kernels it formed at 87-98 % RH. At 98 % RH, aflatoxin developed at 20-40°C in both varieties, but no aflatoxin developed at 15 and 45°C. In 1966 at 30°C, allatoxin formed in Early Runner kernels at RH as low as 85 % and in Florigiant as low as 87 %. At 98 % RH, aflatoxin was produced at 20-35°C in both varieties, with low levels occurring at 40°C in Early Runner and at 15°C in Florigiant. Growth of *A. flavus* was generally correlated with aflatoxin production except at high temperatures of 40-45°C. Free fatty acid formation was generally correlated with the growth of *A. flavus,* but not with aflatoxin production.

463. Diener, U.L., and Davis, N.D. 1968. Effect of environment on allatoxin production in freshly dug peanuts. Tropical Science 10: 22-28.

In Alabama (USA), healthy pods of two groundnut varieties collected from freshly dug-up plants were surface-sterilized and inoculated with spores of an aflatoxin-producing strain of *Aspergillus flavus.* Subsequent incubation for 21 days at 30°C resulted in aflatoxin production in the Early Runner variety when the relative humidity exceeded 83 %, in Florigiant when it exceeded 85 %. Incubation in near-saturated air resulted in aflatoxin formation at temperatures ranging from 12.5 to 42.5°C. Growth of the fungus was generally correlated with aflatoxin formation except at temperatures of 43-45"C when the mycelium was able to grow but did not produce the toxin. Pod shell and seed testa were found to form temporary barriers to penetration by the fungus.

464. du Toit, A.A. 1977. Aflatoxin research in Rhodesia. Rhodesia Agricultural Journal, Research Report no. 1. 33 pp.

Since 1962, a research team in Rhodesia has studied the factors that may affect the incidence and level of aflatoxin contamination of groundnuts and ways of dealing with the aflatoxin problem. This report gives a brief account of the research projects and discusses pertinent results. Among these is the possibility of predicting levels of aflatoxin contamination of the crop resulting from different meteorological, seasonal and cultural factors.

465. El Nur, K., and Ibrahim, G. 1970. *Aspergillus flavus* and aflatoxin production. 1.- Kernel moisture content and kernel contamination. Sudan Agriculture Journal 5(1):5-15.

In the Gezira, the Sudan, field experiments showed that *Aspergillus flavus* and other fungi such as *Penicillium* spp. and *Fusarium* spp., were always present in kernels from broken groundnut pods. On watering before harvest, the kernel moisture content immediately after lifting was 58.7 % for the cultivar Ashford. and 46 % for the cultivar Barberton, but after 7 days in the windrow the moisture content dropped to 4.7 and 3.8 %, respectively. Groundnuts dried immediately after harvest for at least 7 days were considered to be free from aflatoxin.

466. Garren, K.H., Porter, D.M., and Wright, F.S. 1976. Effect of market type and speed of field curing on persistence of *Aspergillus flavus* in peanut seed in Virginia. Proceedings of the American Phytopathological Society 3; 253.

After six months in storage, approximately 15 % of windrow-cured (rapid) and approximately 40 % of stack-cured (slow) seed of the Virginia type groundnut (cv. Florigiant) were infected with *Aspergillus flavus,* whereas with stack curing in the Spanish types Spancross and Starr only approximately 4 and 5 % of the seed, respectively, were infected. The more rapid curing and the tightly adhering pericarp of the small-seeded Spanish-type fruit appears to inhibit invasion by the fungus.

467. Gilman, G.A. 1969. An examination of fungi associated with groundnut pods. Tropical Science 11(1):38-48.

Studies in the Gambia showed that mature kernels inside visibly intact pods of the groundnut varieties 'Senegal' and 'G.O.M.B.' were frequently associated with the fungus *Macrophomina phaseolina* before and directly after harvest. Some mature pods were contaminated with the fungus *Aspergillus flavus* at lifting. The degree of invasion by these fungi was influenced, among other things, by soil moisture, shell and kernel maturity, correct lifting techniques, and the intensity of sun drying. Postharvest invasion increased with higher rainfall intensities. In general, invasion patterns were independent of varieties.

468. Habish, H.A., Abdulla, M.H., and Broadbent, J.H. 1971. The incidence of aflatoxin in Sudanese groundnuts. Tropical Science XIII(4):279-287.

A preliminary survey of the incidence of aflatoxin in the main groundnut production areas of the Sudan is described, correlating the extent of infection with *Aspergillus*

flavus and other kernel fungi with the aflatoxin content of the kernels. In general, the rainfed samples had a higher incidence of kernel contamination than those from the irrigated areas. The majority of the groundnut samples from the irrigated areas were aflatoxin-free, whereas in those from the rainfed areas none was free, and 48 % were classified low-medium (<50 to 50-250 μg kg^{-1} aflatoxin), while 52 % were in the high to very high category (250-1000 µg kg^{-1} to > 1000 µg kg^{-1} aflatoxin).

469. Jackson, C.R. 1965. Growth of *Aspergillus flavus* and other fungi in windrowed peanuts in Georgia. Tropical Science 7(1):27- 34.

Two experiments were conducted on Tifion loamy sand in Georgia (USA) with a view to studying the growth of *Aspergillus flavus* and other fungi in windrowed groundnuts. *A. flavus* invaded groundnut kernels to a slight extent during excellent drying conditions. Other fungi were more abundant. Aflatoxin was absent in kernels and shells from windrowed-groundnuts. Kernels from groundnuts windrowed during cooler weather were rarely invaded by *Aspergillus* spp. Kernels from pods cured against the soil surface were not invaded to a greater extent than those from pods cured in the air. *A. flavus* was present in the pod surface mycoflora in both experiments.

470. Jackson, C.R. 1967a. Influence of drying and harvesting procedures on fungus populations and aflatoxin production in peanut in Georgia. Phytopathology 57: 458-462.

Mature groundnuts (cv. Early Runner), with a moisture content of 45 to 50 % ("green") or 18 to 25 % ("semidry"), were harvested by combining or by hand. Drying rates in the laboratory were then controlled to achieve rapid drying (2 days at 29-35"C, continuous air flow) or slow drying (11 days at 27-35°C, intermittent air flow) until pods reached 8 % moisture. Fungal populations on outer surfaces of pods were estimated by a wash-dilution plate method and surface-sterilized kernels were cultured directly. Aflatoxin content of kernel samples was also determined. Mean numbers of total pod surface and kernel fungi were not affected by harvesting method, but slow drying led to significantly larger numbers than rapid drying. *Aspergillus flavus* was more abundant in kernels from combine-harvested than from hand-harvested pods and more abundant on slowly dried than on rapidly dried pods. Mean aflatoxin B_1 content of slowly dried "green" groundnuts was 1780 µg kg^{-1} for combine-harvested pods, compared with 140 |ig kg^{-1} for hand-harvested pods. For slowly dried "semidry" groundnuts, combined samples had 1160 µg kg^{-1} compared with 140 µg kg^{-1} for hand-harvested samples. Aflatoxin B_1 was absent or found only in trace amounts in rapidly dried groundnuts.

471. Jackson, C.R. 1967b. Some effects of harvesting methods and drying conditions on development of aflatoxins in peanut. Phytopathology 57(11): 1270-1271.

Harvesting methods and drying conditions were studied in relation to fungal invasion and aflatoxin content of groundnut kernels using freshly harvested and partially dried groundnuts. In 1965 and 1966, pods of the groundnut cultivar Early Runner were taken from field plantings near Tifton and Plains, Georgia, USA. At maturity, the plants were mechanically lifted, shaken, and windrowed. Alternate windrows were harvested mechanically with a combine operating at an approximate picking-teeth speed of 300 ft min^{-1}, or manually. In 1965, fresh, mature pods were harvested and placed in driers immediately after windrowing. In other experiments, pods that had dried in the windrows for 2-3 days (semidry) were harvested by the same methods and placed in driers. Combine- and hand-harvested pods from each replication were divided and placed separately in single layers in wire-bottom drier trays. Rapid drying was achieved by using continuous air flow at 29-35°C for 1-2 days. In 1965, green pods required 11 days and semidry pods 7 days to dry to 8 % moisture content. *Aspergillus flavus* was more abundant in kernels from all slowly dried samples, especially the combine-harvested samples in 1965. In 1966, no difference was found in the incidence of *A. flavus* in kernels harvested by the two methods. The frequency of *A. flavus* was generally related to aflatoxin content of kernels. An approximate tenfold difference in aflatoxins levels between harvesting methods was found in both 1965 experiments. The difference in 1966 was much less pronounced. Rapidly dried groundnuts were largely free from aflatoxins and *A. flavus*. The brief windrow periods did not greatly affect aflatoxin content in semidry groundnuts.

472. Manzo, S.K., and Misari, S.M. 1989. Status and management of aflatoxin in groundnuts in Nigeria. Pages 77-90 in Aflatoxin contamination of groundnut : Proceedings of the International Workshop, 6-9 October 1987, 1CRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

A committee to coordinate action on the problem of aflatoxin contamination in Nigeria was formed in 1961 with representatives from four ministries, i.e., the Institute for Agricultural Research (IAR), Zaria. the Nigerian Stored Products Research Institute (NSPRI). and the Northern Nigerian Marketing Board. This Committee was given the responsibility of assessing the extent of the aflatoxin problem in groundnut in the country and of initiating and coordinating all actions leading towards elimination of aflatoxin. IAR investigated the aflatoxin contamination of the groundnut crop up to the stage where the produce was sold by farmers, while NSPRI studied the problem from the time of storage until produce was exported or consumed. IAR investigated the time of invasion of groundnut kernels by *Aspergillus flavus,* and when, and under what conditions it produced

aflatoxin. An interplay of temperature, relative humidity, drought, and erratic rainfall patterns, and maturity of the crop at lifting affected 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, while in the major groundnut growing areas that lie in the drier Northern Guinea and Sudan Savanna the problem is largely 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 groundnuts to cither 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 government 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 with an aflatoxin content that does not exceed the maximum permissible limit of 20 µg kg^{-1} set by the European Economic Community. 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.

473. McDonald, D. 1968. The effect of wetting dried groundnuts on fungal infection of kernels. Samara Agricultural Newsletter 10(1): 4-7.

Investigations were carried out at Samam in 1967 in which windrow-dried pods of the groundnut variety Samaru 38 were anificially wetted and the development of kernel infection by fungi studied. One week after windrow-drying, the dried plants with pods still attached were built into two "kiriga" of 3 ft high by 6 ft in diameter, and to one of these the equivalent of 0.75 in. of rain was applied. On the following day a further 0.5 in. of water was applied. Pods sampled from the wetted "kiriga" showed an increase in kernel fungal infection to 33.5% within 24 h of wetting and the level of infection increased to 67.3% after three days. The initial increase was due to invasion of kernels by *Fusarium* spp. and *Aspergillus flavus,* but the final increase was due largely to heavy infection of kernels by *Macrophomina phaseolina.* Undamaged pods from the dry "kiriga" (control) showed no increase in kernel fungal infection.

474. McDonald, D. 1969. *Aspergillus flavus* on groundnut *(Arachis hypogaea* L.) and its control in Nigeria. Journal of Stored Products Research 5: 275-280.

Groundnuts are produced in two distinct zones in Nigeria; the dry northern zone where 95 % of the crop is grown, and the wetter riverain zone. In the northern zone the crop is normally harvested after the rains have ended, but in the riverain zone harvesting takes place during the rains. Investigations on groundnuts from the 1961 crop showed that *A. flavus* infection of kernels and aflatoxin production occurred in both zones. The condition of the shell was found to have a marked influence on the fungal infection and toxicity of the kernels. Kernels from undamaged pods were rarely infected by *A. flavus,* whereas kernels from perforated pods had a high degree of infection with this and other fungi. Kernels from termite-scarified pods were intermediate in this respect. Kernels from all pod grades showed higher fungal infection in material from the riverain zone than in that from the northern zone, indicating that poor drying conditions could be important. Trials at Mokwa in the riverain zone and at Kano in the northern zone in which various methods of drying were tested demonstrated that rapid drying gave kernels with low fungal infection and little or no toxicity. Slow drying with kernel moisture contents above 20 % for extended periods resulted in heavily infected, toxic kernels. Kernels from undamaged, mature pods were found to be free from fungal infection at lifting, infection by *A. flavus* not normally occurring until 4-6 days after harvest. Over-mature pods and pods from plants that had wilted and died before harvest had kernels infected by fungi at lifting in the northern zone. On the basis of these findings recommendations were provided for management of *A. flavus* in groundnut.

475. McDonald, D. 1970b. Fungal infection of groundnut fruit after maturity and during drying. Transactions of the British Mycological Society 54(3): 461-472.

In trials at Mokwa, Nigeria, shells and seeds of the long-season groundnut variety Samaru 38 were examined for fungal infection. Samples were taken at normal lifting time, during windrow drying, after sun-drying on mats, and after becoming over-mature in the ground. At normal maturity the shells were commonly infected although very few seeds were invaded. Fruits lifted when mature and dried in windrows showed an increase in invasion of seeds. Seeds from fruits picked shordy after lifting, then sun-dried on mats, showed little increase in fungal infection during drying. When fruits were left in the ground after reaching maturity, there was a gradual increase of fungal invasion of the seeds. *Macrophomina phaseoli* and *Fusarium* spp. were the dominant fungi in shells; they were also dominant in seeds of over-mature and windrowed-dried fruit. *Aspergillus* spp. were not abundant in shells or seeds but were frequently present, the toxigenic *Aspergillus flavus* being the most common.

476. McDonald, D., and A'Brook, J. 1963. Growth of *Aspergillus flavus* and production of aflatoxin in groundnuts-Pan III. Tropical Science V(4): 208-214.

Samples of Spanish 205 groundnuts from a series of drying trials conducted at Mokwa, Northern Nigeria, in 1961, were tested for aflatoxin and it was found that some toxicity occurred in sun- dried materials, while material that had been artificially dried shortly after lifting, was free from the toxin. Further trials with the

variety Samaru 38 showed that there were considerable differences in kernel infection with *Aspergillus flavus* and other fungi between samples taken at different stages in the drying processes but no aflatoxin was found in any of the samples tested.

477. McDonald, D., and Harkness, C. 1963. Growth of *Aspergillus flavus* and production of aflatoxin in groundnuts - Part II. Tropical Science V(3): 143-154.

With the discovery of aflatoxin in groundnuts from many producing countries preliminary investigations were made on the 1961 crop in Nigeria. Results of the toxin tests showed that none of the varieties sampled was more resistant to toxin formation than another and that toxic samples appeared with equal frequency in both Northern and Riverain Provinces. Within each provincial group individual sites varied considerably for toxicity of samples and this may have been due in part to different post- harvest drying and storage procedures. The fungal contamination tests showed no evidence of varietal resistance but did indicate considerably more contamination in samples from the Riverain Provinces than in samples from the Northern Provinces. Condition of the shell was found to affect markedly the degree of contamination of the kernels and, as for toxicity, there were considerable differences between individual sites for contamination with *Aspergillus flavus* and other fungi.

478. McDonald, D., and Harkness, C. 1964. Growth of *Aspergillus flavus* and production of aflatoxin in groundnuts - Part IV. Tropical Science VI(1): 12-27.

Investigations on aflatoxin in groundnuts were carried out during 1962 at Mokwa and five other localities in the Riverain Provinces of Northern Nigeria. The results indicated that : (1) *Aspergillus flavus* grew much more successfully on moribund or dead groundnut tissue than it did on living tissue. (2) There was virtually no toxicity in the crop at harvest, the exceptional cases being from broken pods. (3) Contamination of kernels with *A. flavus* and appearance of the toxin did not occur until at least five days after harvest. (4) Kernels from broken pods were more likely to be contaminated with *A. flavus* and to be toxic than were kernels from undamaged pods. (5) Toxicity of the crop varied from year to year at specific localities.

479. McDonald, D., and Harkness, C. 1965. Growth of *Aspergillus flavus* and production of aflatoxin in groundnuts - Pan VIII. Tropical Science VII(3): 122-137.

Trials were carried out at Mokwa Agricultural Research Station, Northern Nigeria, in 1963 to test various natural methods for postharvest drying of groundnuts. Samples of groundnuts were taken from the range of treatments at intervals from harvest until the end of drying, and these were tested for aflatoxin content and kernel contamination with *Aspergillus flavus* and other fungi. Large differences in rates of drying occurred between the various treatments and, in general, the slower the rate of drying the higher was kernel contamination with *A. flavus* and other fungi and the higher the incidence of toxic samples. When pods were hand-picked from the haulms and sun-dried in a layer, with protection from rain, the rate of drying was high and a toxin-free crop resulted None of the treatments in which the pods were dried on the haulms in the field gave a toxin-free crop.

480. McDonald, D., Harkness, C, and Slonebridge, W.C. 1964. Growth of *Aspergillus flavus* and production of aflatoxin in groundnuts - Part VI. Tropical Science VI(3): 131-154.

Trials were carried out at Kano Agricultural Reserch Station, Northern Nigeria in 1963 to test various natural methods for postharvest drying of groundnuts. Samples of groundnuts were taken from the different treatments at intervals from harvest until the end of drying and these were tested for aflatoxin content and kernel contamination with *A. flavus* and other fungi. There was very little difference in rate of drying between the various treatments and no effect on toxicity or fungal contamination was observed. Some kernel samples were found to be toxic at harvest and this was associated with pod damage and with kernel mositure content.

481. Moreau, C. 1976. [Variations of fungal pollution in peanuts and their cakes from harvest to consumption.]. Variations de la pollution fongique des arachides et de leur tourteaux de la recolte a la consommation. Revue de Mycologie 40:97-115.

Mycological analysis of postharvest groundnut samples was carried out. Various soil-fungi were found to infect the pods ; most of them grew through the shell, but not all reached the kernels. The most common were *Aspergillus flavus* and *A. niger.* Where they are treated in oil mills, the originally heavily contaminated kernels are processed through some heat treatments which progressively eliminate all fungal contamination. Nevertheless, recontamination occurs as soon as the pellets are cooled, and a large mold development occurs in the usual conditions of storage. The cakes used to feed cattle are often highly contaminated. Detoxification of cakes by ammoniation destroys the aflatoxin and also has a fungicidal action against many molds, especially *A. flavus.*

482. Moreau, C. 1977. [Fungal pollution in peanuts and their cakes.]. Contamination fongique des arachides et de leurs tourteaux. Revue francaise des Corps Gras 24(1): 27-34.

Mycological analysis of postharvest groundnut samples was carried out. Various soil-fungi were found to infect the pods ; most of them grew through the shell, but not all reached the kernels. The most common were *Aspergillus flavus* and *A. niger.*

Where they are treated in oil mills, the originally heavily contaminated kernels are processed through some heat treatments which progressively eliminate all fungal contamination. Nevertheless, recontamination occurs as soon as the pellets are cooled, and a large mold development occurs in the usual conditions of storage. The cakes used to feed cattle are often highly contaminated. Detoxification of cakes by ammoniation destroys the aflatoxin but also has a fungicidal action against many molds, especially *A. flavus*.

483. Nagaraj, G., and Kumar, K. 1986. Location variations in the aflatoxin content of some Virginia groundnut varieties. Journal of the Oil Technologists' Association of India 18(3): 89-91.

Seven Virginia groundnut varieties, viz. S 230, Kadiri 1, Kadiri 3, Karad 4-11, M13, GAUG 10 and TMV 10 were grown in four locations (Chintamani, Jalgaon, Khargaon, and Junagadh) in India in the rainy season of 1982. After harvest the pods were received at Junagadh during February-March 1983. Pods were shelled and aflatoxin analyses carried out on seeds with a thin-layer chromatography (TEC) method. Aflatoxin B_1 was found in all the groundnut varieties (0.8 to 65.8 μg kg^{-1}). Aflatoxins B_2 and G_1 were found in a few samples from some locations. Among varieties, S 230 had the highest level of aflatoxins at all the locations (49.8 to 85.9 μg kg^{-1}) followed by GUAG 10 (15 to 88.3 μg kg^{-1}) and Kadiri 3 (17.8 to 106.7 μg kg^{-1}). Karad 4-11 had low levels of aflatoxins at all four locations (1.6 to 30.3 µg kg^{-1}) followed by Kadiri 1 (4 to 52 µg kg^{-1}) aflatoxins. M13 and TMV 10 had moderate levels of aflatoxins. Among the locations, Junagadh and Chintamani samples contained higher levels of aflatoxins. Unfavorable postharvest drying conditions at Junagadh and Chintamani probably resulted in higher levels of aflatoxins at these locations. The varieties with more than 3 % phenols in the seed testa generally contained low levels of aflatoxins. The varieties with protein content higher than 25 % contained more aflatoxins, while those having about 22 % proteins contained less aflatoxins except TMV 10 which also had less total phenols. Sugar and oil content did not appear to have any effect on the aflatoxin levels.

484. Norse, D. 1971. Aflatoxin studies. Pages 239-246 in Annual Report of the Department of Agriculture, Malawi, for the year 1967/68, 279 pp.

The incidence of *Aspergillus flavus* and aflatoxin in groundnuts was greater in the hotter, lower altitude areas. *A. flavus* and the toxin were found primarily in broken pods, but they occasionally occurred in apparently undamaged pods. No significant correlation was found between drying treatments and incidence of the fungus in kernels. There was no correlation between superficial shell and kernel infection as long as the shell remained intact.

485. Parker, W.A., Melnick, D., and Mills, W.T. 1971. Influence of peanut harvesting and curing methods on aflatoxin contamination. Proceedings of the American Peanut Research and Education Association 3: 15-28.

A critical evaluation of different methods of harvesting and curing groundnuts was conducted with respect to the incidence of aflatoxin content and overall groundnut quality. Groundnuts (cv. Early Runner) were harvested in bulk according to both the conventional method and according to a New Concept Method. Those groundnuts harvested by the conventional procedure were either subjected to field drying and curing or were dried by artificial means in bins using forced air. The New Concept Method involved deliberately allowing the groundnuts to remain in the soil about one month beyond the ideal harvesting date and then cutting and removing the vines two days prior to digging. The freshly dug kernels were then immediately subjected to artificial curing, employing the same procedure as used for one lot of control groundnuts. *Aspergillus flavus* was found in damaged nut samples obtained by the new harvesting method. Heavy mold colonization was noted on many kernels which remained in the soil. The percentage of rejected (damaged) kernels from the new harvesting method was about four times that experienced with the two control samples after conventional harvesting, those field cured and those artificially cured. Many of the kernels remaining in the soil after the ideal harvest date showed serious hull deterioration and this contributed to loss of natural protection against mold spoilage. A significant increase in aflatoxin content was associated with the increase in mold damage. The artificially cured groundnuts were superior in quality characteristics than those field cured, following conventional harvesting. The mature and damage-free groundnuts, obtained by the New Concept Method, were scored higher in good groundnut flavor after roasting than the control groundnuts obtained by the other two methods. This was attained with no change of direct practical significance in fatty acid composition. However, the advantage of superior flavor of good groundnuts obtained by the New Concept Method was wiped out by the high incidence of mold contamination in the overall crop, associated with increased aflatoxin content (30 to 40 times higher). The standard harvesting procedure, with properly controlled artificial curing thereafter, still offers the best way to obtain quality groundnuts with the least aflatoxin contamination.

486. Pettit, R.E., and Taber, R.A. 1968. Factors influencing aflatoxin accumulation in peanut kernels and the associated mycoflora. Applied Microbiology 16: 1230-1234.

Accumulation of aflatoxin in Spanish groundnut kernel samples from different geographical areas in Texas during 1966, as detected by thin-layer chromatographic method, was relatively low. Analysis of samples obtained from growers using artificial drying equipment (forced and supplemental heat), when windrow conditions were unfavorable for rapid drying, suggests that this practice reduces the possibility of aflatoxin accumulation. In general, groundnuts harvested from land

planted to groundnuts the previous year were more highly infected with fungi and contained more aflatoxin than groundnuts grown on land planted with rye, oats, melons, or potatoes the previous year. Aflatoxin incidence tended to decrease from south to north Texas. These findings verify previous research observations that moist tropical climates are conducive to fungal infection and aflatoxin accumulation. Detection of aflatoxin in sound mature kernels (kernels screened for minimal size) indicates that the practice of screening for removal of small immature kernels and removal of obviously damaged kernels does not completely eliminate aflatoxin accumulation.

487. Pettit, R.E., and Taber, R.A. 1970. Fungal invasion of peanut kernels as influenced by harvesting and handling procedures. Phytopathology 60: 1307.

Groundnuts were dug from field plots at Yoakum and Stcphenville, Texas, during the years 1967 through 1969, and were subjected to the following treatments : field-dried in inverted (pods in the upright position) and random windrows for different time periods; cured on the vines with forced air; combined at kernel moisture levels above 25 % and either flash-dried or stored at 10°C and/or dried at different air flow rates with and without supplemental heat. Following each treatment replicated, 100-kernel samples were surface-sterilized and plated on rose bengal-streptomycin agar. The degree of fungal invasion increased when the average drying rate was less than 0.12 % per hour. This rate was influenced by the air velocity, heavy dew and shower frequency, air temperature, and relative humidity. The best drying treatment was on the vines in bins with forced air, and the second best was where 25 CFM of air were passed through several different volumes of bulk groundnuts. Groundnuts from the inverted windrows were less severely damaged by fungi. Groundnut pods in contact with the soil surface beneath the random windrows were more heavily invaded. High-moisture groundnuts held in bulk containers over 18-24 hours without adequate aeration, and those stored at 10°C for over 48 hours were heavily invaded.

488. Pettit, R.E., Taber, R.A., and Person, N.K. 1971. Microbial infestation of peanuts as related to windrow-curing conditions. Proceedings of the American Peanut Research and Education Association 3:127-136.

Fungal and bacterial damage to groundnuts during windrow-curing was determined by : visual examination of pods and kernels, noting the degree of kernel infection; and determination of germination percentages. Groundnuts removed from the field at digging time and cured on the vines under cover were superior in quality. Infection and physical damage to windrow-cured groundnuts by fungi and bacteria were found to be related to inoculum potential of specific fungal species, degree of pod damage before and during harvest, pod location within the windrows, and climatic conditions during curing. Groundnuts which were cured on the soil surface or inside the windrow during shower periods were more severely infected with bacteria and fungi compared to those cured in the upper part of the windrow. Groundnuts from inverted windrows dried more uniformity under adverse drying conditions and were less severely infected with fungi. Drying groundnuts within the field in random or inverted windrows under high temperatures and/or low humidities caused an increased level of sound splits when shelled. There was an inverse relationship between bacterial infection and percent germination.

489. Pitt, J.I. 1989. Field studies on *Aspergillus flavus* and anatoxins in Australian groundnuts. Pages 223-235 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 Oct 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

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 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 spores g^{-1} to as high as 10^5 spores 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 arc harvested. Invasion will not occur subsequently, aflatoxin is not 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 alter 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.

490. Porter, D.M., and Garren, K.H. 1970. Endocarpic microorganisms of two

types of windrow-dried peanut fruit (*Araehis hypogaea* L.). Applied Microbiology 20(1): 133-138.

The endocarpic microorganisms of groundnut fruit dried in either a random windrow (plants left as they fell from the digger) or an inverted windrow (plants inverted to expose fruit to sunlight) were different from that of freshly dug fruit. *Chaetomium, Penicillium, Trichoderma, Rhizoctonia,* and *Fusarium* were dominant fungi associated with shells (pericarp) of freshly dug fruit. The dominant fungi of shells of windrowed fruit included *Chaetomium, Rhizocionia, Fusarium, Sclerotium,* and *Alltrnaria.* Seeds of freshly dug fruit were dominated by *Penicillium* and *Aspergillus.* The only dominant species in seed of windrowed fruit was *Penicillium.* Microorganisms were isolated from shells and seed of freshly dug fruit at a frequency of 79 % and 52 %, respectively. The percentage of infection was reduced by drying in the field. This was particularly true of the inverted windrows. The proportion of shells and seed infected with a microorganism was reduced 13 % and 36 % respectively, after field drying for 5 to 7 days in random and inverted windrows. Microorganisms were isolated much more frequently from shell pieces (73 %) than from seed (36 %).

491. Porter, D.M., and Wright, F.S. 1969. The effects of field exposure and windrow type on microflora, especially *Aspergillus flavus* associated with peanut fruits. Proceedings of the American Peanut Research and Education Association 1: 96.

Fewer fungi were isolated from groundnut (cv. Virginia 61-R) pods that were windrowed in the up position (44.4 %) than from the down windrow (52.4 %). *Fusarium* spp., *Trichoderma* spp., *Rhizoctonia* spp., *Epicoccum* spp. and *Botrytis* spp. were isolated more frequently from the down windrow than from the up windrow. However. *Chaetomium* spp., *Thielavia* spp., and *Alternaria* spp. were isolated more frequently from the up windrow. More fungi were isolated from pods that were windrowed for 12 days (59.8 %) than those windrowed for 4 days (36.8 *%). Fusarium* spp., *Trichoderma* spp., *Epicoccum* spp., *Phorna* spp.. *Botrytis* spp. and *Altemaria* spp. were isolated more frequently after 12 days than after 4 days. Others including *Chaetomium* spp., *Thielavia* spp. and *Rhizoctonia* spp. were isolated more frequently after 4 days exposure. Incubation for 5 days after combining had little effect on the pod mycofiora. The isolation density of *Aspergillus flavus* was low (3.7 *%)* although pods were inoculated with this fungus immediately after digging. *A. flavus* was isolated more frequently from seed (4.9 %) than from pieces of shell (2.4 %). The isolation density of *A. flavus* from pods (shell and seed) windrowed in the down and in the up position was 4.5 % and 2.9 %, respectively. Isolates of *A. flavus* were obtained almost twice as readily from pods exposed for 4 days as from pods exposed for 12 days. Plating immediately after combining, or 5 days after combining, had little effect on the isolation frequency of *A. flavus.*

492. Porter, D.M., and Wright, F.S. 1971. Proliferation of *Aspergillus flavus* in artificially infested windrow dried peanut fruit in Virginia. Phytopathology 61: 1194-1197.

Artificially infested groundnut fruit, field-dried in random windrows (fruit covered with foliage and not exposed to sunlight), yielded more isolates of *Aspergillus flavus* than fruit dried in inverted windrows (fruit resting on top of the foliage and exposed to direct sunlight). Windrow-dried, small (15 mm long), whole immature groundnut fruits were invaded more often by *A. flavus* after artificial infestation than were pieces of shell and seed of larger (30 mm long) immature and mature fruit. The isolation frequency of *A. flavus* from artificially infested mature fruit was about that of untreated mature fruit. Isolates of *A. flavus* were obtained more readily from shell pieces and seed from infested fruit alter a 4-day exposure period in either windrow than after a 12-day exposure period. Periods of adverse drying conditions enhanced aflatoxin production in seed of mature windrow-dried fruit.

493. Porter, D.M., Wright, F.S., and Steele, J.L. 1972. Relationship of shell damage to colonization of peanut seed by *Aspergillus flavus.* Proceedings of the American Peanut Research and Education Association 4: 207.

Differences in infection of seed by *Aspergillus flavus* were noted when comparisons were made between seed from sound or damaged groundnut fruit with different moisture contents. Seed from pods with visible damage (shell damage detected visually) and invisible damage (shell damage detected by a staining technique) were infected more frequently by *A. flavus* than those from sound fruit (no visible or invisible damage). Seed from invisibly damaged fruit were infected almost as rapidly as seed from visibly damaged fruits. The isolation frequency of *A. flavus* from invisibly and visibly damaged fruit was 23 % and 26 %, respectively. *A. flavus* was isolated at a frequency of 8 % from nondamaged fruit. After incubation at a temperature and relative humidity conducive to the rapid proliferation of *Aspergillus* spp. for a period of 24 to 48 hours, *A. flavus* was isolated just as rapidly from seeds from invisibly damaged fruit as from visibly damaged fruit. Although the isolation frequency of *A. flavus* from seed from sound fruit increased with time, the frequency did not approach that obtained from seed from damaged fruit. Before incubation, the isolation frequency of *A. flavus* from seed from non-inoculated fruit with moisture contents of 10 %, 30 % and 50 % was 4 %, 7 % and 10 %, respectively. Following incubation the isolation frequency of *A. flavus* from seed was greater in partially dried fruit than in fruit with a high moisture content. The isolation frequency of *A. flavus* from seed from partially dried fruit with a moisture content of 10 % and 30 % was 20 % and 25 %. respectively, following incubation.

A. flavus was isolated less frequently (12 %) from seed from fruit with a 50 % moisture content, probably due to competition with other fungi during incubation.

494. Quitco, R., Bautista, L., and Bautista, C. 1989. Aflatoxin contamination of groundnuts at the post-production level of operation in the Philippines. Pages 101-110 in Aflatoxin contamination of groundnut: proceedings of the International Workshop, 6-9 October 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

The results of surveys in the Philippines have shown that farm level aflatoxin significantly increased from harvest to farm storage during the main cropping season. At harvest, groundnuts contained, on average, 3.16 µg kg^{-1} aflatoxin. During windrowing, aflatoxin levels increased at the rate of 1.5 µg kg^{-1} per day. In farm storage aflatoxin contamination continued to increase at the rate of 1 *µg* kg^{-1} per day. Aflatoxin contamination was signifiantly higher during the main cropping season than the second cropping season. At the traders' level, groundnut samples taken from various middlemen contained 35 ug kg^{-1} aflatoxin. On the other hand, samples taken from the wholesalers' newly procured groundnuts contained 188 *µg* kg^{-1} aflatoxin. Groundnuts that had been in wholesalers' warehouse for more than 3 months contained 275 µg kg^{-1} aflatoxin. At the processors' level, raw groundnuts for confectionery use (roasted and fried) contained 7.73 ug aflatoxin kg^{*1}, grounduts intended for peanut butter contained 17.13 µg kg^{-1}, and rejected groundnuts had 120.6 *µg* kg^{-1}. Aflatoxin contamination could start during harvest. Aflatoxin content increased to a significantly high level during trade and processing. This continued increase was attributed to insufficient drying of groundnuts after harvest.

495. Saito, S., Kawasugi, S., Tsuruta, O., and Manabe, M. 1985. *Aspergillus flavus* infection of peanuts in Thailand. Proceedings of the Japanese Association of Mycotoxicology No. 21:34-35.

Groundnut samples were collected from test fields and farmers' fields at harvest during the rainy season and from kernels sold in a market. *Aspergillus flavus* infection was only detected in the samples collected from the market. It is suggested that infection of groundnuts occurs during postharvest drying.

496. Sanders, T.H., Blankenship, P.D., Cole, R.J., and Smith, J.S. 1986. Role of agrometeorological factors in postharvest quality of groundnut. Pages 185-192 in Agrometeorology of groundnut: proceedings of an International Symposium, ICRISAT Sahelian Centre, Niamey, Niger, Patancheru, India : International Crops Research Institute for the Semi-Arid Tropics.

Postharvest quality of groundnut is influenced by the particular set of environmental and cultural practices that influence physiology and maturation. Groundnut composition, although related to environment, changes dramatically as groundnuts mature. There is biochemical basis for inferior quality in immature groundnut. Drought stress and soil temperature influence maturation rate and thus have an indirect effect on postharvest quality. *Aspergillus flavus* invasion and aflatoxin contamination in groundnuts are related to drought stress, soil temperature, and maturity. Small, immature seed are more likely to be contaminated with *A. flavus* than larger, mature seed. The biochemical composition, fungal contamination, and the tendency toward higher moisture content complicate storage of immature seed. Each of these factors predisposes immature seed to rapid quality deterioration in storage. Agrometeorological studies must include an awareness of the interrelationships of environment, maturity, and postharvest quality.

497. Subrahmanyam, P., Reddy, A.N., Sreenivasalu, P., and Kumar, N.R. 1976. An examination of fungi associated with groundnut pods in stacks. Indian Journal of Microbiology 16: 40-42.

Groundnuts were either stacked undried (Stack I) or field-dried to a safe moisture content immediately after harvesting and then slacked (Stack II). The stacks (6 x 3 x 2.5 m) were built on a floor covered with paddy hay to a height of 40 cm. Groundnut pods, withdrawn from the centre of the stack on the day of stacking (day 0) and at 15-day intervals for 2 months, were examined for kernel moisture content and kernel mycoflora. At day 0. 30 and 60, kernels from stack I contained, respectively, 48, 20.5 and 7 percentage moisture; corresponding moisture content for stack 11 were 8, 6.9 and 6.8 %. At day 0, 30 and 60, the % of kernels from stack I infected with fungi was, respectively 40, 100 and 72 % : corresponding levels of fungal infection in stack 11 were 48, 28 and 25 %. Among the fungi, *Aspergillus flavus, A. niger, Fusarium* spp. and *Macrophomina phaseoti* predominated. Those kernels exhibiting visible microbial damage had an unpleasent rancid taste and all yielded *M. phaseoti.*

498. Troeger, J.M., Williams, E.J., and Holaday, C.E. 1969. Aflatoxin incidence in peanuts as affected by harvesting and curing procedures. Proceedings of the American Peanut Research and Education Association 1: 62-67.

Experiments with three groundnut varieties, conducted in the USA, indicated that green or partially dried groundnuts, held with no air flow for 24 hours, were highly susceptible to aflatoxin contamination. Low air flow through the groundnuts did not suppress aflatoxin production. Groundnuts held under N_2 or CO_2 atmospheres developed less aflatoxin, but produced a highly offensive odour. Groundnuts with initial moisture contents below 30 % developed considerably more aflatoxin after having been rewetted and placed in high humidity chambers for five days than groundnuts with initial moisture content above 30 %. The variety Starr Spanish had considerably more aflatoxin than the varieties Early Runner and Florigiant.

Immature grounduts were less susceptible to aflatoxin contamination. Prompt drying is the most effective practice for eliminating aflatoxin contamination of groudnuts.

499. Troeger, J.M., Williams, E.J., and Holaday, C.E. 1970. Aflatoxin incidence in peanuts as affected by harvesting and curing procedures. Oleagineux 25(4): 213-216.

Experiments with three groundnut varieties, conducted in the USA, indicated that green or partially dried groundnuts, held with no air flow for 24 hours, were highly susceptible to aflatoxin contamination. Low air flow through the groundnuts did not suppress aflatoxin production. Groundnuts held under NT, or CO, atmospheres developed less aflatoxin, but produced a highly offensive odour. Groundnuts with initial moisture content below 30 % developed considerably more aflatoxin after having been rewetted and placed in high humidity chambers for five days than groundnuts with initial moisture content above 30 %. The variety Starr Spanish had considerably more aflatoxin than the varieties Early Runner and Florigiant. Immature groundnuts were less susceptible to aflatoxin contamination. Prompt drying is the most effective practice for eliminating aflatoxin contamination of groundnuts.

500. Waliyar, F., and Roquebert, M.F. 1979. [Mycoflora of groundnut pods and seeds in Senegal.]. Mycoflore des gousses et des graines d'arachide au Senegal. Revue de Mycologie 43: 169-186.

Fungi isolated from groundnut pods and seeds are listed. *Aspergillus,* particularly *A. flavus, Penicillium,* and *Fusarium* spp. occurred most commonly.

501. Wilson, D.M., and Flowers, R.A. 1974. Low aflatoxin levels in windrowed peanuts and population changes of the *Aspergillus flavus* group in soil, pods and kernels before and after harvest. Proceedings of the American Peanut Research and Education Association 6: 52-53.

Soil populations of the *Aspergillus flavus* group fungi gradually increased in two groundnut fields during the 1973 growing season. In one field, populations increased from approximately 50 propagules g^{-1} of dry soil in June to about 225 propagules g^{-1} in October. In another field the increase was from 10 in June to 25 propagules g^{-1} in October. Less man 0.1 % of groundnut kernels in windrows had *A. flavus* infection when collected daily from digging to combining (0-7 days). However, the day after combining and drying to 10-12 % moisture *A. flavus* was isolated from 15-25 % of the kernels. Recovery of *A. flavus* from pods by washing with sterile water demonstrated that there were enough propagules on the pod surface to allow this infection. The number of propagules on the surface did not increase from digging to combining in either field. Even though there was a low *A. flavus* kernel infection at harvest, 2 to 15 µg kg^{-1} aflatoxins were found in 21 of the 37 samples collected from digging to combining (0-7 days). The aflatoxins must have been produced by early infections by the A. *flavus* group fungi that did not persist or were not isolated by the technique used.

2.2.4 In Storage

502. Abdalla, M.H. 1974. Mycoflora of groundnut kernels from the Sudan. Transactions of the British Mycological Society 63(2): 353-359.

Fungi isolated from 53 groundnut samples from three production areas (with different soils and irrigation systems) are listed. *Aspergillus niger* and *A. flavus* were isolated from all samples, the former in 29-60 % and the latter in 4-52 % of kernels. *Rhizopus* spp., *Pencillium* spp. and *Macrophomina phaseolina* were fairly prominent in Gezira samples. *A. terreus, A. nidulans* and *Fusarium* spp. were minor components. Further development of fungal growth was checked by the low moisture content of kernels and by favorable postharvest climatic and storage conditions.

503. Abdel-Rahman, A.H.Y. 1982. Effect of fungi on lipid, free fatty acids, and fatty acid composition of stored peanuts. Grasas y Aceites 33(5): 271-272.

The percentages of lipids and free fatty acids did not change in non-inoculated groundnuts during storage for 5 days at 28°C, but increased in groundnuts inoculated with *Aspergillus* and *Rhizopus* spp. Fatty acids did not fluctuate significantly over the storage period, except that percentages of palmitic and stearic acids tended to decrease, while percentages of oleic and linoleic acids increased. Arachidic, eicosenoic, behenic and lignoceric acids levels remained constant.

504. Abdel-Rahman, A.H.Y. 1983. Effect of fungi on thiamin, riboflavin and niacin of stored peanuts at room temperature. Rivista Italiana delle Sostanze Grasse 60(11):703-704.

The contents of thiamin, riboflavin and niacin decreased when stored groundnuts were infected by *Aspergillus* and *Rhizopus* spp.

505. Agboola, S.D., and Opudokun, J.S. 1982. A review of groundnut quality and storage in Nigeria. Pages 397-414 in Proceedings of the International Symposium in Africa on Production, World Oilseeds Market and Intra-African Trade in Groundnuts and Products. 7-11 June 1982, Banjul, The Gambia : African Groundnut Council, Lagos, Nigeria.

Research in Nigeria from 1948 to 1982 has identified the factors that determine quality in Nigerian groundnuts. These include, physical state of groundnuts, their moisture content, and storage conditions with emphasis on temperature, humidity, and infestations by pests, rodents and fungi. Procedures for ensuring purchase of only good quality produce and measures for prevention of damage to the groundnuts during storage are reviewed with particular emphasis on pest control. The problem of aflatoxin contamination of groundnuts is discussed. Recommendations to growers, and those concerned with storage and transport of groundnuts on methods of preventing aflatoxin contamination are summarized.

506. Ahmed, N.E., Younis, Y.M.E., and Malik, K.M. 1989. *Aspergillus flavus* colonization and aflatoxin contamination of groundnut in Sudan. Pages 255-261 in Aflatoxin Contamination of groundnut : proceedings of the International Workshop, 6-9 Oct 1987. ICRISAT Center, India. Patancheru. A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

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 were free from *A. flavus* infection and aflatoxin contamination. *Aspergillus flavus* infected a low percentage (2.7-7 %) of groundnuts left in the soil for 6 weeks after harvest. However, no aflatoxin contamination was detected. Will diseases and insect damage, mainly by while grubs and termites, predisposed seeds to prehavest *A. flavus* infection (56.4-69.8 %) and aflatoxin contamination (18-21 µg kg^{-1}). Groundnuts stored for 3 months in a well-ventilated room with an average temperature of 15°C were infected by *A. flavus* at a low level, but had no aflatoxin contamination. Infection increased with time in storage. Groundnuts harvested 1 week before maturity were not affected by *A. flavus* infection of seeds in storage, and there were no differences among genotypes. Groundnuts stacked in sacks at shelling sites were sampled; 4 % were contaminated with aflatoxin. the average level being 11 µg kg^{-1}. Fifteen % of the samples from oil mill sites were contaminated, the average aflatoxin content being 20 *µg* 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 contaminated samples, with an average aflatoxin content of 10 µg kg^{-1}. This produce is usually allocated for local processing.

507. Aibara, K., Ichinoe, M., Mat-da, K., Itoh, Y., and Nakano, N. 1985. Storage conditions of imported raw shelled peanuts and production of aflatoxins. Journal of the Food Hygienic Society of Japan 26(3): 234-242.

Two bulk samples of imported raw shelled groundnuts naturally contaminated with aflatoxigenic *Aspergillus flavus* were kept under three different conditions in commercial warehouses. During the storage period environmental temperature, relative humidity (RH), water activity, and free fatty acids of the samples, aflatoxin levels and fungal flora were determined at monthly intervals. Samples stored from fall to winter showed no significant accumulation of aflatoxin even though *Aspergilli* including *A. flavus* and Furotium spp. were present. The level of free fatty acids remained unchanged for five months. During summer to fall, groundnut samples contained detectable levels of aflatoxins. However, the variation of aflatoxin levels was small under these conditions after six months of storage. Seasonal changes in RH caused a decrease in fungal species including *A. flavus*. Accumulation of free fatty acids was observed during storage at relatively high temperature. Laboratory studies of aflatoxin production on raw shelled groundnuts were also carried out Groundnuts were inoculated with an aflatoxigenic strain of *A. parasiticus* (ATCC 15517) and incubated under various conditions |0, 15, 30 or 60 days at temperature/RH levels of 15°C/65 %. 25°C/79 %, 25°C/100 %]. Aflatoxins were found after incubation at 100 % RH and 25°C for 2 months. However, growth of the aflatoxigenic fungi and the production of aflatoxins were not observed during storage for the same period at RH <79 % at 25°C.

508. Arnold, W.R., and Pettit, R.E. 1969. Interrelationship between peanut kernel moisture and storage gases with growth of *Aspergillus flavus* and aflatoxin production. Phytopathology 59: 111.

Sound mature groundnuts at kernel moisture levels of 48.9, 38.3. 36.9, 32.8, 25.4, 12.8, and 5.7 % were inoculated with an aflatoxin-producing *Aspergillus flavus* and incubated under compressed air. nitrogen, 2-aminobutane, and a mixture of 87 % CO_2 and 13 % N_2 at 25°C. Fungal growth and aflatoxin production were determined after 9 days. Growth occurred under compressed air at all moisture levels, but was not evident under mixed gas at 12.8 % moisture and below. Growth of *A. flavus* was completely inhibited by the nitrogen gas; consequently no aflatoxin was produced. *Fusarium* species grew under the nitrogen atmosphere, and these kernels developed an offensive odour at all moisture levels. Less growth occurred on kernels stored under 2- aminobutane or washed with a solution of 2-aminobutane; however, the kernels readily absorbed this compound and became dark and odoriferous. These results indicate that nitrogen gas and 2- aminobutane are undesirable for use in preventing fungal growth on groundnuts while they are held in storage. A mixed gas containing a desirable ratio of CO_2, N_2. and O_2 appears to be more desirable than natural air for use as a storage gas.

509. Austwick, P.K.C., and Ayerst, G. 1963. Toxic products in groundnuts: Groundnut microflora and toxicity. Chemistry and Industry 2: 55-61.

Various samples of groundnuts and groundnut meals imported into the UK from six countries (Brazil, Nigeria, Uganda, South Africa, the Gambia, and Zimbabwe) were

examined for microflora and toxicity. The microscopical examination of 29 meal samples showed hyphae in the cotyledon cells of 19, of which 16 were toxic. The proportions of sound, damaged and discolored kernels in the samples were also determined. The proportion of sound kernels was generally high in all samples, and even in the highly toxic sample V.3827, 78 % of the kernels were of this type. Microscopical and cultural examination of 61 sound kernels (undamaged kernels with white flesh cotyledons) showed only three (4.9 %) to be invaded by fungi. The toxicity of 11 samples of these nuts was tested in ducklings and no liver lesions were observed from eight. The remaining three also included damaged and dicolored kernels. Fungi were frequently isolated from kernels with yellow, orange, buff brown or black flesh (74.4 % infected). *Aspergillus flavus* was dominant in the mycoflora followed by *Phoma* spp. and *A. tamarii.* Nine of 19 isolates of *A.flavus* tested, 9 were toxin-producers.

510. Barnes, G.L., Nelson, G.L., Clary, B.L., Moseley, Y.C., and Manbeck, H.B. 1970. Effects of drying, storage gases and temperature on development of mycoflora and aflatoxin in stored high-moisture peanuts. Proceedings of the American Peanut Research and Education Association 2: 42-46.

This paper reports results of research at Oklahoma State University on control of mold development on high moisture groundnut pods by storage in anaerobic and fungitoxic gases at two temperatures during 1968 and 1969. Storage of pods in N_2 or CO_2 to prevent mold growth and aflatoxin production was tested. Nondricd (freshly harvested) and partially dried pods were inoculated with an aflatoxin-producing strain of *Aspergillus flavus* and stored in chambers at 3 and 24°C. During 1968 and 1969, undiluted N_2 and CO_2 were used as test anaerobic-storage gases. During 1969, a mixture of 5 % SO_2 and 95 % N_2 by volume was also tested. Compressed air was used as a check gas each year. The gases were fed through the test chambers for 32 days. Spaced samples were assayed for quality, moisture, mycoflora, and aflatoxins. All air-treated pods at 24°C soon became covered with mycelium of species of *Fusarium, Rhizopus,* and *Mucor,* but the moldy, partially dried pods soon became overgrown with *A.flavus.* In both years, mold development was greatly delayed by CO_2 or N_2. CO_2 prevented mold development for over 2 weeks at 24°C while pods held 36 days at 3°C in CO_2 had their original mold-free appearance and a near-normal odour. While 5 % SO_2 in N_2 prevented mold development and aflatoxin contamination, the treatment caused serious off flavors in both raw and roasted kernels from the treated pods. Oil extracted from SO_2-treated kernels was very dark and had an odour distinctly different from normal. Moldy pods had a fermentation odour. High levels of aflatoxins occurred in kernels from pods stored in air at ambient room temperature; levels increased with time. Higher levels of aflatoxins occurred in high moisture kernels than in those from partially dried pods. All CO_2, N_2 and SO_2 treatments produced aflatoxin-free kernels. Molds were Isolated from all visibly mold-free CO_2 and N_2 treated pods.

511. Barnes, G.L., Nelson, G.L., and Manbeck, H.B. 1970. Effects of drying, storage gases, and temperature on development of mycoflora and aflatoxins in stored high moisture peanuts. Phytopathology 60: 581.

Molds develop on high-moisture groundnuts when these are improperly dried or held in bulk. Storage of pods in N_2 or CO_2 to prevent mold growth and aflatoxin production was tested. Nondricd and partially dried pods were inoculated with an aflatoxin- producing strain *of Aspergillus flavus* and stored in chambers held at 3 and 24°C. Air, N_2 and CO_2 were metered through replicated chambers. Spaced samples were assayed for quality, moisture, mycoflora. and aflatoxins. All air-treated pods at 24°C soon became covered with mycelium of species of *Fusarium, Rhizopus,* and *Mucor,* but the moldy, partially dried pods soon became overgrown with *A. flavus.* Mold development was greatly delayed by CO_2 or N_2. CO_2 prevented mold development for over 2 weeks at 24°C while pods held 36 days at 3°C in CO_2 had their original mold-free appearance and a near-normal odour. Moldy pods had a fermentation odour. High levels of aflatoxins occurred in kernels from pods stored in air at 24°C; levels increased with time. Higher levels of aflatoxin occurred in nondried pods than in partially dried pods. CO_2 and N_2 treatments produced aflatoxin-free kernels. Molds were isolated from all pod samples regardless of treatment. CO_2 and N_2 were fungistatic.

512. Bauduret, P. 1989. Fungal contamination of peanuts sold on markets of Saint-Denis, Reunion Island. Microbiologic-Aliments- Nutrition 7(2): 187-190.

Fungal contamination of 10 samples of groundnuts sold in the 1988 warm rainy season in Reunion Island was examined. *Aspergillus niger* was the most frequent and abundant species in surface and internal mycoflora, followed by *A. glaucus* group. Predominant species on seeds were the *A. glaucus* group, *Syncephalastrum* sp., *A. niger* and *Rhizopus* sp. (15.8, 6.0, 5.6 and 3.6 % of seeds infected, respectively). *A. flavus* was isolated from all samples; 8 were aflatoxigenic (7 seemed to be highly toxigenic). All groundnut samples were negative for aflatoxins.

513. Baur, F.J. 1975. Effect of storage upon aflatoxin levels in peanut materials. Journal of the American Oil Chemists' Society 52(8):263-265.

Samples containing excess amounts of aflatoxin, normal and defatted groundnut meal, raw and roasted peanut butter, were kept exposed to air at 50 % relative humidity and 73°F for up to two years. The aflatoxin contents were not significantly affected by time or storage temperature.

514. Bhat, R.V. 1988. Mould deterioration of agricultural commodities during transit: probelems faced by developing countries. International Journal of Food Microbiology 7(3): 219- 225.

The problem of mold growth and allatoxin contamination in commodities transported over long distances from cultivation regions to consumption centres is discussed. If the contamination occurs during transit, often no insurance coverage for the risk is available. Because of different methods of sampling followed in the exporting and importing countries, it is often difficult to define the exact responsibility of the development of aflatoxin as having taken place during transit. The statistics of the export of allatoxin high-risk commodities like red pepper (chillies), cottonseed and groundnut extractions clearly, for the last decade, demonstrate the extent of loss suffered by the exporting countries because of aflatoxins. The problem of mold damage and mycotoxin contamination can be minimized by improving facilities for storage at port and transit points and on ships.

515. Borut, S.Y., and Joffe, A.Z. 1966. *Aspergillus flavus* Link and other fungi associated with stored groundnut kernels in Israel. Israel Journal of Botany 15: 112-120.

Fifty-one samples of stored groundnut kernels of the 1963 crop and 63 of the 1964, both from different qualities, varieties and regions in Israel, were tested for fungal infection, and for their moisture content and toxicity. Under storage conditions during the seasons relatively low moisture content of the kernels was maintained, always below 9 %. The 71 identified fungal species were similar to those found on groundnut kernels from other countries. *Aspergillus niger* was the most common species, which appeared in large numbers in 97.4 % of the samples. The relative levels of *Rhizoctonia* and *Rhizopus* increased when the samples were exposed to humidity prior to storage. The average number of fungal colonies per sample, the percentage of samples infected with *A. flavus* and the percentage of *A. flavus* in the total mycoflora rose as storage time increased and especially after shelling of pods. *A. flavus* was detected in 78.4 % of the 1963 samples and 63.5 % of the 1964 samples. The percentages of toxic samples for each year were 20 % and 12.7 % respectively. It is assumed that storage conditions in Israel during these years were not conducive to the production of aflatoxins.

516. Chandra, S., Narang, M., and Srivastava, R.K. 1981. Studies on seed mycoflora of oilseeds in India. Part 1. Qualitative and quantitative estimations. International Biodeterioration Bulletin 17(3):71-75.

Fungi isolated from 55 oilseed samples (20 mustard, 10 sunflower, 4 soybean, 4 groundnut, 6 linseed, 5 safflower, 4 sesame and 2 niger) included *Aspergillus fumigatus, A. flavus, A. niger, Fusarium* spp. and *Penicillium* spp.

517. Chen, T.R., Chiou, R.Y.Y., and Tseng, Y.K. 1988. Mycological investigation of raw peanut kernels sampled from the retail stores in Chia-Yun-Nan area. Food Science, China 13(1/2): 71-77.

In summer (June-Aug) and winter (Dec-Feb) 1985-1986, 25 and 51 samples of raw groundnut kernels were obtained from retail stores distributed in Chiayi, Yunlin and Tainan areas of Taiwan. Moisture content, germination, mold count and aflatoxin-producing molds were assayed. Average moisture contents (7.83-8.17 %) did not differ significantly between summer and winter samples. Mold counts of summer samples were much higher than those of winter samples (2.45 vs 1.31 x 10^4 cfu g^{-1}). Percentage samples with *Aspergillus flavus parasiticus* agar (AFPA) positive colonies in summer and winter samples were 24 and 7.8 %, respectively. The germination percentage of the former was lower than that of the latter. All AFPA colonies were confirmed as toxin producers. Mold counts were not linearly dependent upon moisture contents for either sample. No significant difference was noted between mold counts on AFPA and potato-glucose agar media.

518. Chiou, R.Y.Y., Kuvhler, P.E., and Beuchat, L.R. 1984. Hygroscopic characteristics of peanut components and their influence on growth and aflatoxin production by *Aspergillus parasiticus.* Journal of Food Protection 47(10): 719-724.

Sound inshell runner-type groundnuts, manually damaged inshell groundnuts, shells, sound kernels deskinned kernels and skins were stored in separate flasks under an atmospheric relative humidity of 100 % at 28" C. After 5 days, water was absorbed at levels of 1.2, 1.7, 3.9, 0.9, 1 and 9.5 g 100 g^{-1} dry material, respectively. Surface disinfested components were inoculated with spores of an aflatoxin-producing isolate of *Aspergillus parasiticus* (NRRL 2999) and incubated under the same conditions. The time required for visible growth of the fungus was 8, 6, 4, 12, 10 and 3 days, respectively. The time for appearance of the conidiophores was 14, 10, 6, 16, 13 and 6 days. After a 3-week incubation period, aflatoxin levels in groundnut components were 111.4, 159.1, 4.4, 58.7, 99 and 1.5 μg kg^{-1}, respectively.

519. Clarke, J.H., and Niles, E.V. 1979. Fungi and mycotoxins detected in samples from the infestation control service. 1976- 77, and recent laboratory experiments on mycotoxins at PICL. Pages 8-12 in Proceedings of a Third Meeting on Mycotoxins in Animal Disease (Pepin, G.A., Patterson, D.S.P., and Shreeve, B.J., eds.). Pinner, U.K., Ministry of Agriculture, Fisheries and Food.

The fungi most frequently isolated from samples of foods collected during 1976-77 were *Absidia, Aspergillus* (mostly *A. eandidus, A. flavus* and *A. glaucus), Mucor, Paecilomyces, Penicillium* and yeasts. Mycotoxins found in 1976 were ochratoxin in wheat, stcrigmatocystin and ochratoxin in barley, and allatoxin in groundnut kernels and pellets. In 1977, ochratoxin and zearalenone were found in wheat,

ochratoxin, citrinin and zearalenone in barley, ochratoxin in oats and aflatoxins in groundnut pellets and cottonseed cake. Experimental studies of *A. flavus* growth rate and aflatoxin production in sterilized wheat were made. Aflatoxin was found in all samples analyzed whether sterilized by fumigation, autoclaving or irradiation.

520. Dange, S.R.S., Patil, V.J., Ladani, M.G., and Manvar, D.K. 1985. Effect of relative humidity, storage period on fungal invasion and germination of groundnut seeds. Seeds and Farms 11(3):39-41.

Unshelled seeds of groundnuts (cvs. GAUG-1, J 11, and JL 24) stored at 62, 76, 85 or 93 % relative humidity (RM) suffered greater invasion by *Aspergillus niger, A. flavus* and particularly by *Rhizopus* sp. at higher RH. At 62 % R11 seed viability was not reduced. Storage at 85 % RH for 120 days resulted in complete loss of viability, while with 93 % RH for 90 days the highest germination percentage was 5 in cultivar J 11.

521. Diener, U.L. 1960. The mycoflora of peanuts in storage. Phytopathology 50. 220-223.

In quantitative determinations of mycoflora, farmer stock groundnuts from 26 farm-size bins of several types of construction differed in initial moisture, damage, type of bin, and length of time in storage. High mycofloral counts were associated more often with high initial moistures of groundnut kernels than with any other factor. The predominant fungi were several species of *Aspergillus glaucus* group, *A. tamarii,* and *Penicillium citrinum.* Of the *A, glaucus* group, *A. ruber, A. repens,* and *A. restrictus* occurred more frequently and in greater numbers than did *A. chevalieri* and A. *amstelodami.* Other species occurring frequently were *A. candidus, Cladosporium* sp., *Torula sacchari,* and *P. funiculosum.* Storage fungi, found in large populations on stored groundnuts, probably contribute to deterioration as demonstrated with other kinds of stored seed.

522. Doupnik, B.Jr. 1969a. Factors associated with aflatoxins in farmers' stock peanuts in Georgia in 1967. Phytopathology 59: 112-113.

Samples of farmers' stock groundnuts were collected from 6 locations at weekly intervals for 5 weeks during the harvesting season. Seventeen of the 228 samples collected contained aflatoxins. Aflatoxins were present in 2.5 % of the Segregation I (highest quality). 12.1 % of the Segregation II, and 25.7 % of the Segregation 111 samples, and averaged 22, 264. and 324 µg kg^{-1} total aflatoxin respectively. A significant relationship was thus found between the presence and concentration of aflatoxins and grading factors. Samples with high visible damage values were more likely to contain anatoxins than were samples with high invisible damage (rancidity, mold or decay) values. The weekly incidence of aflatoxins was related to the climatological conditions during the 14-day period prior to the date of collection. Mycoflora studies of 73 selected samples showed a relationship between the mean number of fungi per sample and grading factors; the mean number increased as quality decreased. These findings support previous observations that factors which influence groundnut quality also influence the incidence of aflatoxins in farmers' stock groundnuts.

523. Doupnik, BJr. 1969b. Aflatoxins in farmers' stock peanuts : Peanut quality, mycoflora and climatological conditions as influencing factors. Journal of the American Oil Chemists' Society 46: 121 A.

Samples of farmers' stock groundnuts were collected from six locations at weekly intervals for five weeks during the 1967 and 1968 harvesting seasons. Seventeen of 228 samples collected in 1967 and 23 of 356 samples collected in 1968 contained aflatoxins. In 1967, 2.5 % of Segregation 1, 12.1 % of Segregation II, and 25.7 % of Segregation III samples contained aflatoxins and averaged 22, 264, and 324 µg kg^{-1} total aflatoxins respectively In 1968. 3.2 % of Segregation I, 6.3 % of Segregation II, and 11.9 % of Segregation III samples contained aflatoxins and averaged 28, 61, and 583 µg kg^{-1} total aflatoxins respectively. Thus, in both years, significant relationships were found between the presence and concentration of aflatoxins and grading factors. Weekly incidences of aflatoxin contaminated samples were related to climatological conditions during the 14-day period to the collection dates in both years. Mycofloral studies of 73 selected samples in 1967 and of all samples in 1968 showed a relationship between the mean number of fungi/sample, grading factors and aflatoxin contamination. These findings support previous observations that factors which influence groundnut quality also influence aflatoxin contamination in farmers' stock groundnuts.

524. El-Khadem, M. 1990. Aflatoxins in Egyptian peanut. An overview, Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz 97(3): 233-236.

A survey of groundnuts from Egypt showed 19.5 % of unshelled and 49.0 % of shelled samples to contain low levels of aflatoxins B_1, B_2, G_1 and G_2; only B_1 was detected in 3.5 % samples of roasted groundnuts. However, 60 % of *Aspergillus flavus* strains from Egypt produced one or more aflatoxin, and 40 % of toxigenic strains produced high quantities (5000-20000 µg kg^{-1}) of B aflatoxins; the levels found in groundnuts were therefore lower than expected. Studies on the effects of storage temperature and RH on groundnut mycoflora and aflatoxin development showed an RH of 95 % to be optimum for aflatoxin production; since the RH in Egypt is lower than this, this was considered a factor contributing to the low levels encountered. Inoculation of Egyptian groundnuts (cv. Giza 1) with different strains of *A. flavus* showed the shells to act as a barrier towards invasion; this was

considered the main reason for low aflatoxin production. Consequently, it is considered imperative to discard groundnuts with cracked shells before storage.

525. El-Khadem, M., Naguib, M.M., and Abdel Ghani, A.K. 1975. Anatoxins in foodstuffs in Eygpt. 1. Peanut mycoflora and toxicity. Acta Biologica lugoslavica 12(1): 29-36.

Aspergillus flavus, A. niger, Alternaria, Fusarium, Helminthosporium, Mucor, Penicillium, Rhizoctonia, Rhizopus and *Sclerotium* spp. were isolated from groundnut shells, whereas *A. flavus, A. niger, Fusarium, Macrophomina, Penicillium, Rhizoctonia* and *Sclerotium* spp. were the most frequent isolates from kernels. Over 50 % of *A. flavus* strains produced aflatoxins.

526. El-Maghraby, O.M.O., and El-Maraghy, S.S.M. 1987. Mycoflora and mycotoxins of peanut *(Arachis hypogaea* L.) seeds in Egypt. 1 - Sugar fungi and natural occurrence of mycotoxins. Mycopathologia 98: 165-170.

Sixty-four species and two varieties belonging to 19 genera of fungi were isolated from 40 groundnut seed samples collected from different places in Egypt by using a dilution plate method on glucose-Czapek's medium. The most frequent genera were *Aspergillus* (21 species and 2 varieties), *Penicillium* (16 species) and *Fusarium* (6 species). *A. flavus, A. fumigatus, A. niger, P. chrysogenum* and *F. oxysporum* were the most common fungal species. Forty-seven % of the samples were toxic to brine shrimp (*Anemia salina)* larvae. Groundnut samples were contaminated with aflatoxins (11 samples), T-2 toxin (7 samples), diacetoxyscirpenol (3 samples), zearalenone (one sample) and citrinin (one sample). The authors believe that this is the first report of the natural occurrence of zearalenone and trichothecenes in groundnuts.

527. Garren, K.H., and Porter, D.M. 1970. Quiescent endocarpic floral communities in cured mature peanuts from Virginia and Puerto Rico. Phytopathology 60: 1635-1638.

Comparisons were made of the quiescent mycofloral communities in mature, cured groundnut pods from the crops grown in Virginia, USA, with those grown in Puerto Rico. Few fungi were found in seed of undamaged pods from Virginia (VA) and Puerto Rico (PR). After hydration, fungi weTe found in 95 % of seed in discolored shells and in 40 % and 85 %, respectively, of VA and PR seed in unblemished shells. Ten species or genera of fungi were characteristic of one or more of the eight endocarpic communities. Shell communities were more complex. Among the characteristic forms were five toxigenic types and three groundnut pathogens. The PR and VA communities each had three exclusively characteristic forms. Only two forms seemed to invade seeds from shells during hydration. *Aspergillus flavus* was rare in PR samples, and in VA samples was not found before hydration, *A flavus* was a characteristic form of VA unblemished shells and seed. Though no other fungus was found in more than 20 % of VA seed, *A. flavus* was found in up to 30 % and 50 %, respectively, of seed from unblemished and discolord shells. Possibly it was suppressed in discolored VA shells by competition from *Fusarium* spp. and *Rhizopus stolonifer.*

528. Garren, K.H., Porter, D.M., and Wright, F.S. 1976. Effect of market type and speed of field curing on persistence of *Aspergillus flavus* in peanut seed in Virginia. Proceedings of the American Phytopathological Society 3: 253.

After six months in storage, approximately 15 % of windrowed-cured (rapid) and approximately 40 % of stack-cured (slow) seed of the Virginia type groundnut (cv. Florigiant) was infected with *Aspergillus flavus,* whereas with stack curing in the Spanish types Spancross and Starr only approximately 4 and 5 % of the seed, respectively, was infected. The more rapid curing and the tightly adhering pericarp of the small-seeded spanish-type fruit appears to inhibit invasion by the fungus.

529. Gill, L.S., Obi, J.U., and Husaini, S.W.H. 1983. Mycoflora of some Nigerian leguminous seeds. Legume Research 6(1): 29-33.

When seeds of 40 species of leguminosae were examined by the PDA and the blotter method, *Aspergillus clavatus, A. flavus, A. fumigatus, A. giganteus. A, niger* and *A. oryzae* were the most common fungi. *Rhizopus* spp. were found only on groundnut, pigeonpea, Lablab *niger (L. purpureus)* and cowpea.

530. Hasegawa, A., Tanaka, T., Aoki, N., Yamamoto, S., Toyazaki, N., Matsuda, V., and Udagawa, S. 1987. Isolation and identification of *Aspergillus flavus* from imported nuts and their aflatoxin producibility. Proceedings of the Japanese Association of Mycotoxicology No. 25: 21-27.

The occurrence of aflatoxin-producing strains *at Aspergillus flavus* was surveyed in retail samples of five kinds of edible nuts (mostly tree nuts) and compared with 10 samples of groundnuts. All samples had been imported from various countries in 1981 and 1983. *Aspergillus flavus* was present in 128 (46.5 *%)* of 275 tested kernels of the edible nuts, and 49.6 % of the groundnuts. A total of 91 isolates of *A. flavus* were selected and examined for their aflatoxin-producing ability; of 44 isolates of *A. flavus* from other edible nuts. 37 (84.1 *%)* were shown to produce aflatoxin on defatted groundnut meal medium, while 43 of 47 isolates (91.5 %) of *A. flavus* from groundnuts were positive. Most aflatoxigenic isolates from the edible nuts were identified as *A. flavus* subsp. *flavus var. flavus,* and four isolates of these, which were isolated from Hawaiian macadamia nut, produced higher levels of aflatoxins G_1 and G_2 than those of aflatoxins B_1 and B_2 Most isolates from

groundnuts imported from USA produced aflatoxins B and G and were identified as *A.flavus* subsp. *parasiticus* var. *parasiticus*. This survey revealed that imported edible nuts including almond, chickpea, hazelnut, macadamia nut and pistachio were significantly infected with aflatoxigenic *A.flavus*.

531. Jackson, C.R., and Press, A.F. 1967. Changes in rnycoflora of peanuts stored at two temperatures in air or in high concentrations of nitrogen or carbon dioxide. Oleagineux 23(3): 165-168.

Unshelled and shelled groundnuts with initial moisture contents of 7.5 and 6 %, respectively, were stored for 12 months in glass containers at temperatures of 4° and 27"C; the containers were weekly flushed with air, CO_2 or N_2. The composition of pod surface and kernel rnycoflora was not affected by any treatment, the number of propagules remained unchanged at 4° and decreased at 27°C. The data indicated that CO_2 and N_2 may depress aflatoxin production during storage though these atmospheres did not suppress the fungus *Aspergillus flavus,* and that storage of shelled groundnuts for 1 year may involve hazards of deterioration and aflatoxin increase.

532. Joffe, A.Z. 1968. Mycoflora of surface sterilized groundnut kernels. Plant Disease Reporter 52:608-611.

The mycoflora was determined for fresh and stored groundnut kernels removed from disinfested pods, and for kernels from similar pods but disinfested after their removal. Eighty-three fungal species were found on all these kernels, with 35 species (including *Aspergillus flavus)* from surface-sterilized kernels. Forty-six of the species did not appear to have been recorded previously on groundnut pods or kernels. Although the number of colonies yielded by most species was predictably larger on kernels without surface-sterilization, four of the more common species were more frequent after disinfestation: *Aspergillus versicolor, Fusarium solam,* and *Penicillium rubrum* on fresh kernels, and *A. niger* on stored kernels. The high incidence of *A. niger* in stored kernels that were disinfested. as compared with those that were not, is taken to indicate a selective removal of antogonists of this species by mercurial disinfestation.

533. Joffe, A.Z. 1969. The mycoflora of fresh and stored groundnut kernels in Israel. Mycopathologia et Mycologia Applicata 39: 255-264.

The mycoflora of groundnut kernels was studied over a 5 year period in Israel. A total of 132 samples of fresh, and 419 samples of stored kernels were examined. A list of 173 species isolated from these kernels is given, comprising 109 from fresh, and 153 from stored kernels. In three of the four years in which a comparative study of fresh and stored kernels was done, the number of fungal colonies from the former exceeded that from the latter by about 25 %. *Aspergillus flavus* usually occurred only in small quantities. *Aspergillus niger* was the most abundant fungus. On stored kernels it was even more predominant than on fresh kernels. *Penicillium funiculosum* and *P. rubrum* also occurred frequently.

534. Joffe, A.Z. 1970. The presence of aflatoxin in kernels from five years groundnut crops and *of Aspergillus flavus* isolates from kernels and soils. Plant and Soil 33: 91-96.

Aflatoxin was found in up to 6.4 % of fresh groundnut kernels and in up to 32 % of stored kernels, although the intensity of toxicity was always very low (up to 125 μg kg^{-1}). About 90 % of 1626 *Aspergillus flavus* isolates from groundnut kernels, rhizospheres, and geocarpospheres produced aflatoxin in culture. In quantitative tests with 750 isolates, 60% of the isolates produced > 25 μg g^{-1} aflatoxin.

535. Joffe, A.Z., and Lisker, N. 1969. The mycoflora of fresh and subsequently stored groundnut kernels on various soil types. Israel Journal of Botany 18(2): 77-89.

During the first six months of storage *Aspergillus niger* was the dominant fungus in samples of groundnut kernels from the 1965-1966 and 1966-1967 crops on all types of soil. *A. flavus* and *Fusarium* spp. were present in only small amounts , whereas prevalence of *Penicillium* spp. declined in early stages of storage. *Aspergillus niger* appeared to suppress other fungi in the kernel mycoflora. The numbers *of A. niger* colonies in the geocarposphere and within groundnut kernels were positively related, but for other fungi no relationship was observed.

536. Lalithakumari, D., Govindaswamy, G.V., and Vidhyasekaran, P. 1972. Isolation of seed-borne fungi from stored groundnut seeds and their role on seed spoilage. Madras Agricultural Journal 59(1): 1-6.

Twenty species of fungi were isolated from pods and seeds of stored groundnuts collected from differnt areas of Tamil Nadu. Germination was not seriously affected by most of the fungi except *Aspergillus niger* and *Rhizoctonia bataticola* which reduced germination by about 30 % after storage for 6 months. Root development was reduced by 93 to 95 % by *A.flavus* and *A. niger,* shoot development was most reduced (72 %) by *Rhizoctonia bataticola.* The most predominant fungus on the groundnut seeds, *Rhizopus nigricans,* was not pathogenic. Germination was considerably lower when seeds infected with *R. bataticola, A. flavus* and *Cladosporium herbarum* were stored at 95 % relative humidity than at 32.3 % relative humidity. TMTD was more effective than captan or Ceresan in maintaining seed viability. Groundnut seeds stored in jute bags for 3 or 6 months had lower germination values than those stored in wooden boxes, paper or polythene bags.

537. Landers, K.E., Davis, N.D., and Diener, U.L. 1967. Influence of atmospheric gases on aflatoxin production by *Aspergillus flavus* in peanuts. Phytopathology 57: 1086-1090.

Sound, mature kernels of the groundnut cultivar Early Runner were inoculated with *Aspergillus flavus* and incubated under various concentrations of carbon dioxide (CO_2), nitrogen (NV), and oxygen (O_2) at high moisture levels for 2 weeks at 30° C and 6 weeks at 15 C. Observations were made on growth and sporulation of the fungus and assays were conducted on infected kernels to determine the concentrations of aflatoxins and tree fatty acids. No reduction in growth and sporulation of *A. flavus* occurred when the CO_2 concentration was increased from 0.03% (air) to 20%. Fungus growth and sporulation were reduced with each 20% increase in CO_2 from 20% to 80%>. No growth occurred in 100% CO_2. No apparent decrease in growth or sporulation occurred when O_2 was reduced from 20% cent to 50%, regardless of the CO_2 concentration. However, striking reductions occurred when O_2 was reduced from 5 to 1% with 0, 20, or 80% CO_2. Aflatoxin production decreased with increasing concentrations of CO_2 from 0.03 to 100%. In general, reducing the O_2 concentration decreased amounts of aflatoxin. Sizeable decreases in aflatoxin production occurred when O_2 was reduced from 5 to 1 % in combination with 0, 20, or 80%; CO_2. Aflatoxin production was lower in groundnuts stored at 15°C under 20% CO_2 for 6 weeks when O_2 was reduced from 20% to 5%. Aflatoxin was low in groundnuts stored at 15°C for 6 weeks under high concentrations of CO_2. Free fatty acid formation closely paralleled growth, sporulation, and aflatoxin production by the fungus. Striking decreases in percentage of free fatty acids occurred when O_2 was reduced from 5 to 1%.

538. Llewellyn, G.C., Johnson, R.H., and O'Rear, C.E. 1983. A model for evaluating aflatoxin occurrence in shelled peanuts. Pages 638-651 ill Biodeterioration 5. Papers presented at the 5th International Biodeterioration Symposium, Aberdeen, September 1981 (Oxley, T.A., and Barry, S., eds.) John Wiley & Sons, Chicester : UK.

Monthly aflatoxin levels in groundnut lots brought to market, for September 1968-June 1974, were compared to certain monthly environmental characteristics. Graphical and linear regression analyses were employed both separately and in tandem to determine whether a relationship exists between the environmental dam and the aflatoxin levels in postharvest groundnuts. Aflatoxin levels varied with the month of the year in a cyclic fashion, with peaks in May and November. The temperature and the rainfall level were also related to aflatoxin levels in shelled groundnuts.

539. Llewellyn, G.C., O'Rear, C.E., Moll, M.B., and Dashek, W.V. 1986. Aflatoxin contamination of peanuts grown in Virginia for the crop-years 1974-1982. Pages 254-261 in Proceedings of the International Biodeterioration Symposium (Barry, S.. and Houghton. D.R., eds.), August 1984, Washington DC, USA.

An eight year (1974-1982) investigation assessing the relationships between air temperature (AT), precipitation (P) and aflatoxin (AFT) contamination of stored groundnuts is reported. The mean percentages of nuts exhibiting various AFT levels for the 8 years (July-June, 1000 samples $year^{-1}$) were : 80 ± 21, 10 ± 7, 3 + 3, 4 + 6 and 3 + 6 for 0-4, 5-15. 16-25, 26-100 and 100 µg kg^{-1}, respectively. The mean percentage for pooled period USA/FDA regulations was 8 + with 1980/1981 a high (46 %) contamination year. Statistical correlations for either linear (Pearson's) or non-linear (Spearman's) relationships suggested that : (1) the % APTs 15 *µg* kg^{-1} was not related to either AT or P in either a linear or non-linear manner; (2) total AFT levels were related non-linearly to AT but exactly how was not evident; (3) the environmental variable most influencing AFT levels was AT the month that the sample was obtained; and (4) as AT increased, there was an associated decrease in total AFT levels and vice versa.

540. Llewellyn, G.C., O'Rear, C.E., Sherertz, P.C., Ananaba, G., McWright, C.G., and Dashek, W.V. 1988. Aflatoxin contamination of Virginia peanuts for the crop-years 1982-1986. International Biodeterioration 24(4&5): 399-407.

To establish those environmental conditions which promote the growth of aflatoxin-producing *Aspergillus* spp. on groundnuts, a 4-year (1982-1986) investigation was undertaken to examine possible relationships between air temperature (AT), precipitation (P) and aflatoxin contamination of stored groundnuts. The mean percentage of nuts possessing various aflatoxin levels for the years 1982-1986 (July-June) were 74.2- 88.0 for 0-4 µg kg^{-1}, 6.3-14.9 for 5-15 µg kg^{-1}, 2.4-5.9 for 16- 25 µg kg^{-1}, 2.3-6.4 for 26-100 µg kg^{-1}, and 0-4.7 for >100 µg kg^{-1}. The mean percentage for the years which exceeded USDA/FDA regulations were 7.1 (1982-83), 7.6 (1983-84), 11.6 (1984-85) and 17.0 (1985-86). Examination of the mean percentage >15 µg kg^{-1} for each month during these four years revealed that the following months fell within that range; September, November, December, January, Feburary and May (1982-83); July. October. April and June (1983-84); August and June (1984-85); and July, April and May (1985-86). Comparisons of pooled-aflatoxin levels, rainfall and temperature over four years suggested a 'better lit' between mean monthly P and mean % aflatoxin > 15 µg kg^{-1}, than between the latter and mean monthly air temperature. However, application of a predictor equation indicated a correlation between aflatoxin levels and monthly air temperature.

541. Moreau, C. 1976. [Variations of fungal pollution in peanuts and their cakes from harvest to consumption.]. Variations de la pollution fongique des arachides et de leur tourteaux de la recolte a la consommation. Revue de Mycologie 40: 97-115.

Mycological analysis of postharvest groundnut samples was carried out. Various soil-fungi were found to infect the pods ; most of them grew through the shell, but not all reached the kernels. The most common were *Aspergillus flavus* and *A. niger*. Where they are treated in oil mills, the originally heavily contaminated kernels arc processed through some heat treatments which progressively eliminate all fungal contamination. Nevertheless, recontamination occurs as soon as the pellets are cooled, and a large mold development occurs in the usual conditions of storage. The cakes used to feed cattle are often highly contaminated. Detoxification of cakes by ammoniation destroys the aflatoxin and also has a fungicidal action against many molds, especially *A. flavus.*

542. Moreau, C. 1977. (Fungal pollution in peanuts and their cakes.]. Contamination fongique des arachides et de leurs tourteaux. Revue francaise des Corps Gras 24(1): 27-34.

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543. Moubasher, A.H., Abdel-Hafez, S.I., El-Hissy, F.T., and Hassan, S.K.M. 1980. Effect of temperature and moisture content on Egyptian peanut seed-borne fungi. Mycopathologia 70(1):49-54.

In groundnut seeds adjusted to various levels of moisture content (8.5, 13.5, 17.5 and 21 % dry weight basis) and stored for 6 months at 5, 15, 28 and 45°C, the total count of fungi (recovered at 28°C) increased regularly and the germinability declined with the rise in moisture content and with lengthening of storage period. At 5, 15 and 28°C, *Aspergillus fumigatus* was the most dominant species followed by *A. flavus, A. niger, A. terreus, Penicillium* sp., *Pyrenochaeta* sp. and *Scopulariopsis brevicaulis.* The degree of dominance of each species depended on conditions of storage and length of storage.

544. Moubasher, A.H., El-Hissv, F.T., Abdel-Hafez, S.I., and Hassan, S.K.M. 1979. The mycoflora of peanuts in Egypt. Mycopathologia 68(1): 39-46.

Among the fungi isolated from 80 seed and 40 shell samples of groundnuts (from different places) were 16 *Aspergillus* spp., 14 *Penicillium* spp., 2 *Mucor, 2 Rhizopus* spp., 3 *Fusarium* spp., 2 *Gibberella* spp., 2 *Glioclad*ium spp., 2 *Drechslera* spp. and 16 other species. *Aspergillus niger, A. fumigatus, A. flavus, A. terreus, A, ochraceous* and *P. funiculosum* were generally the most common.

545. Nilsson, G. 1972. [Occurrence and distribution of aflatoxins and aflatoxin producing fungi in stock reserves of groundnut cakes.]. Forekomst och fordelning av aflatoxincr och aflatoxinbildande mogelsvamper i beredskapslager av jordnotskakor. Lantbrukshogskolans Meddelanden No. 175:1-14.

There was a 20-fold variation in the aflatoxin content of groundnut cake stored in sacks for about 3 years. The two batches with highest contents had ranges of 630 to 17630 and 1560 to 28440 μg kg^{-1}. Up to 60 % of fungal strains produced aflatoxin. Isolated *Aspergillus flavus* strains produced aflatoxin when grown on moist wheat at 24°C.

546. Nofal, M.A., and El-Said, S.I.A. 1985. Contamination of groundnuts *(Arachis hypogaea* L.) with aflatoxin producing fungi in different sites of south El-Tahir region, Eygpt. Bulletin of Faculty of Agriculture, Cairo University 36(2): 1373-1383.

547. Nwokolo, C, and Okonkwo, P. 1978. Aflatoxin load of common food in savanna and forest regions in Nigeria. Transactions of the Royal Society of Tropical Medicine and Hygiene 72(4): 329- 332.

Aflatoxin was present at a high level in most common foods stored poorly for long periods in Nigeria (viz. groundnuts, dried fish, groundnut oil, guinea corn (sorghum), palm oil. rice, maize, beans, yams, acha, cassava and garri). It may work synergistically with other carcinogens to produce the high incidence of primary liver cancer seen in men under 40 years of age.

548. Patil, S.P., and Shinde, P.A. 1985. Mycotoxin contamination and associated mycoflora of groundnut. Journal of Maharashtra Agricultural Universities 10(1): 99.

In a survey of groundnut samples from markets at 6 locations, 20 per cent of the samples contained aflatoxin. *Aspergillus, Penicillium* and *Rhizopus* spp. predominated among the fungi encountered.

549. Pollet, A., Declert, C, and Chauvier, C. 1987. [Condition and extent of aflatoxin contamination of local groundnut stocks in Cote dTvoire. 1. Preliminary data (1985-1986 season). |. Etat sanitaire et importance des contaminations par aflatoxines des stocks villageois d'arachide constitues en Cote dTvoire. 1. Donnees preliminaires (Campagne 1985-1986). Oleagineux 42(8-9): 327-336.

Groundnut storage problems were studied in the Cote d'Ivoire during 1985 and 1986, from three different aspects : insect pests, fungal infection, and aflatoxin contamination. Sampling was done in all parts of the groundnut-growing areas. Selected from a large population of traditional farmers (only manual practices), 164 local stocks were sampled three times throughout the storage season, in December 1985, and in Feburary and April 1986. Other samples were taken at the same time from, different town and village markets. Several parameters such as pest damage (penetrated and/or scarified pods, non-damaged pods), fungal infection, germination of seeds, and aflatoxin contamination levels were studied. Generally, the market samples were less contaminated than the samples from the local stocks. With a few exceptions the stocks studied were always contaminated with detectable aflatoxin levels. Nine % of all the local stocks had more than 250 µg kg^{-1} of total aflatoxins, but 65 % of the samples never reached the European Economic Community (EEC) norm of 10 μg kg^{-1} of aflatoxin (against 4 % and 82 %, respectively for related market samples. Highly significant correlations were found between aflatoxin contamination and different parameters such as scarified, penetrated and broken pods, time in storage and quantity of remaining groundnut stocks, prior to sampling. Corresponding regressions could explain more than 25 % of the whole variance (from a particular study of 75 local storage areas).

550. Pollet, A., Declert, C, Wiegandt, W., Harkema, J., and Lisdonk, E.de. 1989. Traditional groundnut storage and aflatoxin problems in Cote d'Ivoire : ecological approaches. Pages 263-268 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 Oct 1987, ICRISAT Center, India. Patancheru 502 324, A.P., India . International Crops Research Institute for the Semi-Arid Tropics.

Groundnut storage problems were studied in C6te d'Ivoire during two successive storage periods (1985/86 and 1986/87) 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 infected than samples 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 μg kg^{-1}, with 4.4 % above 1000 μg kg^{-1}. It was also found that 73 % of these samples were above the European Economic Community (EEC) safety level of 10 µg kg^{-1}. Significant correlations were found between aflatoxin contamination and different storage and meterological variables. These included physical characteristics and age of the pods, and the influence of the prevailing atmospheric conditions.

551. Quebral, F.C. 1976. Mold : deterioration in stored grains and grains product. Grains Journal 1(1):12-13, 53-54.

In the Philippines, where rice is harvested by hand, there is frequently a delay between harvesting and threshing, and grain with up to 30 % moisture content may be left in the field for lengthy periods prior to storage. This leads to the development of discoloration, bad odours, and ultimately to molds and mycotoxins. Reports are given on a survey recently concluded in which samples of paddy, groundnuts, soyabeans and copra were collected and examined for molds. The principal fungus recorded was *Aspergillus flavus.* The importance of mycotoxins and their effect on man and animals is discussed, with special reference to aflatoxin, and its occurrence as recorded during the survey.

552. Quitco, R., Bautistu, L., and Bautista, C., 1989. Aflatoxin contamination of groundnuts at the post-production level of operation in the Philippines. Pages 101-110 in Aflatoxin contamination of groundnut: proceedings of the International Workshop, 6-9 October 1987. ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

The results of surveys in the Philippines have shown that farm level aflatoxin significantly increased from harvest to farm storage during the main cropping season. At harvest, groundnuts contained, on average, 3.16 µg kg^{-1} aflatoxin. During windrowing. aflatoxin levels increased at the rate of 1.5 µg kg^{-1} day^{-1}. In farm storage aflatoxin contamination continued to increase at the rate of 1 µg kg^{-1} day^{-1}. Aflatoxin contamination was signifiandy higher during the main cropping season than the second cropping season. At the traders' level, groundnut samples taken from various middlemen contained 35 µg kg^{-1} aflatoxin. On the other hand, samples taken from the wholesalers' newly procured groundnuts contained 188 µg kg^{-1} aflatoxin. Groundnuts that had been in wholesalers' warehouses for more than 3 months contained 275 µg kg^{-1} aflatoxin. At the processors' level, raw groundnuts for confectionery use (roasted and fried) contained 7.73 ug aflatoxin kg^{-1}, grounduts intended for peanut butter contained 17.13 µg kg^{-1}, and rejected groundnuts had 120.6 µg kg^{-1}. Aflatoxin contamination could start during harvest. Aflatoxin content increased to a significantly high level during trade and processing. This continued increase was attributed to insufficient drying of groundnuts after harvest.

553. Saito, M., and Singh, R.B. 1976. Reports on study of mycotoxins in foods in relation to liver diseases in Malaysia and Thailand. Institute of Medical Science, University of Tokyo, Tokyo, Japan, 85 pp.

Part I of this report deals with a survey of mycotoxins contamination in food and its relation to hepatoma in Malaysia. The topics covered include : (a) Biostatistical aspect of liver cancer in Malaysia (b) A semi-quantitative study on frequency of

food intake in Sekinchan, (c) Results of mycological survey of Malaysian foods, and (d) Analysis of mycoloxins in foods in Malaysia. The incidence of liver cancer is higher in Malaysia than in Japan and is highest in the Chinese male population. In the three communities rice, oil, fat and vegetables are the main foods. The Malays frequently eat fresh fish, beans and bawang and use chili kering; the Chinese frequently eat bawang meats, fresh fish and beans; the Indians frequently use milk and its products and dry curry powder. *Aspergillus* spp. were the major fungal species found, especially in milled rice. *Aspergillus candidus* was most frequent in rice (52 % of samples) and *A. niger* moderately abundant. *A. flavus* was isolated from 13-21 % of rice samples and *A. fumigatus* from 3-21 %. Curry powder was highly contaminated with *A. flavus; A. niger, Penicillium citrinum* and mucoraceous fungi were also dominant. *Aspergillus flavus* was found in groundnuts, cereal powders, beans and dried fish and *A. niger* was widespread in some foodstuffs. No mycotoxins were detected in rice, moldy soybeans or tempeh. Aflatoxin was detected in groundnut samples. Extracts of rice, beans and tempeh samples showed varying toxicity to HeLa cells. All except one groundnut sample showed low toxicity. Of 30 strains of *A. flavus* isolated, 8 produced aflatoxin, and 3 of 4 strains of *A. versicolor* produced sterigmatocystin. All 91 strains of *A. candidus* examined produced terphenyllin but not xanthoascin. Part 11, on studies in Thailand, includes (i) Mycological survey on market foods of Thailand, (ii) Distribution of aflatoxin-producing fungi in agricultural soils of Southeast Asia, and (iii) A glimpse into Reyes syndrome in Khon Kaen, a north-eastern district of Thailand. The dominant fungi isolated from 36 samples of eight types of foods were : *A. candidus, A. flavus, A. niger, Eurotiwn* and *Penicillium* spp. from rice; *A. flavus, A. niger, Mucor* and *Rhizopus* spp. from groundnuts; *Fusarium* spp. from beans and maize; *A. niger* from pepper; *A. niger* and *Fusarium* spp. from cassava starch; *A. flavus* and *A.niger* from chilli, chilli powder, shrimp and shell fish. *A. flavus* was isolated from 15 of 50 soil samples from Malaysia and 29 of 106 from Thailand. Of these 44 strains, 16 produced aflatoxin.

554. Schroeder, H.W., and Boiler, R.A. 1971. Invasion of farmers' stock peanuts by strains of *Aspergillus flavus* in a controlled environment in the laboratory. Proceedings of the American Peanut Research and Education Association 3: 35-42.

Farmers' stock groundnuts with sound pods and with damaged pods were inoculated in two separate tests with spores of *Aspergillus flavus;* an aflatoxin-producing strain (P-70-51i) and a white- spored mutant strain (AF-2) capable of producing little or no aflatoxin. After one week incubation in a relative humidity of 90 % at 25° C, *A. flavus* was recovered from surface-sterilized shelled kernels as follows : (i) inoculated with P-70-51i, 10 % of kernels from sound pods and 62 % of kernels from damaged pods, (ii) inoculated with AF-2, 6 % of kernels from sound pods and 78 % of kernels from damaged pods. Tests of *A. flavus* isolates from (i) and (ii) indicated that nearly all strains were similar to corresponding parent cultures. Species of the *Aspergillus glaucus* group became prevalent after 2 weeks in storage. *Penicillium* spp. became a significant segment of the mycoflora after 4 weeks. Aflatoxins were detected in (i) after 2 weeks. Levels of the toxins remained low until 4 weeks, then reached 70 μg kg^{-1} in sound pods and over 7000 μg kg^{-1} in damaged pods. In (ii), aflatoxin B_1 was detected in groundnuts from damaged pods after 1 week at a level of 4 μg kg^{-1}. After 2 weeks, 21 μg kg^{-1} were detected in groundnuts from sound pods compared to a trace level from broken pods. The moisture content of the groundnuts varied from 13.0 to 14.9 % in 8 determinations made after 3 and 5 weeks, respectively.

555. Sim, T.S., Teo, T., and Sim, T.F. 1985. A note on the screening of dried shrimps, shrimp paste and raw groundnut kernels for aflatoxin-producing *Aspergillus flavus.* Journal of Applied Bacteriology 59(1);29-34.

All the 33 samples of dried shrimps, shrimp paste and raw groundnut kernels were contaminated with fungi. *Aspergillus* and *Penicillium* spp. were the predominant isolates from dried shrimps and raw groundnut kernels but no *Aspergillus* spp. were present in peanut butter or shrimp paste samples. No aflatoxins were detected in the food samples although some were visibly moldy and some had high mold counts. The occurrence of aflatoxin-producing strains of *A. flavus* in dried shrimps and raw groundnut kernels warrants further investigation of these foods and their products as potentially significant sources of aflatoxins.

556. Smith, J.S.Jr., and Cole, R.J. 1981. Relationship between soldiers and aflatoxin contamination during storage of farmers stock peanuts. Proceedings of the American Peanut Research and Education Society 13(1):46-52.

Soldiers (columns of moldy groundnuts) and samples of the groundnuts surrounding the soldiers were gathered from several warehouses in which farmers stock groundnuts were stored. The sound mature kernels and sound split kernels as well as loose shelled kernels from each soldier and sample were analyzed using the minicolumn method to determine aflatoxin contents. Two lots of groundnuts, one officially graded Segregation 1 and one officially graded Segregation 3, were used to create artificial soldiers in the warehouse by soaking samples before storage. After a 90-day storage period the samples were removed, shelled, and aflatoxin determinations were made. Results showed that moisture accumulations in farmers stock groundnuts from roof leaks and condensation drips create ideal conditions for *A. flavus* growth and aflatoxin production.

557. Subrahmanyam, P., and Rao, A.S. 1976. Fungi associated with concealed damage of groundnut. Transactions of the British Mycological Society 66(3): 551-552.

During a survey of groundnut lots in oil mills for aflatoxins interior yellowing of cotyledons, with a thin mat of fungal hyphae intermixed with black fruiting bodies in the intercotyledonary space, was observed when some seeds were split open, but only in the winter crop. Such seeds tasted rancid and germination was very low. The fungi associated with such damaged seeds were *Aspergillus niger* (60 % of total isolations), *A.flavus. Macrophomina phaseolina, Fusarium* spp., *Rhizopus* spp. and other *Aspergillus* spp.

558. Thapar, V.K., Paul, S., and Sharma, S.S. 1986. Evaluation of aflatoxins susceptibility to different moisture contents in groundnut at different stages of postharvest operations. Journal of Research - Punjab Agricultural University (India) 23 (2):267-271.

In Punjab, India, samples of groundnuts were collected from fanners' fields and from stores of grain markets to evaluate susceptibility of aflatoxins to different moisture contents in kernels, in relation to methods of postharvest handling of groundnuts. The moisture contents of kernels were kept under laboratory conditions at five levels ranging from 10 to 16% with corresponding relative humidities (RH) ranging from 82 to 95%. Groundnut samples from fanners' fields were more vigorous than those from stores of grain markets indicating poor storage conditions. There was a definite conelation between ambient RH, moisture content of kernels and aflatoxin contamination. Aflatoxins developed at high RH (>82%) and corresponding moisture contents of kernels (>10%). However, aflatoxins (B_1, B_2, G_1 and G_2) were present in varied proportions at different moisture contents of kernels.

559. Thasnakorn, P. 1976. Detection of aflatoxin in ground roast peanut. Siriraj Hospital Gazette 28(3): 375-382.

Aflatoxin B, (at 10-1120 µg kg^{-1}) was detected in 22 of 29 samples of groundnut from commercial sources. *Aspergillus Jlavus* was isolated from 11 of the aflatoxin-contaminated samples.

560. Troeger, J.M., Williams, E.J., and Holaday, C.K. 1969. Aflatoxin incidence in peanuts as affected by harvesting and curing procedures. Proceedings of the American Peanut Research and Education Association 1: 62-67.

Experiments with throe groundnut varieties, conducted in the USA, indicated that green or partially dried groundnuts, held with no air flow for 24 hours, were highly susceptible to aflatoxin contamination. Low air flow through the groundnuts did not suppress aflatoxin production. Groundnuts held under N_2 or CO_2 atmospheres developed less aflatoxin, but produced a highly offensive odour. Groundnuts with initial moisture contents below 30 % developed considerably more aflatoxin after having been rewetted and placed in high humidity chambers for five days than groundnuts with initial moisture content above 30 %. The variety Starr Spanish had considerably more aflatoxin than the varieties Early Runner and Florigiant. Immature grounduts were less susceptible to aflatoxin contamination. Prompt drying is the most effective practice for eliminating aflatoxin contamination of groudnuts.

561. Troeger, J.M., Williams, E.J., and Holaday, C.E. 1970. Aflatoxin incidence in peanuts as affected by harvesting and curing procedures. Oleagineux 25(4): 213-216.

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562. Tsai, A.H., and Yeh, C.C. 1985. Studies on aflatoxin contamination and screening for disease resistance in groundnuts. Journal of Agricultural Research of China 34(1): 79-86.

In a study of four Virginia and four Spanish groundnut varieties at 2 sites, preharvest pod damage, by insects or microorganisms, led to increased aflatoxin contamination during storage. Virginia varieties were more easily damaged than Spanish varieties at both sites. Of 350 varieties and lines screened for reaction to seed colonization by *Aspergillus Jlavus* in laboratory tests, 16 proved resistant. The commercial cultivars Tainan 9, Tainan 10, Tainung 4 (all Spanish types) and Penghu 2 (Virginia type) were highly susceptible.

563. Udagawa, S. 1976. Distribution of mycotoxin-producing fungi in foods and soil from New Guinea and Southeast Asia. Proceedings of the Japanese Association of Mycotoxicology No. 2: 10-15.

Aspergillus Jlavus, A. niger, Penicillium citrinum, P. cyclopium, P. Juniculosum, P. paraherquei, Fusarium and *Rhizopus* spp. were isolated from groundnut samples in Papua New Guinea and *A.Jlavus, A. terreus, A. niger, Fusarium, Nectria* and *Mucor* spp. were isolated from maize. The *A.flavus* isolates produced varying amounts of aflatoxins B_1, B_2, G_1 and G_2. The fungi isolated from milled rice from Malaysia

were *A. candidus, A. flavus, A. fumigatus, A. niger, A. versicolor, Chaetomium globosum, Eurotium, Fusarium, Mucorales* and *P. citrinum.* Other food samples yielded *A. clavatus, A. flavus, A. niger, A. versicolor, P. citrinum, P. meleagrinum, Fusarium* spp. and *Mucorales. A. flavus* was also isolated from soil samples from Malaysia and Thailand.

564. Valentine, A.A. 1990. Aflatoxin contamination in some feeds and feeding stuffs : Highlights of some nutritional, physiopathological and economic implications. Food Chemistry 37:145-153.

A number of feeds and feedingstuffs, along with the postharvest factors predisposing them to aflatoxin contamination are outlined. Based on field reports, the nutritional and physiopathological implications of aflatoxins as an environmental pollutant and feed contaminant in Nigeria are discussed. Viewed from the context of the positive response of the farming population to the call for local sourcing of a substantial proportion of industrial raw materials (backward integration) by various governments, the economic implications of postharvest deterioration of large tonnages of food crops (now being experienced nation-wide) are highlighted. Various ameliorative measures (both short and long term) are suggested to minimize both postharvest deterioration and the imminence of the scourge of aflatoxicosis, especially in the poultry industry.

565. Vidal Gaona, G., and Zenteno Zcvada, M. 1982. The mycoflora of groundnuts *(Arachis hypogaea* L.) destined for human consumption. Anales del Instituto de Biologia Universidad Nacional Autonoma de Mexico, Botanica 47-53: 229-238.

Groundnut samples from Mexico FD, and several localities in Jalisco, Oaxaca and Guerrero were tested using malt salt agar (6 % NaCl, MSA) and malt agar (MA) media for *Aspergillus flavus.* MSA was more favorable for the development of the mycoflora than *MA.* Groundnuts from Mexico FD showed both high and low numbers of fungi and those retaining the testa were more susceptible to fungal colonization. Since *Aspergillus glaucus* was present in all the tests and *A. flavus* in few samples and in low quantity, the RH equilibrium of the fruits was deduced to be less than 75 %. Although the proportion of *A. flavus* involved in the colonization was small, the use of tests to detect mycotoxin levels is recommended.

566. Welty, R.E., and Cooper, W.E. 1969. Prevalence and development of storage fungi in peanut *(Arachis hypogaea)* seed. Mycopathologia Mycologia et Appiicata 35: 290-296.

Comparisons were made on the storage fungi present on groundnut seed samples stored for 6 months at 22-28°C and at different moisture contents. At 4.5 % *Aspergillus* species remained constant but *Penicillium* spp. decreased; at 8.9 *% A. flavus,* A *repens,* and *P.* spp. decreased but *A. amstelodami* and *A. ruber* increased; and at 11.5 % all seed yielded *A. ruber* after 4 months but other fungi decreased to 0-2 %. In a sample in which the moisture content increased slowly from 4 to 28 % the dominant fungi changed monthly; optimum moisture contents were : for/ A. *ruber* 12-15 *%, A. repens* 18.5 *%, A. flavus* and *P.* spp. 20 %. and *A. amstelodami* 20-28 %. At > 18 % *Fusarium* spp. increased very rapidly.

567. Wilson, D.M., and Jay, E. 1976. Effect of controlled atmosphere storage on aflatoxin production in high moisture peanuts (groundnuts). Journal of Stored Products Research 12(2): 97-100.

Shelled groundnuts remoistened to 16.7 % were stored for four weeks at about 27°C in air (0.03 % CO_2, 21 % O_2, *78 % N_2) and in three modified atmospheres : (1) 13.6 % CO_2. 0.3 % CO, 0.6 % O_2, 84.7 % N_2; (2) 12.2 % CO_2, 3.1 % CO, 0.3 % O_2, 83.5 % N_2; and (3) 13.6 % CO_2,* 0.1 % CO, 1.5 % O_2, 83.9 % N_2. Aflatoxins, % free fatty acids, and % seed infection by the *Aspergillus flavus* group were determined weekly. Groundnuts in air and in the atmosphere containing 1.5 % O_2 accumulated high levels of aflatoxin in 1 and 2 weeks, respectively. In the other two controlled atmospheres aflatoxin B_1 did not exceed 21 fig kg^{-1} and the % of free fatty acids increased only slightly. None of the treatments eliminated seed infection by the *A. flavus* group. After 4 weeks groundnuts in all treatments had visible fungal growth on seeds.

568. Wilson, D.M., Jay, E., Hale, O.M., and Huang, L. 1977. Controlled atmosphere storage of high-moisture corn and peanuts to control aflatoxin production. Ibid 54: 495-500.

Groundnuts with 16.7 % moisture stored for 4 weeks in air and a modified atmosphere with 1.5 % O_2 were contaminated with high levels of aflatoxins. Groundnuts in modified atmospheres with 0.6 % and 0.3 % O_2 had 21 μg kg^{-1} allatoxin B^1 or less. *Aspergillus flavus* survived in all treaunents. When containers of freshly harvested uninoculated U.S. No.2. IRN no. 4-02-931 maize with 18.8 % moisture were purged with 14.3 % CO_2, 0.5 % O_2 and 85.2 % N_2, scaled and stored for 36 and 109 days at 25°C, *A. flavus* and *Fusarium moniliforme* survived and anatoxin contamination was < 15 μg kg^{-1}. The 5-week product was compared with 18.8 % moisture maize treated with 0.8 % propionic acid and with dried maize in feeding experiments. The modified atmosphere maize and dried maize diets were preferred.

569. Wilson, D.M., Jay, E., and Hill, R.A. 1985. Micronora changes in peanuts (groundnuts) stored under modified atmospheres. Journal of Stored Products Research 21(1):47-52.

Pilot-scale experiments were designed to determine if long-term storage of groundnuts is practical in modified atmospheres with minimal deterioration through molding, aflatoxin contamination, and insect infestation, without use of refrigerator or pesticides. Two lots of U.S. No. 1 groundnuts (248 kg) were stored at 26 + 2°C for 1 year in 1978-1979 and in 1980-1981, in atmospheres either of 60 % CO_2 simulated burner gas, 99 % N_2 or in refrigerated and non-refrigerated ambient air. In 2 larger tests, the CO_2 atmosphere was used with 1996 and 6451 kg of groundnuts in different non-refrigerated bins. When the CO_2 was not recirculated in the 1996 kg bin the humidity and moisture increased and moisture migration occurred. The groundnuts at the top molded, predominantly with yeast species and *Penicillium roqueforti.* Species of the genus *Eurotium (Aspergillus glaucus* group), the *A. flavus* group, and various *Penicillium* spp. were also isolated. These mycofloral changes were different from those seen in ambient air or refrigerated storage when moisture increases occurred. No molding and only minor change in the microflora was observed in the bin with 6451 kg of groundnuts with CO_2 recirculation and humidity control. Only minor changes in the microflora occurred in the control treatments. No aflatoxins accumulated in any treatment.

570. Wilson, D.M., Mixon, A.C., and Troeger, J.M. 1977. Aflatoxin contamination of peanuts resistant to seed invasion by *Aspergillus flavus.* Phytopathology 67(7):922-924.

Harvested shelled and unshelled groundnuts of genotypes PI 337409 and PI 337394F, previously reported to be resistant to invasion and colonization by *Aspergillus* spp. of the *A. flavus* group, were held under conditions of high humidity and the aflatoxin content that developed was compared with that in similarly stored nuts of PI 343360, susceptible to *A. flavus,* and the commercial cultivar Florunner. Each line developed natural seed infection of 2-3 % by *Aspergillus* spp. All genotypes developed appreciable amounts of aflatoxin after 9-10 days' storage at 87-95 % RH and 23-26°C. Sound nuts of PI 3373941- also developed aflatoxin after 9 days of 80 + 2 % RH (the lowest RH) and 23°C. It is concluded that genotypes invaded or colonized with difficulty in the field may be readily infected in storage under conditions of high RH and temperature favorable to colonization by the fungus.

2.2.5 In Transit

571. Bhat, R.V. 1988. Mould deterioration of agricultural commodities during transit: probelems faced by developing countries. International Journal of Food Microbiology 7(3):219- 225.

The problem of mold growth and aflatoxin contamination in commodities transported over long distances from cultivation regions to consumption centres is discussed. If the contamination occurs during transit, often no insurance coverage for the risk is available. Because of different methods of sampling followed in the exporting and importing countries, it is often difficult to define the exact responsibility of the development of aflatoxin as having taken place during transit. The statistics of the export of aflatoxin high-risk commodities like red pepper (chillies), cottonseed and groundnut extractions clearly, for the last decade, demonstrate me extent of loss suffered by the exporting countries because of aflatoxins. The problem of mold damage and mycotoxin contamination can be minimized by improving facilities for storage at port and transit points and on ships.

2.2.6 Aflatoxin-Producing Potential of *Aspergillus flavus* Isolates from Groundnuts

572. Armbrecht, B.H., Hodges, F.A., Smith, H.R., and Nelson, A.A. 1963. Mycotoxins. I. Studies on aflatoxin derived from contaminated peanut meal and certain strains of *Aspergillus flavus.* Journal of the Association of Official Agricultural Chemists 46(5): 805-817.

Aspergillus flavus strains were obtained from British and domestic sources, and were cultured on natural substrates. Aflatoxin was extracted from groundnut meal and from wheat. The amounts of toxic substances present were estimated by paper chromatography. Concentrates were obtained by precipitation. One concentrate was reduced. Toxicity tests on ducklings confirmed British findings.

573. Austwick, P.K.C., and Ayerst, G. 1963. Toxic products in groundnuts: Groundnut microflora and toxicity. Chemistry and Industry 2: 55-61.

Various samples of groundnuts and groundnut meals imported into the UK from six countries (Brazil, Nigeria, Uganda, South Africa, the Gambia, and Zimbabwe) were examined for microflora and toxicity. The microscopical examination of 29 meal samples showed hyphae in the cotyledon cells of 19, of which 16 were toxic. The proportions of sound, damaged and discolored kernels in the samples were also determined. The proportion of sound kernels was generally high in all samples, and even in the highly toxic sample V.3827. 78 % of the kernels were of this type. Microscopical and cultural examination of 61 sound kernels (undamaged kernels with white flesh cotyledons) showed only three (4.9 %) to be invaded by fungi. The toxicity of 11 samples of these nuts was tested in ducklings and no liver lesions were observed from eight. The remaining three also included damaged and dicolored kernels. Fungi were frequently isolated from kernels with yellow, orange, buff brown or black flesh (74.4 % infected). *Aspergillus flavus* was dominant in the

mycoflora followed by *Phoma* spp. and *A. tamarii*. Nine of 19 isolates of *A.flavus* tested, 9 were toxin-producers.

574. Bauduret, P. 1989. Fungal contamination of peanuts sold on markets of Saint-Denis, Reunion Island. Microbiologie-Aliments- Nutrition 7(2):187-190.

Fungal contamination of 10 samples of groundnuts sold in the 1988 warm rainy season in Reunion Island was examined. *Aspergillus niger* was the most frequent and abundant species in surface and internal mycoflora, followed by *A. glaucus* group. Predominant species on seeds were the *A. glaums* group, *Syncephalastrum* sp., *A. niger* and *Rhizopus* sp. (15.8, 6.0, 5.6 and 3.6 % of seeds infected, respectively). *A. flavus* was isolated from all samples; 8 were aflatoxigenic (7 seemed to be highly toxigenic). All groundnut samples were negative for aflatoxins.

575. Blaha, J., and Lohnisky, J. 1990. Aflatoxin production by *Aspergillus flavus* isolated from Vietnamese feeds. Tropical Science 30: 33-40.

Two experiments were conducted for the determination of aflatoxin production by strains of *Aspergillus flavus* isolated from Vietnamese yellow maize and groundnut oil cakes. Strains were inoculated onto sterilized (irradiated) maize, wheat and feed mixture (Experiment 1). The *A.flavus* strain isolated from groundnut oil cakes was inoculated onto unirradiated maize and feed mixture (Experiment 2). The moistened and dry samples of inoculated feeds were stored for 50 days at 20°C and 30°C. The feed samples were analyzed for aflatoxin B_1 before the experiments and then after 10, 20, 30, 40 and 50 days. The feed samples were microbiologically examined before the experiments and then after 30 and 50 days. *A. flavus* grew more intensively in the moistened samples (about 20 % moisture) and produced much more aflatoxin B_1 than in dry samples. The strain of *A. flavus* isolated from groundnut oil cakes produced more aflatoxin B,. Most feed samples attained peak production of toxin between 20 and 40 days in storage, depending on moisture and temperature (max 520.6 mg kg^{-1}). The growth and aflatoxin production ability *of A. flavus* was lower in the mixture of fungi obtained from unirradiated feeds.

576. Borut, S.Y., and Joffe, A.Z. 1965. *Aspergillus flavus* Link aflatoxins and toxicity of groundnuts in Israel. Israel Journal of Botany 14: 198.

Research was done in Israel on the occurrence of aflatoxin-producing fungi in soils and in groundnut kernels. *Aspergillus flavus* was prevalent in low levels in soils of the groundnut fields in Israel. *Aspergillus flavus* infection of groundnut kernels increased with storage time. 80 % of 55 stored groundnut samples tested from 1963 crop and 64 % of 63 stored samples from 1964 crop were infected with the fungus while only 20 % of the 1963 crop and 12.7 % of the 1964 crop were contaminated with aflatoxin. 71.2 % of 330 *A. flavus* isolates tested were toxigenic. The percentage of aflatoxigenic strains among soil isolates was higher than that of the kernel isolates.

577. Chen, T.R., Chiou, R.Y.Y., and Tseng, Y.K. 1988. Mycological investigation of raw peanut kernels sampled from the retail stores in Chia-Yun-Nan area. Food Science, China 13(1/2): 71-77.

In summer (June-Aug) and winter (Dec-Feb) 1985-1986, 25 and 51 samples of raw groundnut kernels were obtained from retail stores distributed in Chiayi, Yunlin and Tainan areas of Taiwan. Moisture content, germination, mold count and aflatoxin-producing molds were assayed. Average moisture contents (7.83-8.17 %) did not differ significantly between summer and winter samples. Mold counts of summer samples were much higher than those of winter samples (2.45 vs 1.31 x 10^4 cfu g^{-1}). Percentage of samples with *Aspergillus flavus* parasiticus agar (AFPA) positive colonies in summer and winter samples were 24 and 7.8 %, respectively. The germination percentage of the former was lower than that of the latter. All AFPA colonies were confirmed as toxin producers. Mold counts were not linearly dependent upon moisture contents for either sample. No significant difference was noted between mold counts on AFPA and potato-glucose agar media.

578. Codner, R.C., Sargeant, K., and Yeo, R. 1963. Production of aflatoxin by the culture of strains of *Aspergillus flavus-oryzae* on sterilized peanuts. Biotechnology and Bioengineering 5(3): 185-192.

By growing *Aspergillus parasiticus* (C.M.I. 15957) under controlled conditions on sterilized groundnuts, an average of 265 mg kg^{-1} aflatoxin was produced. Other strains from the *Aspergillus flavus-oryzae* group tested gave lower yields of aflatoxin and one such strain produced aflatoxin from which certain normal components were absent. The aflatoxin produced on sterilized groundnuts by any particular strain of *A. flavus-oryzae* was shown by thin-layer chromatography to contain the same major components as were produced by that strain on unsterilized whole groundnuts.

579. Diener, U.L., and Davis, N.D. 1965. Toxin-producing ability of *Aspergillus flavus* strains grown on peanuts and on artificial medium. Phytopathology 55: 497.

Strains of *Aspergillus flavus* obtained from groundnuts, corn, and other seed and food materials or from other research workers were tested for aflatoxin-producing potential on groundnut substrate and on an artificial medium. Strains varied widely in the amount of aflatoxins produced; the proportion of aflatoxins B_1 and G_1 varied with the strain.

580. Diener, U.L., and Davis, N.D. 1966. Aflatoxin production by isolates of *Aspergillus flavus*. Phytopathology 56: 1390-1393.

Species of the *Aspergillus flavus* group were isolated from groundnuts, corn, feed, and other sources. Also, isolates of *A. flavus* from groundnuts, cereals, soybeans, and other crops were obtained from other investigators. Isolates were screened for aflatoxin production on groundnuts and in a nutrient solution. About 80 % of the *A. flavus* isolates produced aflatoxin to some degree. Ninety % of the isolates produced primarily aflatoxin B_1, whereas about 10 % produced both aflatoxins B_1 and G_1. Optimal temperature for aflatoxin B_1 production by *A. flavus* on groundnuts and in nutrient solution was 25°C for an incubation period of 7 to 9 days. Optimal temperature range for aflatoxin production by *A. parasiticus* on both media was 25 to 30°C. Aflatoxin levels were high throughout the 7- and 21-day incubation periods. The proportion of aflatoxin B_1 to aflatoxin G_1 varied with the temperature.

581. Diener, U.L., and Davis, N.D. 1969. Aflatoxin formation by *Aspergillus flavus.* Pages 13-54 in. Aflatoxin : Scientific Background, Control, and Implications (Goldblatt, L.A., ed.). Academic Press, New York : USA.

This paper comprehensively reviews aflatoxin production by *Aspergillus flavus* isolates from various agricultural commodities, and the factors affecting aflatoxin production on natural substrates in nature, in vitro, and in synthetic and semi-synthetic media by the fungus.

582. Diener, D.L., and Davis, N.D. 1977. Aflatoxin formation in peanuts by *Aspergillus flavus.* Bulletin of the Alabama Agricultural Experimental Station (No. 493), 49 pp. Auburn, Alabama, USA : Auburn University.

This review discusses factors influencing production of aflatoxin in groundnuts including: the aflatoxin-producing fungus *Aspergillus flavus,* the substrate, relative humidity, moisture, temperature, time, aeration, the pod, and damage to the kernel.

583. Diener, U.L., Davis, N.D., Salmon, W.D., and Prickett, C.O. 1963. Toxin producing Aspergillus isolated from domestic peanuts. Science 142: 1491-1492.

Nine species of fungi isolated from stored domestic groundnuts were grown on sterilized groundnuts and were incorporated into diets fed to ducklings. Symptoms of acute toxicity resulted only alter consumption of one of the diets and this one contained material incubated with the fungus, *Aspergillus flavus.*

584. Doupnik, B.,Jr. 1969. Aflatoxin production by isolates within the Aspergillus flavus group obtained from farmers stock peanuts. Phytopathology 59: 1024.

Isolates within the *Aspergillus flavus* group obtained from Spanish, Runner, and Virginia-type groundnuts, were screened for aflatoxin production in a culture medium. Of 244 isolates, 161 (66 %) produced aflatoxins; 30 isolates produced only aflatoxin B_1, 50 produced aflatoxins B, and B_2, 25 produced aflatoxins B_1 and G_1, 7 produced aflatoxins B_1, B_2, and G_1, 36 produced aflatoxins B_1, G_1 and G_2, 13 produced all four aflatoxins. Quantitatively, 16 yielded less than 1 $\mu g\ g^{-1}$ total aflatoxins, 16 yielded from 1-10 $\mu g\ g^{-1}$, 48 yielded from 10-100 $\mu g\ g^{-1}$, 77 yielded from 100-500 $\mu g\ g^{-1}$, and 4 yielded over 500 $\mu g\ g^{-1}$. Eighty-five % of the lower yielding isolates (less than 100 $\mu g\ g^{-1}$) produced only aflatoxins Bs, whereas 85 % of the higher yielding isolates produced both aflatoxins Bs and Gs. In general, the isolates produced 30-40 times more B_1 than B_2 or G_2, and 2 limes more B_1 than G_1. Aflatoxin production was not related to the source of an isolate or to its ability to sporulate on the test medium.

585. El-Khadem, M. 1990. Aflatoxins in Egyptian peanut. An overview. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz 97(3): 233-236.

A survey of groundnuts from Egypt showed 19.5 % of unshelled and 49.0 % of shelled samples to contain low levels of aflatoxins B_1, B_2, G_1 and G_2; only B_1 was detected in 3.5 % samples of roasted groundnuts. However, 60 % of *Aspergillus flavus* strains from Egypt produced one or more aflatoxin, and 40 % of toxigenic strains produced high quantities (5000-20000 $\mu g\ kg^{-1}$) of B aflatoxins; the levels found in groundnuts were therefore lower than expected. Studies on the effects of storage temperature and RH on groundnut mycoflora and aflatoxin development showed an RH of 95 % to be optimum for aflatoxin production; since the RH in Egypt is lower than this, this was considered a factor contributing to the low levels encountered. Inoculation of Egyptian groundnuts (cv. Giza 1) with different strains of *A. flavus* showed the shells to act as a barrier towards invasion; this was considered the main reason for low aflatoxin production. Consequently, it is considered imperative to discard groundnuts with cracked shells before storage.

586. El-Khadem, M., Naguib, M.M., and Abdel Ghani, A.K. 1975. Aflatoxins in foodstuffs in Eygpt. 1. Peanut mycoflora and toxicity. Acta Biologica lugoslavica 12(1): 29-36.

Aspergillus flavus, A. niger, Altcrtiaria, Fusarium, Helminthosporium, Mucor, Penicillium, Rhizoctonia, Rhizopus and *Selerotium* spp. were isolated from groundnut shells, whereas *A. flavus. A. niger, Fusarium, Macrophomina, Penicillium, Rhizoctonia* and *Sclerotium* spp. were the most frequent isolates from kernels. Over 50 % of *A. flavus* strains produced aflatoxins.

587. Fandialan, I.M., and Hag, L.L. 1973. Aflatoxin production of *Aspergillus flavus* isolates from rough rice, corn, soybean, peanut and copra. Philippine Agriculturist 57: 254-263.

Rice was the best substrate for aflatoxin production with from 0.036 to 15 $\mu g\ g^{-1}$ produced depending on the original source of the *Aspergillus flavus* culture. The

other substrates were maize (traces to 3 μg g^{-1}), groundnut (none to 0.75 μg g^{-1}), copra (none to 1.35 μg g^{-1}) and soybean (none to 0.75 μg g^{-1}).

588. Fonseca, H., Martinelli Filho, A., Nery, H.del., and Koncatto. E. 1974. [Aflatoxin-producing *Aspergillus* species in the Araraquarense region, Sao Paulo.]. Especies de *Aspergillus* produtoras de aflatoxina na regiao Araraquarense, SP. Anais da Escola Superior de Agriculture "Luiz de Queiroz" 31: 519-536.

Of a total of 107 cultures of *Aspergillus flavus. A, oryzae* var. *effusus, A. oryzae, A. parasiticus* and *A. ochraceus* from groundnuts 33 (31 %) (32 *A. flavus* and 1 *A. oryzae)* produced aflaioxin, four only in the culture medium and two only in the mycelium. Toxin production was much greater in the mycelium than in the culture medium.

589. Gangawane, L.V., and Reddy, B.R. 1988. Distribution and survival of toxigenic strains of *Aspergillus flavus* to fungicides in Marathwada. Toxicon 26(1): 21.

Production of aflatoxin by *Aspergillus flavus* isolates from groundnut pods, kernels and soils from the Marathwada area ranged from 131 to 1240 μg kg^{-1} okra seeds. Of 40 isolates tested, 2 isolates (AF-28 and AF-29) were most toxigenic. There was variation in the sensitivity of isolates to fungicides; AF-29 was resistant. Under selection pressure of carbendazim the population of the resistant strain was increased. At equal proportions (50:50) 100 % survival was noted from second passage isolates only. The same was the case with thiophanate methyl. Survival ability of the resistant strain among the population of geocarposphere fungi under fungicidal pressure was also maximum. The resistant strain, however, could not compete with *A. fumigatus* under carbendazim pressure from the 4th passage at equal proportions in me mixture. Soil type, cultivation pattern of groundnut and irrigation all related to the distribution of toxigenic strains. It is concluded that chemical management with fungicides in the field is possible, but at the same time prediction for development of resistance can be provided.

590. Hasegawa, A., Tanaka, T., Aoki, N., Yamamoto, S., Toya/aki, N., Matsuda, Y., and Udagawa, S. 1987. Isolation and identification of *Aspergillus flavus* from imported nuts and their aflatoxin producibility. Proceedings of the Japanese Association of Mycotoxicology No. 25: 21-27.

The occurrence of aflatoxin-producing strains of *Aspergillus flavus* was surveyed in retail samples of five kinds of edible nuts (mostly tree nuts) and compared with 10 samples of groundnuts. All samples were imported from various countries in 1981 and 1983. *Aspergillus flavus* was present in 128 (46.5 %) of 275 tested kernels of the edible nuts, and 49.6 % of the groundnuts. A total of 91 isolates of *A. flavus* were selected and examined for their aflatoxin-producing ability; of 44 isolates of *A. flavus* from other edible nuts, 37 (84.1 %) produced aflatoxin on defatted groundnut meal medium, while 43 of 47 isolates (91.5 %) of *A. flavus* from groundnuts were positive. Most aflatoxigenic isolates from the edible nuts were identified as *A. flavus* subsp. *flavus* var. *flavus,* and four isolates of these, which were isolated from Hawaiian macadamia nut, produced higher levels of aflatoxins G_1 and G_2 than those of aflatoxins B_1 and B_2. Most isolates from groundnuts imported from USA produced aflatoxins B and G and were identified as *A. flavus* subsp. *parasiticus* var. *parasiticus.* This survey revealed that imported edible nuts including almond, chickpea, hazelnut, macadamia nut and pistachio were significantly infected with aflatoxigenic *A. flavus.*

591. Hesscltine, C.W. 1976. Mycotoxin research in India. Mycopathologia 58(3): 157-163.

This paper briefly reviews mycotoxin research in India. Research on various aspects of the groundnut aflatoxin problem, and on oilier mycotoxins is highlighted. Aspects discussed include occurrence of aflatoxins in various agricultural commodities, toxicity of aflatoxins to humans, resistance to aflatoxin production in natural substrates, detoxification, and mycology of aflatoxin-producing strains of *Aspergillus flavus.*

592. Hesseltine, C.W., Shorwell, O.L., Ellis, J.J., and Stubblefield, R.D. 1966. Aflatoxin formation by *Aspergillus flavus.* Bacteriological Review 30: 795-805. The natural occurrence of aflatoxin in agricultural commodities and factors affecting aflatoxin production in nature are reviewed. Aflatoxin production by strains of *Aspergillus flavus-oryzae* is also discussed. Research on aflatoxins at the Northern Regional Research Laboratory (NRRL), USA, is presented.

593. Jesenska, Z. 1987. [*Aspergillus flavus* and its toxigenic variants in soil.]. *Aspergillus flavus* und dessen toxigene varianten in Boden. Mykosen 30(11): 548-552.

A total of 33 soil samples from five different establishments for processing imported groundnuts, cocoa, coconuts, coffeebeans and similar imported foodstuffs, were examined. The soil samples were placed directly on Sabouraud's glucose agar with 7.5 % of NaCl. Of 619 strains of *Aspergillus flavus,* 312 were positive on APA medium (for aflatoxin producing *Aspergillus* spp.) and 304 were negative. A total of eight strains were found to produce aflatoxin B,. The risk of contamination of foodstuffs from this source in Central Europe is very low.

594. Joffe, A.Z. 1969. Aflatoxin produced by 1,626 isolates of *Aspergillus flavus* from groundnut kernels and soil in Israel. Nature 221: 492.

A population of 1,626 isolates *of Aspergillus flavus* obtained from groundnut kernels and from soil of groundnut fields in various regions of Israel were quantitatively and qualitatively tested for their potential to produce allatoxin. About 90 % of the isolates produced aflatoxin. Most of the aflatoxigenic isolates produced anatoxins B_1 and B_2, but only 8.4 % produced aflatoxins B_1, B_2, G_1 and G_2. The quantitative tests, performed only on 750 isolates, showed that some isolates produced as much as 150 mg kg^{-1} aflatoxin B_1, while others produced 50 mg kg^{-1} only. However, more than 60 % of the isolates produced aflatoxin in excess of 25 mg kg^{-1}. This indicates the enormous potential range of aflatoxin production by the isolates.

595. Joffe, A.Z. 1970. The presence of aflatoxin in kernels from five years groundnut crops and *of Aspergillus flavus* isolates from kernels and soils. Plant and Soil 33: 91-96.

Aflatoxin was found in up to 6.4 % of fresh groundnut kernels and in up to 32 % of stored kernels, although the intensity of toxicity was always very low (up to 125 µg kg^{-1}). About 90 % of 1626 *Aspergillus flavus* isolates obtained from groundnut kernels, rhizospheres, and geocarpospheres produced aflatoxin in culture. In quantitative tests with 750 isolates, 60 % of the isolates produced > 25 $\mu g\ g^{-1}$ aflatoxin.

596. Kang, M.S. 1970. Pathogenesis of groundnut by *Aspergillus flavus.* Ph.D. Dissertation (Plant Pathology), Punjab Agricultural University, Ludhiana, India.

Pathogenesis of groundnut by *Aspergillus flavus* was investigated. Aflatoxigenic isolates of *A. flavus* were pathogenic to groundnut seedlings and produced the characteristic disease syndrome of aflaroot. Injury to the cotyledons was considered essential for infection by *A. flavus.* The nontoxigenic isolates of *A. flavus* tested were not pathogenic to groundnut. Biochemical changes in the diseased and healthy groundnut seedlings are described. The culture filtrates of toxigenic isolates of A. *flavus* had deleterious effects on germination of seeds of some crop plants and produced chlorosis in the cotyledonary leaves of seedlings of *Abelmoschus esculentus.* Of 21 isolates of *A .flavus* from groundnuts tested, 18 were aflatoxigenic. All the aflatoxigenic isolates produced only aflatoxin B_1.

597. Lafont, P., and Lafont, J. 1971. (Aflatoxin production *of* isolates of *Aspergillus flavus* Link of different origins.]. Production d'aflatoxine par des souches d'*Aspergillus flavus* Link de differentes origines. Mycopathologia et Mycologia Applicata 43(3-4): 323-328.

The aflatoxin production of 124 isolates *of Aspergillus flavus* was studied. Among these isolates two principal groups were characterized : one with a high toxigenesis, the other with low or no toxigenesis. The percentage of highly toxigenic isolates was higher among *A. flavus* from groundnuts than *A. flavus* from wheal, maize, and animal feedstuffs.

598. Nakazato, M., Saito, K., Kikuchi, V., Ibe, A., Fujinuma, k., Nishijima, M., Nishima, T., Morozumi, S., Wauke, T., and Hitokoto, H. 1985. Aflatoxin formation by *Aspergillus flavus* and *Aspergillus parasiticus.* Journal of the Food Hygienic Society of Japan 26(4): 380-384.

The formation of aflatoxicols by nine strains of *Aspergillus flavus* isolated from moldy maize naturally contaminated with both aflatoxins and aflatoxicol and by two strains of A. *parasiticus* was investigated. All of the aflatoxin-producing *A. flavus* and *A. parasiticus* strains produced both aflatoxins and aflatoxicols. The accumulation of aflatoxins and aflatoxicols in maize grits, polished rice and groundnuts infected with an aflatoxin-producing strain of *A. flavus* was investigated. In each substrate, the greatest amount of aflatoxin was detected after incubation for 6 to 10 days, followed by a rapid increase in aflatoxicols A and B.

599. Nilsson, G. 1972. [Occurrence and distribution of aflatoxins and aflatoxin producing fungi in stock reserves of groundnut cakes.]. Forekomst och fordelning av aflatoxiner och aflatoxinbildande mogelsvamper i beredskapslager av jordnotskakor. Lantbrukshogskolans Meddelanden No. 175: 1-14.

There was a 20-fold variation in the aflatoxin content of groundnut cake stored in sacks for about 3 years. The two batches with highest contents had ranges of 630 to 17630 and 1560 to 28440 µg kg^{-1}. Up to 60 % of fungal strains produced aflatoxin. *Aspergillus flavus* strains produced aflatoxin on moist wheat at 24°C.

600. Ogundero, WW. 1987. Temperature and aflatoxin production by *Aspergillus flavus* and *A. parasiticus* strains from Nigerian groundnuts. Journal of Basic Microbiology 27(9): 511-514.

The abilities of 4 isolates of *Aspergillus parasiticus* and 6 of *A. flavus* to produce aflatoxins on Nigerian groundnuts at incubation temperatures of 10-35°C were studied. While all *A. parasiticus* isolates produced aflatoxins B_1, B_2, G_1 and G_2, only aflatoxins B_1 and B_2 were produced by 2 isolates of *A. flavus.* The optimum temperature for aflatoxin production by the 2 species was 30°C; there was negligible production at 10°C.

601. Rao, K.S., Madhavan, T.V., and Tulpule, P.G. 1965. Incidence of toxigenic strains of *Aspergillus flavus* affecting groundnut crop in Coastal districts in India. Indian Journal of Medical Research 53:1196-1202.

Of 288 samples of groundnuts from six coastal districts of Andhra Pradesh State,

India, it was possible to isolate strains of *Aspergillus flavus* from 29. Of the 29 strains 6 produced aflatoxin B_1 when cultured, as estimated by thin-layer chromatography (TLC) and in tests with ducklings. Aflatoxin contamination was found in 36 samples. Toxic samples were more prevalent in some districts than others. Samples commonly included 6 to 14 % of pods with shell damage, but this damage was not related to toxicity.

602. Sarnaik, S., Godbole, S.H., and Pradnya Kanekar. 1988. Incidence of toxigenic *Aspergillus flavus* in marketed edible vegetable oils. Current Science, India 57(24): 1336-1337.

Twenty-six groundnut oil samples (12 unrefined, 7 refined and 7 from ration shops) were collected from the local market and assessed for *Aspergillus flavus* contamination. Three unrefined and three ration oil samples contained the fungus. Five of these six isolates produced aflatoxins in a nutrient medium. One isolate from unrefined groundnut oil was not toxigenic. When the ability of all isolates to produce aflatoxin in sterile groundnut oil was tested, the isolates did not produce any toxin during a 3-month period.

603. Schroeder, H.W., and Ashworth, L.J.Jr. 1966. Aflatoxins : Some factors affecting production and location of toxins in *Aspergillus flavus-oryzae*. Journal of Stored Products Research 1: 267-271.

The quantity and quality of aflatoxins produced by two *Aspergillus flavus-oryzae* isolates from rough rice and Spanish groundnut were compared with a known, highly toxigenic *A. flavus* strain. Total aflatoxin yields on sterile groundnut, rough rice, and shredded wheat after 18 days at 30° ranged from 4000 to 892,000 µg k g . In shake cultures growing on 4% suspensions of these substrates, yields ranged from 17000 to 426,000 μg kg^{-1}. Total aflatoxin yields and the proportion of the four primary aflatoxins reflected interactions between strains, substrates, and metliods of culture. In shake cultures, from 52 to 80% of the aflatoxins were located in the mycelium. Conidia collected from a shredded wheal substrate after 18 days were found to contain aflatoxins at concentrations ranging from 700 to 56,100 µg kg^{-1}.

604. Sreenivasamurthy, V., Jayaraman, A., and Parpia, H.A.B. 1965. Aflatoxin in Indian peanuts : Analysis and extraction. Pages 251-260 in Mycotoxins in Foodstuffs (Wogan, G.N., ed). The Massachusetts Institute of Technology : The M.I.T. Press.

Of the nearly 150 isolates of *Aspergillus flavus* from groundnuts tested, only four produced aflatoxin B_1. These isolates differed from the type strain of *A. flavus* M001 (obtained from the Tropical Products Institute, London) in some morphological characteristics. Of the several salt solutions tested. 1 % $CaCl_2$ was found most promising in extracting aflatoxin from the contaminated groundnut cake. In the preparation of the protein isolates, precipitation of the protein in $CaCl_2$ solution helped to remove nearly 80 % of the toxin from the protein. Certain apparently healthy groundnuts showed bright bluish-violet fluorescence under ultraviolet light. Extracts of such groundnuts on thin-layer chromatography (TLC) plates showed the fluorophor at the same Rf as that of aflatoxin B_1. A simple solvent system of benzene : cyclohexane : acetic acid (3:5:2, v/v/v) on a paper chromatogram was as good for resolutions of the aflatoxin complex as the alumina TLC with chloroform and methanol as a solvent.

605. Sripathomswat, N., and Thasnakorn, P. 1981. Survey of aflatoxin-producing fungi in certain fermented foods and beverages in Thailand. Mycopathologia 73(2): 83-88.

Aflatoxin-producing fungi were found in fermented foods and beverages: fermented rice (kaomak), soybean sauce (taotjo), peanut butler, soy sauce (shoyu), Thai red and white wine, and rice sugar wine. These foods were extracted directly and tested for aflatoxins by thin-layer chromatography (TLC) and high pressure liquid chromatography (HPLC). Four strains of aflatoxin- producing fungi were isolated from peanut butter, taotjo, and shoyu. Direct extracts of 10 % of the peanut butter samples tested and 5 % of the kaomak contained high levels of aflatoxins.

606. Strzelecki, EX., and Cader-Strzelecka, B. 1988. Aflatoxigcnicity of *Aspergillus flavus* strains in food products. Microbios Letters 39(153): 37-40.

A total of 79 *Aspergillus flavus* strains isolated from samples of swine, cattle and poultry feed, groundnut and soya meals, wheat, barley, rye, ground pea. dry cracover sausage, cowshed ceiling and laboratory air and 6 control strains were tested for aflatoxin production using thin-layer chromatography (TLC) and UV detection methods. Of 19 aflatoxin-positive strains, 13 were isolated from feedstuffs (19 % swine feeds, 10 % poultry feeds, 43 % groundnut meal and 29 % wheat). Aflatoxin production was very low at 0.08 µg g^{-1}, low at 0.12-0.32 µg g^{-1} and high at 18.7-21.8 µg g^{-1}. In strains from feedstuffs, aflatoxin production was very low in 54 %, low in 31 % and high in 15 % of strains.

607. Subrahmanyam, P., and Rao, A.S. 1974. Occurrence of aflatoxins and citrinin in groundnut *(Arachis hypogaea* L.) at harvest in relation to pod condition and kernel moisture content. Current Science 43(22): 707-710.

Groundnut pods were collected from fields on the day of harvest in November 1972, graded into undamaged and damaged pods and seed moisture contents determined. High levels of aflatoxin were found in seeds with low moisture (< 30 %), which occurred under rainfed conditions and low levels or no toxins in those with high moisture content (under irrigation). In all instances damaged seeds contained the toxins. Seed moisture content and pod damage appeared to be the major factors

contributing to fungal infection and toxin accumulation before harvest. The accumulation of a yellow pigment in some seeds, especially in damaged pods, was identified as citrinin. Only *Aspergillus flavus* isolates produced aflatoxins while isolates of *Penicillium citrinum, P. jenseni* and *A. terreus* produced citrinin.

608. Subrahmanyam, P., and Rao, A.S. 1974. A note on aflatoxin production by some Indian strains of *Aspergillus flavus.* Indian Journal of Microbiology 14(1):

Of 240 isolates of *Aspergillus flavus* isolated from groundnut samples, only 72 produced anatoxins. Sixteen isolates produced only aflatoxin B_1, 8 isolates produced aflatoxins B_1 and G_1 and others produced aflatoxins B_1, G_1, B_2, and G_2. Aflatoxin production was higher when groundnut, copra and castor were used as substrates, whereas it was progressively lower in sunflower, safflower, niger seed and gingelly, respectively.

609. Surekha, M., and Reddy, S.M. 1989. Influence of temperature and humidity on biodeterioration and aflatoxin production in groundnut fodder by *Aspergillus flavus.* Journal of Toxicology, Toxin Reviews ((1-2)):291-297.

The influence of temperature and relative humidity (RH) on biodeterioration of groundnut fodder and aflatoxin production by *Aspergillus flavus* was investigated. Although aflatoxin production was observed at all incubation temperatures tested, its production was maximum at 25°C. The amount of aflatoxin production then decreased significantly with increase in incubation temperature. When a marginal increase in total protein and ash content was recorded, cellulose, lignin, starch and total nitrogen content decreased significantly due to *A. flavus* infestation. This was maximum at 25°C and decreased both with an increase or decrease of incubation temperature. Groundnut fodder showed significant weight loss due to *A. flavus* infestation and this was maximum at 25°C. Relative humidity of more or equal to 90 % was conducive for aflatoxin production. *A. flavus* could produce aflatoxins (trace amounts) even at 30 % RH.

610. Taber, R.A., and Schroeder, H.W. 1967. Aflatoxin-producing potential of isolates of the *Aspergillus flavus-oryzae* group from peanuts (*Arachis hypogaea).* Applied Microbiology 15(1):140-144.

Seventy-eight samples of farmers' stock groundnuts, representing groundnuts grown in nine different geographical areas in Texas during 1964, were assayed for aflatoxin and examined for associated microflora. Only two samples contained more than 50 µg kg^{-1} aflatoxin. Infestation by members of the *Aspergillus flavus-oryzae* group varied from 35 to 100 % of the kernels per area and from 1 to 100 % of the kernels per sample. 213 isolates of *A. flavus* were examined for their abilities for aflatoxin production on groundnut and rice substrates. Aflatoxin production by individual isolates ranged from 0 to 349.1 µg kg^{-1} on a groundnut substrate. In general, the isolates produced 8 to 10 times more aflatoxin B_1 than aflatoxin B_2, and no isolate produced aflatoxins G_1 or G_2.

611. Varsavsky, E.. Sommer, S.E., and Cabrera, A.L.L.de 1973. [Aflatoxins : their detection in groundnuts and soil in Cordoba Province (Argentina)]. Aflatoxins : su deteccion en mani y suelos de la Provincia de Cordoba (Argentina). Revista de Agronomia y de Veterinaria 2(6):30.

Of 41 isolates from groundnut samples, 16 were *Aspergillus flavus,* 10 of which produced aflatoxins. Of 27 isolates from soil samples five were *A.flavus,* two of wluch produced aflatoxins.

612. Wildman, J.D., Stoloff, L., and Jacobs, R. 1967. Aflatoxin production by a potent *Aspergillus flavus* Link isolate. Biotechnology and Bioengineering 9: 429-437.

An aflatoxin-producing isolate of *Aspergillus flavus* was a consistent producer of aflatoxin on all substrates which supported the growth of *A.flavus.* In competition with six other selected molds, this isolate was dominant over all but a species of *Penicillium.* Aflatoxin production was directly related to *A. flavus* growth irrespective of substrate or competition.

3. BIOCHEMICAL CHANGES IN GROUNDNUTS AFTER COLONIZATION BY THE *ASPERGILLUS FLAWS* GROUP OF FUNGI

3.1 REVIEW

The importance of aflatoxin contamination of groundnuts when colonized by *Aspergillus flavus* group fungi is discussed in other sections. Here we focus upon the biochemical changes that occur when these fungi colonize groundnuts and groundnut products.

Much of the research on biochemical changes induced in groundnuts by either *A. flavus* or *A. parasiticus* has been done in the USA (Diener and Davis 1977, Pattee and Sessoms 1967, Cherry et al. 1972, Cherry et al. 1975, Cherry et al. 1978, Deshpande and Pancholy 1979). In infected groundnuts there is reduction of dry matter and oil content, increase in fatty acids, and deterioration of seed quality and nutritive value (Diener 1973).

Pattee and Sessoms (1967) showed a positive relationship between growth of *A. flavus* and rapid increase in free fatty acid (FFA) in groundnut kernels. The decrease in oil content was accompanied by a hydrolytic rancidity (high FFA) that made groundnuts unacceptable for utilization in edible products (Farag et al. 1980).

Studies conducted by Cherry and coworkers (Cherry et al. 1975, Cherry et al. 1978) have shown that infection and colonization of groundnut kernels by *A. flavus* and *A. parasiticus* cause degradation of the major storage proteins to small molecular weight components and quantitative depletion of the small protein components. Changes in enzyme activity and quantity of ether-soluble oil, and reduction in content of the basic amino acids (lysine, histidine, and arginine) were also reported (Cherry et al. 1978. Cherry et al. 1972, Cherry and Beuchat 1976). In artificial inoculation studies. Deshpande and Pancholy (1979) showed that *A.* flavus-resistant genotypes (PI 337394F and PI337409) underwent less biochemical change than A. flavus-susceptible cultivars. Other reports indicated that decreases in vitamins (thiamin, riboflavin and niacin) in groundnuts are associated with infection and colonization by *A. flavus* (Abdel-Rahman 1983). *A. flavus* infestation and aflatoxin contamination also affects protein quality of groundnut meal (Ostrowski-Meissner 1984).

The ability of the aflatoxigenic fungi to metabolize groundnut carbohydrates, proteins and oil and to reduce quality of kernels has been clearly demonstrated in laboratory experiments. But there is limited information on the effects of *A. flavus* and other fungi, individually or collectively, on quality of groundnut kernels when stored under suboptimal conditions for extended periods of time. In commercial storage, insects, fungi, humidity and temperature contribute to seed deterioration. Therefore, it may be difficult to determine the specific effects of *A. flavus* group fungi. Fortunately, storage practices recommended for control of *A. flavus* infection and colonization are generally effective in minimizing deterioration associated with Other factors.

It is obvious that seed viability is affected by physical damage of the embryo and cotyledons by the invading fungal mycelium and that biochemical changes of the stored carbohydrates, proteins and oil reduce the value of these nutrients to the embryo and young seedling. In some cases the mycelium of the fungus remains viable when the seed is sown and may contribute to either seed rot or seedling disease (aflaroot).

3.2 BIBLIOGRAPHY

3.2.1 BIOCHEMICAL CHANGES IN GROUNDNUTS AFTER COLONIZATION BY THE *ASPERGILLUS FLAWS* GROUP OF FUNGI

613. Abdel-Rahman, A.H.Y. 1982. Effect of fungi on lipid, free fatty acids, and fatty acid composition of stored peanuts. Grasas y Aceites 33(5):271-272.

The percentages of lipids and free fatty acids did not change in non-inoculated groundnuts during storage for 5 days at 28°C, but increased in groundnuts inoculated with *Aspergillus* and *Rhizopus* spp. Fatty acids did not fluctuate significantly over the storage period, except that percentages of palmitic and stearic acids tended to decrease, while percentages of oleic and linoleic acids increased. Arachidic, eicosenoic, behenic and lignoceric acids levels remained constant.

614. Abdel-Rahman, A.H.Y. 1983. Effect of fungi on thiamin, riboflavin and niacin of stored peanuts at room temperature. Rivista Italiana delle Sostanze Grasse 60(11): 703-704.

The contents of thiamin, riboflavin and niacin decreased when stored groundnuts were infected by *Aspergillus* and *Rhizopus* spp.

615. Beuchat, L.R., Young, C.T., and Cherry, J.P. 1975. Electrophoretic patterns and free amino acid compositions of peanut meal fermented with fungi. Canadian Institute of Food Science and Technology Journal 8(1): 40-45.

Groundnut meal was fermented with 9 different fungi at 28°C for periods of time ranging up to 98 h. Qualitative changes in groundnut protein resulting from fungal proteolysis were followed electrophoretically using polyacrylamide gel. During fermentation the large molecular weight globulins observed in gel patterns of non-fermented samples were hydrolyzed to smaller components. The extent of peptone formation varied with the fungal strain used for fermentation. Total amino acid composition and free amino acid content of the femients were determined. Only small changes in the total amino acid profile of groundnuts occurred upon fermentation; the percentage of amino acids as free and the proportions of specific amino acids within this fraction varied greatly among the ferments as well as between ferments and non-fermented controls.

616. Cherry, J.P. 1982. Seed protein degradation by storage fungi [Peanuts, *Aspergillus].* American Chemical Society Symposium Series 206: 93-107.

Quantitative protein assays showed that groundnut seeds inoculated with *Aspergillus parasiticus* or *A. uryzae* and incubated for time intervals ranging up to 18 days showed decreases in percentages of proteins during the early stages (from 0 to 4-6 days) of the incubation period. During longer incubation periods, fungus-infected seeds showed a progressive decrease in soluble protein that leveled off at the later stages of colonization by *A. parasiticus.*

617. Cherry, J.P. 1983. Protein degradation during seed deterioration. Phytopathology 73(2): 317-321.

In this review biochemical changes that occur in fungus-infected groundnut seeds are discussed with particular reference to degradation of proteins in groundnut seeds infected with *Aspergillus parasiticus.*

618. Cherry, J.P., and Beuchat, L.R. 1976. Distribution of oil or other diethyl ether extractable material in various fractions of peanuts infected with selected fungi. Journal of me American Oil Chemists' Society 53: 551-554.

Percentage oil or other diethyl ether soluble material was determined in lyophilized ground whole seeds and in buffer (sodium phosphate: pH 7.9, 1-0.01) soluble and insoluble extracts of high quality Florunner groundnuts not inoculated or inoculated with *Aspergillus parasiticus, Aspergillus oryzae, Rhizopus oligosporus,* or *Neurospora silophila* and held for various time intervals ranging up to 18 days. During the test period, percentage ether extractable material increased in buffer soluble fractions of groundnut infected with the fungi and decreased in insoluble preparations. The trends of these quantitative changes at various test intervals were similar for seeds infected with *A. parasiticus* and *R. oligosporus.* Likewise, percentages were similar for seed infected with *N. silophila* and *A. oryzae.* The percentage ether soluble material in whole seeds infected with these fungi either decreased slightly or did not change during the test periods. Data suggest that techniques developed to separate oil and protein fractions from high quality groundnuts using aqueous extraction processes may not be suitable for isolating these components from molded or partially decomposed seeds.

619. Cherry, J.P., Beuchat, L.R., and Koehler, P.E. 1978. Soluble proteins and enzymes as indicators of change in peanuts infected *with Aspergillus flavus.* Journal of Agricultural and Food Chemistry 26: 242-245.

Buffered extracts of groundnut seeds infected with 4 atlatoxigenic and 5 nonaflatoxigenic strains of *Aspergillus flavus* were examined electrophoretically for soluble proteins and selected enzymes. Quantitatively, soluble proteins in extracts of infected seeds were significantly lower after 4 days than those in control seeds; however, the number of low molecular weight proteins in infected seeds increased.

Enzyme patterns of extracts from seeds infected with aflatoxigenic *A. flavus* did not differ distinctively from patterns of seeds infected with nonaflatoxigcnic strains. Esterase, leucine, aminopeptidase, gluconate and alcohol dehydrogenase, and alkaline and acid phosphatase patterns in extracts could be distinguished between infected and control seeds.

620. Cherry, J.P., Mayne, R.Y., and Ory, R.L. 1974. Proteins and enzymes from seeds of *Arachis hypogaea* L. IX. Electrophoretically detected changes in 15 peanut cultivars grown in different areas after inoculation with *Aspergillus parasiticus.* Physiological Plant Pathology 4: 425-434.

Dehulled and testa-free seeds from 15 groundnut cultivars in the USA were all colonized by *Aspergillus parasiticus* when kept in moist chambers at 30"C. Gel electrophoretic patterns of proteins and seven enzymes of colonized seeds, cotyledons and axial tissues showed the same sequence of changes from 0 to 5 days after inoculation. The changes were distinct from a standard profile of healthy kernels. During this period high molecular weight globuline degraded to smaller components, proteins decreased quantitatively, enzymes changed in composition, and aflatoxins were formed. Many of the new isozymes in colonized groundnuts were also observed in zymograms from extracts of fungal tissue only, collected from the exterior surface of seeds. Transformations such as the rapid catabolism of groundnut proteins coincided with changes in isozyme patterns during colonization by *A. parasiticus.*

621. Cherry, J.P., Ory, R.L., and Mayne, R.Y. 1972. Proteins from peanut cultivars *(Arachis hypogaea)* grown in different areas. VI. Changes induced in gel electrophoretic patterns by *Aspergillus* contamination. Proceedings of the American Peanut Research and Education Association 4(1): 32-40.

Gel electrophoretic studies were employed to develop "standard" gel patterns of total proteins and enzymes from crude extracts of individual seeds of a commerical groundnut cultivar (Virginia market type; Virginia 56R) for use in comparative biochemical investigations. Changes in these "standard" patterns due to growth of *Aspergillus parasiticus* on the groundnuts were easily delected. Within two to three days after inoculation of groundnuts with the fungus, large molecular weight proteins in the upper half of the gels rapidly decreased. At the same time, many new, small molecular weight proteins appeared in the lower half of the gels. After 5 days of fungal development, most of the small molecular weight proteins were difficult to detect in the electrophoretic patterns. Simultaneously with these changes in the total protein patterns, new and more complex zymograms were observed for several enzymes compared to their "standard" patterns. Examination of these enzymes in the fungal tissue collected from the external surfaces of groundnuts, or grown separately in Czapek's solution, indicated that most of the new isoenzymes in contaminated seed extracts were derived from the invading mold. The implications of these changes from the "standard" protein and enzyme patterns of groundnuts and their relation to the development of A. *parasiticus* on these seeds are discussed.

622. Cherry, J.P., Young, C.T., and Beuchat, L.R. 1975. Changes in proteins and free and total amino acids of peanuts *{Arachis hypogaea L.)* infected with *Aspergillus parasiticus.* Canadian Journal of Botany 53(22): 2639-2649.

Protein and amino acid composition of groundnuts inoculated with *Aspergillus parasiticus* were compared with those of noninfected seeds during an 18-day test period to determine metabolic changes within this interrelationship. The levels of buffer soluble proteins of infected groundnuts decreased rapidly to quantities much lower than those of noninfected seeds shortly after inoculation. Simultaneously the levels of insoluble proteins increased to quantities greater than those contained in soluble fractions. Gel electrophoresis of soluble extracts from inoculated groundnuts showed that proteins were hydrolyzed to many small molecular weight components, which eventually dissappeared as fungal growth progressed. A corresponding increase in quantity of most free amino acids was observed shortly alter inoculation. Major changes in free amino acid content coincided with substantial alterations of proteins in both soluble and insoluble fractions. These data suggest that inoculation with *A. parasiticus* initiated a sequence of events whereby proteins were hydrolyzed first to small polypeptides and/or insoluble components, then to free amino acids. After extended periods of infection, levels of free amino acids varied from day to day, suggesting that differential utilization of these components by the fungus was taking place. Quantities of total amino acids in whole seeds and soluble and insoluble fractions were different for noninoculated and inoculated groundnuts. Distinct differences were particularly noted among samples of these three fractions of inoculated seeds. Differences in total amino acid contents apparently reflect qualitative and quantitative changes in proteins and/or polypeptides present in various fractions examined during the infection period.

623. Deshpande, A.S. 1978. Effect of *Aspergillus flavus* infestation on the nutritional quality of peanuts. Ph.D Thesis. Florida A & M University, USA.

Seeds of six commercially grown groundnut cultivars (Altika, Dixie Runner, Early Bunch, Florigiant, Florunner and NC-Fla 14) and four lines were inoculated with an aflatoxigenic strain of *Aspergillus flavus,* and incubated at 37° C and > 90% relative humidity for 4, 7, or 14 days. After the incubation period, groundnuts were analyzed for oil, protein, free fatty acids, changes in amino acid composition, and aflatoxin production. Seeds of these cultivars/lines had varying degrees of resistance to colonization by the fungus. Early Bunch, NC-Fla 14, and UF 77412 were highly susceptible, while Altika, Dixie Runner and Florigiant showed moderate resistance

and Florunner, PI 337409 and PI 337394F showed high resistance to *A. flavus* colonization. The significant biochemical changes in groundnuts resulting from *A. flavus* colonization included : reduction of oil and protein content, rapid increase in free fatty acids, aflatoxin production, and changes in the amino acid composition. There was a significant decrease in the basic amino acids, an increase in glutamic acid and no change in aromatic amino acids.

624. Deshpande, A.S., and Pancholy, S.K. 1979. Colonization and biochemical changes in peanut seeds infected with *Aspergillus flavus.* Peanut Science 6(2): 102-105.

Seeds from three commercially grown groundnut cultivars and two "resistant" genotypes had varying degrees of resistance to colonization by the fungus *Aspergillus flavus.* Groundnut genotypes PI 337409 and PI 337394F had significantly higher resistance to colonization than other cultivars. Some of the biochemical changes in groundnuts resulting from *A. flavus* infection included : reduction of oil and protein content, rapid increase in free fatty acids and changes in the amino acid composition.

625. Diener, U.L. 1973. Deterioration of peanut quality caused by fungi. Pages 523-557 in Peanuts : Culture and Uses. American Peanut Research & Education Association, Stillwater. OK, USA.

This paper reviews seed infection by *Aspergillus flavus* and other fungi, and aflatoxin contamination of groundnuts from the time the groundnuts are growing in the field until they are marketed, stored and shelled. Causes of aflatoxin contamination and methods to control the aflatoxin problem are discussed.

626. Diener, U.L., and Davis, N.D. 1977. Aflatoxin formation in peanuts by *Aspergillus flavus.* Bulletin of the Alabama Agricultural Experimental Station (No. 493), 49 pp. Auburn, Alabama. USA : Auburn University.

This review discusses factors influencing production of aflatoxin in groundnuts including : the aflatoxin-producing fungus *Aspergillus flavus,* the substrate, relative humidity, moisture, temperature and time, aeration, the pod, and damage to the kernel.

627. Farag, R.S., El-Leithy, M.A., Basyony, A.E., and Daw, Z.V. 1986. Effects of varied substrates on aflatoxin production by *A. parasiticus.* Journal of the American Oil Chemists' Society 63(8): 1024-1026.

Sterilized and nonsterilized wheat kernels, soybean seeds, sesame seeds, groundnuts and faba beans were infected by an aflatoxigcnic strain of *Aspergillus parasiticus.* The chemical composition, aflatoxin content and fatty acid patterns of the seeds were determined. Aflatoxins B_1, B_2, G_1 and G_2 were detected, and the amounts of aflatoxins B_1 and G_1 were greater than those of B_2 and G_2. When infected by the fungus, sterilized seeds contained greater amounts of aflatoxins than did nonsterilized seeds. The highest and lowest toxicity indices were recorded for sterilized wheat and soybeans, respectively. Sesame, groundnut and soybeans exhibited intermediate toxicity indices. In every instance the amounts of aflatoxins produced were related significantly to the carbohydrate and lipid : protein ratio, and not to the polyunsaturated fatty acids of the seeds.

628. Farag, R.S., Tana, R.A., and Khalil, F.A. 1980. Effect *of Aspergillus flavus* infection on the cottonseed, peanut and maize oils. Grasas Y Aceites 31: 411-415.

Some physical and chemical properties were studied in groundnut, cottonseed and corn germ oils extracted from seeds inoculated with *A. flavus* and from healthy seeds. Results, shown graphically (UV visible and IR spectra) and in a table (refractive index, color, acid, saponification, iodine and peroxidase values), revealed serious deterioration in corn oil due to fungal infection, with great rise in the Lovibond red color and in peroxidase value. Similar but less pronounced changes were observed in the other 2 oils. All 3 oils showed greatly increased acid value. These results cast doubt on industrial use of oil extracted from infected seeds for food processing. Spectrophotometric data were examined for possible elucidation of chemical changes in the oils from infected seeds.

629. Ostrowski-Meissner, H.T. 1984. Effect of contamination of foods by *Aspergillus flavus* on the nutritive value of protein. Journal of the Science of Food and Agriculture 35(1): 47-58.

The effect of aflatoxin on the protein quality of groundnut meal and fish meal was measured. Total protein efficiency, protein efficiency ratio (PER), and net protein utilization, examination of the histopathology of the liver, ileal digestion of amino acids and plasma amino acids concentration were used as bioassays together with chemical score (CS), dye-binding capacity (DBC). essential amino acid index (EAAI), and discriminant computed PER (DC-PER) as chemical methods. In one trial, aflatoxin-frce groundnut meal was compared with infected groundnut meal at graded levels of toxin when fed to chickens and ducklings. In another trial, various mixtures of groundnut meal and fish meal at a constant aflatoxin level (280 μg kg^{-1}) were given to compare the effects of aflatoxin on proteins of differing quality. Ducks were more sensitive to the toxin than chickens, as indicated by deterioration of protein quality, and the effects on growth and the histological appearance of the liver were magnified on diets of low quality groundnut meal, but not of high quality fish meal. Contamination of groundnut meal resulted in progressive increase in DBC and, to a lesser extent, in DC-PER, while EAAI and CS were not affected. The

importance of these findings lies in the problem of mold contamination of animal feedstuffs in humid, tropical conditions which may affect the sensitive animals, and may not be detected by chemical methods of measuring protein quality or by bioassay on chickens, if the levels of contamination are low.

630. Ostrowski-Meissner, H.T., Siswohardjono, W., Sutler-man, D., and Barchia, I. 1984. The effect of contamination of peanut meal with *Aspergillus flavus* on protein quality as measured chemically and in bioassays on chickens and ducklings. Tropical Animal Production 9(1): 35-44.

The protein quality of groundnut oil meal either aflatoxin-free or contaminated with aflatoxins was evaluated using chemical score and predicted discriminant computed protein efficiency ratio (DC PER) or protein efficiency ratio (PER), net protein utilization (NPU), total protein efficiency (TPE), plasma total (PTAA) and essential (PEAA) amino acids, and nitrogen retention with both chickens and ducks. When ducklings were used.becausc of their high sensitivity to aflatoxins, results were much lower with the contaminated than with the aflatoxin-free groundnut meal. Bioassays on chickens and chemical assays were insensitive in predicting an increased utilization of dietary proteins by ducks given the meal contaminated with aflatoxins. In the humid tropics where most stored foods are infested with *Aspergillus flavus,* a reduction in the utilization of protein from foods contaminated with the fungus may be expected for organisms sensitive to aflatoxins. Such a reduction may not be detected if chemical assays or bioassays with test animals of low sensitivity to aflatoxins are used.

631. Pattee, H.E., and Sessoms, S.L. 1967. Relationship between *Aspergillus flavus* growth, fat acidity, and aflatoxin content in peanuts. Journal of the American Oil Chemists' Society 44: 61-63.

The influence of fungal growth, under standardized conditions, on fat acidity in large-seeded Virginia-type groundnuts inoculated with *Aspergillus flavus,* and relationships between fat acidity and aflatoxin were studied. Fat acidity increased quadratically and was highly correlated with visible fungal growth. A lag in aflatoxin production in relation to fat acidity increase was noted; fat acidity reached 60 mg KOH per 100 g kernels before aflatoxin became detectable. This relationship suggests that a rapid method of determining fat acidity might be used to screen groundnut samples for the possible presence of aflatoxin.

632. Szerszen, J.B., and Pettit, R.E. 1990. Changes in isozyme patterns of *Aspergillus flavus* group spp. infected peanut cotyledons from plants grown under drought stress. Proceedings of the American Peanut Research and Education Society 22: 34.

Isozyme profiles of buffer-extractable cotyledonary proteins from *Aspergillus flavus* and *A. parasiticus* infected groundnut kernels from five cultivars grown under drought stress and normal irrigation were assayed clectrophoretically by means of microprocessor-controlled IEF-PAGE (pl 3-9) and discontinuous native-PAGE (gradient 8-25). Drought stress was imposed 100 days after planting until harvest. Testa-free viable kernels, hydrated previously to 25 % of moisture, were inoculated with conidia of the aspergilli ($7x10^6$ conidia mL^{-1}). and sampled every 6 h during 72 h of incubation (dark, 32°C, 95 % RH). Total protein profiles of non-infected cotyledons from drought-stressed and irrigated plants were identical. Both fungi caused qualitative and quantitative changes of ADH, ACPH, ALPH, EST, LAP, PER, 6-PGD. MDH. and G-6PD within 12-72 h of incubation Aspergilli-infected cotyledons from drought-stressed plants exhibited differences in banding patterns and activities of ACPH, ALPH, ESI, MDH. and G-6 PD, when compared to infected cotyledons from plants grown under irrigation. Drought-stressed cultivarTX 79836 showed the most isozyme changes among cultivars tested. Drought stress can predispose viable groundnut kernels to altered enzymatic reactions occurring during early stages of infection by *Aspergillus flavus* group spp.

633. Vaidya, A., and Dharam Vir. 1989. Changes in the oil in stored groundnut due to *Aspergillus niger* and *A. flavus.* Indian Phytopathology 42(4): 525-529.

Aspergillus niger and *A. flavus* caused spoilage of stored groundnut and brought about reduction in quality and quantity of oil during pathogenesis. Postharvest treatment with propionic acid and sodium metabisulphite as spray and dip were effective against infection by these fungi.

4. FACTORS AFFECTING PRODUCTION OF AFLATOXIN IN GROUNDNUTS IN THE LABORATORY

4.1 REVIEW

In the early years of research on aflatoxins there was much interest in determining what natural substrates were capable of supporting aflatoxin production when colonized artificially or naturally by toxigenic strains of *Aspergillus flavus* and/or *A. parasiticus.* There was also an interest in producing aflatoxins for experimental purposes.

Many natural substrates have been used to produce large quantities of aflatoxin in the laboratory. Typically, natural substrates are rehydrated, sterilized, inoculated with strains of the fungi *Aspergillus flavus,* and/or *A, parasiticus,* and then incubated at 25°-30°C for 5-14 days. Aflatoxins have been produced by the aflatoxigenic fungi when grown on every major cereal, grain, and oil-seed (Hesseltine et al. 1966, Diener and Davis 1966, Diener and Davis 1977). Crushed groundnuts, whole groundnut kernels, groundnut germ and groundnut meal support substantial amounts of aflatoxins by aflatoxin-producing isolates *of A. flavus* and/or *A. parasiticus.* Various investigators obtained high levels of aflatoxin production by culturing *A. flavus* isolates of varying toxin-producing abilities on groundnut substrates (Table 3). Differences in aflatoxin production on uniform substrates are attributed to such factors as fungal strain, temperature, moisture in the substrate and/or surrounding relative humidity, aeration, length of the incubation period, and the method of aflatoxin analysis (Hesseltine et al. 1966, Diener and Davis 1977).

Relative Humidity and Moisture

Important factors in growth and aflatoxin production by *A. flavus* are moisture content and relative humidity (RH) equilibrium of the substrate (Austwick and Ayerst 1963). Diener and Davis (1967) found that a relative humidity of 85 ± 1% at 30°C for 21 days was the limiting condition for aflatoxin production in heat-killed, sound- and broken- mature groundnut kernels, immature kernels, and kernels from unshelled Early Runner groundnuts. Allatoxin production in living kernels from freshly dug, surface-sterilized Early Runner pods showed a similar limiting RH. Dickens and Pattee (1966) found that aflatoxin developed in 2 days at moisture contents between 15 and 30% at 32.2°C and in 4 days at moisture contents between 20 and 31% at 21.1°C in freshly dug groundnuts.

Temperature and Time

It is difficult to separate the effects of temperature and time from those of RH and moisture. Minimal and maximal temperatures for growth are affected by moisture, oxygen concentration, availability of nutrients, and other factors (Diener and Davis 1977). Diener and Davis (1977) reported minimum temperature for growth *of A. flavus* to be 6-8"C, optimum 36-38°C, and maximum 44-46°C.

Optimum production of aflatoxin by *A. flavus* on sterilized groundnuts can be obtained by incubation at 30°C for 5-7 days, at 25° C for 7-9 days, or at 20° C for 11-13 days (Diener and Davis 1966). Research by Schroeder and Hein (1967) supports these results.

In critical studies, Diener and Davis (1967) found that the lower limiting temperature for aflatoxin production by *A. flavus* was 13° ± 1°C for a 21-day incubation at 98 ± 1% RH. The upper limiting temperature was 41.5° ± 1.5°C. Minimum time for aflatoxin production under optimal conditions is two and a half days after inoculation.

Temperature also influences the proportion of aflatoxin B_1 to G_1 produced by *A. flavus* in groundnuts and in a semisynthetic medium (Diener and Davis 1966; Schroeder and Hein 1967).

Aeration

The proportions of aunosphcrie gases present influence growth, sporulation, and aflatoxin formation by *A. flavus* in groundnuts (Landers et al. 1967, Sanders et al. 1968). Landers ct al. (1967) observed no visible change in *A. flavus* growth and sporulation when *CO,* concentration was increased from 0.03% (concentration in air) to 20%; however, aflatoxin formation was reduced by 75%. Aflatoxin production decreased proportionately with an increase in CO_2 levels ranging from 20 to 80%, and aflatoxin was not produced at 100% CO_2. In general, reducing the O_2 concentration decreased aflatoxin production, but the largest decreases occurred when O_2 was reduced from 5 to 1% with 0, 20, or 80% CO_2.

Proportions of N_2 to O_2 as high as 99% - 1% did not inhibit fungus growth, as evidenced by production of aflatoxin (Landers ct al. 1967). Sanders et al. (1968) found that *A. flavus* could produce aflatoxin at 25°C under 99%; RH in an atmosphere containing 20% CO_2, but when temperature was reduced to 17"C and RH to 86-92% aflatoxin production was inhibited. Similarly, aflatoxin production was inhibited at 25°C by 60% CO_2 al 86 and 92% RH and by 40% CO_2 at 86% RH, but not at a higher RH (92%) and temperature (30°C). The importance of aeration

to aflatoxin production in natural substrates was previously demonstrated by Hesscltine et al. (1966), who recorded 3- to 100-fold increases in aflatoxin B_1 with shake cultures when compared with stationary cultures of the fungus cultured on corn, groundnut, rice, sorghum, soybean, and wheat.

Most studies of in vitro aflatoxin production in natural substrates have involved the use of autoclaved seeds. This is logical in terms of eliminating problems from resident seed microflora which could interfere with aflatoxin production by artificial inoculations with (he toxigenic fungi. It is emphasized that the autoclaving of natural substrates can induce biochemical changes in the substrates that may either enhance or decrease aflatoxin by the toxigenic cultures. It is desirable to utilize healthy natural substrates in studies on aflatoxin production.

Table 3. Maximum aflatoxin production by different isolates of *Aspergillus flavus* and *A. parasiticus*

Investigator(s)	Substrate	Fungus isolate	Aflatoxins
Codner et al. (1963)	Groundnut	*A. parasiticus* - 15957	894
Vogel et al. (1965)	Crushed groundnut	*A. flavus* - NRRL 3000	1000[a]
Cucullu et al. (1966)	Groundnut germ	Unknown	4000
Schroeder (1966)	Spanish groundnut	*A. parasiticus* -64-R8	650
Boiler and Schroeder (1966)	Spanish groundnut	*A. flavus* -F262	894
Diener and Davis (1966)	Groundnut	*A, parasiticus* - Ala-6	364
Taber and Schroeder (1967)	Spanish groundnut	*A. flavus*	363[b]
Schroeder and Hein (1967)	Groundnut	*A. flavus it* 10	680
Hesseltine et al. (1968)	Groundnut	*A. flavus* -NRRL 3145	488

[a]aflatoxin B_1 only; [b]aflatoxins B_1 and B_2

4.2 BIBLIOGRAPHY

4.2.1 FACTORS AFFECTING PRODUCTION OF AFLATOXIN IN GROUNDNUTS IN THE LABORATORY

634. Bell, D.K., and Uoupnik, B. 1971. Infection of groundnut pods by isolates of *Aspergillus flavus* with different aflatoxin-producing potentials. Transactions of the British Mycological Society 57(1): 166-169.

Investigations were made into growth and competitive behavior of six isolates of *Aspergillus flavus* with different aflatoxin producing potentials, when inoculated onto fumigated, rehydrated groundnut pods (cv. Starr). Inoculated pods were placed in shallow pans in a controlled environment of 99% relative humidity at 27°C in the dark for 7 days. Six replicate batches of 100 pods were inoculated with each isolate or pair of isolates. There were no differences in invasive potential between the isolates. Double-isolate inoculations of 'nil' - 'high', 'low' - 'high', and 'medium' - 'medium' pairs of isolates produced lower amounts of aflatoxins in kernels than single-isolate inoculations of the two 'high' isolates and of the higher 'medium' isolate. Results indicated that one isolate did not suppress completely infection by another. However, competition for infection between isolates in double-isolate inoculations was not determined directly by re-isolation of each isolate because there were insufficient diagnostic characteristics.

635. Dickens, J.W., and Pattee, H.E. 1966. The effects of time, temperature and moisture on aflatoxin production in peanuts inoculated with a toxic strain of *Aspergillus flavus.* Tropical Science 8(1):11-22.

Effects of time, temperature and moisture on the production of aflatoxin in groundnuts inoculated with an aflatoxin-producing strain of *Aspergillus flavus* were studied. Moisture contents between 15 and 30% were conducive to aflatoxin production. At least 48 and over 100 hours from the time of inoculation were required for aflatoxin to develop in kernels at 32°C and 21°C, respectively, at all moisture levels. Groundnut curing usually involves partial drying in windrows and subsequent drying in bulk curing bins. Unless considerable mold growth occurs before the start of bulk curing, aflatoxin is not likely to develop during the curing process if recommended practices are followed. In cool weather, even prolonged periods in the windrows at high moisture contents did not cause aflatoxin development in groundnuts previously inoculated with *A. flavus* isolates.

636. Diener, U.L., and Davis, N.D. 1966. Aflatoxin production by isolates of *Aspergillus flavus.* Phytopathology 56: 1390-1393.

Species of the *Aspergillus flavus* group were isolated from groundnuts, corn, feed, and other sources. Also, isolates of *A. flavus* from groundnuts, cereals, soybeans, and other crops were obtained from other investigators. Isolates were screened for aflatoxin production on groundnuts and in a nutrient solution. About 80% of the A. flavus isolates produced aflatoxin to some degree. Ninety per cent of the isolates produced primarily aflatoxin B_1, whereas about 10% produced both aflatoxins B_1 and G_1. Optimal temperature for aflatoxin B_1 production by *A. flavus* on groundnuts and in nutrient solution was 25°C for an incubation period of 7 to 9 days. Optimal temperature range for aflatoxin production by *A parasiticus* on both media was 25" to 30"C. Aflatoxin levels were high throughout the 7- and 21-day incubation periods. The proportion of aflatoxin B_1 to aflatoxin G_1 varied with the temperature.

637. Diener, U.L., and Davis, N.D. 1967. Limiting temperature and relative humidity for growth and production of aflatoxin and free fatty acids by *Aspergillus flavus* in sterile peanuts. Journal of the American Oil Chemists' Society 44(4): 259-263.

Sound mature kernels, immature kernels, and unshelled groundnuts (cv. Early Runner) were heat-treated and inoculated with spores of an aflatoxigenic strain of *Aspergillus flavus,* and incubated at 97-99% relative humidity (RH) at different temperatures ranging from 5 to 55°C and also at 30°C with RH ranging from 55 to 99%. Samples were removed after 7 and 21 days and assayed for aflatoxin, free fatty acids, and kernel moisture content. The limiting RH for aflatoxin production was 85 $\pm$ 1% RH for 21 days at 30°C. The limiting low temperature for visible growth and aflatoxin production by the fungus was 13 $\pm$ PC for 21 days at 97-99% RH. Damaged kernels, however, developed some aflatoxin in 21 days at 12°C. The maximum temperature for aflatoxin production was 41.5 $\pm$ 1.5°C for 21 days at 97-99% RH. Fungus growth and sporulation at 43°C were equal to that at 40"C. but no aflatoxin was produced. Moisture content of immature kernels was higher at equilibrium with the same RH than the moisture content of sound mature kernels, damaged kernels, or kernels from unshelled groundnuts. There appeared to be no proportional quantitative correlation between synthesis of aflatoxin and production of free fatty acids in heat-treated groundnuts, but no aflatoxin was produced without a simultaneous increase in free fatty acids.

638. Diener, U.L., and Davis, N.D. 1969a. Aflatoxin formation by *Aspergillus flavus.* Pages 13-54 in Aflatoxin : Scientific Background. Control, and Implications (Goldblatt, L.A., ed.). Academic Press, New York : USA.

This paper comprehensively reviews aflatoxin production by *Aspergillus flavus*

isolates from various agricultural commodities, and the factors affecting aflatoxin production on natural substrates in nature, in vitro, and in synthetic and semi-synthetic media by the fungus.

639. Diener, U.L., and Davis, N.D. 1969b. Production of aflatoxin on peanuts under controlled environments. Journal of Stored Products Research 5: 251-258.

The influence of temperature, relative humidity (RH), nature of the substrate, atmospheric gases, and other factors on growth and aflatoxin production by *Aspergillus flavus* in groundnuts (cv. Early Runner) was investigated under controlled environments. Sound or broken mature kernels, immature kernels, and unshelled groundnuts were inoculated with spores of an aflatoxigenic strain of Aspergillus flavus and incubated at 98 ± 1% RH at temperatures ranging from 10 to 45°C, and also 30 ± 0.5°C in RH ranging from 70 to 99%. The substrate was heat-treated (for 12-14 h at 99% RH and 85°C). cured groundnuts, or surface-sterilized pods of freshly dug groundnuts, or nonsterile cured groundnuts. Surface-sterilized, sound mature kernels were used in studies with atmospheric gases. Samples were removed after 7, 21, 42, or 84 days and assayed for aflatoxin, free fatty acids, and kernel moisture content. The limiting RH for aflatoxin production was 83 ± 1% or higher at 30°C, varying with the substrate and length of the incubation period. The lower limiting temperature was 11-12 ± 1°C, whereas the upper limiting temperature was 40.5 ± 0.5°C at 98 + 1% RH. Groundnuts in shell were slightly less susceptible to invasion by the fungus and to aflatoxin formation than were shelled or heat-treated groundnuts. Aflatoxin production in sound mature kernels decreased with increasing concentrations of CO_2 from 0.03 to 100%. Reducing O_2 concentrations generally reduced aflatoxin production. Notable decreases in aflatoxin production resulted when O_2 was reduced from 5 to 1% in combination with 0, 20, or 80% CO_2. Lowering temperature or RH below optimal levels also reduced aflatoxin production. Free fatty acid formation paralleled fungus growth rather than aflatoxin formation.

640. Diener, U.L., and Davis, N.D. 1977. Aflatoxin formation in peanuts by *Aspergillus flavus.* Bulletin of the Alabama Agricultural Experimental Station (No. 493), 49 pp. Auburn, Alabama, USA : Auburn University.

This review discusses factors influencing production of aflatoxin in groundnuts including the aflatoxin-producing fungus *Aspergillus flavus,* the substrate, relative humidity, moisture, temperature and time, aeration, the pod, and damage to the kernel.

641. Farag, R.S., El-Leithy, M.A., Basyony, A.E., and Daw, Z.Y. 1986. Effects of varied substrates on aflatoxin production by *A. parasiticus.* Journal of the American Oil Chemists' Society 63(8):1024-1026.

Sterilized and nonsterilized wheat kernels, soybean seeds, sesame seeds, groundnuts and faba beans were infected by an aflatoxigenic strain of *Aspergillus parasiticus.* The chemical composition, aflatoxin content and fatty acid patterns of the seeds were determined. Aflatoxins B_1, B_2, G_1 and G_2 were detected, and the amounts of aflatoxins B_1 and G_1 were greater than those of B_2 and G_2. Sterilized seeds infected by the fungus contained greater amounts of aflatoxins than nonsterilized seeds. The highest and lowest toxicity indices were recorded for sterilized wheat and soybeans, respectively. Sesame, groundnut and soybeans exhibited intermediate toxicity indices. In every instance the amounts of aflatoxins produced were related significantly to the carbohydrate and lipid : protein ratio, and not to the polyunsaturated fatty acids of the seeds.

642. Hesseltine, C.W., Shotwell, O.L., Ellis, J.J., and Stubblefield, R.D. 1966. Aflatoxin formation by *Aspergillus flavus.* Bacteriological Review 30: 795-805.

This paper briefly reviews the natural occurrence of aflatoxin in agricultural commodities and factors affecting aflatoxin production in nature. Aflatoxin production by strains of *Aspergillus flavus-oryzac* is also discussed. Research on aflatoxins at the Northern Regional Research Laboratory (NRRL), USA, is presented.

643. Landers, K.E., Davis, N.D., and Diener, U.L. 1967. Influence of atmospheric gases on aflatoxin production by *Aspergillus flavus* in peanuts. Phytopathology 57: 1086-1090.

Sound, mature kernels of the groundnut cultivar Early Runner were inoculated with *Aspergillus flavus* and incubated under various concentrations of carbon dioxide (CO_2), nitrogen (N_2), and oxygen (O_2) at high moisture levels for 2 weeks at 30° C and 6 weeks at 15°C. Observations were made on growth and sporulation of the fungus and assays were conducted on infected kernels to determine the concentrations of aflatoxins and free fatty acids. No reduction in growth and sporulation *of A. flavus* occurred when the CO_2 concentration was increased from 0.03% (air) to 20%. Fungus growth and sporulation were reduced with each 20% increase in CO_2 from 20% to 80%. No growth occurred in 100% CO_2. No apparent decrease in growth or sporulation occurred when O_2 was reduced from 20% to 50%, regardless of the CO_2 concentration. However, striking reductions occurred when O_2 was reduced from 5 to 1% with 0, 20, or 80% CO_2. Aflatoxin production decreased with increasing concentrations of CO_2 from 0.03 to 100%. In general, reducing the O_2 concentration decreased amounts of aflatoxin. Sizeable decreases in aflatoxin production occurred when O_2 was reduced from 5 to 1% in combination with 0, 20, or 80% CO_2. Aflatoxin production was lower in groundnuts stored at 15°C under 20% CO_2 for 6 weeks when O_2 was reduced from 20% to 5%. Aflatoxin was low in groundnuts stored at 15°C for 6 weeks under high concentrations of CO_2. Free

fatty acid formation closely paralleled growth, sporulation, and aflatoxin production by *the* fungus. Striking decreases in percentage of free fatty acids occurred when O_2 was reduced from 5 to 1%.

644. Moreno Romo, M.A., Fernandez, G.S., and Cartagena Madel, C.R. 1985. Experimental short time production of aflatoxin by *Aspergillus parasiticus* in mixed feeds as related to various moisture contents. Mycopaihologia 92: 49-52.

Production of aflatoxins in mixed feeds at 22, 28 and 37°C was studied in relation to various moisture contents. Growth *of Aspergillus parasiticus* was not observed in meals with a moisture content of 15%. When incubated at 22°C and 19.4% moisture content, aflatoxin was detected on the fourth day. When incubated at 28°C and 29.3% moisture content, 113 μg g^{-1} of aflatoxin was produced within 4 days. The ratio of aflatoxin B_1 to aflatoxin G_1 increased as the temperature increased.

645. Obidoa, (.)., and Ndubuisi, I.E. 1981. The role of zinc in the aflatoxigenic potential of *Aspergillus flavus* NRRL 3251 on foodstuffs. Mycopaihologia 74: 3-6.

The production of total aflatoxins (B_1, B_2, M_1 and M_2) in ten tropical foodstuffs (with and without zinc enrichment) inoculated with *Aspergillus flavus* strain NRRL 3251 was examined to determine the effect of zinc on aflatoxin production. Production of aflatoxin was not linearly correlated with zinc levels of the food substrates. The data presented indicate that optimal zinc requirement for maximal aflatoxin production was substrate specific. Aflatoxin production was high on groundnut, melon, and plantain without zinc enrichment. These results indicated a poor correlation between levels of aflatoxin produced and the zinc content of the foodstuffs.

646. Ogundero, V.W. 1987. Temperature and aflatoxin production by *Aspergillus flavus* and *A. parasiticus* strains from Nigerian groundnuts. Journal of Basic Microbiology 27(9): 511-514.

The abilities of 4 isolates *of Aspergillus parasiticus* and 6 of A. *flavus* to produce aflatoxins on Nigerian groundnuts at incubation temperatures of 10-35°C were studied. While all *A. parasiticus* isolates produced aflatoxins B_1, B_2, G_1 and G_2, only aflatoxins B_1 and B_2 were produced by 2 of the isolates of *A. flavus.* The optimum temperature for aflatoxin production by the 2 species was 30°C; there was negligible production at 10°C.

647. Park, K.Y. 1984. Aflatoxin : factors affecting aflatoxin production. Journal of the Korean Society of Food and Nutrition 13(1):117-126.

Physical (moisture, temperature, water activity, oxygen concentration, incubation time, presence of other microbes) and biochemical (carbon source, sugars available) factors affecting aflatoxin production on food by *Aspergillus flavus* and *A. parasiticus* are reviewed.

648. Pattee, H.E., Sessoms, S.L., and Dickens, J.W. 1966. Influence of biologically modified atmospheres on aflatoxin production by *Aspergillus flavus* growing on peanut kernels. Oleagineux 21: 747-748.

Effects of atmospheric gases on the production of aflatoxin in groundnuts inoculated with an aflatoxin-producing strain of *Aspergillus flavus* were studied. Sizeable decreases in aflatoxin production occurred when O_2 was reduced from 21 to 3%.

649. Sakai, T., Sugihara, K., and Kozuka, H. 1984. Growth and aflatoxin production of *Aspergillus parasiticus* in plant materials. Journal of Hygienic Chemistry 30(2): 62-68.

The relationship between fungal growth and aflatoxin production by *Aspergillus parasiticus* was investigated. Thirteen types of powdered plant material were used, including nuts, beans, roots and seeds, classified into three groups according to chemical composition, viz., (i) high glucide or starch content (5 types), (ii) high lipid content (5 types) and (iii) high protein content (3 types). The mold grew actively on (i) and (ii). Aflatoxin production was particularly active on (ii); maximum total aflatoxin content (μg g^{-1}) for (i), (ii) and (iii) was in the range 0.1-172, 4.4-1223 and 0.9-10.2 respectively. However, aflatoxin production in castor seeds (group ii) was low despite rapid fungal growth, suggesting that some constituent(s) of castor seeds may inhibit aflatoxin production. Graphs plotting substrate consumption against aflatoxin production for rice, castor seeds, pine nuts, groundnuts and soybeans are included.

650. Sanders, T.H., Davis, N.D., and Diener, U.L. 1968. Effect of carbon dioxide, temperature, and relative humidity on production of aflatoxin in peanuts. Journal of the American Oil Chemists' Society 45: 683-685.

Effects of carbon dioxide (CO_2) in combination with reduced relative humidities (RH) and temperatures on growth and aflatoxin production by *Aspergillus flavus* in groundnuts were investigated. Sound mature kernels of the variety Early Runner were surface-disinfested, inoculated with *A. flavus,* and incubated at various temperatures, RH, and CO_2 concentrations. Visible growth, aflaioxin production, and free fatty acids (FFA) formation by *A flavus* was inhibited at approximately 86% RH by 20% CO_2 at 17°C and by 60 and 40% CO_2 at 25°C. Aflatoxin and FFA levels decreased as RH decreased from approximately 99% to 86%. At constant temperature, increase in CO_2 concentration caused decrease in aflatoxin and FFA,

and, at a given CO_2 concentration, lowering the temperature decreased aflatoxin and FFA levels.

651. Schindler, A.F., Palmer, J.G., and Eisenberg, W.V. 1967 Aflatoxin production by *Aspergillus Jlavus* as related to various temperatures. Applied Microbiology 15(5): 1006-1009.

Two aflatoxin-producing isolates of *Aspergillus Jlavus* were grown for 5 days on Won media at 3. 7, 13, 18, 29. 35, 41, 46. and 52°C. Maximal production of aflatoxin occurred at 24°C. Maximal gTowth of *A. flavus* isolates occurred at 29 and 35°C. The ratio of the production of aflatoxin B_1 to aflatoxin G_1 varied with temperature. Aflatoxin production was not related to growth rate of *A. flavus;* one isolate at 4°C. at almost maximal growth of *A. Jlavus,* produced no aflatoxins. At 5 days, no aflatoxins were produced at temperatures lower than 18ºC or higher than 35°C. Color of chloroform-extracts appeared to be directly correlated with aflatoxin concentrations. *A. Jlavus* isolates grown at 2, 7, and 41°C for 12 weeks produced no aflatoxins. At 13°C, both isolated produced aflatoxins in 3 weeks, and one isolate produced increasing amounts with time. The second isolate produced increasing amounts through 6 weeks, but at 12 weeks lower concentrations of aflatoxins were recovered than at 6 weeks.

652. Schroeder, H.W., and Ashworth, L.J.Jr. 1966. Aflatoxins : Some factors affecting production and location of toxins in *Aspergillus flavus-oryzae.* Journal of Stored Products Research 1: 267-271.

The quantity and quality of aflatoxins produced by two *Aspergillus Jlavus oryzae* isolates from rough rice and Spanish groundnut were compared with a known, highly toxigenic *A. Jlavus* strain. Total aflatoxin yields on sterile groundnut, rough rice, and shredded wheat after 18 days at 30° ranged from 4000 to 892,000 $\mu g\ kg^{-1}$. In shake cultures growing on 4% suspensions of these substrates, yields ranged from 17000 to 426,000 $\mu g\ kg^{-1}$. Total aflatoxin yields and the proportion of the four primary aflatoxins reflected interactions between strains, substrates, and methods of culture. In shake cultures, from 52 to 80% of the aflatoxins were located in the mycelium. Conidia collected from a shredded wheat substrate after 18 days were found to contain aflatoxins at concentrations ranging from 700 to 56,100 $\mu g\ kg^{-1}$.

653. Schroeder, H.W., and Hein, H.Jr. 1967. Aflatoxins: Production of the toxins in vitro in relation to temperature. Applied Microbiology 15(2): 441-445.

The production or accumulation of aflatoxins in vitro by four isolates of *Aspergillus Jlavus* on three substrates (acid-delinted cottonseed, groundnut, and rough rice) was studied in relation to temperature in the range of 10 to 40°C. Within the first 10 days after incubation, the optimal temperature range for aflatoxin production was between 20 and 35°C. Only small amounts of the toxins were produced at 10 and 40°C. Within the optimal temperature range, the time required for toxin production and for significant accumulation decreased as the temperature increased. More aflatoxin G was produced or accumulated in relation to aflatoxin B at low temperatures (within the optimal range), and aflatoxins G were metabolized rapidly at the higher temperatures.

654. Wildman, J.D., Stoloff, L., and Jacobs, R. 1967. Aflatoxin production by a potent *Aspergillus Jlavus* Link isolate. Biotechnology and Bioengineering 9: 429-437.

An aflatoxin-producing isolate *of Aspergillus Jlavus* was found to be a consistent producer of aflatoxin on all substrates which supported the growth of the mold. In competition with six other selected molds, this isolate was dominant over all but a species of *Penicillium.* Aflatoxin production was directly related to *A. Jlavus* growth irrespective of substrate or competition.

655. Wilson, D.M., and Bell, D.K. 1984. Aflatoxin production by *Aspergillus flavus* and *A. parasiticus* on visibly sound rehydrated peanut, com and soybean seed. Peanut Science 11(1): 43-45.

Groundnut, corn and soybean seed were separately inoculated with 14 isolates of *Aspergillus Jlavus* and *A. parasiticus.* The seeds were hand sorted to remove all visibly damaged seeds and were fumigated under vacuum with 2.2% cyano (methylmercury) guanidine at 37°C for 48-96 h. All fumigated seed had a minimum of 95% germination and a maximum of 5% residual contamination by fungi and bacteria. Corn and groundnut samples (100 g per flask) were rehydrated to 28% moisture and separately inoculated with all isolates; soybean samples (100 g per flask) were rehydrated to 28% moisture and separately inoculated with four A *flavus* and two *A parasiticus* isolates. Samples were incubated for 10 days at 30°C and analyzed for aflatoxins. Aspergillus parasiticus isolates produced aflatoxins B_1, B_2, G_1 and G_2, while *A. Jlavus* isolates produced afaltoxins B_1 and B_2. Mean B_1 production for 12 isolates was 34 mg kg^{-1} in groundnut seed and 3.6 mg kg^{-1} in corn seed. Two *A. Jlavus* isolates produced 3.8 to 5.4 mg kg^{-1} B_1 in groundnut seed, and 2.2 mg kg^{-1} in corn seed. Overall, the mean B_1 production was about 10 times higher on groundnut seed than on corn seed. However, more G, was produced on soybean seed than B_1. The isolate and the substrate are apparent limiting factors in aflatoxin production. Groundnut seed accumulated more aflatoxin than corn or soybean seed when inoculated with the same isolates and incubated under similar conditions.

5. AFLATOXINS IN GROUNDNUTS AND GROUNDNUT PRODUCTS

5.1 REVIEW

Recognition of the aflatoxin problem in 1960 led to a number of country surveys to determine the incidence of aflatoxins in agricultural products and imported products. Special emphasis was placed on contamination of groundnut kernels and groundnut products and a considerable amount of information was collected. Some of these data have been published and will be quoted in this review, but there are many unpublished data in government and commercial archives that could be of interest but may be difficult to access. One problem encountered when trying to obtain an accurate record of the extent and degree of aflatoxin contamination of groundnuts and groundnut products is the variation in efficiency of sampling and in the sensitivity and applicability of the analytical procedures. In some countries only a few samples have been collected from farms or from trade groundnuts, whereas in others there has been regular monitoring of groundnuts and groundnut products from the farm and final consumer products.

In this section of the review we have arranged the information on aflatoxins in groundnuts and groundnut products according to regions and countries. This was necessary because some publications contained data important to both exporters and importers. In the database the publications are grouped according to specific products.

Aflatoxins in Groundnuts and Groundnut Products in Africa

Aflatoxins have been reported in groundnuts and groundnut products in only one country of North Africa - Egypt; in five countries in East Africa - Burundi, Ethiopia, Kenya, the Sudan and Uganda; in six countries in West Africa - the Gambia, Ghana, Cote d'Ivoire, Niger, Nigeria and Senegal; and from seven in Southern Africa - Malawi, Mozambique, Swaziland, Zaire, Zambia. Zimbabwe, and the Republic of South Africa.

Egypt

In Egypt, the crop is cultivated in the governorates of Assuit, El-Behera, El-Minia, El-Sharkitt, Giza, Sohage, and Ismailia. Approximately 40 % of the national production (25000 t) is exported, and the remainder is consumed locally as confectionery nuts, edible oil, peanut butter, and animal feeds.

Only two studies have reported aflatoxins in some groundnut samples. Girgis et al. (1977) found low levels of aflatoxin (3-12 µg kg^{-1}) in 2 of six samples of groundnuts analyzed. Abdel-Hamid (1985) reported aflatoxin contamination in 44 % of 95 samples of various foodstuffs and feedstuffs, including groundnuts, from various parts of Egypt; groundnuts from Ismailia showed the highest contamination (400 µg kg^{-1} aflatoxin B_1).

Burundi

Constant et al. (1984) examined the geographical distribution of aflatoxins in human foods in Burundi. Food products most frequently and heavily contaminated included groundnuts (maximum aflatoxin content 425 µg kg^{-1}), cassava (325 µg kg^{-1}) and maize (148 (µg kg^{-1}); aflatoxin contamination was most common in the low altitude production areas.

Ethiopia

A single report from Ethiopia gave mean levels of aflatoxin B_1 of 34.7 and 105 µg kg^{-1} in samples of groundnuts and peanut butter, respectively (Besrat and Gebre 1981).

Kenya

In a foods and feeds survey conducted in Nairobi, only one sample of raw groundnuts contained aflatoxin in the amount of 1050 µg kg^{-1} (Muraguri et al. 1981). Other nuts and oil samples were free of aflatoxin.

The Sudan

Groundnut is an important crop in the Sudan, with production of over 1 million tons of in-shell nuts. Of the estimated 1 million ha of groundnuts grown in the Sudan, about 200,000 ha are grown in the irrigated Gezira region along the Nile south of Khartoum, and 800,000 ha are grown in the Western rainfed region.

Several investigators conducted surveys for aflatoxin levels in groundnuts and groundnut products (Habish et al. 1971, Elamin et al. 1988, Ahmed et al. 1989, Singh et al. 1989). In a 1969-70 survey, samples from the rainfed groundnut production areas of the Sudan were collected from one of the principal auction markets about six weeks after the beginning of the harvest. Samples from the

irrigated areas were taken from the field about two weeks after the beginning of the harvest. Of 110 samples from the irrigated areas, 9 contained anatoxin; levels in five samples were below 50 μg kg^{-1}, one contained 50 to 250 |ig kg ' and three contained > 1000 (μg kg^{-1}. In contrast, aflatoxin was detected in 62 of the 63 samples collected from the rainfed areas (Kosti and El Obeid); levels in 24 samples were less than 50 *μg* kg^{-1}, 27 contained between 50 and 1000 μg kg^{-1}, and 11 had levels > 1000 (ig kg^{-1} (Habish et al. 1971). The high incidence of contamination in the rainfed samples was attributed to poor drying during the six or seven days immediately following harvest. Postharvesl washing to remove soil from groundnuts grown under irrigation in heavy clay soils was identified as a cause for ailatoxin contamination (Habish et al. 1971).

Elamin et al. (1988) reported that the samples collected from the rainfed region (Western Sudan) showed incidence of ailatoxin contamination ranging from 100% of samples in El Hamdi (mean aflatoxin level < 10 μg kg^{-1}) to only 10 % in Casgeal (mean aflatoxin level < 1 μg kg^{-1}). None of the 100 samples collected from the irrigated region (Central Sudan) was contaminated with ailatoxin. They also found that groundnut products (groundnut paste, red and grey roasted pods) collected from Khartoum North (Bahri) had higher levels of aflatoxin (6-15 μg kg^{-1}) than those collected from Khartoum and Umdorman (< 5 *μg* kg^{-1}). Grey and red roasted pods showed higher levels of aflatoxins than did the groundnut paste.

Surveys were also conducted in the Gezira and Rahad schemes to determine the level of aflatoxin contamination in irrigated groundnuts (Ahmed et al. 1989). No aflatoxin was detected in the 500 samples collected from groundnuts heaped in the traditional manner. Of the 200 samples collected from sacks stacked at shelling sites in Central Sudan, only 4 % were contaminated, with the average ailatoxin content was 11 μg kg^{-1}. Of 120 samples from oil mills' stores, 15 % were contaminated with an average aflatoxin content of 20 μg kg^{-1} (Ahmed et al. 1989).

In 1984 a survey was conducted in a rainfed area (El Obeid) and an irrigated area (Wad Medani) of the Sudan, to evaluate farmers' stock groundnuts for ailatoxin contamination (Singh et al. 1989). None of the samples collected from farm households in the regions contained more than 20 μg kg^{-1} of total anatoxins. However, samples collected during 1983 and 1984 from markets in Khartoum and Wad Medani contained anatoxins up to 945 μg kg^{-1} in raw groundnuts, and up to 994 μg kg^{-1} in groundnut paste (Singh et al. 1989). A possible reason for the high levels of aflatoxins in these groundnuts and groundnut paste appears to be the use of groundnuts gleaned after the main harvest, a common practice in the Sudan. The gleaned groundnuts are consumed by farm families or sold in the markets (Singh el al. 1989). Paste prepared from carefully sorted and cleaned groundnuts had a lower level of aflatoxins (19 μg kg^{-1}) than the commonly sold groundnut paste (Singh et al. 1989).

The occurrence of aflatoxins in Sudanese food items, including groundnuts, is a recognized problem. A study conducted by the Liverpool School of Tropical Medicine, UK, indicated that groundnuts contained aflatoxin B_1 up to a level of 59666 μg kg^{-1}, B_2 to 370 μg kg^{-1}. and G2 to 23 μg kg^{-1}. Peanut butter contained anatoxin B_1 at up to 26300 μg kg^{-1}, G_1 to 84500 μg kg^{-1} and B_2 to 9720 μg kg^{-1} (Hendrickse et al. 1982).

Studies reported the occurrence of all four aflatoxins in Sudanese groundnuts, but levels of aflatoxin B_1 were generally higher than those of B_2, G_1, and G_2.

Uganda

Groundnut is the most important legume crop in Uganda, with production levels ranging from 70,000 to 200,000 metric tons. Groundnuts are produced mainly on light sandy loams, but appreciable amounts are also produced on clay loams. They are mainly consumed locally.

In a survey conducted in 1966-1967, samples of foods were collected from randomly selected homes and markets from all parts of Uganda. Of 152 samples of groundnuts analyzed, 18 % contained aflatoxin, 8 % had low levels (< 100 *μg* kg^{-1}), 5 % had levels in the range of 100 to 1000 μg kg^{-1}, and 5 % had levels > 1000 μg kg^{-1} (Alpert et al. 1971). This study did not relate the incidence of aflatoxin in groundnuts to climatic conditions or cultural practices, but the incidence of aflatoxin contamination in foods was generally highest in two provinces, one of which had a dry climate..

West Africa

Ghana

In a study of groundnuts in Ghana (Mintali and Hunter 1978), 80 samples of groundnut, representing both the Northern and Volta Region types, collected from markets in and around Accra were tested for aflatoxin contamination. The Volta type groundnuts contained significantly higher levels of aflatoxin (10-216 μg kg^{-1}; mean 62 (μg kg^{-1}) than the Northern nuts (3-133 μg kg^{-1}; mean 27 μg kg^{-1}); 65 % of the Volta type and 30 % of the Northern type samples had levels > 30 μg kg^{-1}. The high incidence and levels of aflatoxin in the Volta type groundnuts was attributed to improper drying during harvest in the Volta region.

Cote d'Ivoire

Surveys have been conducted in Cote d'Ivoire to determine the level of aflatoxin contamination in groundnuts during two successive storage periods (1985/86 and

1986/87) (Pollet et al. 1989). Over the two storage periods, 434 samples of groundnuts from farms in the groundnut-growing areas, and 72 samples from town and village markets were examined. Overall, aflatoxin levels were slightly higher in the market samples 18 % of which had levels of aflatoxin in excess of 50 µg kg^{-1}, while 14 % of farmers' samples had similar levels of aflatoxin. Over the 2-year survey period, 7.9 % of the 434 local stocks had > 250 µg kg^{-1} aflatoxin, with 4.4 % containing above 1000 µg kg^{-1}. Significant differences were evident between the two storage periods in relation to percentages of samples with aflatoxin levels above 250 µg kg^{-1} (5.3 % in 1985/86; 10.6 % in 1986/87). Rainfall and humidity during storage appeared to influence aflatoxin contamination. Significant correlation between the geographical locations of the sampling sites and the risks of aflatoxin contamination have been recorded for the locations in the central region (Bouake, Bouafle and Bondoukou) of Cote d'Ivoire (Pollet et al. 1989).

Nigeria

Groundnut is a very important food and cash crop, especially in the northern part of Nigeria (Manzo and Misari 1989). The crop is grown in two main zones : (1) the northern zone with a drier climate, an annual rainfall of 750 to 900 mm, and a rainy season from June to September; and (2) the riverain zone with a humid climate, and annual rainfall of 1000 to 1250 mm, and a rainy season from April to November.

Many studies have determined levels of aflatoxin in Nigerian groundnuts and groundnut products (Crowther 1973, McDonald 1976, Nwokolo and Okonkwo 1978, Abalaka and Elegbede 1982, Ajulo and Opadokun 1974).

Researchers at the Nigerian Stored Products Research Institute (NSPRI) have carried out regular surveys of groundnuts supplied to the Kano oil mills, and also of groundnut cake and other products. They found that over the years 1962-1968 levels of aflatoxin in cake were low, averaging 250 µg kg^{-1} with a range of 50-1000 µg kg^{-1} (Halliday and Kazaure 1967, McDonald 1976).

Samples of groundnuts for aflatoxin analyses were collected in 1973 from major storage sites, from lots delivered to oil mills, and from vendors in the Kano city market (Crowther 1973). Aflatoxin levels of 35 samples taken from storage sites ranged from 50 to 200 µg kg^{-1} (mean, 130 µg kg^{-1}). The range in levels in 51 samples from groundnuts delivered to oil mills was 50 to 1000 pg kg^{-1} (mean, 150 µg kg^{-1}). Seven samples purchased from vendors had levels ranging from 50 to 60 µg kg^{-1}

Handpicked selected groundnuts (HPS) from Kano State rarely contain more than 50 µg kg^{-1} aflatoxin. The occurrence of a number of highly toxic samples of HPS nuts in consignments tested by the NSPRI in Kano in 1971 led to that Institute, in cooperation with the Institute for Agricultural Research (1AR), carrying out a survey of groundnuts held by Licensed Buying Agents and the Northern States Marketing Board. Of 98 samples collected from sites within live of the northern states, 94 were contaminated with aflatoxin. Fifty-four per cent of the samples had levels of aflatoxin in excess of 30 µg kg^{-1} (McDonald 1976). This bad situation in 1971 was attributed to the sudden and early ending of the rains in that year. It was noted that many farmers left their crops in the ground long alter the rains had ended, a practice known to favor aflatoxin development.

In the riverain area, however, HPS groundnuts in 1973 contained > 70 µg kg^{-1} aflatoxin. Rejected nuts contained about 400 µg kg^{-1} aflatoxin and were sold in the local market (Crowther 1973).

Groundnut cake from 5 oil mills in Kano were also sampled and analyzed. Aflatoxin levels were between 270 and 400 µg kg^{-1} and averaged 380 µg kg^{-1}, excluding one sample that had > 1000 µg kg^{-1}. This high level was in a sample from a mill which received groundnuts from the riverain zone. In comparison, analyses of groundnut cake from these oil mills in 1966 showed an average aflatoxin content of 250 pg kg^{1} and a range from 10 to 1000 µg kg^{-1} (Crowther 1973). Nwokolo and Okonkwo (1978) reported aflatoxin B_1 in groundnut cake at levels between 600 and 1100 µg kg^{-1}, and in crude groundnut oil from 200 to 500 pg kg^{-1}.

Investigations by Abalaka and Elegbede (1982) revealed that edible oils including groundnut oil were significantly contaminated with aflatoxins.

Recently. Akano and Atanda (1990) reported aflatoxin levels in groundnut cake products sampled from four markets (Shasha, Bodija, Dugbe and Oje) in Ibadan, Oyo State. All samples tested during April-November 1988 contained aflatoxin; levels of allatoxin B_1 ranged from 20 to 455 µg kg^{-1}.

Senegal

Groundnut is the major cash crop in Senegal, and represents from 1/3 to 1/2 of the Senegalese exports. Over 1 million tons are produced annually; most of the crop produce is crushed for oil and nearly all of the groundnut cake is exported for animal feed. Most of the groundnuts are grown in the West-Central part of the country north of the Gambia, with some grown in the north, east and southern areas. Rainfall averages from 900 mm in the south to 400 mm in the north of this area. Senegal has a hot, generally dry climate. Rainfall occurs between July and early October and dry weather prevails for the remainder of the year. Groundnuts are harvested during the first week of October.

Aflatoxin contamination of groundnut is a major problem in Senegal. The

significance of the problem in relation to public health and to the future of the export trade has been recognized.

Aflatoxin content of groundnut cake from Zigninchor ranged from 70 to 350 µg kg^{-1} in 1970; from 140 to 700 µg kg^{-1} in 1971; from 70 to 140 µg kg^{-1} in 1973 and from 140 to 750 µg kg^{-1} in 1973 (Report de Mission dans les Regions du Sine-Saloume et de la Casamance. 1975. Institute de Technologie Alimentaire, Senegal).

Analyses of 46 crude oil samples collected from the groundnut basin (Kaolack and Gossas areas) revealed that 78% of samples contained aflatoxin B_1. The average aflatoxin concentration of the samples from Kaolack and Gossas was 1.5 µg kg^{-1} (maximum 4 µg kg^{-1}) and 9 µg kg^{-1} (maximum 35 µg kg^{-1}), respectively (Pettit 1989).

Southern Africa

Malawi

Groundnut is an important cash and food crop in Malawi. The main groundnut cultivars in Malawi are long-duration, large- seeded confectionery types. They are grown in the plateau areas (>1000 m altitude) which are cool and moist in the growing season. Some cultivars are cultivated for oil extraction in the medium altitude areas (<500-1000 m altitude) along the lake shore where it is hot and humid with erratic rainfall in the growing season. In the main groundnut-producing areas, there is no rain after April and the dry weather favors rapid drying of pods.

The Agricultural Development and Marketing Corporation (ADMARC) is the main marketing organization for all agricultural produce in Malawi. ADMARC buys shelled confectionery groundnuts and in-shell oil nuts from farmers. Malawi exports 25000 to 40000 tons of HPS nuts every year to the U.K.. Germany. Holland, and South Africa. ADMARC monitors aflatoxin levels in all confectionery export consignments. Generally. Malawean HPS groundnuts contain low levels of aflatoxin *(*<5 µg kg^{-1}), chiefly because the groundnuts are hand-graded.

Some 38000-60000 tons of nuts are crushed for oil every year; 2280-4800 tons are processed as groundnut cake meal. Anatoxins (73-300 µg kg^{-1}) occur in oil nuts (Personal communication-Mr. Mkandawire).

Mozambique

Groundnuts are produced in Mozambique for local consumption and as a cash crop for export

In a survey of foods conducted in the Inhambane district, groundnuts provided the main source of dietary protein but also contained the main source of aflatoxin in the diet. Analysis of 153 samples of groundnuts collected from households revealed a mean aflatoxin level of 1036 µg kg^{-1}. In-shell groundnuts contained less aflatoxin than shelled nuts. The average level in the in-shell nuts was 233 µg kg^{-1}, whereas the shelled nuts contained an average of 1838 µg kg^{-1}. However, aflatoxin levels in prepared foods were relatively low as compared with those found in stored groundnuts, suggesting a considerable degree of selection by the housewife prior to the preparation of food (Van Rensburg et al. 1975).

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 µg kg^{-1}, aflatoxin B_1 being the main contaminant (Casadei et al. 1982).

Swaziland

Groundnut is an important source of dietary protein in Swaziland (Peers et al. 1976).

In an epidemiological study of primary liver cancer, samples of food and prepared meals were collected from homes in all parts of Swaziland during 1972 to 1973 and analyzed for aflatoxin (Peers et al. 1976). Of 93 samples of groundnuts, 11 contained aflatoxin. Groundnuts stored for five or six months contained about live limes as much aflatoxin as those tested shortly after entering storage.

Zaire

Only a single report is available and this indicated aflatoxin levels in excess of 250 µg kg^{-1} in low quality nuts (Brudzynski et al. 1977).

Zambia

Groundnut is an important food and cash crop in Zambia. Most of the groundnuts are consumed locally, the quantity exported fluctuating considerably. Very little of the crop is used for edible oil. Monitoring kernels for aflatoxin contamination has been limited to export quality control.

Quality control of export materials is handled by the Eastern Co-operative Union (ECU) Limited, Chipata, a parastatal organization handling all Zambian groundnut kernel exports. From 1977/78 to 1985/86, a total of 28410 samples were analyzed;

a mean of 6.3 % samples had aflatoxin levels of > 5 µg kg^{-1} (Kannaiyan et al 1989).

Zimbabwe

Groundnuts are a controlled product in the country and must be sold through the Grain Marketing Board (GMB). Estimated deliveries to the Board are 20000 tons annually, of which some 5000 tons are exported annually as confectionery nuts, and about 2000 tons of confectionery nuts are consumed in the country. The remainder of the delivered nuts arc crushed for oil.

Investigations of aflatoxin contamination of groundnuts were started in 1962 following the discovery by the Veterinary Research Laboratories of two cases of aflatoxicosis in poultry (Bushnell 1965). There was a low incidence of aflatoxin contamination in the 1962/63 Southern Zimbabwean groundnut crop (50-250 µg kg^{-1} aflatoxin B_1), whereas with the 1963/64 crop, an appreciable number of samples were contaminated with > 250 µg kg^{-1} aflatoxin B_1. Since then, results of a 10-year survey have been published (du Toit 1977). From 1964/65 and 1973/74, a total of 4667 samples were analyzed; a mean of 67 % samples had aflatoxin levels ranging from 5-50 µg kg^{-1} (38 % samples), 50-250 µg kg^{-1} (20 %), 250-1000 µg kg^{-1} (8 %) and > 1000 µg kg^{-1} (1 %). The levels of aflatoxins in groundnuts varied considerably from season to season and were highest in the 1972-73 season and lowest in the 1966-67 season. In general, aflatoxins B_1 and G_1 were found in contaminated samples; contamination with aflatoxin G_1 was lower than that for B_1 in practically all cases, but showed similar seasonal variations (du Toit 1977). Some interesting points were raised enabling the prediction of aflatoxin contamination in the Zimbabwean groundnut crop from consideration of differing climatic conditions and agronomic practices. Aflatoxin contamination probably occurred prior to harvest. In seasons where end-of-scason rainfall was low, aflatoxin contamination was high, while more favorable growing seasons had lower levels. Variations of aflatoxin levels in groundnuts from individual GMB depots were observed. GMB depots drawing their groundnuts from the cooler, high-rainfall areas had groundnuts with less aflatoxin contamination than those serving the hotter and drier areas (du Toit 1977).

Siwela and Caley (1989) reported results of surveys carried out from 1982/83 to 1986/87. Aflatoxins were analyzed in groundnuts stored for either local or export sales. Four hundred and tony-one samples of seven groundnut varieties were collected for analysis. Sixty-eight per cent of the samples had total levels of aflatoxins B_1 and G_1 of up to 25 µg 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 µg kg^{-1} aflatoxin in 79 % of the samples, plus aflatoxin G_1 in 67 % of the samples.

South Africa

A survey over the years 1963-1964 of groundnuts and groundnut products from various parts of the Republic of South Africa showed that the samples obtained from the North-Western Transvaal (Bushveld areas) were the most heavily contaminated with aflatoxin (aflatoxin content > 2000 µg kg^{-1}). The samples from the Northern Cape Province, Orange Free State and Natal, except for 16 samples with 100-2000 µg kg^{-1}, were free of aflatoxin (Sellschop et al. 1965). A high degree of aflatoxin contamination in the groundnut samples from the North-Western Transvaal was attributed mainly to the damage caused to the maturing groundnut pods by termites, and to protracted droughts followed by late rains. Eleven of 16 groundnut cake samples (implicated in livestock deaths) contained > 2000 µg kg^{-1} aflatoxin. Seventeen of 101 peanut butter samples tested contained aflatoxin; 12 had aflatoxin contents of between 100 and 500 µg kg^{-1} and 5 had 25 µg kg^{-1} (Sellschop et al. 1965).

A study reported in 1988 of groundnuts and peanut butter samples collected from supermarkets revealed only low levels of aflatoxin (up to 10 µg kg^{-1}) in about 30% of the samples (Lotter and Krohm 1988).

Aflatoxins in Groundnuts and Groundnut Products in Asia

Countries in Asia that have reported detection of aflatoxins in groundnuts and groundnut products are India, Indonesia, Malaysia, Pakistan, the People's Republic of China, the Philippines, Taiwan and Thailand.

India

India is the world's largest producer of groundnut, with an annual production of between 6 and 7 million metric tons. Most of the groundnuts produced are consumed locally, with only 5% being exported. Groundnuts are the primary source of vegetable oil. Only 1.5% of the production is consumed directly as confectionery and other products.

Limited surveys have been conducted to determine aflatoxin levels in groundnuts and groundnut products in several groundnut- producing states of India. A study reported in 1965 of groundnut samples collected from six coastal districts of Andhra Pradesh State showed that 36 (12.5%) of the 288 samples were contaminated with aflatoxin (Rao et al. 1965). Toxic samples were more prevalent in some districts than others. Samples commonly included 6 to 14% of pods with shell damage, but this condition was not related to toxicity. Aflatoxin levels ranging from 1000 to 5000 µg kg^{-1} were found.

A 1965-1967 survey of groundnuts and groundnut cake samples obtained from districts of Gujarat, Andhra Pradesh and Tamil Nadu States showed that 20-40% of the groundnuts and 82% of the cakes contained fairly high levels of aflatoxin (Anonymous 1967).

Nearly 50% of 500 samples of groundnuts collected in a 1967-68 survey of the West Coast crop contained aflatoxin Bl at levels between 100 and 250 µg kg^{-1} (Wagle 1970).

In a survey of foods and animal feeds conducted in Tamil Nadu, aflatoxins were detected in 4 of 91 samples of groundnut candies and in 28 of 134 samlpes of groundnut cake (Neelakantan et al. 1981).

Nagaraj and Kumar (1986) reported aflatoxin levels in kernel samples of seven Virginia groundnut cultivars collected from the 1982 rainy season crops grown at four locations, viz., Chintamani, Jalgaon, Khargon and Junagadh. All cultivars were contaminated, with levels of aflatoxin B_1 ranging from 0.8 to 65.8 µg kg^{-1}. Junagadh and Chintamani samples had the highest levels of allatoxin, probably because of unfavorable postharvest drying conditions.

In Uttar Pradesh State, most samples of raw and roasted groundnuts tested were toxic, levels of aflatoxin B_1 ranging from 33-440 µg kg^{-1} in raw groundnuts and from 10-85 µg kg^{-1} in roasted groundnuts (Singh et al. 1982). Only 5 of 26 samples of groundnuts collected from local markets in Maharashtra State showed aflatoxin contamination (Patil and Shinde 1985). A study of groundnut samples collected from Ahmednagar city and nearby villages revealed that 75 samples were contaminated with aflatoxin B_1. Among the contaminated samples, 37 had aflatoxin B_1 in excess of 120 µg kg^{-1} (Kshemkalyani and Patel 1988).

Various studies have reported the occurrence of aflatoxins in commercial groundnut cake used for cattle feed in Andhra Pradesh, Gujarat, Tamil Nadu and Punjab States (Fulsoundar and Shukla 1978, Ghewande et al. 1989, Nusrath and Nahdi 1983, Nahdi and Nusrath 1985, Choudary and Rao 1982, Balasubramanian 1985. Patel et al. 1981, Pal et al. 1979, Reddy et al. 1986, Phutela and Kabra 1979). In a survey of feeds conducted in Tamil Nadu, aflatoxin B_1 was detected in 66% of 101 samples. Of the 13 feed ingredients analyzed, only groundnut oilcake contained aflatoxin B_1 and that at levels between 330 and 2670 µg kg^{-1} (Balasubramanian 1985). Aflatoxins (1400-3600 µg kg^{-1}) have been reported to occur in groundnut cake samples implicated in aflatoxicosis in livestock and poultry birds (Choudary and Rao 1982).

A few studies have highlighted the occurrence of aflatoxins in unrefined groundnut oil samples in the states of Karnataka, Uttar Pradesh and Andhra Pradesh (Dwarakanath et al. 1969, Amla et al. 1974, Peers and Linsell 1973, Sreenivasamurthy 1975. Pal et al. 1979, Giridhar and Krishnamurty 1977); levels of aflatoxins were in the range of 20-400 µg kg^{-1} (Dwarakanath et al. 1969, Amla et al. 1974). Some samples contained > 2000 µg kg^{-1} (Sreenivasamurthy 1975, Giridhar and Krishnamurty 1977). Nearly 70%) of the contaminated oil samples from Uttar Pradesh contained in excess of 2000 µg kg^{-1} aflatoxin B_1 (Pal et al. 1979). Refined groundnut oil or hydrogenated oil samples did not contain any aflatoxins.

Indonesia

Groundnut is a major food legume in Indonesia, with annual estimated production of 0.5 million tons of unshelled nuts from 550000 ha. Although Indonesia exports some of its groundnuts, most are used domestically, primarily for human consumption as boiled nuts or in other food products. There is also a delicacy called "oncom" made by fermenting groundnut presscake (bungkil), a by-product of groundnuts that have been pressed to extract the oil (Machmud 1989).

Surveys for aflatoxins in groundnuts and groundnut products were initiated in 1970. Of the marketable groundnuts tested, around 70% were contaminated with aflatoxin at levels from 40 to 4100 µg kg^{-1} (Muhilal et al. 1971). Interestingly, groundnut samples obtained from distributors or subdistributors contained virtually no aflatoxin, while those obtained from the retailers contained considerable levels of aflatoxin. It was also reported that almost all the groundnuts and groundnut products (groundnut oil, "oncom", and peanut butter) sampled from markets, stores, and food manufacturers contained aflatoxins (from trace amounts to over 1000 µg kg^{-1}), except in fried groundnuts in which aflatoxin was not detectable. Manufacturers of groundnut food products tend to use the lower grades of groundnuts for products in which the appearance of the kernels is not important

Analysis of groundnuts exported in 1975/76 showed some consignments of exported kernels (990 tons) to contain aflatoxin at over 5 µg kg^{-1} (Muhilal and Nurjadi 1977). Most Indonesian groundnut exports are to the Netherlands; those containing more than 5 µg kg^{-1} of aflatoxin B_1 are not acceptable.

The humid tropical conditions of Indonesia and the prevailing agricultural practices favor molding of foodstuffs, and groundnuts are commonly infested by *A. flavus,* the mold responsible for aflatoxin production (Husaini et al. 1974).

The People's Republic of China

During 1973-77, 1689 samples of groundnut kernels and 1172 samples of groundnut oil from 24 provinces were analyzed for contamination with aflatoxin B, (PHI

1983). The percentages of samples contaminated were 26.3% for kernels and 47.3% for oil. The percentage of contaminated samples decreased with increase in latitude, being high in southern China (41.7% in groundnuts samples; 68.1% in groundnut oil samples), moderate in the Yangtze valley, and negligible in northern China (no contamination in groundnuts; 4.7% in groundnut oil samples) (PHI 1983, Daren 1989). In southern China, the warm and humid weather favors postharvest aflatoxin contamination. Daren (1989) reported aflatoxin B_1 contamination in various samples of stored groundnuts from several provinces, with levels of aflatoxin in the range of 3-500 µg kg^{-1}.

Malaysia

High levels of aflatoxin in samples of groundnuts and peanut butter have been reported (Mat and Nazarifah 1986). Of 16 samples of groundnut oil (9 of refined oil and 7 of unrefined oil) from local markets analyzed, five samples of unrefined oil contained aflatoxin at levels of 8-16 µg kg^{-1} (Chong and Beng 1965).

Nepal

Only limited surveys have been carried out to determine aflatoxin levels in groundnuts. During 1980-1986, 764 samples of various food commodities from hills and Terai region were analyzed for aflatoxin contamination. One hundred and forty-eight samples of different feed ingredients from different Feed Industries of Kathmandu area were also aoalyzed. Of 67 samples of groundnuts analyzed, 2 were heavily contaminated with aflatoxin. Peanut butter samples (31.7 %) were also contaminated with aflatoxin (Karmacharya 1984, 1988). Maize and groundnut cake, used as feed ingredients, were mostly contaminated.

Pakistan

A few researchers have conducted surveys for aflatoxin contamination in groundnuts and groundnut products (Begum et al. 1985, Shah et al. 1981, Sheikh et al. 1983). In a survey of various foodstuffs, Shall et al. (1981) found aflatoxins in one of 16 samples of roasted groundnuts tested (total aflatoxin content 800 µg kg^{-1}). Begum et al. (1985) reported that all samples of roasted groundnuts obtained from local markets in Khuzdar were contaminated with aflatoxins. Many samples from local markets in Lahore also showed aflatoxin contamination (aflatoxin content 200-800 µg kg^{-1}). None of the samples from Quetta, Murree, and Rawalpindi had any detectable aflatoxin contamination.

The Pakistan Council of Scientific and Industrial Research (PCS1R) Central Laboratories, Karachi, reported aflatoxin levels of up to 300 µg kg^{-1} in 150 samples of groundnuts collected from various parts of Sind and Baluchistan provinces (Rana 1989).

Philippines

Groundnuts are grown on a small scale throughout the Philippines, but the primary production area is in northern Luzon in the Cagayan and Isabelle provinces. There arc about 90000 ha grown annually with a total production of 45 000 tons. Over 90%. of the production is used as human food, mainly in the form of snack items and peanut butter.

Surveys for aflatoxins in groundnuts and groundnut products were initiated in 1968 by the Food and Nutrition Research Institute (FNRI), since then, there has been regular monitoring. All processed groundnuts sampled in the Philippines contained levels of aflatoxin ranging from trace amounts to more than 1000 µg kg^{-1} (Santamaria et al. 1985). Results of the 1985 surveys showed that the farm level aflatoxin significantly increased from harvest to farm storage during the main cropping season (Quitco et al. 1989). At harvest, groundnuts contained, on average, 3.16 µg kg^{-1} aflatoxin. Groundnut samples taken from middlemen contained an average of 35 µg kg^{-1} aflatoxin, but samples from the wholesalers' newly procured groundnuts contained an average of 188 µg kg^{-1} aflatoxin. Groundnut samples stored in warehouses for more than 3 months contained 275 µg kg^{-1} aflatoxin. At the processors' level, raw materials for confectionery groundnuts (roasted and fried) contained 7.73 µg kg^{-1} aflatoxin while those intended for peanut butter contained 17.13 µg kg^{-1}, and rejected groundnuts had 120 µg kg^{-1} (Quitco et al. 1989).

Taiwan

In a survey of foods for aflatoxin in 1966, samples of groundnuts from stores of three of eight oil mills surveyed contained aflatoxin B_1 levels in the range of 40 to 430 µg kg^{-1}. Groundnut cake samples collected from 4 of 12 oil mills contained aflatoxin B_1 at levels of from 80 to 290 µg kg^{-1} (Tung and Ling 1968).

Thailand

Groundnut is an important crop in Thailand, with an annual production of around 137000 tons. The major growing areas are in the North and Central Plains and in the Northeast. Groundnuts are utilized in various types of confectionery products and for oil. Oil cake is used for animal feed.

Aflatoxin levels in groundnuts were determined in a survey of all Thai foods during a 2-year period from 1967 to 1969. Food samples were collected from over

100 towns and villages during both rainy and dry seasons. Of 219 samples of groundnuts analyzed, aflatoxin was detected in 49% of samples at an average level of 1530 µg kg^{-1}. The highest level was 12300 µg kg^{-1}. Contamination was most frequent in samples collected in the rainy season, when the average concentration of total aflatoxins in all groundnut samples was almost twice the level observed in the dry season (Shank et al. 1972).

Several other studies have reported aflatoxin contamination (aflatoxin levels 10-1120 µg kg^{-1}) in samples of groundnuts and peanut butter from commercial sources (Thasnakom 1976, Sripathomswat and Thasnakorn 1981, Sommartya et al. 1988, Imwidthaya et al. 1987).

Israel

Groundnut is an important crop in Israel where approximately 30 000 metric tons of groundnuts are produced annually.

Only limited surveys have been conducted to determine aflatoxin levels in groundnuts. During 1964-1967. 186 samples of freshly harvested groundnuts and 419 samples of stored kernels were analyzed for aflatoxin contamination (Joffe 1970). Only 5 samples of fresh kernels contained aflatoxin, while 56 samples of stored kernels were contaminated with aflatoxin; levels of aflatoxin were low in most contaminated samples. Only 6 samples (5 of stored kernels and one of fresh kernels) contained aflatoxin levels in excess of 50 µg kg^{-1} (range 60-125 µg kg^{-1}).

Jordan

Only a single report is available and this indicated aflatoxin levels in the range of 98-1056 |ig kg^{-1} in only 3 of 40 groundnut samples analyzed (Jarrar et al. 1983).

Tunisia

Of 65 samples of groundnuts analyzed, only 4 contained aflatoxin and these had levels between 6 and 46 µg kg^{-1} (Boutrif et al. 1977).

Japan

There is little mention of aflatoxin contamination of locally produced groundnuts. Most concern has been given to aflatoxin contamination of imported groundnuts and groundnut products.

In a survey of commercial foods conducted in Tokyo during 1982-1986, 3 samples of peanut butter were contaminated with aflatoxin (Tabata and Kamimura 1988).

Aflatoxins in Groundnuts and Groundnut Products in South America

Two countries, Brazil and Argentina, have reported analyses of groundnut and groundnut products for aflatoxin.

Argentina

Groundnut is an important food and cash crop in Argentina. The main groundnut-growing area is in Cordoba province. About 300 000 metric tons of shelled groundnuts are produced annually. About 75% of the crop is used for edible oil production.

Only a single report is available and this indicated aflatoxin levels in excess of 50 µg kg^{-1} in groundnut samples from Cordoba markets (Jodral 1974).

Brazil

Groundnut is an important crop in Brazil where approximately 500 000 tons of groundnuts are produced annually. The principal groundnut-growing area is in the state of Sao Paulo. Most of the crop is used for edible oil production. About 10% of the produce is consumed as roasted and salted nuts.

Several surveys have been conducted to determine aflatoxin levels in grounduts and groundnut products in Brazil. Very high levels (> 1000 µg kg^{-1}) and incidences (60-90%) of aflatoxin contamination in groundnuts and groundnut products (groundnut cake, groundnut bran and groundnut flour) have been reported (Fonseca 1968, 1976 a. b, c, Fonseca et al. 1983, Sabino et al. 1982. Scussel and Rodriguez-Amaya 1985, Sabino et al. 1989). Some samples contained > 10000 µg kg^{-1} (Fonseca 1976b, c).

Sabino (1989) reported aflatoxin levels in groundnuts from the rainy season and the dry season crops in the West, Northwest and Northeast regions of Sao Paulo State. The survey of groundnuts from the rainy season crop in West Sao Paulo State showed that 61.2% of 152 samples were contaminated with aflatoxin; 9% samples contained levels above 5000 µg kg^{-1} of aflatoxins B_1 and G_1, and the average level of total aflatoxins in all contaminated samples was 2391 µg kg^{-1}. Analysis of 111 samples from northeast Sao Paulo State showed mat 44% were contaminated with aflatoxin, 7% contained > 5000 µg kg^{-1} aflatoxins B_1 and G_1. and the average level

of total aflatoxins in all these contaminated samples was 2664 ug kg^{-1}. Of 50 samples from northwest Sao Paulo Slate, 60% were contaminated with aflatoxin; 10% of samples contained > 5000 µg kg^{-1} aflatoxins, and the average level of total anatoxins in all the contaminated samples was 1971 µg kg^{-1}.

Of 83 samples from the dry season crops. 23% had aflatoxin levels of more than 30 µg kg^{-1}. However, a few samples from West Sao Paulo State contained high levels of aflatoxins (991-33500 µg kg^{-1}).

High levels of aflatoxins in the Brazilian groundnuts are attributed to poor postharvest drying conditions.

Aflatoxins in Groundnuts and Groundnut Products in North America

The United States and Mexico are the principal producers of groundnuts in North America. Reports on aflatoxin incidence are available only from the USA.

USA

Groundnut is an important food and cash crop in the USA. The crop is grown in three principal regions of the USA - the Southeast, the Southwest and the Virginia-North Carolina region. Total production is over 2 million tons (in-shell groundnuts). About 300000 tons of shelled nuts are exported. Major domestic use is as human food.

Soon after aflatoxin contamination was discovered in the domestic crop, a program was initiated to control the aflatoxin content in marketed groundnuts. A system of analyses and certification was developed by the U.S. Department of Agriculture (USDA), the Food and Drug Administration (FDA) and the groundnut industry which provides comprehensive data on the crop as farmers' stock groundnuts (in shell) or as shelled nuts.

Eadie and O'Rear (1967) reported that 6.1% of groundnut samples from the 1964-1965 Virginia-North Carolina crop contained aflatoxin, and 23 of 51 samples of peanut butter were contaminated will) aflatoxin. Contaminated lots of groundnuts were reduced to 5.3% in 1965-1966 and to 4.4% in 1966-1967; no peanut butter sample was contaminated in 1965-1966.

Taber and Schroeder (1967) found aflatoxin in farmer' stock groundnuts throughout Texas, but levels of aflatoxin rarely exceeded 50 µg kg^{-1}.

Pettit and Taber (1968) reported results of aflatoxin analyses of 334 groundnut samples from 155 individual farms in South, Evast and North Texas. Twenty-eight per cent of samples were contaminated with aflatoxin. but only 2.7% of these contained levels of aflatoxin > 30 µg kg^{-1}. None of the North Texas samples contained significant levels of aflatoxin. Aflatoxin accumulation was highest in kernels from those areas which had near tropical environmental conditions during the harvest period.

Doupnik (1969) found aflatoxins in 17 of 228 samples of farmers' stock groundnuts collected from six locations in Georgia Stale. Aflatoxins were detected in 2.5% of Segregation I groundnuts (highest quality groundnuts), 12.1% of Segregation II, and 25.7% of Segregation 111 samples, and averaged 22, 264, and 324 µg kg^{-1} total aflatoxin respectively, indicating a strong relationship between levels of aflatoxin and grading factors.

Stoloff (1980) has provided comprehensive data on aflatoxin contamination levels in the US groundnuts over the years 1967- 1978, indicating a general improvement over time in the occurrence of aflatoxin contamination, presumably attributable to improved agronomic, warehousing, and inspection practices.

Aflatoxin levels in peanut butters (various national brands of peanut butter) and confectionery products containing groundnuts were determined in surveys conducted in the 3-year period 1982-1984 (Gilbert and Shepherd 1985). Most of the samples tested contained aflatoxin but at levels well below the voluntary guideline limit for total aflatoxin (30 µg kg^{-1}). Only a few samples contained high levels of aflatoxin (318-345 µg kg^{-1}).

Aflatoxins in Groundnuts and Groundnut Products in Central America

Groundnut production is relatively small in Central America where approximately 100000 tons of nuts (in-shell) are produced annually.

Analyses for aflatoxin have been reported from Gautemala (Campos and Olszyna-Marzys 1979). In a survey of farmers' groundnuts conducted in Jamaica and St. Vincent, 8 of 160 samples had aflatoxin contamination ranging from 8 to 7526 µg kg^{-1} (Singh et al. 1989). Four of the toxic samples were from the St. Elizabeth area of Jamaica, and four from growing areas adjacent to Kingstown, St. Vincent. Analyses of nine groundnut products collected from markets in St. Vincent in 1984 revealed high levels of aflatoxins (97-469 µg kg^{-1}) in 4 products, viz., roasted groundnuts, salted and unsalted groundnuts, and peanut butter (Singh et al. 1989).

Chang-Yen and Felmine (1987), in Trinidad, reported only low levels of

aflatoxin (< 5 μg kg^{-1}) in one of 64 bulk samples of raw groundnuts and peanut butter tested.

Aflatoxins in Groundnuts and Groundnut Products in Australasia

Australia

Groundnut is an important food and cash crop in Australia. The crop is mainly grown in the state of Queensland. In Queensland, the traditional area for groundnut production is the Burnen region, northwest of Brisbane. A second major area is the Atherton Tableland, west of Cairns in the far northern part of the state. Most groundnuts are grown under rainfed conditions. Total production is over 50000 metric tons. Most of the produce is used for confectionery and table purposes. A small quantity of oil is produced from groundnuts unfit for these purposes.

Surveys for aflatoxins in groundnuts were initiated in 1979 (Pitt 1989). High levels of aflatoxin (45-1680 µg kg^{-1}) in groundnuts produced in the Burnett region were reported in 1980 and 1981 (Pitt 1989). Similar levels of aflatoxin occurred in groundnuts sampled from several farms in the Burnett region in 1986 (Pitt 1989).

Aflatoxins (500-22000 µg kg^{-1}) have been reported in groundnut meals implicated in aflatoxicosis in livestock (Bryden et al. 1980, Connole et al. 1981, Ketterer et al. 1982, McKenzie et al. 1981).

Aflatoxins in Groundnuts and Groundnut Products in Europe

Production of groundnuts in Europe is very limited, small areas being planted to the crop in some mediterranean countries, Hungary and the U.S.S.R. Most attention has been given to aflatoxin contamination of imported groundnuts and groundnut products. Countries in Europe with reported detection of allatoxins in imported groundnuts and groundnut products are Czechoslovakia. Finland, Federal Republic of Germany, German Democratic Republic, France, Norway, Spain, Sweden, the U.K., U.S.S.R. and Yugoslavia (Fukal et al. 1987, Korpinen 1971, Pensala et al. 1977, Seibold and Ruch 1977, Yndestad and Underdal 1975, Sanchis et al. 1986, Josefsson et al. 1975. Jewers 1982, Tutelyan et al. 1989, Haberle et al. 1978). All European countries have regulations on the acceptable limits for aflatoxin in groundnuts and groundnut products. Papers relating to regulatory aspects of aflatoxins in trade are discussed in a separate section.

Most of the samples of imported groundnuts and groundnut products analyzed in the Federal Republic of Germany, Spain, Sweden, and Yugoslavia contained aflatoxin levels at lower than the permissible level (5 µg kg^{-1}) . A few samples of groundnuts imported into Finland, Norway, Spain, U.S.S.R., and Yugoslavia contained fairly high levels of aflatoxin (50-3650 μg kg^{-1}) (Korpinen 1971, Pensala et al. 1977, Haberle et al. 1978. Yndestad and Unerdal 1975, Sanchis et al. 1986, Tutelyan el al. 1989).

Testing of groundnut imports into the U.K. from South America and Africa for toxic substances was carried out to determine the cause of the "Turkey X" disease outbreaks of 1960. This led to the discovery of aflatoxins. Since then, analyses of imported groundnuts and groundnut products has been a regular practice in the U.K.

The U.K. surveys showed significant levels of aflatoxin in a number of samples of imported groundnuts (Jewers 1982). Thirty-one of 159 samples examined in the period 1977-1978 had aflatoxin levels in excess of 30 µg kg^{-1} (the maximum permitted level in the U.K.). This emphasized the need for regular surveillance by importers and processors of edible nuts for the presence of aflatoxin.

High levels of aflatoxins in some imported groundnut meals have been reported (Harvey 1980).

Concluding Remarks

Because the incidence of aflatoxin contamination is closely associated with weather conditions, considerable emphasis has been given to effects of rainfall and temperature. Data from regions where aflatoxin contamination has been found could possibly be useful in predicting aflatoxin formation in areas where surveys have not been conducted. Although aflatoxin incidence in groundnuts has been reported from all regions of the world where the crop is grown, only a few satisfactory monitoring and surveillance activities have been conducted. The mycotoxin problem is obviously not limited to these countries, and the incidence and levels of contamination is likely to be even higher in countries that have neglected this problem. It is fortunate that several important groundnut-producing countries have recognized the problem and are supporting research and monitoring/control activities aimed at mitigating the problem. It is significant that several countries are working towards acceptance of only aflatoxin-free products.

5.2 BIBLIOGRAPHY

5.2.1 AFLATOXINS IN GROUNDNUTS AND GROUNDNUT PRODUCTS

656. Abalaka, J.A., and Elegbede, J.A. 1982. Allatoxin distribution and total microbial counts in an edible oil extracting plant. I. Preliminary observations. Food and Chemical Toxicology 20(1): 43-46.

All samples of groundnut kernels, groundnut and cottonseed pellets and groundnut and cottonseed oils (crude and refined) screened contained aflatoxins B_1, B_2, G_1 and G_2, Aflatoxin B_1 was measured quantitatively by thin-layer chromatography and was present in the samples at 9-860 µg kg^{-1}.

657. Anonymous. 1967. Annual Report, 1966-67. Regional Research Laboratory. Hyderabad, India.

From 1965 to 1967, a survey of aflatoxin contamination in groundnuts and groundnut cake was carried out in three districts each of Gujarat, Andhra Pradesh and Tamil Nadu states. About 600 samples were analyzed for aflatoxin contamination. Results showed that 20-40 % of the groundnuts and 82 % of samples of the cake contained fairly high levels of aflatoxin. The contamination was greater in samples collected from Andhra Pradesh and Tamil Nadu states than in samples collected from Gujarat state.

658. Crowther, P.C. 1973. Report to the Government of Nigeria on aflatoxin in groundnut and groundnut products. UNDP Report No. TA 3221. 33 pp.

A survey carried out during 1972-73 in Nigeria on the allatoxin problem in groundnuts and groundnut products has shown that the basic need is to reduce the levels of toxin in the groundnut crop. Present extension procedures giving advice to farmers on allatoxin control are too diffuse and it is suggested that a film be produced dealing with the problem and its control and shown incentives must be worked out to recompense the fanner for carrying out these recommendations. In order to check on the efficacy of these extension and incentive processes, it is suggested that the Nigerian Stored Products Research Institute, Kano should improve their aflatoxin testing facility.

659. Eka, O.U. 1977. Studies on the susceptibility of traditional and imported foods to aflatoxin contamination. West African Journal of Biological and Applied Chemistry 20: 13-20.

Samples of foods and foodstuffs purchased from local markets around Zaria, Nigeria, were investigated for aflatoxin content. The findings indicated that a large number of them were susceptible to contamination by aflatoxin since they could support both the growth of *Aspergillus flavus* and the production of aflatoxin by aflatoxin-producing strains of the fungus. The most susceptible foods were groundnut based foods, rice meals and cereal based foods such as koko and dan wake. The need for routine screening of certain local foods to determine the actual level of aflatoxin in them is suggested.

660. Fonseca, H. 1968. [Contribution to the study of the occurrence of aflatoxin in groundnut *(Arachis hypogaea* L.) meal, bran and flour in Sao Paulo State.]. Contribuicao ao estudo da ocorrencia de aflatoxina em tortas, farelos e farinhas de amendoim *(Arachis hypogaea* L.) no estado de Sao Paulo. Anais de Escola Superior de Agncultura "Luiz de Queiroz" 25: 47-49.

The aflatoxins B and G contents of 264 samples of groundnut meal, bran and flour from 40 oil-extracting mills were determined. All the samples were contaminated, with allatoxin B_1 content being >1000 µg kg^{-1} in 89.78 % of the samples. Allatoxin levels were much higher in samples from 'wet weather harvests' in March and May than from dry weather harvests' in July and September. In one region of the Sao Paulo Slate, allatoxin G level was high, possibly indicating a different strain of *Aspergillus flavus* or a high zinc content in the soil enabling the fungus to produce more aflatoxins.

661. Fonseca, H. 1976a. | Study of aflatoxin in groundnut from harvest to processing in the Monte Alto region, Sao Paulo.], listudos da aflatoxina no amendoim, da colheita a industrializacao, na regiao de Monte Alto, S.P. Anais da Escola Superior de Agncultura "Luiz de Queiroz" 33: 375-384.

The presence of aflatoxin in groundnuts was studied at the time of sale by the grower, during storage, and after oil extraction. Of 40 samples of groundnut and 20 of groundnut flour, 90 % contained aflatoxin. Allatoxin B_1 at >1000 µg kg^{-1} was found in 90 % of the samples, the level rising from the time of sale to milling, then falling after oil extraction.

662. Fonseca, H. 1976b. [Study of aflatoxin in groundnut from harvest to processing in the Santa Adelia region, Sao Paulo.]. Estudo da aflatoxina no amendoim, da colheita a industrializacao, na regiao de Santa Adelia, A.P. Anais da Escola Superior de Agncultura "Luiz de Queiroz" 33: 385-393.

The presence of aflatoxin in groundnuts was examined at the time of sale by the grower, during storage, and after oil extraction. Of 40 samples of groundnut and 20 of groundnut flour, all but one contained aflatoxin. Aflatoxin B_1 levels were very

high in 31.6 %, and high in 55 % of the samples. Aflatoxin levels fell from the time of sale to milling. Five samples had >10 000 µg kg^{-1}.

663. Fonseca, H. 1976c. [Study of aflatoxin in groundnut from harvest to processing in the Fernandopolis region. Sao Paulo.]. Estudo da aflatoxina no amendoim, da colheita a industrializacao, na regiao de Fernandopolis. S.P. Anais da Escola Superior de Agricultura "Luiz. de Queiroz" 33: 395-405.

The presence of aflatoxin in groundnuts was examined at the time of sale by the grower, during storage, and after oil extraction. Of 40 samples of groundnut and 20 of groundnut flour, all but one contained aflatoxin. Aflatoxin levels were very high in 38.3%. and high in 51.7% of the samples. Aflatoxin levels rose from the time of sale till milling, then fell. Five samples had >I0 000 µg kg^{-1} aflatoxin.

664. Fonseca, H., Nogueira, J.N., Graner, M., Oliveira, A.J., Caruso, J.G.B., Boralli, C, Calori, M.A., and Khatounian, C.A. 1983. Natural occurrence of mycotoxins in some Brazilian foods 11. Pages 53-54 in Proceedings of the Sixth International Congress of Food Science and Technology. Vol. 3 (McLoughlin, J.V., and McKenna, B.M., eds.). Boole Press Ltd., Dublin : Irish Republic.

Surveys were carried out for two years to determine the natural occurrence of mycotoxins (aflatoxin, ochratoxin and zearalenone) in some common Brazilian foods including raw and salted roasted groundnuts, pacoca (a very popular groundnut candy), peanut butter, maize, soybean, cured cheese, salami and copa (cured and dried pork). Eight characteristic regions. Vale do Paraiba, Mogiana, Paulista Velha, Araraquarense, Noroeste, Paulista Nova, Sorocabana and Vale do Ribeira/Litoral Sul, were surveyed, and 742 samples were analyzed. Estimation of toxins was made by thin- layer chromatography (TLC) by comparison with standards. Six samples of corn (4.7 %) were contaminated with aflatoxin. ranging from 41 to 2000 (ig kg^{+1} of aflatoxin B_1; 81 samples of pacoca (63.3 %), ranging from < 20 to 1187 µg kg^{-1}; 80 samples of peanut butter (62.5 %), from < 20 to 275 µg kg^{-1}; 17 samples of raw groundnuts (17.7 %), from 19 to 3125 µg kg^{-1}; and 9 samples of roasted salted groundnuts (9.4 %) from < 30 to 4250 *µg* kg^{-1}. From the first to the second year, the percentage of contaminated samples increased slightly for raw groundnuts (from 15.6 to 18.8 *%)* and decreased for pacoca (67.2 to 59.4 *%)* for roasted salted groundnuts (12.5 to 7.8 *%),* corn (6.2 to 3.1 *%),* and for peanut butter (84.4 to 40.6 %). The average content of aflatoxin of the contaminated samples increased for raw groundnuts (from 166.7 to 641.7 µg kg^{-1}) and roasted salted groundnuts (410 to 980 µg kg^{-1}) and decreased tor pacoca (165.6 to 108.8 µg kg^{-1}), peanut butter (91.3 to 50.4 *µg* kg^{-1}), and corn (853.3 to 700 µg kg^{-1}).

665. Gilbert, J., and Shepherd, M.J. 1985. A survey of aflatoxins in peanut butters, nuts and nut confectionery products by HPLC with fluorescence detection. Food Additives and Contaminants 2(3): 171-183.

A preliminary survey of peanut butter in 1982 indicated that 31 of 32 samples of major national brand-named products contained aflatoxin B_1 up to 10 µg kg^{-1}, and that 59 % of those were below the limit of detection (2 µg kg^{-1}). Of 25 peanut butter samples from specialist 'Health Food' outlets, 64 % contained up to 10 µg kg^{-1}, and the remainder contained 16-318 µg kg^{-1}, with one sample having 345 µg kg^{-1} aflatoxin. Surveys of 'Health Food' products in 1983 and 1984 confirmed that manufacturers were still experiencing some difficulty in complying with the 30 (ig kg^{-1} voluntary guideline limit for total aflatoxin. In 1984. 228 retail samples of nuts and nut confectionery products comprising groundnuts (shelled, unshelled, roasted and salted), mixed nuts, almonds (both unblanched and ground), Brazil nuts (in shell), hazelnuts (in shell), chocolate-coated groundnuts, peanut britde and coconut ice were examined. Results showed that 74 % of the samples contained aflatoxin B_1 at up to 0.5 µg kg^{-1}, and 3.1 %, mainly groundnuts and Brazil nuts, exceeded the guideline tolerance for total aflatoxin. Total aflatoxin was greatest in unshelled groundnuts, 4920 µg kg^{-1}, and in a composite sample of visibly molded Brazil nuts, 17926 µg kg^{-1}.

666. Hendrickse, R.G., Coulter, J.B.S., Lamplugh, S.M.. Maclarlane, S.B.J., Williams, T.E., Omer, M.I.M., and Suliman, G.I. 1982. Aflatoxins and Kwashiorkor : a study in Sudanese children. British Medical Journal 285(6345): 843-846.

Blood and urine samples from 252 children were investigated for their aflatoxin content by high-performance liquid chromatography (HPLC). Aflatoxins were detected more often and at higher concentrations in sera from children with kwashiorkor than in other malnourished and control groups. Aflatoxicol was detected in the sera of children with kwashiorkor and marasmic kwashiorkor but not in the controls and only once in a marasmic child. These differences were significant. Urinary aflatoxin was most often detected in children with kwashiorkor but the mean concentration was lower than in the other groups. Aflatoxicol was not detected in urine in any group. It is suggested either that children with kwashiorkor have greater exposure to aflatoxins or that their ability to transport and excrete aflatoxins is impaired by the metabolic derangements associated with kwashiorkor. Aflatoxins were detected by HPLC in groundnuts (B_1 59666 µg g^{-1}, B_2 370, G_2 23), limed groundnuts (B_1 3517, G_1 2816, G_2 6), chickpeas (B_1 876 µg g^{-1}), dried okra (G_2 12675 µg g^{-1}) and peanut butter (B_1 26300, B_2 9720, G_1 84500 µg g^{-1}). obtained from local markets.

667. Hermana. 1973. Studies on aflatoxin in Indonesia. Bogor, Indonesia : Nutrition Research Institute. 9 pp.

This report reviews aflatoxin contamination problem in groundnuts and groundnut products in Indonesia. Possible relationship between aflatoxin ingestion and hepatocellular carcinoma in humans is also discussed.

668. Husaini, Pang, R.T.L., Tarwotjo, I., and Karyadi, D. 1974. Dietary aflatoxin contents, improving agricultural practices and its possible relation to human hepatocellular carcinoma in Indonesia. Bogor, Indonesia : Nutrition Research Institute. 14 pp.

Aflatoxin contents of some Indonesian foods are reviewed. Aflatoxin contamination problems in groundnuts and groundnut products arc discussed. Possible relationship between dietary aflatoxin and hepatocellular carcinoma in humans is also discussed.

669. Imwidthaya, S., Anukarahanonta, T., and Komolpis, P. 1987. Bacterial, fungal and aflatoxin contamination of cereal and cereal products in Bangkok. Journal of the Medical Association of Thailand 70(7): 390-395.

Samples of cereals and cereal products (150) were collected from various markets in the Bangkok area from July 1983 to April 1985. These included 40 samples of rice products, 50 samples of groundnuts and groundnut products, 20 samples of soybean and soybean products. 20 samples of other beans, and 20 samples of maize. Forty-three samples of groundnuts and groundnut products contained aflatoxins (aflatoxin B, at 40-780 μg kg^{-1} and aflatoxin G_1 at 10-160 μg kg^{-1}). *Aspergillus flavus* and *A. niger* were isolated from these samples. A total of 40 % of a fermented soybean product (tao-chiew) contained aflatoxins (aflatoxin B_2 20 μg kg^{-1} and aflatoxin G_1 20-100 μg kg^{-1}); 20 % of salted bean curd derived from soybean contained aflatoxins (aflatoxin B_1 at 210 pg kg^{-1} and aflatoxin G_1 at 110-270 pg kg^{-1}); 40 % of soybean sauce samples contained aflatoxin B, (20-170 μg kg^{-1}) and aflatoxin G, (40 μg kg^{-1}); 10 % of rice samples contained aflatoxin B_1 (10 μg kg^{-1}) and aflatoxin G_1 (20 μg kg^{-1}). Bacteria found included only *Bacillus* spp. According to the standard safety level for aflatoxin offered by the World Health Organization (WHO), the groundnut, fermented soybean, salted bean curd and maize were not safe for consumption but the rice was sale. This research project also investigated the ability *of A. niger* to produce aflatoxins in glutinous rice and groundnut, and revealed that *A. niger* could produce both aflatoxins B_1 and G_1 in these substrates.

670. Jclinek, C.F., Pohland, A.E., and Wood, G.E. 1989. Worldwide occurrence of mycotoxins in foods and feeds - An update. Journal of the Association of Official Analytical Chemists 72(2):223-230.

This paper reviews the worldwide levels and occurrence of mycotoxins in various commodities since 1976. Comparatively few countries have lowered the acceptable levels for aflatoxins in susceptible commodities. However, intensified efforts are needed to establish control of aflatoxin levels in global food supply, particularly in groundnuts, tree nuts, com, and animal feeds. Extensive deoxynivalenol (DON) contamination of grains, especially wheat, was demonstrated. Co-contamination of grains by *Fusarium* toxins, especially DON and nivalenol, with zearalenone to a lesser extent, was reported. However, more information on co-occurrence of *Fusarium* toxins in cereals should be developed. When contamination of feeds by ochratoxin A was significant, this toxin occurred in swine kidney and smoked meats in high levels. On the basis of occurrence and/or toxicity, patulin and penicillic acid contamination of foods does not appear to be of real concern. More recent developments suggest, however, that expanded monitoring studies of *Alternaria* toxins, moniliformis citrinin, cyclopiazonic acid, penitrem A, and ergol alkaloids are indicated.

671. Krogh, P., and Hald, B. 1969. [Occurrence of aflatoxin in imported groundnut products.]. Forekomst of aflatoksin i importerede jordΦdprodukter. Nord. Vet. Med. 21: 398-407.

Groundnut products imported into Denmark for feed manufacturing were investigated for aflatoxin contents. Fifty-two samples were analyzed for aflatoxin contamination using two methods : thin- layer chromatography (TLC) and a minocolumn method. A few. selected samples were subjected to confirmatory tests including U.V. spectrography and bioassay (duckling test). The investigations showed that 86.5 % of the samples were contaminated with aflatoxin, and 82.7 % contained a content of total aflatoxin > 100 pg kg^{*1}. The highest toxin conteni was found in sample no. 20, with a total content of 3465 μg kg^{-1} and 2520 μg kg^{-1} aflatoxin B_1. Aflatoxin occurred in all kinds of groundnut products (meal, expcllers, cakes and whole nuts). Aflatoxin was detected in batches from each of the 10 groundnut exporting countries (Argentina, Brazil, Congo, Ghana. Indonesia, Kenya, Nigeria, Senegal, the Sudan, and Uganda). All four aflatoxins (B_1, B_2, G_1 and G_2) were only detected in 38 % of the contaminated samples. In the present investigations the sensitivity of the Holaday minicolumn test was in the range of 100-200 μg kg^{-1}.

672. Krogh, P., Hald, B., and Korpinen, E.L. 1970. [Occurrence of aflatoxin in groundnut- and copra-products imported into Finland]. Forekomst af aflatoksin i jordnΦd- og kokosprodukter indf0rt til Finland. Nord. Vet. Med. 22: 584-589.

Groundnut products and copra products imported into Finland for feed manufacturing as well as for other purposes, were investigated for aflatoxin content. Twenty-four samples of groundnut products, two samples of feedstuff containing groundnut, and 16 samples of copra products were analyzed using a thin-layer chromatography (TLC) method. Seventy-five % of the groundnut samples were contaminated with aflatoxin, and 38 % contained more than 100 μg kg^{-1}. The

highest toxin content was found in sample No. 18. with aflatoxin B_1 content of 2 222 µg kg^{-1} out of a total content of 4 056 µg kg^{-1}. Twenty-three % of the contaminated groundnut samples contained all four aflatoxins B_1, B_2, G_1 and G_2. Among the copra samples, 62.5 % were contaminated, with a comparatively lower aflatoxin content. The highest toxin content was found in sample No. 8, which contained 100 µg kg^{-1}. Aflatoxins G_1 and G_2 were not detected in the copra samples.

673. Kuhn, G. 1986. [On the occurrence of aflatoxin-forming moulds in foods. |. Zum vorkommen aflatoxin-bildender schimmelpilze in lebensmitteln. Nahrung 26(1):31-35.

Aspergillus flavus and other molds were isolated from 900 food samples tested. Aflatoxin was found in groundnut, groundnut products, nuts and their raw products (marzipan).

674. Laub, E., and Woller, R. 1977. [Occurrence of aflatoxins B_1, B_2, G_1 and G_2 in commercial foodstuff samples. |. Vorkommen der aflatoxine B_1, B_2, G_1 und G_2 in Lebensittelproben des handels. Deutsche Lebensmittel-Rundschau 73(1): 8-10.

Of the 1000 food samples examined, aflatoxins were found in groundnut products, maize products, almond products and nuts. Of 316 samples of groundnut, 35 (111 %) contained aflatoxins; 23 (7.3 %) had high levels (> 10 µg kg^{-1} aflatoxin B_1 or > 20 µg kg^{-1} total aflatoxin).

675. Ling, K.H., Tung, C.M., Sheh, I.F., Wang, J.J., and Tung, T.C. 1968. Aflatoxin B, in unrefined peanut oil and peanut products in Taiwan. Journal of Formosan Medical Association 67(7): 309- 314.

A survey of unrefined groundnut oil, kernels, and peanut butter for aflatoxin contamination was carried out during 1966-1967. Twenty-seven samples of unrefined oil from oil mills located in the Yunglin county and 23 unrefined oil from the markets in Taipei were analyzed. Few samples of groundnut kernels and cake were obtained from the same oil mills. Various kinds of peanut butter were purchased from markets in Taipei. Both the incidence and levels of aflatoxin contamination were as follows : peanut butter > groundnut cake > kernels > oil. Only very low levels of aflatoxin were found in unrefined oil. High levels of aflatoxin were found in peanut butter.

676. Lohiya, G., Nichols, L., Hsieh, D., Lohiya, S., and Nguyen, H. 1987. Aflatoxin content of foods served to a population with a high incidence of hepatocellular carcinoma. Hepatology, Baltimore 7(4): 750-752.

A total of 36 samples of foods collected during August 1985. December 1985 and March 1986 and served to mentally retarded clients with a high incidence of hepatocellular carcinoma, were analyzed for aflatoxin. Aflatoxin was not detected (< 5 µg kg^{-1}) by thin-layer chromatography in 35 food samples containing groundnuts, maize, wheat or milk. One peanut butler sample contained 20 µg kg^{-1} aflatoxin. Aflatoxin content of these foods was at or below the level permitted by the Food and Drug Administration. It is concluded that aflatoxin is probably not responsible for liver disease in this population.

677. Machmud, M. 1989. Groundnut aflatoxin problems in Indonesia. Pages 215-222 in Aflatoxin contamination of groundnut : Proceedings of the International Workshop, 6-9 October 1987, ICRISAT Center. India. Patancheru. A.P. 502 324. India : International Crops Research Institute for the Semi-Arid Tropics.

Aflatoxin research in Indonesia was initiated in 1969. Of the marketable groundnuts tested, 60-80 per cent 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 presscake significantly reduced aflatoxin contamination. Clinical 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 x groundnut x environment interactions favoring aflatoxin production.

678. Mat, Isa Awang, and Nazarifah, I. 1986. Aflatoxin contamination in agricultural commodities. Teknologi Makanan, Malaysia 5(1): 54-58.

Aflatoxin contamination in live agricultural commodities namely groundnut, dried cocoa beans, copra, pepper (black and white) and paddy and rice was determined. Shelled groundnuts and their products such as peanut butter and satay gravy were highly susceptible to aflatoxin contamination, but the "menglembu" type groundnut was free of aflatoxin. Copra, dried cocoa beans and pepper (black and white) were the other highly susceptible commodities, but stored paddy and rice were safe but a few samples contained aflatoxin. The extent of contamination in several other commodities is also mentioned and general control methods are discussed.

679. McDonald, D. 1976. Aflatoxins : Poisonous substances that can be present in Nigerian groundnuts. Samaru Miscellaneous Paper 53, Institute for Agricultural Research, Samaru, Ahmadu Bello University, Zaria, Nigeria, 14 pp.

This paper outlines events leading up to the discovery of aflatoxin, describes briefly research done on the groundnut aflatoxin problem in the northern states of Nigeria. considers the implications of aflatoxin in relation to animal and human health, and

discusses measures for elimination of aflatoxin from Nigerian groundnuts, or to at least greatly reduce incidence of aflatoxin.

680. Muhilal, Karyadi, D., and Prawiranegara, D.D. 1971. Study on aflatoxin contents of peanut and peanut products. Gizi Indonesia 2: 162.

Samples of groundnuts and groundnut products obtained from distributors and retailers in different markets in different areas of Bogor. Nine of 16 samples of groundnuts contained aflatoxins at levels from 40 to 4500 µg kg^{-1}. Groundnut samples obtained from distributors or sub-distributors were free of aflatoxins or contained only low levels of aflatoxin. while those obtained from the retailers contained substantial levels of aflatoxins. Six of seven samples of black 'oncom', made of groundnut presscake, contained aflatoxins at levels from traces to 1800 µg kg^{-1}. Two samples of groundnut oil tested contained aflatoxins at levels of 1000 and 1270 µg kg^{-1}. Fried groundnuts prepared in various ways were free of aflatoxins. Groundnut presscake samples contained aflatoxins ranging from traces to 760 (ig kg^{-1}. One peanut butter sample was negative for aflatoxin and the other one contained 40 µg kg^{-1} aflatoxin.

681. Muhilal, and Nurjadi, Ir. 1977. Status report on mycotoxin in Indonesia. Presented at the International Conference on Mycotoxins, 19-27 Sep 1977, Nairobi, Kenya : FAO/WHO/UNEP. 44 pp.

Aflatoxin contamination of various foodstuffs in Indonesia is reviewed. Aflatoxins were determined in various samples of groundnuts and groundnut products obtained in 1970 and 1976 from collectors, sub-distributors and retailers in different markets in Bogor. West Java, Indonesia. Groundnut samples obtained from collectors or sub-distributors were free of aflatoxins or contained only traces of aflatoxins, but those obtained from the retailers contained considerable levels of aflatoxins (traces to 4 100 µg kg^{-1}). However, levels of aflatoxins were lower in retail groundnuts in 1976 (o to 67 µg kg^{-1}) than in 1970. Aflatoxins were also detected in samples of sweetened groundnut cake (average aflatoxin B_1 170 µg kg^{-1}; aflatoxin G_1 83 µg kg^{-1}), groundnut sauce (average aflatoxin B_1 83 µg kg^{-1}; G_1 49 µg kg^{-1}), and peanut butter (average B_1 13 µg kg^{-1}) samples. Fried groundnuts were free of aflatoxin. Aflatoxins were also detected in some consignments of exported groundnut kernels (990 tons) at a level of > 5 µg kg^{-1}. Only a few samples of rice, corn, spices and cassava were contaminated with aflatoxins.

682. Neelakantan, S., Balasubramanian, T., Jasmide, I., and Balasaraswathi, R. 1981. Presence of aflatoxins in foods and feeds available in the Tamil Nadu region. Madras Agricultural Journal 68(3): 189-195.

The results of a survey to detect aflatoxins in foods and food products, and in feeds and feed ingredients in the Tamil Nadu region of India are presented. Aflatoxins were detected in 4 of 91 samples of groundnut candies, 1 of 18 dried date samples, 2 of 67 scented supari, 1 of 54 broken processed arecanut, 6 of 37 lime pickles. 4 of 43 dried vegetables, 1 of 6 apples, 2 of 6 dried coconut, 2 of 30 samples of cottonseed, 28 of 134 groundnut oil cake samples, 4 of 83 gingelly oil cake samples, 3 of 96 coconut oil cake samples, 7 of 38 animal feeds, 19 of 60 poultry feeds and 4 of 10 oilseed cake mixtures.

683. Nizami, H.M., and Zuberi, SJ. 1977. Aflatoxin and liver cancer in Karachi, a preliminary survey. Journal of the Pakistan Medical Association 27(6): 351-352.

Twelve of 28 food samples were contaminated with aflatoxin, viz. rice, broken rice, raw grams, almonds, groundnuts, peanut butter, brown beans, white beans, cardamom black, pistachio, maize flour and raw groundnuts. The consumption of foods containing aflatoxins may account for the incidence of liver cancer in Karachi, Pakistan.

684. Pcnsala, O., Niskanen, A., and Lahtinen, S. 1977. The occurrence of aflatoxin in nuts and nut products imported to Finland for human consumption during the years 1974-1976. Nordisk Veterinaermedicin 29 (7-8): 347-355.

Samples of nuts and nut products examined on entry to Finland revealed 4.2 % contamination with aflatoxin, of which 97 % involved groundnuts. Aflatoxins B', and B_2 predominated, and aflatoxins G_1 and G_2 were found only in combination with aflatoxins B_1 and B_2. Levels of aflatoxin < 5 µg - 1000 µg kg^{-1} were found. Sliced and crushed nuts had the highest levels of aflatoxins. The producer's and consumer's risks are assessed, sampling methods are reviewed and reaffirmation is made of the acceptable quality level of 5 µg aflatoxin kg^{-1} nuts.

685. PHI (Public Health Institute). 1983. Aflatoxin contamination situation in grains in China. Development of Food Hygiene 1(1):204-208.

From 1973 to 1977, a general survey of aflatoxin contamination in foodstuffs was carried out in 24 provinces. More than 14 000 samples of maize, groundnut, rice, wheat, and various legumes were analyzed for aflatoxin B_1 contamination. The contamination in groundnut samples was 26.3% and in groundnut oil samples 47.3%. The contamination in groundnut samples was high in south- central China, moderate in east and northwest China, and negligible in northeast China. The percentage of aflatoxin- contaminated samples gradually decreased as latitude increased.

686. Prado, G. 1983. [Incidence of aflatoxin B, in foods.). Incidencia de aflatoxina B_1 em alimentos. Revista de Farmacia e Bioquimica, Belo Horizonte 5(2): 147-157.

Aflatoxin B_1 was determined in samples of groundnuts and their products, wheat, corn and manioc flour, collected in commercial establishments in Belo Horizonte in 1983, with a thin-layer chromatography (TLC) method. Aflatoxin B_1 was detected in 44 % of the samples of groundnut and groundnut products; aflatoxin levels were above the tolerance level permitted by Brazilian legislation (30 µg kg^{-1} aflatoxins B_1+G_1).

687. Pregnoiatto, W., and Sabino, M. 1970. [Search and dosage of aflatoxin in peanuts and derivatives and other cereals.]. Pesquisa e dosagem de aflatoxina em Amendoim e derivados e em outros cereais. Revista do Instituto Adolfo Lutz, Sao Paulo 29/30: 65-71.

Of 32 foods (for human consumption) and 130 rations (for animals) analyzed for aflatoxin, 20 foods and 104 rations contained aflatoxin. Besides groundnut flour and other groundnut products, manioc flour was highly contaminated with aflatoxin. Sodium bisulfite (1:10000) prevented the development of *Aspergillus flavus* in the groundnut flour.

688. Pruthi, J.S. 1978. Mycotoxins in foods and feeds - their detection, estimation, preventive and curative measures. Bulletin of Grain Technology 16(1): 51-68,

This review covers several aspects including a survey of aflatoxin contamination of food grains and of groundnut oil, human and animal health hazards from mycotoxins, techniques for detection and estimation of aflatoxins, factors affecting aflatoxin formation, preventive or control measures.

689. Sabino, M., Inomata, E.I., and Lamardo, L.C.A. 1982. [Variation of the content of aflatoxin B_1 in peanut paste and peanut sweet bars consumed in Sao Paulo State, Brazil.|. Variacao dos niveis de aflatoxina B_1 em pasta de amendoim c pacoca consumidas no Estado de Sao Paulo. Revista do Instituto Adolfo Lutz, Sao Paulo 42(1/2): 39-44.

Aflatoxin B_1 was detected in 50 samples of groundnut paste and groundnut sweet bars for sale in various parts of the state of Sao Paulo. Aflatoxin levels ranged from 10 to 278 µg kg^{-1}.

690. Sabino, M., Zorzetto, M.A.P., Pedroso, M.O., and Milanez, T.V. 1989. Incidence of aflatoxins in peanut and peanut products consumed in Sao Paulo city during the period 1980 to 1987. Revista do Instituto Adolfo Lutz, Brazil 49(1): 41-44.

Aflatoxins were determined in 1374 samples of groundnuts and groundnut products sold for consumption in Sao Paulo city. Aflatoxins were detected in 576 samples and the levels of aflatoxin contamination varied from year to year. Of these samples, 68.75% had > 30 µg kg^{-1}, the maximum permitted by Brazilian legislation.

691. Scussel, V.M., and Rodriguez-Amaya, D.B. 1985. [Aflatoxin levels in peanut and peanut products commercialized in Campinas in the period 1980-1982. |. Teores de aflatoxinas em amendoim c seus produtos comercializados em Campinas em 1980-1982. Boletim da Sociedade Brasileira de Ciencia e Tecnologia de Alimentos Campinas 19(2): 109-119.

Samples of groundnuts and groundnut products (raw shelled and unshclled groundnuts, fried salted groundnuts with or without skin, soygroundnut, sugar-coated colored groundnuts, chocolate- coated groundnuts, peanut butter, ground groundnut bar and candied groundnut bar), purchased at random from different supermarkets and smaller stores during the period from the second semester of 1980 to the first sememster of 1982, were analyzed for aflatoxin with the Romer method. Of the 241 samples analyzed. 128 had aflatoxins, and 92 of these contained levels above the tolerance limit permitted by Brazilian legislation (30 µg kg^{-1} aflatoxins B_1+G_1). Ground groundnut bar, raw shelled groundnuts, soygroundnut and fried salted groundnuts with the skin had greater contamination levels with 61 %, 55 % and 42 % respectively of their samples with aflatoxin levels above the limit. The highest levels of aflatoxins B_1 and G_1 detected were I 282 and 476 µg kg^{-1} for the ground groundnut bar, 1 904 and 69 *µg* kg^{-1} for raw shelled groundnuts and 1 026 and 366 µg kg^{-1} for soygroundnut, respectively.

692. Seibold, R., and Ruch, W. 1977. [Aflatoxin content of mixed feed of dairy cows.J. Der aflatoxingehalt im milchviehmischfutter. Kraftfutter 60(5):182, 184-185.

Of 60 samples of groundnut-free feed compounds for dairy cows, only one contained more than 20 µg kg^{-1} aflatoxin, the maximum level permitted under new Federal German regulations. In feed compounds including groundnut products, however, average aflatoxin levels far exceeded the maximum permitted level. The new regulations are intended to prevent aflatoxin contamination of milk and thus possible human health hazards.

693. Sellschop, J.P.F., Kriek, N.P.J., and Du Precz, J.C.G. 1965. Distribution and degree of occurrence of aflatoxin in groundnuts and groundnut products. South African Medical Journal 39: 771-774.

Distribution and degree of occurrence of aflatoxins in various samples of groundnuts and groundnut products in the Republic of South Africa are described. Over 1000 samples of groundnuts and groundnut products were examined during 1963 and another 1000 samples of producers' grades were investigated during 1964. The samples obtained from the North- Western Transvaal, or so-called Bushveld areas,

were most seriously contaminated with aflatoxin; all of the 75 samples containing > 2000 µg kg^{-1} aflatoxin were from the receiving depots in the North-Western Transvaal. The samples from the Northern Cape Province. Orange Free State and Natal, except for 5 samples with 500-2000 µg kg^{-1} and 11 samples with 100-500 µg kg^{-1}, were free from aflatoxin. Similar results were found in the 1964 survey for anatoxin contamination; however, only 23 samples from the North-Western Transvaal areas contained > 2000 µg kg^{-1}. High degree of anatoxin contamination in the groundnut samples from the North-Western Transvaal was ascribed mainly to (1) the damage caused to the maturing groundnut pods and kernels by termites, and (2) protracted droughts followed by late rains. Lower or inferior grade groundnuts contained higher levels of aflatoxins than the higher or superior ones. No aflatoxins were detected in any of the samples of hand-picked selected (HPS) kernels examined in 1963 and 1964. Groundnut cake samples implicated in livestock deaths were also examined for aflatoxin contamination. Eleven of the 16 samples of this groundnut cake contained > 2000 |ig kg' anatoxin. Of 101 peanut butter samples tested. 17 were contaminated with aflatoxin. Of these 17 samples, 12 had an aflatoxin contents of 100-500 µg kg^{-1} and 5 had up to 25 µg kg^{-1}.

694. Sim, T.S., Tew, T., and Sim, T.F. 1985. A note on the screening of dried shrimps, shrimp paste and raw groundnut kernels for aflatoxin-producing Aspergillus *flavus.* Journal of Applied Bacteriology 59(1): 29-34.

All of 33 samples of dried shrimps paste, peanut butter and raw groundnuts were contaminated with fungi. *Aspergillus* and *Penicillium* species were the predominant types in dried shrimps and raw groundnuts, but no *Aspergillus* spp. were present in peanut butter or shrimp paste samples. Among 81 *Aspergillus* isolates obtained from dried shrimps and raw groundnuts, 10 *were A. flavus/A. parasiticus,* of which 5 were potential aflatoxin- producing *A. flavus* strains. No aflatoxins were detected in the food samples, but some were moldy and some had high mold counts.

695. Singh, B., Khalid, A.S., Magboul, B., Okezie, B.O., Anderson, J.C., Wheelock, G.C., Jones, 11., and Caples, V. 1989. Aflatoxin contamination of groundnuts with special reference to Sudan and some Caribbean countries. Pages 245-253 in Aflatoxin Contamination of Groundnut: Proceedings of the International Workshop, 6-9 Oct 1987, ICRISAT Center, India. Patancheru, A.P. 502 324. India : International Crops Research Institute for the Semi-Arid Tropics.

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 µg kg^{-1} of aflatoxin. However, samples collected from the markets in Khartoum and Wad Medani contained up to 945 µg kg^{-1} in raw groundnuts, up to 517 µg kg^{-1} in roasted groundnuts, and up to 994 µg kg^{-1} in groundnut paste. Groundnut paste prepared alter 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 µg kg^{-1} of aflatoxins. Roasted groundnuts and peanut butter samples collected from markets in Jamaica and Trinidad did not contain detectable amounts of aflatoxins. However, groundnut products collected from St. Vincent had very high levels of aflatoxin varying from 1 to 469 µg kg^{-1}.

696. Stoloff, L. 1976. Incidence, distribution and disposition of products containing aflatoxins. Proceedings of the American Phytopathological Society 3: 156-172.

The incidence, distribution, and disposition of products containing aflatoxins are discussed. Susceptible commodities include groundnuts, com, sorghum, rye, wheat, rice, barley, oats, soybeans, pecans, walnuts, almonds, filberts and figs. Anatoxins in groundnuts and com are discussed in detail.

697. UK Ministry of Agriculture, Fisheries and Food, Steering Group on Food Surveillance Mycotoxins. 1987. Food Surveillance Paper No. 18.

This report of the Working Party on Naturally Occurring Toxicants in Food. Sub-Group on Mycotoxins, describes surveillance for mycotoxins since 1979. During the survey aflatoxin content in milk decreased; 75 and 4 % of bulk-dried milk samples contained aflatoxins in 1977-79 and 1981-83, respectively. Aflatoxins were also detected in nuts and nut products, particularly in groundnuts. Ochratoxin was detected in trace amounts in pigs' kidney and nuts. During 1982. 44, 24 and 16 % of samples of UK- grown barley, wheat and oats contained deoxynivalenol between 20 and 100 µg kg^{-1} but only 4 % of all UK-grown cereals between 1980 and 1982 contained deoxynivalenol at more than 100 µg kg^{-1}. Legislative control of mycotoxins in the U.K. food supply is summarized. A maximum permitted limit of 10 µg kg^{-1} for total aflatoxins (B_1, B_2, G_1 and G_2) in nuts and nut products is proposed.

698. Wei, D.L., and Wei, R.D. 1980. High pressure liquid chromatographic determination of aflatoxins in peanut and peanut products of Taiwan. Proceedings of the National Science Council. Taiwan 4(2): 152-155.

Analysis was carried out on 401 samples of locally-consumed groundnut and groundnut products including peanut butter, groundnut cake, tried or roasted groundnuts, and oil, using a high pressure liquid chromatography (HPLC) method. The incidence of aflatoxin contaminated samples was 23.4 %. Aflatoxin B_1 was found in all positive samples.

699. Wood, G.E. 1989. Aflatoxins in domestic and imported foods and feeds. Journal of the Association of Official Analytical Chemists 72 (4): 543-548.

Data generated from compliance programmes on aflatoxins in foods and feeds, enforced by the FDA, are summarized for the fiscal year 1986. Commodities sampled included groundnuts and groundnut products, maize and maize products, tree nuts, cottonseed, milk, spices, manufactured products and miscellaneous foods and feeds. Correlations were highest between aflatoxin contamination and geographical areas for maize/maize products and cottonseed/cottonseed meal. Higher incidences of aflatoxin contamination in maize and maize products designated for human consumption were observed in samples collected in the southeastern states (32 and 28 %, respectively). A higher incidence of contamination was observed in maize designated for animal feed from Arkansas-Texas (74 %) than from the southeastern states (47 %). Only 3 % of feed maize from corn belt states contained detectable aflatoxins. All aflatoxin-contaminated cottonseed was collected in the Arizona-California area; 80 % of cottonseed meal analyzed from this area also contained detectable levels of aflatoxins. No aflatoxin MI was detected in any of the 182 samples of fluid milk and milk products examined. It is concluded that the percentage of samples that contain measurable levels of aflatoxins is expected to vary with commodities from year to year and the 1986 information can be used as a baseline for comparison to determine the effectiveness of control efforts exerted by the food and feed industries.

700. Yndestad, M, and Underdid, B. 1975. [Aflatoxin in foods on the Norwegian market.]. Aflatoksin i naeringsmidler pa det norske marked. Nordisk Verterinaermedicin 27(1): 42-48.

A survey of the aflatoxin content in some food products available on the market in Norway is reported. During autumn 1973, samples of Brazil nuts, groundnuts, peanut butter, hazelnuts, walnuts, mixed nuts, cocoa, cocoa products and dried milk were bought from stores in the Oslo area. In addition, samples of Brazil nuts and groundnuts were taken from import stores. Aflatoxins were detected in 6 of 34 samples of bought groundnuts, 1 of 3 of sorted and pooled groundnuts, 1 of 16 mixed nuts, 1 of 14 walnuts, 1 of 3 cashew nuts, 18 of 27 Brazil nuts and 1 of 40 cocoa products. Levels of aflatoxins B_1, B_2, G_1 and G_2 were 4200, 1600, 3100 and 600 µg kg^{-1}, respectively, for one sample of Brazil nuts and 400, 50, 350 and 30 µg kg^{-1} for a sample of groundnuts. Other aflatoxin levels were generally much lower. No aflatoxin was detected in peanut butter, hazelnuts and dried milk.

5.2.2 Groundnut kernels

701. Abdcl-Hamid, A.M. 1985. Detection of aflatoxins in Egyptian feedstuffs. Annals of Agricultural Science, Moshtohor 23(2): 649-657.

Of 95 samples of various feedstuffs, which were tested for aflatoxins B_1, B_2, G_1 and G_2 by thin-layer chromatography (TLC), 44.2 % were positive (including maize, rice crack, rice germ, rice germ cake, rice bran, wheat bran, cotton seed, cotton seed cake, and groundnut and mixed feed for broilers, egg production, calf fattening and milk production). Most of the samples (90.4 %) were contaminated with <100 µg kg^{-1} total anatoxins. Groundnuts from "Ismailia" had the highest contamination (400 µg kg^{-1} aflatoxin B_1) and the ratio of aflatoxins in kernels and shells was 1:7. Soybeans were contaminated with 5 µg kg^{-1} aflatoxin B_1, while horse beans and fish meal were not contaminated. Aflatoxin B_1 was present in only 76.1 % of the positive samples, and the concentration ratio of aflatoxins B_2:G_1:B_1 was 1:2.3:22.4.

702. Abdel-Rahim, A.M., Osman, N.A., and Idris, M.O. 1989. Survey of some cereal grains and legume seeds for aflatoxin contamination in the Sudan. Zentralblatt fur Mikrobiologie 144(2): 115-121.

Aflatoxins were detected in some cereal and leguminous seeds (including maize, wheat, haricot bean, broad bean, lupin, groundnuts and cowpea) collected from 2 cities in the Sudan (Wad Medani and Khartoum). Levels of aflatoxins in these seeds were below the reported hazard threshold. Studies on the effect of seed grading on aflatoxin contamination of 4 crops (dura, maize, haricot bean and broad bean) showed that sound intact seeds contained low or no aflatoxins, compared with significantly higher levels detectable in damaged and moldy seeds. Effect of temperature on production of 4 aflatoxin components (B_1, B_2, G_1 and G_2) was studied in 2 isolates of *Aspergillus flavus* (MS III from maize and HCB 33 from haricot bean). Optimum temperature was 30°C for MS III and 20°C for HCB 33. Large amounts of aflatoxins B_1, B_2 and G_1 were produced by the isolates, while aflatoxin G_2 was produced only by HCB 33 at optimum temperature.

703. Ahmed, N.E., Younis, Y.M.E., and Malik, K.M. 1989. *Aspergillus flavus* colonization and aflatoxin contamination of groundnut in Sudan. Pages 255-261 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 Oct 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

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,

were free from *A.flavus* infection and aflatoxin contamination. *Aspergillus flavus* infected 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 μg kg^{-1}). Groundnuts stored for 3 months in a well-ventilated room with an average temperature of 15°C were infected by *A.flavus* at a low level, but had no aflatoxin contamination. Infection increased with time in storage. Groundnuts harvested 1 week before maturity were not affected by *A. flavus* infection of seeds in storage, and there were no differences among genotypes. Groundnuts stacked in sacks at shelling sites were sampled; 4 % were contaminated with aflatoxin, the average level being 11 μg kg^{-1}. Fifteen per cent of the samples from oil mill sites were contaminated, the average aflatoxin content being 20 μg 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 contaminated samples, with an average aflatoxin content of 10 μg kg^{-1}. This produce is usually allocated for local processing.

704. Albert, L.A. 1990. Environmental contaminants in Mexican food. Pages 541-577 in Food Contamination from Environmental Sources (Nriagu. J.O., and Simmons, M.S., eds.). John Wiley and Sons : New York.

Existing data indicated that 10-20 % of the corn tortillas sold in Mexico City are contaminated with aflatoxins. A large part of imported corn is also contaminated with aflatoxins. Results of surveys of Mexican foods from 1979-1981 found the frequency of aflatoxin contamination of groundnuts, processed groundnuts, beans, sorghum and corn to be 47, 7-31, 5, 3 and 1 %, respectively.

705. Alisauskas, V.A. 1974. Determination of aflatoxin in grain and feedstuffs. Food Technology in Australia 26(6): 233-237.

Ninety-two samples of grain and common feed ingredients were analyzed in Australia using thin-layer chromatography (TLC) and a rapid minicolumn screening method. Three samples contained aflatoxins; a sorghum grain sample containing 30 μg kg^{-1} aflatoxin B_1, and two samples of moldy groundnuts containing 2500 μg kg^{-1} aflatoxin B_1 and 400 μg kg^{-1} aflatoxin B_2, and 200 μg kg^{-1} B_1 and 38 μg kg^{-1} B_2, respectively. A non-destructive detection technique involving scanning the grain with a UV lamp and examination of fluorescence is reported.

706. Alpert, ME., Hutt, M.S.R., Wogan, G.N., and Davidson, C.S. 1971. Association between aflatoxin content of food and hepatoma frequency in Uganda. Cancer Research 28(1): 253-260.

Aflatoxins were estimated in 480 food samples stored for consumption between harvests and collected from different parts of Uganda in 1966-67. Among these samples, 29.6 % contained detectable amounts of aflatoxins and 3.7 % contained more than 1 μg kg^{-1}. The frequency of aflatoxin contamination was particularly high in provinces with a high incidence of hepatoma, or where cultural and economic factors favored the ingestion of moldy foods.

707. Baquete, E.F., and Freire, M.J. 1989. Present status and perspectives of aflatoxin research in Mozambique. Pages 93-94 in. Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 Oct 1987, ICRISAT Center, India. Pautancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

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 Investigacion Veterinaria (INIV) and the Laboratorio Nacional para la Higiene de Agua y Alimentos (LNHAA) are involved in the analysis of food products, for both animals and humans. In 1981, 17 food products, comprising a total of 313 samples were tested and 16 samples were contaminated with aflatoxin B_1. 10 with B_2, 4 with G_1. and 3 with G_2. 87-100 % of the groundnut, beer, rice, and maize samples tested were contaminated. The aflatoxin levels in the groundnut samples ranged from 3 to 5500 μg kg^{-1}, aflatoxin B_1 being the main contaminant. An analysis program is investigating the possible correlation between consumption of contaminated food and the possible presence of aflatoxin M, in human breast milk. The possibility of further work involving the INIV, LNHAA, and the Faculdade de Agronomia. Universidade Edurado Mondlane, Groundnut Improvement Project is being studied to include an agronomic component and formulate practical recommendations for small farmers and traders.

708. Begum, N., Adil, R., and Shah, F.H. 1985. Contamination of groundnuts with aflatoxins. Pakistan Journal of Medical Research 24(3): 129-131.

Groundnut samples collected from different areas in Pakistan were tested for aflatoxin contamination. Romer's method was used for the analysis of aflatoxins. Fifteen per cent of the groundnut samples from Lahore markets were contaminated with aflatoxins, while all samples from Khuzdar showed aflatoxin contamination. Among the contaminated samples, levels of aflatoxins (B_1 and B_2) ranged from 80-800 μg kg^{-1}, except for one sample that had 24 μg kg^{-1}. Raw groundnut samples did not have any aflatoxins.

709. Bcsrat, A., and Gebre, P. 1981. A preliminary study on the aflatoxin content of selected Ethiopian foods. Ethiopian Medical Journal I9(2): 47-52.

Injera, a fermented, pancake-shaped food prepared from *Eragrostis tef,* showed no aflatoxin contamination as commonly prepared and handled; fermentation of the dough or storage of injera for prolonged periods did not increase afiatoxin B_1 contamination. Preparations of red pepper powder and its paste showed some afiatoxin contamination (mean 32 µg kg^{-1} for powder, 1 paste sample had 102.2 pg kg ' afiatoxin B_1). Samples of groundnuts and peanut butter had afiatoxin B_1 at mean values of 34.7 and 105 µg kg^{-1}, respectively.

710. Bessard, C.M., Soubeyrand, J., Chauvier, C., and Pollet, A. 1990. Alimentary contamination and hepatic presence of aflatoxins in an individual with first-stage liver cancer in Ivory Coast. Journal of Toxicologic Clinique et Experimentale 10(1): 41-44.

Of 78 groundnut samples analyzed in Ivory Coast, 22 contained > 20 pg kg^{-1} afiatoxin B, and 27 contained > 200 µg kg^{-1} allatoxins B_1 B_2 G_1 or G_2, At post-mortem, allatoxins were detected in liver samples from a patient with liver cancer : B_1 11.8 ug kg^{-1}; B_2 1.9 ug kg^{-1}; G_2 0.1 µg kg^{-1}.

711. Blankenship, P.D., Holaday, C.E., and Butler, J.L. 1973. Some results concerning the occurrence of afiatoxin in selected sizes of peanut kernels. Proceedings of the American Peanut Research and Education Association 5: 160-163.

A group of 60 samples from aflatoxin-contaminatcd groundnuts was provided by the Federal-State Inspection Service from six widely separated grading points in Southwest Georgia. Another group of 28 samples was collected from various warehouses in Georgia, Alabama, and Florida. Fach sample of groundnuts was shelled and the kernels divided into four subsamples of different sizes. Standard slotted-hole grading screens having either 20/64-, 18/64 , or 16/64-inch width slots were used to make the size separations. Analysis of the subsamples for aflatoxin showed that 85 % of the subsamples in the group of 60 samples contained measurable amounts of afiatoxin and the kernels that fell through the 18/64-inch screen and rode the 16/64-inch screen had a significantly higher average concentration of afiatoxin than the other kernels. Afiatoxin at > 20 µg kg^{-1} was detected in 65 % of the subsamples in the 28-sample set. The smaller size kernels contained higher levels and had more frequent occurrence of afiatoxin man me larger size kernels.

712. Borut, S.Y., and Joffe, A.Z. 1965. *Aspergillus flavus* Link aflatoxins and toxicity of groundnuts in Israel. Israel Journal of Botany 14: 198.

Research was done in Israel on the occurrence of aflatoxin-producing fungi in soils and in groundnut kernels. *Aspergillus flavus* was prevalent in low levels in soils of the groundnut fields in Israel. *Aspergillus flavus* infection of groundnut kernels increased with storage time. 80 % of 55 stored groundnut samples tested from 1963 crop and 64 % of 63 stored samples from 1964 crop were infected with the fungus while only 20 % of the 1963 crop and 12.7 % of the 1964 crop were contaminated with allatoxin. 71.2 % of 330 *A. flavus* isolates tested were toxigenic. The percentage of aflatoxigenic strains among soil isolates was higher than that of the kernel isolates.

713. Boutrif, E., Jemmali, M., Campbell, A.D., and Pohland, A.E. 1977. Aflatoxin in Tunisian foods and foodstuffs. Annales de la Nutrition et de l'Alimentation 31: 431-434.

1076 samples of different commodities taken from stores and dealers throughout the country were screened for afiatoxin contamination. Results showed that 13 samples were contaminated with afiatoxin, with 3 of 38 corn samples containing 48-62 pg B_1 kg^{-1}, and 8-22 µg G_1 kg^{-1}, 2 of 6 corn samples 30-35 µg B_1 kg^{-1}, 1 of 19 pistachio nut samples 22 µg B_1 kg^{-1} 1 of 35 pea samples 25 pg B_1 and 42 pg G_1 kg^{-1}, 2 of 248 cheese samples 1 µg B_1 and 6.2-10.6 µg M_1 kg^{-1} and 4 of 65 groundnut samples 6-46 µg B_1 and 0.38 µg G_1 kg^{-1}. No positive samples were found in wheat, barley, lentils, almonds, sesame and sunflower seeds, bean and milk (45 samples).

714. Brudzynski, A., Pee, W.van, and Kornas/ewski, W. 1977. The occurrence of aflatoxin B_1 in peanuts, corn and dried cassava sold at the local market in Kinshasa, Zaire: its coincidence with high hepatoma morbidity among the population. Zeszyty Problemowe Postepow Nauk Rolniczych 189: 113-115.

Of 12 maize samples analyzed. 1 had allatoxin B_1 at over 200 µg kg^{-1}, and 33 % of dried cassava samples were contaminated with afiatoxin. Select and low quality groundnuts had 80 and 39 % of samples not contaminated. Of the low quality samples 33 % contained more than 250 µg kg^{-1}. Results are related to local incidence of hepatoma and to the high humidity of the climate.

715. Burdaspal, P.A., and Gorostidi, A. 1989. Contamination of groundnuts and oilier nuts by afiatoxin. Alimentaria 26(199): 51- 53.

In 2 surveys during 1985 and 1986 highest incidence of anatoxin B_1, B_2, G_1 and G_2, contamination in 422 samples of unshelled and shelled groundnuts, pistachio nuts and brazil nuts from different regions of Spain was in unshelled groundnuts (9.1 and 10.6 % of samples in the 2 surveys). Incidence in shelled groundnuts was 4.9 and 4.0 %, respectively. No aflatoxins were detected in pistachio nuts. The highest value, 2478 µg kg^{-1} was in an unshelled groundnut sample.

716. Bushnell, D.G. 1965. The incidence of aflatoxin in the Rhodesian groundnut crop. Rhodesia Agricultural Journal, Bulletin no. 2287. 62 : 94-96.

There was a low incidence of aflatoxin contamination in the 1962/63 Southern Rhodesian groundnut crop (50-250 µg kg^{-1} aflatoxin B_1). whereas with the 1963/64 crop, an appreciable number of samples were contaminated with more than 250 µg kg^{-1} aflatoxin B_1. This incidence occurred before marketing and was not aggravated by storage conditions used in Rhodesia. Although fungal infection might occur before harvesting, the major portion of aflatoxin contamination occurred in the period between lilting of the crop and delivery of the shelled groundnuts to the Grain Marketing Board. There was no evidence that the distribution of contamination was dependant upon the rainfall in the harvesting period. There appeared to be a higher level of afflatoxin B_1 contamination in areas with high air temperatures (65°F and above) in the harvesting period. The aflatoxin research program for the 1965 crop is described.

717. Campos, M.de, and Olszyna-Marzys, A.E. 1979. Aflatoxin contamination in grains and grain products during the dry season in Gautemala. Bulletin of Environmental Contamination and Toxicology 22(3): 350-356.

Seventeen per cent of 264 samples (of maize, rice, cottonseed, beans, groundnuts, coffee, cocoa, wheat, sorghum, sesame and other foods) were contaminated with aflatoxins; 8 % exceeded the 20 µg kg^{-1} level. The highest level (240 µg kg^{-1}) was in silo- stored yellow maize from a hot and humid area. This maize sample showed visible fungal contamination. Aflatoxin B_1 alone or in combination with other allaloxins was found in all positive samples. Aflatoxin G, was found in maize, groundnut, rice, beans and meat meal, while aflatoxin G_2 was found in rice and beans.

718. Casadci, E., Pereira, C.R., and Bruheim, S. 1982. Contaminacao em aflatoxinas nos produtos de base em Mocambique. Revistas Medica de Mozambique 1(1): 23-31.

Allatoxin contamination levels in groundnut samples collected in Mozambique ranged from 3 to 5500 µg kg^{-1}.

719. Chang-Yen, I., and Felmine, J. 1987. Aflatoxin levels in selected bulk foods and feeds in Trinidad. Tropical Agriculture 64(4): 283-286.

Anatoxins B_1, B_2, G_1 and G_2 were determined in bulk samples of raw groundnuts, peanut butter, wheat flour, and chicken, pig and dairy feeds. Samples were taken in accordance with an established sampling plan, and dry samples were subsampled with the Dickens Subsampling Mill. A minicolumn procedure was used to screen 64 samples, eight of which were further analysed by thin-layer and high-performance liquid chromatography. Of the eight samples, only one contained aflatoxin G_1 (2µg kg^{-1}).

720. Constant, J.L., Kocheleff, P., Carteron, B., Perrin, J., Bedere, C., and Kabondo, P. 1984. (Geographical distribution of aflatoxins in human food in Burundi.]. Distribution geographique des aflatoxines dans l'alimentation humaine au Burundi. Science des Aliments 4(2): 305-315.

Aflatoxin contamination of foods was found to be greater in areas of low altitude. Food products most frequently and heavily contaminated included groundnuts (maximum aflatoxin 425 µg kg^{-1}), cassava (325 µg kg^{-1}) and maize (148 µg kg^{-1}). The highest incidence of hepatoma in Burundi coincides with the areas of highest aflatoxin contamination.

721. Daren, Xiao. 1989. Research on aflatoxin contamination of groundnut in the People's Republic of China. Pages 95-100 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 Oct 1987. ICR1SAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

During 1973-1977, 1689 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,. 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. Screening of groundnut germplasm for resistance to *Aspergillus flavus* is in progress.

722. DiProssimo, V.P. 1976. Distribution of aflatoxins in some samples of peanuts. Journal of the Association of Official Analytical Chemists 59(4): 941-944.

Of 18 samples of groundnuts analyzed, 5 contained higher levels of aflatoxin G than aflatoxin B. One sample of roasted nuts contained aflatoxins (21 to 26 *µg* kg^{-1}) with about 20 times as much G as B aflatoxins.

723. Doupnik, B.Jr. 1969. Factors associated with aflatoxins in farmers' stock peanuts in Georgia in 1967. Phytopathology 59: 112-113.

Samples of farmers' stock groundnuts were collected from 6 locations at weekly intervals for 5 weeks during the harvesting season. Seventeen of the 228 samples

collected contained aflatoxins. Aflatoxins were present in 2.5 % of the Segregation I (highest quality), 12.1 % of the Segregation 11, and 25.7 % of the Segregation III samples, and averaged 22, 264, and 324 μg kg^{-1} total aflatoxin respectively. A significant relationship was thus found between the presence and concentration of aflatoxins and grading factors. Samples with high visible damage values were more likely to contain aflatoxins than were samples with high invisible damage (rancidity, mold or decay) values. The weekly incidence of aflatoxins was related to the climatological conditions during the 14-day period prior to the date of collection. Mycofloral studies of 73 selected samples showed a relationship between the mean number of fungi per sample and grading factors; the mean number increased as quality decreased. These findings support previous observations that factors which influence groundnut quality also influence the incidence of aflatoxins in farmers' stock groundnuts.

724. Doupnik, B.Jr. 1969. Aflatoxins in farmers' stock peanuts : Peanut quality, mycoflora and climatological conditions as influencing factors. Journal of the American Oil Chemists' Society 46: 121A.

Samples of farmers' stock groundnuts were collected from six locations at weekly intervals for five weeks during the 1967 and 1968 harvesting seasons. Seventeen of 228 samples collected in 1967 and 23 of 356 samples collected in 1968 contained aflatoxins. In 1967, 2.5 % of Segregation I. 12.1 % of Segregation II, and 25.7 % of Segregation III samples contained aflatoxins and averaged 22, 264, and 324 µg kg^{-1} total aflatoxins respectively. In 1968, 3.2 % of Segregation I, 6.3 % of Segregation II, and 11.9 % of Segregation III samples contained aflatoxins and averaged 28, 61, and 583 µg kg^{-1} total aflatoxins respectively. Thus, in both years, significant relationships were found between the presence and concentration of aflatoxins and grading factors. Weekly incidences of aflatoxin contaminated samples were related to climatological conditions during the 14-day period prior to the collection dates in both years. Mycofloral studies of 73 selected samples in 1967 and of all samples in 1968 showed a relationship between the mean number of fungi/sample, grading factors and aflatoxin contamination. These findings support previous observations that factors which influence groundnut quality also influence aflatoxin contamination in farmers' stock groundnuts.

725. Eadie, T., and O'Rcar, C.E. 1967. The occurrence of aflatoxin in Virginia-North Carolina peanuts and peanut products. Virginia Journal of Science 18: 140-141.

Aflatoxin levels in groundnut samples from the Virginia-North Carolina crop were determined in a survey carried out in the 3- year period 1964-1967. About 6% of samples from the 1964-1965 crop contained aflatoxin, and 23 of 51 samples of peanut butter were contaminated with aflatoxin. Contaminated lots of groundnuts were reduced to 5.3% in 1965-1966 and to 4.4% in 1966-1967. No peanut butter sample was contaminated in 1965-1966.

726. Elamin, N.H.H., Abdel-Rahim, A.M., and Khalid, A.E. 1988. Aflatoxin contamination of groundnuts in Sudan. Mycopathologia 104(1): 25-31.

Groundnut samples, collected soon after harvest, from different districts in the irrigated region (Central Sudan) were free from aflatoxins. Samples collected from the rainfed region (Western Sudan) showed incidence of aflatoxin contamination ranging from 100% samples in El Hamdi to only 10% samples in Casgeal. Damaged pods were highly contaminated with *Aspergillus flavus* and aflatoxins. Sound intact pods had lower fungal contamination and were almost free of aflatoxins. Groundnut products (groundnut paste, red and grey roasted pods) collected from Khartoum North (Bahri) had higher levels of aflatoxin than those collected from Khartoum and Umdorman. Grey and red roasted pods showed higher levels of aflatoxins than the groundnut paste. None of the three varieties (MH383, Barberton, Ashford) of groundnut tested was completely resistant to *A. flavus* contamination and aflatoxin production. A temperature of 30°C and 86.3 % relative humidity were the optimum conditions for both *A. flavus* growth and aflatoxin production in groundnuts.

727. El-Khadem, M. 1990. Aflatoxins in Egyptian peanut. An overview. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz 97(3): 233-236.

A survey of groundnuts from Egypt showed 19.5 %of unshelled and 49.0 %of shelled samples to contain low levels of aflatoxins B_1, B_2, G_1 and G_2; only B, was detected in 3.5 % samples of roasted groundnuts. However, 60 % of *Aspergillus flavus* strains from Egypt produced one or more aflatoxin, and 40 %of toxigenic strains produced high quantities (5000-20000 μg kg^{-1}) of B aflatoxins; the levels found in groundnuts were therefore lower than expected. Studies on the effects of storage temperature and RH on groundnut mycoflora and aflatoxin development showed an RH of 95 %to be optimum for aflatoxin production; since the RH in Egypt is lower than this, this was considered a factor contributing to the low levels encountered. Inoculation of Egyptian groundnuts (cv. Giza 1) with different strains of *A. flavus* showed the shells to act as a barrier towards invasion; this was considered the main reason for low aflatoxin production. Consequently, it is considered imperative to discard groundnuts with cracked shells before storage.

728. Emerole, G.O., Uwaifo, A.O., Thabrew, M.I., and Bababunmi, E.A. 1982. The presence of aflatoxin and some polycylic aromatic hydrocarbons in human foods. Cancer Letters 15(2): 123-129.

Total aflatoxin content, measured spectrophotometrically, of crops and spices grown locally was 1600 ± 500 µg kg^{-1} for manihot flour, 4 0 0 1 0 0 for yam flour, 700±

150 for red pepper, 1400 ± 400 for millet, 400 + 100 for soybeans. 1120 ± 500 for maize, 500 ± 150 for black eye beans. 4 0 + 1 0 for rice and 1700 ± 400 for groundnuts. Results are discussed in relation to the relatively high incidence of cancer in Tropical Africa.

729. Fong, L.Y.Y., Ton, C.C.T., Koonanuwatchaidet, P., and Huang, D.P. 1980. Mutagenicity of peanut oils and effect of repeated cooking. Food and Cosmetics Toxicology 18(5): 467-470.

Four samples *of* groundnut oils obtained from local markets in Hong Kong were tested for mutagenicity using the *Salmonella/microsamal* test system, in the presence of microsomes, dimethylsulphoxide (DMSO) extracts of the samples were mutagenic to *Salmonella typhimurium* strains TA 98 and TA 100, but the mutagenic activity of the oils decreased after they had been repeatedly cooked. However, when the extracts were pre-incubated with the bacteria (TA 100) and microsomes the mutagenic activity of the cooked oils was greater than that of the uncooked oil, suggesting the possible contamination of the cooked oils by N-nitrosarnines. Mutagenic activity was also detected in random samples of seven other brands of uncooked oils obtained from local markets. By absorption spectrophotometry, aflatoxin B_1 levels in 3 of these oils were in the range 98-150 µg kg^{-1}. DMSO-extracts of 10 samples of poor-grade market groundnuts were also mutagenic and the groundnuts contained 95-1055 µg kg^{-1}aflatoxin B_1.

730. Fonseca, H., Nogueira, J.N., Graner, M., Oliveira, A.J., Caruso, J.G.B., Boralli, C., Calori, M.A., and Khatounian, C.A. 1983. Natural occurrence of mycotoxins in some Brazilian foods. II. Pages 53-54 in Proceedings of the Sixth International Congress of Food Science and Technology. Vol. 3 (McLoughlin, J.V., and McKenna, B.M., eds.). Boole Press Ltd., Dublin : Irish Republic.

Surveys were carried out for two years to determine the natural occurrence of mycotoxins (aflatoxin, ochratoxin and zearalenone) in some common Brazilian foods including raw and salted roasted groundnuts, pacoca (a very popular groundnut candy), peanut butter, maize, soybean, cured cheese, salami and copa (cured and dried pork). Eight characteristic regions, Vale do Paraiba. Mogiana, Paulista Velha, Araraquarense, Noroeste, Paulista Nova, Sorocabana and Vale do Ribeira/Litoral Sul, were surveyed, and 742 samples were analyzed. Estimation of toxins was made by thin-layer chromatography (TLC) by comparison with standards. Six samples of corn (4.7 %) were contaminated with aflatoxin, ranging from 41 to 2000 *µg* kg^{-1} of aflatoxin B_1; 81 samples of pacoca (63.3 %), ranging from < 20 to 1187 µg kg^{-1}; 80 samples of peanut butter (62.5 %), from < 20 to 275 µg kg^{-1}; 17 samples of raw groundnuts (17.7 %), from 19 to 3125 µg kg^{-1}; and 9 samples of roasted salted groundnuts (9.4 %) from < 30 to 4250 µg kg^{-1}. From the first to the second year, the percentage of contaminated samples increased slightly for raw groundnuts (from 15.6 to 18.8 %) and decreased for pacoca (67.2 to 59.4 %) for roasted salted groundnuts (12.5 to 7.8 *%),* com (6.2 to 3.1 *%),* and for peanut butter (84.4 to 40.6 *%).* The average content of aflatoxin of the contaminated samples increased for raw groundnuts (from 166.7 to 641.7 µg kg^{-1}) and roasted salted groundnuts (410 to 980 µg kg^{-1}) and decreased for pacoca (165.6 to 108.8 µg kg^{-1}). peanut butter (91.3 to 50.4 µg kg^{-1}), and corn (853.3 to 700 µg kg^{-1}).

731. Fritz, W. 1983. [Occurrence of selected mycotoxins in foods.]. Untersuchungen zum vorkommen ausgewahlten mykotoxine in lebensmitteln. Zeitschrift fur die Gesamte Hygiene und ihre Grenzgebiete 29(11): 650-654.

The concentrations of aflatoxins B_1, B_2. G_1, G_2 and M_1. ochratoxin A, patulin and byssochlaminic acid in a variety of foods were determined by fluorescence spectral analysis. In groundnuts concentrations of up to 1600 pg aflatoxin kg^{-1} were found. Of 198 apparently uncontaminated milk samples, aflatoxin B_1 was detected in 1. and aflatoxin M_1 was identified in 4 of 60 milk samples examined. No aflatoxin M_1 was detected in 22 milk product samples analyzed. Of 49 moldy cereal samples, 2 contained ochratoxin A but no aflatoxins were detected. Both samples of raw coffee examined contained ochratoxin A. Patulin was delected in 19 of 110 samples of fruits and fruit products, in particular commercial apple juice. Byssochlaminic acid was not identified in fruits or fruit products. The toxicological implications of mycotoxins in foodstuffs and the possible ways in which levels in foods could be reduced are discussed.

732. Fukal, L., Prosek, J., and Sova, Z. 1987. The occurrence of aflatoxins in peanuts imported into Czechoslovakia for human consumption. Food Additives and Contaminants 4(3): 285-289.

Results of 492 analyses for aflatoxin in raw shelled groundnuts imported into Czechoslovakia during 1982-1984 are presented. In most (55.3%) of the samples no aflatoxin was detectable by the analytical method used (sensitivity limit of radioimmunochemical method 0.8 µg kg^{-1}). Further analyses showed that 239 of 410 samples of roasted groundnuts contained aflatoxin below the detection limit. Only 1.9% of samples had aflatoxin present at more than 5 µg kg^{-1}. The highest concentrations of aflatoxin were in a raw sample (202.1 µg kg^{-1}) and in a roasted sample (32.6 µg kg^{-1}).

733. Gelda, C.S., and Luyt, L.J. 1976. Survey of total aflatoxin content in peanuts, peanut butter and other foodstuffs. Page 2 in. Abstracts of the Third International I.U.P.A.C. Sponsored Symposium on Mycotoxins in Foodstuffs, 16-18 September 1976, Paris, France.

A study employing the Best Foods (BF) method was carried out for a period of six

years to determine the aflatoxin content in groundnuts, peanut butter and other foodstuffs. The study was divided into three groups. In Group 1 a total of 3928 samples were analyzed; 1679 samples of raw groundnuts, 2092 samples of peanut butter, and 157 samples of processed groundnuts; 5.8 %, 0.1 %, and 1.3 % of the samples analyzed in these groups contained a total aflatoxin content over 25 μg kg^{-1}, respectively. Group II consisted of oilier nuts : cashew, almonds, walnuts, pecans, filberts, Brazil nuts, and pistachios, of which 641 samples were analyzed. Two per cent contained aflatoxin over 25 μg kg^{-1}. Group III, other foodstuffs, consisted of sunflower seeds, cocoa beans, beans, corn, mushrooms, and coffee beans. The total number of samples analyzed were 244 and only 0.4 % contained aflatoxin at over 25 μg kg^{-1}. On the basis of the samples analyzed it is evident that in the past six years the incidence of aflatoxin occurring at levels of less than 5 μg kg^{-1} in groundnuts and groundnut products has been 90.1 % of the samples analyzed. In the other nuts tested, 91.7 % of the samples contained less than 5 μg kg^{-1} of aflatoxin. The test data of samples of foodstufs other than nuts showed that 95.9 % of the samples contained aflatoxin at a level of less than 5 μg kg^{-1}.

734. Gelda, C.S., and Luyt, L.J. 1977. Survey of total aflatoxin content in peanuts, peanut butter, and other foodstuffs. Annales de la Nutrition et de l'Alimentation 31(4/5/6): 477-483.

A study using the 'Best Foods' (BF) method was done over 6 years to determine aflatoxin contents in groundnuts, peanut butter and other foods. Results showed that of 1679 samples of raw groundnuts, 98 (5.8%) contained > 25 μg kg^{-1} total aflatoxins, 89.8 % < 15 μg kg^{-1}. Of 157 samples of processed nuts, 2 (1.3%) contained > 25 μg kg^{-1} and the rest < 15 μg kg^{-1}. Of 2092 samples of peanut butter, 3 (0.14%) contained > 25 μg kg^{-1} and 12 (0.57%) contained 15-25 μg kg^{-1}. Of 120 samples of cashews, 10 (8.3%) contained > 15 μg kg^{-1} as well as 1 of 78 samples of almonds, 2 (1.3%) of 156 samples of walnuts, 10 (5.7 %) of 175 samples of pistachios. None of the samples of pecans, filberts and Brazilnuts tested contained aflatoxin. Of 244 samples of other foods, only one sample each of sunflower seeds and corn contained > 15 μg kg^{-1}. None of the samples of cocoa beans, beans, mushrooms tested had aflatoxin contamination. Test data of foods other than nuts had aflatoxin levels of < 5 μg kg^{-1} in 95.9% of samples.

735. Gemeinhardt, H., and Krug, G. 1973. [The microflora and aflatoxin B_1 content of groundnut samples.]. Zur mykoflora und zum aflatoxin B_1-gehalt von erdnussproben. Zentralblatt fur Bakteriologie. Parasitenkunde, Infektionskrankheiten und Hygiene, Zweite Abteilung 128(1/2): 42-50.

Of 100 imported groundnut samples examined, 27 contained aflatoxin B_1 at 5-800 fig kg^{-1}. *Aspergillus flavus, A. niger* and some mucoraceae were isolated from both aflatoxin-positive and aflatoxin-negative samples.

736. Gilbert, J., and Shepherd, M.J. 1985. A survey of aflatoxins in peanut butters, nuts and nut confectionery products by HPLC with fluorescence detection. Food Additives and Contaminants 2(3): 171-183.

A preliminary survey of peanut butler in 1982 indicated that 31 of 32 samples of major national brand-named products contained aflatoxin B_1 at up to 10 μg kg^{-1}, and that 59 % of those were below the limit of detection (2 μg kg^{-1}). Of 25 peanut butter samples from specialist 'Health Food' outlets, 64 % contained up to 10 μg kg^{-1}, and the remainder contained 16-318 μg kg^{-1}, with one sample having 345 μg kg^{-1} aflatoxin. Surveys of 'Health Food' products in 1983 and 1984 confirmed that manufacturers were still experiencing some difficulty in complying with the 30 μg kg^{-1} voluntary guideline limit for total aflatoxin. In 1984, 228 retail samples of nuts and nut confectionery products comprising groundnuts (shelled, unshelled, roasted and salted), mixed nuts, almonds (both unblanched and ground). Brazil nuts (in shell), hazelnuts (in shell), chocolate-coated groundnuts, peanut britde and coconut ice were examined. Results showed that 74 % of the samples contained aflatoxin B_1 at up to 0.5 μg kg^{-1}, and 3.1%, mainly groundnuts and Brazil nuts, exceeded me guideline tolerance for total aflatoxin. Total aflatoxin was greatest in unshelled groundnuts, 4920 μg kg^{-1}, and in a composite sample of visibly molded Brazil nuts, 17926 μg kg^{-1}.

737. Girgis, A.N., El-Sherif, S., Rofael, N., and Nesheim, S. 1977. Aflatoxins in Egyptian foodstuffs. Journal of the Association of Official Analytical Chemists 60(3): 746-747.

Six samples each of wheat, maize, lentils, beans, fenugreek, groundnuts and cottonseed cake from various areas of Egypt were analyzed for aflatoxins at the time of collection and after 12 months in storage. Aflatoxin was found at low levels (3-12 μg kg^{-1} total aflatoxins) in 14 of 42 samples (1 each of maize, lentils and beans, 2 of groundnuts, 3 of fenugreek and 6 of cottonseed cake).

738. Haberlc, V., Balenovic, J., and Briski, B. 1978. [Contents of aflatoxin in imported groundnuts, coffee, barley, wheat and walnut kernels.]. Kontrola sadrzaja aflatoksina u uvezenom arasidu, kavi, jeemu, psenici i jezgri oraha. Hrana i Ishrana 19(9/10): 451-460.

Aflatoxins were estimated in 295 samples of groundnuts, coffee, barley, wheat and walnuts imported into Yugoslavia. Levels were from 1 to 25 μg kg^{-1} (mean 3.16 μg kg^{-1}). Only 20 % of samples had more than 5 μg kg^{-1} aflatoxin.

739. Habish, H.A., Abdulla, M.H., and Broadbent, J.H. 1971. The incidence of aflatoxin in Sudanese groundnuts. Tropical Science XIII(4): 279-287.

A preliminary survey of the incidence of aflatoxin in the main groundnut production areas of the Sudan is described, correlating the extent of infection with *Aspergillus jlavus* and other kernel fungi with the aflatoxin content of the kernels. In general, the rainfed samples had a higher incidence of kernel contamination than those from the irrigated areas. The majority of the groundnut samples from the irrigated areas were aflatoxin-free, whereas in those from the rainfed areas none was free, and 48 % were classified low-medium (<50 to 50-250 µg kg^{-1} aflatoxin), while 52 % were in the high to very high category (250-1000 µg kg^{-1} to > 1000 µg kg^{-1} aflatoxin).

740. Hamid, A., and Shah, F.H. 1984. Elaboration of aflatoxins on raw groundnuts in Pakistan. Pakistan Journal of Scientific and Industrial Research 27(3): 167-168.

Of 50 raw groundnut samples analyzed, 3 were contaminated with allatoxin. Testing of fungi isolated from samples showed that 37.5 % of the isolates of *Aspergillus flavus* were capable of producing aflatoxins in vitro. None of the samples was resistant to the production of aflatoxin following inoculation with aflatoxin-producing isolates.

741. Haydar, ML, Benelli, L., and Brera, C. 1990. Occurrence of aflatoxins in Syrian foods and foodstuffs : a preliminary study. Food Chemistry 37: 261-268.

Of 19 food commodities (63 samples) analyzed for aflatoxin, 15 commodities including wheat, legumes, nuts, dried fruits, vegetables, cheese and some local popular foods showed no contamination. Aflatoxins were detected in groundnuts, dried figs, lentils and the dairy product koshk. The level of aflatoxin was highest in dried figs with aflatoxin B_1 at 11.8 µg kg^{-1}.

742. Horvath, E., Biro, Z_1 Andrassy, K., and Horvath, I. 1982. | Aflatoxin in foods of plant and animal origin.]. Allatoxin novenyi es allati elelmiszerekben. Orvosi Hetilap 123(20): 1235- 1239.

Aflatoxin at up to 200 µg kg^{-1} was detected in 16-20 % of imported groundnut samples analyzed during 1976-1978. In 1979 a highly contaminated Egyptian groundnut lot raised the positivity to > 80 %, with a maximum contamination of 1000 µg kg^{-1}. The efficiency of recovery was 75-90 % with Pons' method and 60-71 % by the Beljaars method. Aflatoxins were not detected in samples of Hungarian flour and vegetables for soup. In an examination of samples of milk from large-scale dairy farms, allatoxin M_1 at 0.06-0.08 µg/L was detected in 2 of 20 samples in 1977 and 2 of 30 samples in 1978. The aflatoxin B_1 content of groundnut-base feed at one farm was 60 µg $kg._1$. The 30 milk samples analyzed in 1979 did not contain aflatoxin. In 1977 one of 20 powdered milk samples examined contained aflatoxin M, at 200 *µg* kg^{-1}. Egg samples examined during 1978-79 contained no aflatoxin. The methods used in milk and egg analyses had extraction efficiencies of 80-90% and 75-80 %. respectively.

743. Imwidthaya, S., Anukarahanonta, T., and Komolpis, P. 1987. Bacterial, fungal and aflatoxin contamination of cereal and cereal products in Bangkok. Journal of the Medical Association of Thailand 70(7): 390-395.

Samples of cereals and cereal products (150) were collected from various markets in the Bangkok area from July 1983 to April 1985. These included 40 samples of rice products, 50 samples of groundnuts and groundnut products, 20 samples of soybean and soybean products. 20 samples of other beans, and 20 samples of maize. Forty-three samples of groundnuts and groundnut products contained aflatoxins (aflatoxin B, at 40-780 µg kg^{-1} and aflatoxin G, at 10-160 µg kg^{-1}). *Aspergillus flavus* and *A. niger* were isolated from these samples. A total of 40% of a fermented soybean product (tao-chiew) contained aflatoxins (aflatoxin B_1 20 µg kg^{-1} and aflatoxin G, 20-100 µg kg^{-1}); 20 % of salted bean curd derived from soybean contained aflatoxins (aflatoxin B_1 at 210 *µg* kg^{-1} and aflatoxin G_1 at 110-270 µg kg^{-1}); 40% of soybean sauce samples contained aflatoxin B, (20-170 µg kg^{-1}) and aflatoxin G_1 (40 µg kg^{-1}); 10 % of rice samples contained aflatoxin B_1 (10 µg kg^{-1}) and aflatoxin G_1 (20 µg kg^{-1}). Bacteria found included only *Bacillus* spp. According to the standard safety level for aflatoxin offered by WHO, groundnut, fermented soybean, salted bean curd and maize are not safe for consumption but rice is safe. This research project also investigated the ability of *A. niger* to produce aflatoxins in glutinous rice and groundnut and revealed that *A. niger* could produce both aflatoxins B_1 and G_1 in these substrates.

744. Jarrar, B.M, Salhab, A., Natour, R., and Mahasneh, A. 1983. Incidence of aflatoxins in some foodstuffs in Jordan. Dirasat (Natural Sciences) 9(2): 233-243.

Of 430 samples of 22 different foods collected in Jordan between January 1978 and January 1979, 5 were contaminated with aflatoxin, 3 of 40 groundnut samples, 1 of 50 rice samples, and 1 of 15 samples of dried milk. Groundnuts contained aflatoxin B_1 at 98-1056 µg kg^{-1}, rice contained aflatoxin B_1 at 29 *µg* kg^{-1} and dried milk contained aflatoxin M_1 at 15 µg kg^{-1}. Aflatoxigenic fungi isolated from the food samples were mainly *Aspergillus flavus,* but some *A. parasiticus* was also detected. Some soil samples collected from 20 different areas of Jordan contained aflatoxigenic fungi.

745. Jewers, K. 1982. Mycotoxins in food - the application of survey and quality control. Royal Society of Health Journal 102(3): 114-118.

The methodology for conducting a survey of mycotoxin contamination of foods and feedstuffs, including sampling, analysis and treatment of data obtained is presented.

Results of a survey of the aflatoxin content of a small proportion of groundnuts from the 1977/78 season imported into the U.K. are given. Thirty-one of the 159 samples exceeded the maximum permitted level of aflatoxin in raw groundnuts (30 µg kg^{-1}). The highest level of aflatoxin contamination was in samples from the Gambia and India (> 400 µg kg^{-1} in 1 sample each). Results of a survey of the aflatoxin M_1 content of samples of producer-retailer milk in the U.K.. commissioned in 1977 by the Ministry of Agriculture, Fisheries and Food are presented. The survey showed that 11.9 % of the milk samples tested contained > 0.1 µg L^{-1} aflatoxin M_1 (the limit permitted by European Community Regulations). A parallel study of random samples of dairy feed indicated that 8 % contained > 30 µg kg^{-1} aflatoxin B_1. In another study the average level of aflatoxin in a 12 ton batch of groundnut cake obtained from a farmer in the U.K. was 1540 µg kg^{-1}, which would result in feedstuffs containing aflatoxin well in excess of the permitted level.

746. Jodral, V.M 1974. Study of aflatoxins in groundnuts. Archivos de Zootechnia 23(89): 1-8.

Samples of groundnuts from Cordoba market contained aflatoxins B_2 and M_1. Groundnuts containing 50 µg kg^{-1} aflatoxin B_1 were considered unfil for human consumption.

747. Joffe, A.Z. 1970. The presence of aflatoxin in kernels from five years groundnut crops and of *Aspergillus flavus* isolates from kernels and soils. Plant and Soil 33: 91-96.

Aflatoxin was found in up to 6.4% of fresh groundnut kernels and in up to 32% of stored kernels, although the intensity of toxicity was always very low (up to 125 µg kg^{-1}). About 90% of 1626 *Aspergillus flavus* isolates from groundnut kernels, rhizospheres, and geocarpospheres produced aflatoxin in culture. In quantitative tests with 750 isolates, 60% of the isolates produced > 25 µg g^{-1} aflatoxin.

748. Josefsson, E., Nilsson, G., and Akerstrand, K. 1975. [Occurrence of moulds and mycotoxins in foods in Sweden.). Forekomst av mogelsvampar och mykotoxiner i livsmedel i Sverige. Lakartidningen 72(51): 5074-5077.

The total number of molds and of species of fluorescent *Aspergillus* and *Penicillium* varied widely in samples of rye and wheat flour. The fluorescent strains formed ochratoxin. Aflatoxin was found in certain nuts, especially Brazil nuts and groundnuts, imported into Sweden.

749. Kannaiyan, J., Sandhu, R.S., and Phiri, A.L. 1989. Aflatoxin and *Aspergillus flavus* contamination problems of groundnuts in Zambia. Pages 65-70 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 Oct 1987, ICRISAT Center, India Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

In Zambia, groundnut kernels 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 *Aspergillus flavus* seed infection. Seed treatment with Benlate(R)/Labilite(R) at 3 g kg^{-1} seed controlled *A. flavus* in groundnut seed and improved crop stand.

750. Karmaeharyu, S. 1984. Aflatoxin contamination in foods & feeds of Nepal. Pages 135-144 in First National Seminar on Food Industry and Food Technology, 23-26 April 1984, Dharan Campus, Institute of Science and Technololgy, Nepal : Tribhuvan University.

Four hundred and sixty five samples of various food commodities and 94 samples of different feeds and feed ingredients were analyzed for aflatoxin contamination. These samples were collected from different parts of Nepal. Mostly maize and groundnut samples were contaminated with aflatoxins B, and B_2. Of 101 samples, 10 samples of maize used as food were contaminated with aflatoxin at levels > 30 µg kg^{-1}, while only 2 of 67 samples of groundnuts were highly contaminated. Among feed samples, 25 of 58 poultry feed samples were highly contaminated. Maize and groundnut cake were mostly contaminated used as feed ingredients. The other commodities used as foods and feeds had aflatoxin levels well within the sale limit (30 µg kg^{-1}).

751. Karmacharya, S. 1988. Studies on the occurrence of aflatoxins in various food and feed commodities of Nepal. Pages 426-433 in Proceedings of the National Conference on Science and Technology, 24-29 April 1988, Kathmandu. Nepal.

Studies on aflatoxin contamination in various food samples of Nepal were carried out during the period 1980-1986. About 764 samples of various food commodities from hills and Terai region were analyzed for aflatoxin contamination. One hundred and forty-eight samples of different feed ingredients from different Feed Industries of Kathmandu were also analyzed. Of the total number of respective samples analyzed, 19 % of maize, 29.5 % of maize Hour, 17.9 % to 33.8 % of groundnuts, 31.7 % of peanut butter and 9.4 % of wheat flour were contaminated with aflatoxin, but tolerance limit (30 µg kg^{-1}) exceeded in 4.5 % to 15.9 % of these samples. Rainy season is the most prone period for aflatoxin contamination. In case of feed, about 50 % of poultry feed, 26.7 % of cattle feed and 20 % of pig feed samples were contaminated with aflatoxins. Twenty % of the poultry feed samples had aflatoxins above the tolerance limit (10 to 50 µg kg^{-1} depending upon the age of the animals).

752. Kershaw, S.J. 1985. Aflatoxins in imported edible nuts: Some data 1982-84. Journal of Food Technology 20(5) : 647-649.

Data from analysis of 188 groundnut samples imported into the U.K. during 1982-1984 showed that most samples (140/188; 74%) had aflatoxin content < 5 µg kg^{-1}.

753. Kim, Y.H., Hwangbo, J.S., and Lee, S.R. 1977. Detection of aflatoxins in some Korean foodstuffs. Korean Journal *of* Food Science and Technology 9: 73-80.

Fifty-four samples of Meju (a naturally inoculated soybean substrate for soy sauce and paste fennentation), 125 samples of Doenjang (a Korean style fermented soybean paste), both produced at the household level, and 31 samples of groundnuts were collected from 8 major cities of South Korea and subjected to aflatoxin assay by the AOAC method. Results showed that frequencies of occurrence of aflatoxins in Meju, Doenjang, and groundnuts were 7.4% (4 out of 54), 8.8 % (11 out of 125) and 0 (none out of 31) respectively. A Doenjang sample from Busan had the highest content of aflatoxins; concentrations of aflatoxins B_1, B_2, G_1 and G_2 were 66, 13, 0 and 5 µg kg^{-1}, respectively, while in other samples only aflatoxin G_2 was detected. Aflatoxin B_1, isolated from the Doenjang sample from Busan, was confirmed by TLC, derivative formation and chicken embryo bioassay.

754. Korpinen, E.L. 1971. Occurrence of aflatoxin in groundnuts, some other nuts and industrial proteins imported into Finland. Nordisk Hygienisk Tidskrift 53(2): 60-69.

Of the 182 samples of groundnuts and other nuts examined, aflatoxin was detected in 6 samples of groundnuts (all of poor quality) at levels of 2-50 µg kg^{-1}.

755. Krug, C., and Kusche, P. 1973. |Aspects of aflatoxin contamination of foodstuffs concerned with preventive measures in health protection!. Aspekte der aflatoxinkontamination von lebensmitteln als aufgabe des vorbeugenden gesundheitsschutzes. Zeitschrift fur die gesamte Hygiene und ihre Grenzgebiete 19(5); 342-348.

A thin-layer chromatographic (TLC) method for semi-quanutative determination of aflatoxin B_1 in foodstuffs, suitable for routine analysis, is described. The application of the method to the analysis of groundnut kernels is discussed. Of groundnut samples imported into Germany in 1970-1971, 10% of samples had aflatoxin. Measures for improving health control of imported groundnuts are discussed.

756. Kshemkalyani, S.B., and Patel, G.S. 1988. Investigation of aflatoxin contamination in commercial groundnuts. Journal of Food Science and Technology 25(6): 364-365.

Groundnuts stored and sold in Ahmednagar city and nearby villages were analyzed, 75 of samples had aflatoxin B_1. Among the contaminated samples, 37 had aflatoxin B_1 at over 120 µg kg^{-1}. Three samples had aflatoxins B_1 and B_2. Storage in open containers with other grains (at 70-80% relative humidity) was observed in most of the shops.

757. Laub, E., and Wollcr, R. 1977. [Occurrence of aflatoxins B_1. B_2, G_1 and G_2 in commercial foodstuff samples.]. Vorkommen der aflatoxine B_1, B_2, G1 und G_2 in Lebcnsmittelproben des handels. Deutsche Lebensmittel-Rundschau 73(1): 8-10.

Of 1000 food samples examined, aflatoxins were found in groundnut products, maize products, almond products and nuts. Of 316 samples of groundnut, 35 (11.1 %) contained aflatoxins; 23 (7.3 %) had high levels (> 10 µg kg^{-1} aflatoxin B_1 or > 20 µg kg^{-1} total aflatoxin).

758. Lopez, A., and Crawford, M.A. 1967. Aflatoxin content of groundnuts sold for human consumption in Uganda. Lancet 2: 1351- 1354.

The content of aflatoxin was estimated in groundnuts sold for human consumption in Uganda. About 15 % of the samples examined contained > 1000 µg kg^{-1} aflatoxin B_1 and 2.5 % contained > 10000 µg kg^{-1}. The level of aflatoxin contamination seemed to be highest at the end of the rains and before the new crops were harvested.

759. Lotter, L.H., and Krohm, H.J. 1988. Occurrence of aflatoxins in human foodstuffs in South Africa. Bulletin of Environmental Contamination and Toxicology 40(2): 240-243.

During 1985 and 1986, samples of sorghum beer, sorghum cereal, groundnuts, peanut butter and maize meal, purchased from supermarkets, were analyzed for aflatoxins using HPLC and fluorescence detection methods. Of the samples analyzed during 1985. approximately one-third were contaminated with aflatoxins, although none were above the legal limit of 10 µg kg^{-1} aflatoxins. In 1986, the numbers of contaminated samples increased, but the level of contamination remained low, with only one sample exceeding the legal maximum.

760. Lovelace, C.E.A., and Aalbersberg, W.G.L. 1989. Aflatoxin levels in foodstuffs in Fiji and Tonga islands. Plant Foods and Human Nutrition 39: 393-399.

Of 33 groundnut samples analysed, 50 % of Fijian samples were contaminated with aflatoxin and 9 % of Tongon samples contained aflatoxin. Local copra, cassava and corn samples examined were also contaminated with aflatoxin with corn contaminated at a high level.

761. Maeda, K. 1983. Aflatoxin inspection of imported raw shelled peanuts in Japan. Proceedings of the Japanese Association of Mycotoxicology No. 17: 26-31.

Samples of groundnut imported into Japan during 1972-1981 were investigated for aflatoxin content. Of 2612 samples of Virginia type groundnuts examined, 13 contained aflatoxin B_1 (12 contained < 10 µg kg^{-1} and one > 10 µg kg^{-1} aflatoxin B_1). Of 9412 samples of Spanish-Valencia type groundnuts examined, 474 contained aflatoxin B_1 (310 contained < 10 µg kg^{-1} and 164 > 10 µg kg^{-1} aflatoxin B_1). Samples containing > 10 µg kg^{-1} were from Brazil, the Sudan, Indonesia, India, Paraguay, and the USA.

762. Manzo, S.K., and Misari, S.M. 1989. Status and management of aflatoxin in groundnuts in Nigeria. Pages 77-90 in Aflatoxin contamination of groundnut : Proceedings of the International Workshop, 6-9 October 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

A committee to coordinate action on the problem of aflatoxin contamination in Nigeria was formed in 1961 with representatives from four ministries, i.e., the Institute for Agricultural Research (IAR), Zaria, the Nigerian Stored Products Research Institute (NSPRI), and the Northern Nigerian Marketing Board. This Committee was given the responsibility of assessing the extent of the aflatoxin problem in groundnut in the country and of initiating and coordinating all actions leading towards elimination of aflatoxin. IAR investigated the aflatoxin contamination of the groundnut crop up to the stage where trie produce was sold by farmers, while NSPRI studied the problem from the time of storage until produce was exported or consumed. IAR investigated the time of invasion of groundnut kernels by *Aspergillus flavus,* and when, and under what conditions it produced aflatoxin. An interplay of temperature, relative humidity, drought, and erratic rainfall patterns, and maturity of the crop at lifting affected 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, while in the major groundnut growing areas that lie in the drier Northern Guinea and Sudan Savanna the problem is largely 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 groundnuts to either 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 government 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 with an aflatoxin content that does not exceed the maximum permissible limit of 20 *µg* kg^{-1} set by the European Economic Community. 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.

763. Masimango, N., Ramaut, J.L., and Remade, J. 1977. |Anatoxins and toxigenic fungi in foodstuffs from Zaire.]. Aflatoxins et champignons toxinogeenes dans des denrees alimentaires Zairoises. Revue des Fermentations et des Industries Alimentaires 32: 164-170.

Groundnuts (3 samples), bananas (2), millet (3), maize flour (10), sorghum (2), cassava flour (13), sweet potatoes (6). potatoes (3) and germinated sorghum (6) were examined. Aflatoxins were detected in 19 samples; 5 had more than 1 µg g^{-1} and a sample of sweet potato had 17 µg g^{-1}. Values were generally greatest in the samples with highest water contents. Most fungi were of *Aspergillus* spp. and *A. flavus* was most common. Studies in vitro showed that *A. flavus* was not the only species to synthesize aflatoxin.

764. Mat, Isa Awang, and Nazarifah, I. 1986. Aflatoxin contamination in agricultural commodities. Teknologi Makanan, Malaysia 5(1): 54-58.

Aflatoxin contamination in five agricultural commodities namely groundnut, dried cocoa beans, copra, pepper (black and white) and paddy and rice was determined. Shelled groundnuts and their products such as peanut butter and satay gravy were highly susceptible to aflatoxin contamination, but the "menglembu" type groundnut was free of aflatoxin. Copra, dried cocoa beans and pepper (black and white) were the other highly susceptible commodities, but stored paddy and rice were safe but a few samples contained aflatoxin. The extent of contamination in several other commodities is also mentioned and general control methods are discussed.

765. Mintah, S., and Hunter, R.B. 1978. The incidence of aflatoxin found in groundnuts *(Arachis hypogaea* L.) purchased from markets in and around Accra, Ghana. Peanut Science 5(1): 13-16.

Eighty samples of groundnut, representing both the Northern and Volta Region types, were obtained from markets in and around Accra, Ghana, and analyzed for aflatoxin. Sample size was 0.5 kg. Mean levels found for each type (with ranges in

parentheses) were Northern 27 μg kg^{-1} (3-133 μg kg^{-1}) and Volta 62 μg kg^{-1} (10-216 μg kg^{-1}), 65 % of Volta type and 30 % of the Northern type samples had levels > 30 μg kg^{-1}, the maximum recommended level by FAO/WUO. The Volta type groundnuts contained on an average significantly higher levels of aflatoxin than the Northern nuts. The Volta region conditions are much wetter at the time when the first season groundnuts are harvested. Damaged kernels had significantly higher levels of aflatoxin than undamaged kernels. No undamaged kernels contained aflatoxin > 30 *μg* kg^{-1}. When damaged kernels were removed from the samples the remaining whole kernels had aflatoxin levels below the tolerance level established by FAO/WHO.

766. Muhilal, and Karyadi, D. 1984. Aflatoxin in nuts and grains. Pages 311-316 in Health and ecology in grain post-harvest technology (Scrnple, R.L., and Frio, A.S., eds.). A.S. College, Laguna, Philippines.

Groundnuts obtained from distributors were aflatoxin-free, while about 30% of those bought from small retailers in the market were contaminated with aflatoxin (7 to 2000 μg kg^{-1} aflatoxin B_1). All samples of rice, green grain and soybean tested were aflatoxin- free. Eleven of 20 samples of com were contaminated with aflatoxin, with average values of 25.8 μg kg^{-1} of aflatoxin B_1, and 24 8 μg kg^{-1} of aflatoxin G,. One sample of moldy candle nut had 426 μg kg^{-1} of aflatoxin. The results of this limited survey on aflatoxins in nuts and grains have indicated that groundnut and corn are the commodities which are most susceptible to contamination with aflatoxin. Guidance on postharvest technology should be given to the farmers, distributors, and retailers concerning proper drying and storage of the crops to minimize mold growth.

767. Muraguri, N., Omukosio, L.C., Kenji, G.M., and Condier, G.A. 1981. A survey of mycotoxins in human and animal foods - Part 1. East African Medical Journal 58(7): 484-488.

A total of 195 samples of human food and 12 samples of animal feed were analyzed for aflatoxins. Aflatoxins were detected in 40 of the human food samples, mainly maize and maize products. Most of the contaminated samples contained < 100 μg kg^{-1} aflatoxin, but 1 maize flour sample from a gunny bag in a shop contained 140 *μg* kg^{-1} aflatoxin. Contamination in wheat flour did not exceed 25 μg kg^{-1} aflatoxin.and for rice, the few samples tested contained only trace amounts of aflatoxin. Except for 1 sample of raw groundnuts, containing 1050 μg kg^{-1} aflatoxin. other nuts and oils were relatively free of contamination.

768. Nagaraj, G., and Kumar, K. 1986. Location variations in the aflatoxin content of some Virginia groundnut varieties. Journal of the Oil Technologists' Association of India 18(3): 89-91.

Seven Virginia groundnut varieties, viz. S 230. Kadiri 1, Kadiri 3, Karad 4-11, M_{13}, GAUG 10 and TMV 10 were grown in four locations (Chimamani, Jalgaon, Khargaon, and Junagadh) in India in the rainy season of 1982. After harvest the pods were received at Junagadh during February-March 1983. Pods were shelled and aflatoxin analyses carried out on seeds with a thin-layer chromatography (TLC) method. Aflatoxin B_1 was found in all the groundnut varieties (0.8 to 65.8 *μg* kg^{-1}). Aflatoxins B_2 and G_1 were found in a few samples from some locations. Among varieties, S 230 had the highest level of aflatoxins at all the locations (49.8 to 85.9 μg kg^{-1}) followed by GUAG 10 (15 to 88.3 μg kg^{-1}) and Kadiri 3 (17.8 to 106.7 μg kg^{-1}). Karad 4 -11 had low levels of aflatoxins at all four locations (1.6 to 30.3 μg kg^{-1}) followed by Kadiri 1 (4 to 52 μg kg^{-1}). M_{13} and TMV 10 had moderate levels of aflatoxins. Among the locations, Junagadh and Chintamani samples contained higher levels of aflatoxins. Unfavorable postharvest drying conditions at Junagadh and Chintamani probably resulted in higher levels of aflatoxins at these locations. The varieties with more than 3 % phenols in the seed testa generally contained low levels of aflatoxins. The varieties with protein content higher than 25 % contained more aflatoxins, while those having about 22 % proteins contained less aflatoxins except TMV 10 which also had less total phenols. Sugar and oil content did not appear to have any effect on the aflatoxin levels.

769. Natarajan, K.R., Rhee, K.C., Cater, C.M., and Mattil, K.F. 1975. Distribution of aflatoxins in various fractions separated from raw peanuts and defatted peanut meal. Journal of the American Oil Chemists' Society 52: 44-47.

The present investigation is the first definitive study of the distribution of aflatoxins in a wet-milling process of raw groundnuts. The results show that the majority of aflatoxins originally present in the groundnuts remained in the solid fractions, particularly the protein fractions, during wet-milling. In the protein concentrate preparation, the concentrates carried 81-89 % of the total aflatoxin; crude oil, 5-8 %; and whey fraction, 3-14 %. In the case of protein isolate preparation, 51-56 % of the total toxin remained with the isolates, 22-26 % with the residue, 11-17 % with the whey, and 7- 8 % with the crude oil. Distribution of aflatoxins in the preparation of protein isolates from defatted groundnut meal showed that 55-65 % of the total toxin originally present in the meal remained with the protein isolates, 20-28 % with the residue, and 10-20 % with the whey fraction. Changes in extraction pHs for the preparation of protein isolates either from raw groundnuts or defatted meal did not alter the distribution pattern. A new approach based upon the charge- transfer (electron acceptor-donor) complex formation is suggested to shift this aflatoxin distribution from protein products to disposable whey or residue fraction from the processing of raw groundnuts and defatted meal for protein products.

770. Okonkwo, P.O., Umerah, G., and Nwokolo, C. 1977. Procedures to reduce

aflatoxin levels in common foods. West African Journal of Pharmacology and Drug Research 4(1): 62-63.

A study of major foods in Nigeria showed that groundnuts and dried fish were highly contaminated with aflatoxins (900 µg kg^{-1} in groundnuts ; 600-700 *µg* kg^{-1} in dried fish) whereas various grains, millets, guinea corn and rice were moderately contaminated (300-150 µg kg^{-1}). Low risk foods such as yams became contaminated with aflatoxin after storage, particularly when wrapped in cellophane or even in newspaper. Immersion of foods in a dilute (1 %) solution of NaCl 16.5 % and sodium hypochlorite 1 % (Milton) reduced the level of aflatoxin by almost 50 %. Exposure to direct sunlight caused a marked decline (30-16 %) in aflatoxin levels of foods.

771. Patil, S.P., and Shinde, P.A. 1985. Mycotoxin contamination and associated mycoflora of groundnut. Journal of Maharashtra Agricultural Universities (India) 10(1): 99.

A survey was carried out to determine the extent of aflatoxin contamination of groundnuts in Maharashtra. Aflatoxin contamination was detected in 5 of 26 samples of groundnuts collected from six markets. Species of *Aspergillus, Rhizopus* and *Penicillium* were predominant in the seed samples.

772. Peers, F.G., Oilman, (J,A., and Linsell, C.A. 1976. Dietary aflatoxins and human liver cancer : A study in Swaziland. International Journal of Cancer 17(2): 167-176.

A study in Swaziland to assess the possible relationship of aflatoxin contamination and the incidence of primary liver cancer is reported. Aflatoxin ingestion levels were determined in food from plate samples collected over a one year period. A significant correlation between the calculated ingested daily dose and the adult male incidence of primary liver cancer in different parts of Swaziland was established. Samples of foodstuffs other than plate samples also reflected the correlation of aflatoxin contamination and liver cancer. This study extends and amplifies the findings of an earlier study in the Murang's district in Kenya and supports the hypothesis that aflatoxin ingestion is a factor in the genesis of primary liver cancer in Africa.

773. Pettit, R.E., and Taber, R.A. 1968. Factors influencing aflatoxin accumulation in peanut kernels and the associated mycoflora. Applied Microbiology 16(8): 1230-1234.

Levels of aflatoxin in Spanish groundnut kernel samples from different geographical areas in Texas during 1966 were low. Of the 334 samples tested, 239 (71.6 %) contained no aflatoxin and only 2.7 % contained > 30 µg kg^{-1} and 25.8 % contained trace-29 µg kg^{-1}. Analysis of samples obtained from growers using artificial drying equipment (forced air and supplemental heat), when windrow conditions were unfavorable for rapid drying, suggests that this practice reduces the possibility of aflatoxin accumulation. In general, groundnuts harvested from land planted to groundnuts the previous year were more highly infected by fungi and contained more aflatoxin than groundnuts grown on land planted to rye, oats, melons, or potatoes the previous year. Aflatoxin incidence tended to decrease from south to north Texas. Detection of aflatoxin in sound mature kernels (kernels screened for minimal size) indicates that the practice of screening for removal of small immature kernels and removal of obviously damaged kernels does not completely eliminate aflatoxin contamination.

774. Pitt, J.I. 1989. Field studies on *Aspergillus flavus* and aflatoxins in Australian groundnuts. Pages 223-235 in Aflatoxin contamination of groundnut: proceedings of the International Workshop, 6-9 Oct 1987, ICRISAT Center, India. Patancheru. A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

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 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 spores g^{-1} to as high as 10^5 spores 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, aflatoxin is not 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 alter 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 arc not yet of practical utility.

775. Pollet, A., Declert, C, Wicgandt, W., Harkema, J., and Lisdonk, E.de. 1989. Traditional groundnut storage and aflatoxin problems in Cote d'Ivoire : ecological approaches. Pages 263-268 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 Oct 1987. ICRISAT Center. India. Patancheru 502 324, A.P., India : International Crops Research Institute for the Semi-Arid Tropics.

Groundnut storage problems were studied in Cote d'Ivoire during two successive storage periods (1985/86 and 1986/87) 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 infected than samples 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 µg kg^{-1}, with 4.4 % above 1000 µg kg^{-1}. It was also found that 73 % of these samples were above the European Economic Community (EEC) safety level of 10 µg kg^{-1}. Significant correlations were found between aflatoxin contamination and different storage and meteorological variables. These included physical characteristics and age of the pods, and the influence of the prevailing atmospheric conditions.

776. Prado, G. 1983. [Incidence of aflatoxin B, in foods. |. Incidencia de aflatoxina B_1 em alimentos. Revista de Farmacia e Bioquimica, Belo Horizonte 5(2): 147-157.

Aflatoxin B_1 was determined in samples of groundnuts and their products, wheat, corn and manioc flour, collected in commercial establishments in Belo Horizonte in 1983, using a thin-layer chromatography (TLC) method. Aflatoxin B_1 was detected in 44 % of the samples of groundnut and their products; aflatoxin levels were above the tolerance level permitted by Brazilian legislation (30 µg kg^{-1} aflatoxins B_1+G_1).

777. Quitco, R., Bautista, I., and Bautista, C. 1989. Aflatoxin contamination of groundnuts at the post-production level of operation in the Philippines. Pages 101-110 in Aflatoxin contamination of groundnut: proceedings of the International Workshop. 6-9 October 1987, ICRISAT Center, India. Patancheru. A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

The results of surveys in the Philippines have shown that farm level aflatoxin significantly increased from harvest to farm storage during the main cropping season. At harvest, groundnuts contained, on average, 3.16 µg kg^{-1} aflatoxin. During windrowing, aflatoxin levels increased at the rate of 1.5 *µg* kg^{-1} day^{-1}. In farm storage aflatoxin contamination continued to increase at the rate of 1 µg kg^{-1} day^{-1}. Aflatoxin contamination was signifiantly higher during the main cropping season than the second cropping season. At me traders' level, groundnut samples taken from various middlemen contained 35 µg kg^{-1} aflatoxin. On the other hand, samples taken from the wholesalers' newly procured groundnuts contained 188 µg kg^{-1} aflatoxin. Groundnuts that had been in wholesalers' warehouses for more than 3 months contained 275 µg kg^{-1} aflatoxin. At the processors' level, raw groundnuts for confectionery use (roasted and fried) contained 7.73 µg aflatoxin kg^{-1}, grounduts intended for peanut butter contained 17.13 µg kg^{-1}, and rejected groundnuts had 120.6 µg kg^{-1}. Aflatoxin contamination could start during harvest. Aflatoxin content increased to a significantly high level during trade and processing. This continued increase was attributed to insufficient drying of groundnuts after harvest.

778. Rana, I.A. 1989. Aflatoxin contamination of groundnuts in Pakistan. Pages 111-114 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 Oct 1987. ICRISAT Center, India. Patancheru, A.P. 502 324. India : International Crops Research Institute for the Semi-Arid Tropics.

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 groundnuts from areas other than Khuzdar were contaminated. The aflatoxin content of the contaminated samples varied from 24 to 800 *µg* kg^{-1}. All the tested samples of roasted groundnuts from Khuzdar were contaminated with aflatoxins.

779. Rao, K.S., Madhavan, T.V., and Tulpule, P.G. 1965. Incidence of toxigenic strains of *Aspergillus flavus* affecting groundnut crop in Coastal districts in India. Indian Journal of Medical Research 53: 1196-1202.

Of 288 samples of groundnuts from six coastal districts of Andhra Pradesh State, India, it was possible to isolate strains of *Aspergillus flavus* from 29. Of the 29 strains 6 produced aflatoxin B_1 when cultured, as estimated by thin-layer chromatography (TLC) and in tests with ducklings. Aflatoxin contamination was found in 36 samples. Toxic samples were more prevalent in some districts man others. Samples commonly included 6 to 14 % of pods with shell damage, but this damage was not related to toxicity.

780. Sabino, M. 1989. National monitoring and control program on mycotoxins in Brazil. Pages 115-120 in Aflatoxin contamination of groundnut: proceedings of the International Workshop. 6-9 Oct 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

Analyses of groundnut samples collected in the surveys of rainy season (313 samples) and dry season (83 samples) crops in the State of Sao Paulo showed that on an average 48-74 % of the samples collected from the west and northeast regions contained 5-22500 µg aflaioxin B_1 kg^{-1}. This 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.

781. Sanchis, V., Sala, N., Palomes, A., Santamarina, P., and Burdaspal, P.A. 1986. Occurrence of aflatoxin and aflatoxigenic molds in foods and feed in Spain. Journal of Food Protection 49(6): 445-448.

A survey was carried out to obtain data on the occurrence of aflatoxins and aflatoxigenic mold contamination of foods in Spain. 338 samples of cereal grains, mixed feeds, edible nuts, wheat flour for bread-making, biscuits, sliced bread, soybeans and breakfast cereals were analyzed. Aflatoxins were detected in 4 of 27 samples of mixed feeds at levels below 5 µg kg^{-1}; one sample of groundnuts was contaminated with 120 µg aflatoxin B_1 kg^{-1} and 22 µg aflatoxin B_2 kg^{-1}. Aflatoxins B_1 and B_2 were also detected in a lot of whole maize flour, averaging 8 µg kg^{-1} and 3 µg kg^{-1} respectively. All of 288 samples tested showed fungal contamination to varying extent. Corn samples were most frequently contaminated with *Aspergillus flavus* (54.5 %). Isolates of *A. flavus* from corn samples also showed a higher proportion of aflatoxigenic strains (17.2 *%)* as compared with those from other sources.

782. Sanders, T.H., McMeans, J.L., and Davidson, J.I.Jr. 1984. Aflatoxin content of peanut hulls. Journal of the American Oil Chemists' Society 61(12): 1839-1841.

The degree of aflatoxin contamination in groundnut hulls (shells) used in cattle feed was determined by analyzing inoculated hand- shelled hulls and hulls from groundnuts known to contain aflatoxin. Hulls adjusted to 20 % moisture, inoculated with *Aspergillus flavus* and incubated for 7 days at 25°C supported growth of the fungus but not aflaioxin production. Groundnuts from 20 selected lots with visible *A. flavus* contamination contained 13-353 µg kg^{-1} aflatoxin. The machine-shelled hulls from these lots were analyzed and 3 lots contained no detectable aflatoxin, 13 lots contained 4-88 µg kg^{-1} and 4 lots contained 116 µg kg^{-1}. Aflatoxin concentrations of 53-87 µg kg^{-1} were detected in hulls when groundnuts containing relatively high levels of aflatoxin (up to 26800 µg kg^{-1} in damaged kernels) were carefully machine-shelled. Hulls from the same samples obtained by hand-shelling contained no detectable aflatoxin. Separating hulls over a 4.76 mm screen appeared to provide a means of removal of most aflatoxin-containing particles in groundnut hulls.

783. Santamaria, P.A., Pixarro, A.C., and Jackson, C.R. 1985. Aflatoxin contamination on raw agricultural crops and their by- products in the Philippines. Philippine Phytopathology 8(1&2):12-19.

Raw agricultural crop produce and their by-products collected from different areas of the Philippines contained varying amounts of aflatoxin and degrees of *Aspergillus flavus* infection. Some of the test samples showed no visible signs of *A. flavus,* but contained high levels of aflatoxin. Conversely, other samples yielded the fungus in agar plates but contained little or no aflatoxin. Corn grains on the cob contained high levels of aflatoxin. Corn grown in the Visayas and Mindanao had more aflatoxin than those grown in Northern Luzon. Dried groundnuts from the Visayas and Mindanao generally contained low levels of aflatoxin. Sorted out groundnuts with wrinkled kernels unfit for commercial processing, however, had as much as 144 µg kg^{-1} aflatoxin. The processes in copra-making like drying and storage predispose the coconut meat to infection by *A. flavus* and other micro-organisms. However, a finished product, copra pellet, contained no aflatoxin after treatment with hexane.

784. Sebunya, T.K., and Yourtee, D.M. 1990. Aflatoxigenic aspergilli in foods and feeds in Uganda. Journal of Food Quality 13(2): 97-107.

The occurrence of aflatoxins and aflatoxigenic fungi in staple Ugandan food crops and poultry feeds derived from these foodstuffs was investigated. Fifty-four samples of maize, groundnuts, soybean and poultry feed were analyzed for aflatoxigenic fungi, *Aspergillus flavus* and *A. parasiticus,* and 25 of the samples were also screened for aflatoxins B_1 and G_1, zearalenone, sterigmatocystin. ochratoxin A, citrinin, vomitoxin and diacetoxyscirpenol. Aflatoxigenic *A. flavus/A. parasiticus* was detected from the majority of maize (77 %), groundnuts (36 % human food and 83.3 % animal feed) and poultry feed (66.6 %), but not from soybean samples. Two of the 25 samples (maize and poultry feed) contained detectable levels of aflatoxin B_1 (20 µg kg^{-1}). Zearalenone (3 samples) and vomitoxin (2) were detected in maize.

785. Sellschop, J.P.F., Kriek, N.P.J., and Du Preez, J.C.G. 1965. Distribution and degree of occurrence of aflatoxin in groundnuts and groundnut products. South African Medical Journal 39: 771- 774.

Distribution and degree of occurrence of aflatoxins in various samples of groundnuts and groundnut products in the Republic of South Africa are described. Over 1000 samples of groundnuts and groundnut products were examined during 1963 and another 1000 samples of producers' grades were investigated during 1964. The samples obtained from the North-Western Transvaal, or so-called Bushveld areas, were most seriously contaminated with aflatoxin; all of the 75 samples containing > 2000 µg kg^{-1} aflatoxin were from the receiving depots in the North-Westem

Transvaal. The samples from the Northern Cape Province, Orange Free State and Natal, except for 5 samples with 500-2000 µg kg^{-1} and 11 samples with 100-500 µg kg^{-1}, were free from allatoxin. Similar results were found in the 1964 survey for aflatoxin contamination; however, only 23 samples from the North-Western Transvaal areas contained > 2000 µg kg^{-1}. High degree of aflatoxin contamination in the groundnut samples from the North-Western Transvaal was ascribed mainly to (1) the damage caused to the maturing groundnut pods and kernels by termites, and (2) protracted droughts followed by late rains. Lower or inferior grade groundnuts contained higher levels of aflatoxins than the higher or superior ones. No aflatoxins were detected in any of the samples of hand-picked selected (HPS) kernels examined in 1963 and 1964. Groundnut cake samples implicated in livestock deaths were also examined for allatoxin contamination. Eleven of the 16 samples of this groundnut cake contained > 2000 µg kg^{-1} aflatoxin. Of 101 peanut butter samples tested, 17 were contaminated with aflatoxin. Of these 17 samples, 12 had an allatoxin contents of 100-500 µg kg^{-1} and 5 had up to 25 µg kg^{-1}.

786. Shah, F.H.. Begum, N., Adil, R., and Sheikh, A. 1981. Aflatoxins in food and feedstuffs. Pakistan Journal of Medical Research 20(2): 40-43.

Two of 97 food samples (roasted groundnuts and maize) contained allatoxin at 800 and 500 µg kg^{-1}, respectively. Aflatoxin was also detected in 13 of 74 feed samples (5 poultry feed samples at 5-1000 µg kg^{-1}, 1 sample of cottonseed meal at 8 µg kg^{-1} and 7 samples of maize gluten at 450-1140 µg kg^{-1}).

787. Shah, F.H., and Hamid, A. 1989. Aflatoxins in various foods and feed ingredients. Pakistan Journal of Science and Industrial Research 32: 733-736.

Cereals, pulses, nuts and other food and feed ingredients from different regions of Pakistan were screened for aflatoxin contamination. Samples of wheat, rice, pulses and beans showed no aflatoxin contamination although toxigenic strains of *Aspergillus flavus* were present in some samples. Aflatoxins B_1 and B_2 were present in maize and maize products at up to 800 µg kg^{-1} and in groundnuts, pistachio nuts and walnuts in the range 400-800 µg kg^{-1}. Poultry feed and its ingredients contained 8-1140 µg kg^{-1} aflatoxin.

788. Singh, T., Tyagi, R.P.S., and Varma, B.K. 1982. Study of the occurrence of aflatoxin B_1 in foodgrains. Journal of Food Science and Technology 19: 35-37.

Of the 125 samples of foodgrains and oilseeds analyzed, aflatoxin B_1 was found in 23.2% of samples. Among the 15 samples of each foodgrain analyzed, aflatoxin B_1 was found in 2 samples of rice (20 and 50 µg kg^{-1}), 6 samples of sorghum (22.8-550 µg kg^{-1}), 10 samples of raw groundnuts (33-440 µg kg^{-1}), 5 samples of roasted groundnuts (10-85 µg kg^{-1}), and in 6 samples of maize (15-680 µg kg^{-1}).

789. Siwela, A.H., and Caley, A.D. 1989. Aflatoxin contamination of stored groundnuts in Zimbabwe. Pages 59-63 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 Oct 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

Aflatoxins were analyzed in groundnuts stored for either local or export sales in the period 1982/83 to 1986/87. Of the 441 samples of seven groundnut varieties analyzed. 68 % had total concentrations of aflatoxins B_1 and G_1 of up to 25 µg kg^{-1} In me 1986/87 groundnut crop, the Flamingo and Makulu Red varieties, which constitute the bulk of export sales, had up to 25 µg kg^{-1} aflatoxin B_1 in 79 % of the samples, plus aflatoxin G^1 in 67 % of the samples. Overall, the Egret variety was the most susceptible to aflatoxin contamination during this period.

790. Sommartya, T., Jatumanusiri, T., Konjing, C., and Maccormac, C. 1988. *Aspergillus flavus* in peanut in Thailand will) special reference to aflatoxin contamination, and detoxification. Proceedings of the Japanese Association of Mycotoxicology Supplement No.1: 71-72.

Groundnut kernels collected from north-eastern plantations in Kalasin Province, Thailand, in August 1987 were roasted, split open and the seed coal, cotyledons and embryos were analyzed for aflatoxins by a Velasco aflatoxin meter. Results indicated accumulation of aflatoxin in embryos (274 µg kg^{-1}) and ground cotyledons (275 µg kg^{-1}). Of various food preservatives studied, sodium bisulfite was the best detoxification agent in ground groundnuts, with effective dose as low as 100 mg kg^{-1}.

791. Sova, Z., and Reisnerova, M. 1989. Determination of aflatoxins in biological materials. Sbomik Vysoke Skoly Zemedelske v Praze, Fakulta Agronomicka. B No. 50: 7-18.

Numerous samples of foods, animal tissues, faeces, Lepidoptera, and edible and poisonous fungi were analysed for aflatoxins by radioimmunoassay. Among the foods, high aflatoxin concentrations were recorded in : groundnuts (> 40 µg kg^{-1}), and mold- contaminated grapefruit juice (33.1 µg kg^{-1}). The other samples (cheese, currant juice, coffee, sweets, bread, maize, oats, wheat, barley) had low aflatoxin concentration (< 10 µg kg^{-1}). Allatoxin concentrations in the fungi ranged from 0 to 7.3 µg kg^{-1}.

792. Sreenivasamurthy, V. 1975. Mycotoxins in foods. Proceedings of the Nutrition Society of India No. 19: 1-6.

Causes of the high aflatoxin levels in foods, in particular in groundnuts and

groundnut oil, are discussed. A survey of 22 market samples of unrefined groundnut oil showed aflatoxin levels ranging from 0 to 2600 μg kg^{-1}. With the increase in export of handpicked seeds, the quantity of aflatoxin-contaminated seeds getting crushed for edible oil seems to have increased considerably in recent years.

793. Stanton, D.W. 1977. A survey of some foods for aflatoxins. Food Technology in New Zealand 12(4): 25.

Groundnut kernels, barley, rice, peanut butter, soybeans, soybean oil, sunflower seed oil, wheal Hour, soya flour, wholemeal flour, pearl barley and whole millet samples were analyzed for aflatoxin. Aflatoxin B_1 was detected only in two groundnut samples (at 5 and 6 μg kg^{-1}).

794. Sylos, C M., and Rodriguez-Amaya, D.B. 1989. Inexpensive, rapid screening method for aflatoxins in peanuts and peanut products. Journal of the Science of Food and Agriculture 49(2): 167-172.

The extraction and cleanup steps of the AOAC minicolumn technique were modified to provide an inexpensive, rapid screening procedure for aflatoxins in groundnuts and groundnut products. A total of 52 samples of groundnuts and groundnut products were collected from shops and markets in Campinas, Brazil, and were analysed for aflatoxins by the old and the modified methods. The results by both methods were the same : 28 samples were negative, four contained <20 μg kg^{-1} aflatoxin, 12 contained 20-50 μg kg^{-1}, three had 50-100 μg kg^{-1} and five had >100 μg kg^{-1}. The detection limit in both minicolumn methods was 10 μg kg^{-1}.

795. Taber, R.A., and Schroeder, H.W. 1967. Aflatoxin-producing potential of isolates of the *Aspergillus flavus-oryzae* group from peanuts *(Arachis hypogaea).* Applied Microbiology 15(1): 140-144.

Seventy-eight samples of farmers' stock groundnuts, representing groundnuts grown in nine different geographical areas in Texas during 1964, were assayed for aflatoxin and examined for associated microflora. Only two samples contained more than 50 μg kg^{-1} aflatoxin. Infestation by members of the *Aspergillus flavus-oryzae* group varied from 35 to 100 % of the kernels per area and from 1 to 100 % of the kernels per sample. 213 isolates of *A.flavus* were examined for their abilities for aflatoxin production on groundnut and rice substrates. Aflatoxin production by individual isolates ranged from 0 to 349.1 μg kg^{-1} on a groundnut substrate. In general, the isolates produced 8 to 10 times more ailatoxin B_1 than aflatoxin B_2, and no isolate producing allaioxins G_1 or G_2 was found.

796. Thasnakorn, P. 1976. Detection of aflatoxin in ground roast peanut. Siriraj Hospital Gazette 28(3): 375-382.

Aflatoxin B_1 (at 10-1120 μg kg^{-1}) was detected in 22 of 29 samples of groundnut from commercial sources. *Aspergillus flavus* was isolated from 11 of the aflatoxin-contaminated samples.

797. Thurm, V. 1977. Organization and results of mycotoxin control in the GDR. Zeszyty Problemowe Postepow Nauk Rolniczych No. 189: 149-154.

The examination of foods for mycotoxins in the German Democratic Republic is discussed, with particular reference to aflatoxins in groundnuts, and resulting aflatoxins M_1 and M_2 in milk from cows fed contaminated groundnut meal. Sampling procedures for imported groundnuts are described and the official methods for screening for aflatoxin content and quantitative determination of aflatoxins in groundnuts are summarized. Contaminated groundnuts are used for producing groundnut oil. 23% of groundnut samples analyzed contained aflatoxins (mainly B_1 and G_1). It is recommended that sampling be extended to cover other foods and other mycotoxins, and that animal feed should be sampled as a preventive measure against contamination of milk and meat.

798. Topsy, K. 1977. Aflatoxins from *Aspergillus* in soybean and other legumes. Annales de la Nutrition et de l'Alimentation 31: 625- 634.

Laboratory studies of soybeans, groundnuts, and their mixtures with various legumes (lentils, dried garden peas, Bengal gram (chick pea), green peas, red peas and broad beans showed that *Aspergillus flavus* and aflatoxins B_1 and G_1 were present in soybeans and groundnuts, but not in the other legumes. Growth of *A.flavus* and aflatoxin formation was inhibited in mixtures of other legumes with soybeans, but promoted in mixtures of groundnuts with soybeans, suggesting that soybeans should be included in the national food monitoring programme.

799. Tutelyan, V.A., Eller, K.I., and Sobolev, V.S. 1989. A survey using normal-phase high-performance liquid chromatography of aflatoxins in domestic and imported foods in the USSR. Food Additives and Contaminants 6(4): 459-465.

A highly sensitive normal-phase HPLC method was developed to study me occurrence of aflatoxins in Soviet imported and domestic foodstuffs. The detection limit was 0.1 μg kg^{-1} and the coefficients of variation were 11 % and 8.5 % at contamination levels of 10 and 100 μg kg^{-1} aflatoxin B_1, respectively. A survey of the occurrence of aflatoxins B_1, B_2, G_1 and G_2 in domestic and imported cereals and nuts (totalling 4532 samples) collected during 1985-87, showed that 26.9 % of imported groundnuts, 2.2 % of maize and 28.3 % of cottonseed were contaminated by aflatoxins at levels exceeding the maximum tolerance level established in the USSR (5 μg kg^{-1} for aflatoxin B_1 in foodstuffs of all types excluding baby foods), maximum concentrations were 3650, 600 and 153 μg kg^{-1}, respectively.

800. Tutelyan, V.A., Sobolev, V.S., Rybakova, N.V., and Eller, K.I. 1989. A survey using normal phase HPLC of aflatoxins in domestic and imported foods and dairy products in the USSR. Journal of Toxicology, Toxin Reviews 8(1-2): 375-387.

An improved normal phase HPLC method was developed to study the occurrence of aflatoxins B_1, B_2, G_1, G_2 and M_1 in domestic and imported foods. Ether-methanol-water (95:4:1) mobile phase and fluorometrie detector with silica gel packed flow cell were used. The detection limit of the method was 0.1 µg kg^{-1} for aflatoxin B_1, coefficients of variation were 11 and 8.5 % at contamination levels 10 and 10 µg kg^{-1} of aflatoxin B_1, respectively. Recoveries of added aflatoxins B_1, B_2, G_1, and G_2 for maize ranged from 78 to 88 %. This method allowed the determination of aflatoxins B_1, B_2, G_1, G_2 and M_1, B_2a. M_2, as well as other aflatoxin metabolites. The method was used in monitoring aflatoxin contamination of foods, the first stage of which is a preliminary screening of samples by TLC (the detection limit is 1 µg kg^{-1} for aflatoxin B_1). A survey of the occurrence of aflatoxins B_1, B_2, G_1, G_2 in Soviet domestic and imported cereals, nuts, beans and oilseeds harvested in 1985-87 (> 4300 samples) as well as aflatoxin M_1 in domestic dairy products (> 250 samples) was carried out using HPLC and TLC methods. It was shown that 26.9 % of imported groundnuts, 2.8 % of maize and 6.2 % of barley were contaminated with aflatoxins at levels exceeding the maximum tolerated level established in the USSR (5 µg kg^{-1} for aflatoxin B_1 in foods of all kinds excluding baby foods). Maximum concentrations were 3600, 155 and 8 µg kg^{-1}. respectively. As much as 28.3 % of domestic cottonseed samples, which were chosen for analysis due to toxic effects on animals, were also contaminated with aflatoxins.

801. Van Rensburg, S.J., Kirsipuu, A., Coutinho, L.P., and Van der Watt, J.J. 1975. Circumstances associated with the contamination of food by aflatoxin in a high primary liver cancer area. South African Medical Journal 49: 877-883.

In Mozambique, methods of food production, harvesting, storage and preparation were studied to detect points of aflatoxin contamination. Groundnuts, the main dietary source of protein, provided the most aflatoxin contamination, traditional agricultural methods promoting fungal infection and growth. Aflatoxin production also occurred in the main carbohydrate sources, cassava and maize, during storage. Western-type foods had particularly low aflatoxin contents. A simple educational program could reduce the incidence of primary liver cancer associated with aflatoxin poisoning; westernisation of some living habits could also reduce the incidence of the disease.

802. Varsavsky, E., and Sommer, S.E. 1977. Determination of aflatoxins in peanuts. Annales de la Nutrition et de rAlimentation 31: 539-544.

503 samples of groundnuts (mainly of lower commercial grades) from 2 seasons were analyzed for *Aspergillus* and aflatoxin contamination. Results showed 7.6 and 18.5 % respectively of aflatoxin contaminated samples in the 2 seasons. A relation appeared between % contamination and acidity of the oil, with, from each season, 19 and 47 % respectively of samples having > 2 % acidity. About 50 % of positive samples contained 100-1000 µg aflatoxin B_1 kg^{-1}, and 30 % > 1000 µg B_1 kg^{-1}. Of 6 strains of *A.flavus* isolated, only 2 were toxigenic : 1 was isolated from a positive and 1 from a negative sample.

803. Wagle, N.G. 1970. Detection and estimation of aflatoxin in groundnuts and groundnut products. Indian Standards Institute Bulletin 22: 299-301.

During 1967-68, a survey of aflatoxin contamination in groundnuts was carried out in West coast areas (Saurashira and Bombay). Of 500 samples of groundnuts analyzed, nearly 50 % of samples contained aflatoxin B_1 in the range 100-250 µg $k g^{-1}$

804. Yndestad, M., and Underdal, B. 1975. [Aflatoxin in foods on the Norwegian market.I. Aflatoksin i naeringsmidler pa det norske marked. Nordisk Verterinaermedicin 27(1): 42-48.

A survey of the aflatoxin content in some food products available on the market in Norway is reported. During autumn 1973, samples of Brazil nuts, groundnuts, peanut butter, hazelnuts, walnuts, mixed nuts, cocoa, cocoa products and dried milk were bought from stores in the Oslo area. In addition, samples of Brazil nuts and groundnuts were taken from import stores. Aflatoxins were detected in 6 of 34 samples of bought groundnuts, 1 of 3 of sorted and pooled groundnuts, 1 of 16 mixed nuts, 1 of 14 walnuts, 1 of 3 cashew nuts, 18 of 27 Brazil nuts and 1 of 40 cocoa products. Levels of aflatoxins B_1, B_2, G_1 and G_2 were 4200, 1600, 3100 and 600 µg kg^{-1}, respectively, for one sample of Brazil nuts and 400, 50, 350 and 30 µg kg^{-1} for a sample of groundnuts. Other aflatoxin levels were generally much lower. No aflatoxin was detected in peanut butter, hazelnuts and dried milk.

5.2.3 Groundnut oil

805. Ajulo, E., and Opadokun, J.S. 1974. Aflatoxin contamination of unrefined groundnut oil. Nigerian Stored Products Research Institute Annual Report : 39-41.

Studies by me Nigerian Stored Products Research Institute showed that while refined groundnut oil had negligible aflatoxin content, crude groundnut oils had traces of aflatoxin present.

806. Amla, I., Murthy, V.S., Jayaraj. A.P., and Parpia, H.A.B. 1974. Aflatoxin and Indian childhood cirrhosis-a review. Journal of Tropical Pediatrics and Environmental Child Health 20(1): 28-33.

Aflatoxin B, was detected in the urine of 7 % of 255 cases of childhood cirrhosis during 1964-1969 and in 25 % of breast milk samples from their mothers. The excretion pattern coincided with that of primates injected with C^{14} labelled anatoxin. Of 25 samples of parboiled rice collected from the homes of patients with cirrhosis, 2 showed both fungal growth and an aflatoxin B_1 fluorescent spot on chromatographic examination, and all 25 samples of unrefined groundnut oil showed a similar fluorescent spot. Aflatoxin administered to rats caused muscle fragmentation changes, fibrosis and fatty infiltration characteristic of 56 cases of childhood cirrhosis. Growth retardation was noticed both in 24 cirrhotic children and in the litters of lactating rats fed with aflatoxin.

807. Chong, Y.H., and Beng, C.G. 1965. Aflatoxins in unrefined groundnut oil. Medical Journal of Malaya 20(1): 49-50.

Sixteen samples of groundnut oil (9 refined and 7 unrefined oil) purchased from local markets were analyzed for aflatoxins. Five samples of unrefined oil contained 8-16 µg kg^{-1} aflatoxin B_1. Aflatoxin G_1 was also present in approximately the same concentration range.

808. Daren, Xiao. 1989. Research on aflatoxin contamination of groundnut in the People's Republic of China. Pages 95-100 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 Oct 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

During 1973-1977, 1689 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,. 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. Screening of groundnut germplasm for resistance to *Aspergillus flavus* is in progress.

809. Dwarakanath. C.T., Sreenivasamurthy, V., and Parpia, H.A.B. 1969. Aflatoxin in Indian peanut oil. Journal of Food Science and Technology 6(2): 107-109.

Samples of unrefined groundnut oil, refined oil and hydrogenated fats, collected from local markets in Mysore, were analyzed for aflatoxin. Samples of unrefined oil contained aflatoxins B_1 and B_2, while samples of refined oil and hydrogenated fats had no aflatoxin contamination. Aflatoxin content of unrefined oils from freshly harvested groundnuts and from those stored for six months after harvest ranged from 20-200 µg kg^{-1} (average 100 *µg* kg^{-1}) and 60-260 µg kg^{-1} (average 140 µg kg^{-1}) respectively. Only one of the samples had an aflatoxin content as high as 400 µg kg^{-1}. Heating unrefined groundnut oil to 150°C for 10 minutes caused 50 % destruction of aflatoxins. Food materials fried in aflatoxin contaminated-unrefined oil absorbed aflatoxins; the quantity increased with the amount of oil absorbed by the food material. Addition of casein or arecanut to the oil and heating caused considerable reduction in aflatoxins, but the added material imparted undesirable color and flavor to the oil.

810. Fong, L.Y.Y., and Chan, W.C. 1981. Long-term effects of feeding aflatoxin-contaminated market peanut oil to Sprague-Dawley rats. Food and Cosmetics Toxicology 19(2): 179-183.

Groundnut oils obtained from Hong Kong markets were frequendy contaminated with aflatoxins. A purified diet in which aflatoxin-contaminated market groundnut oil (aflatoxin B_1 110 µg kg^{-1}) was used as the fat source was given to Sprague-Dawley rats for 22 months from weaning, its estimated allatoxin B, content was 5 to 7 µg kg^{-1}. Controls were given a diet of identical composition except that Manzola maize oil (aflatoxin-free) was used. Of 76 rats given aflatoxin, 3 had sarcomat, one in the liver, one in the wall of the colon and one in the subcutaneous tissue of the groin; 18 rats given groundnut oil showed parenchymal liver damage and different degrees of fatly change and one showed pre-malignant changes in liver cells. Of 90 control rats, none developed malignant tumours. The liver-to-body weight ratios for experimental and control rats were 2.93 and 2.62, respectively. The difference between those values was significant, reflecting the degree of fatty change in the livers of experimental rats compared with that in the controls. Over 90 % of Hong Kong households use groundnut oils for cooking purposes, and these results indicate a possible health hazard in the use of contaminated groundnut oil.

811. Fong, L.Y.Y., Ton, C.C.T., Koonanuwatchaidet, P., and Huang, D.P. 1980. Mutagenicity of peanut oils and effect of repeated cooking. Food and Cosmetics Toxicology 18(5): 467-470.

Four samples of groundnut oils obtained from local markets in Hong Kong were tested for mutagenicity using the *Salmonella/microsomal* test system. In the presence of microsomes, dimethylsulphoxide (DMSO) extracts of the samples were mutagenic to *Salmonella typhimurium* strains TA 98 and TA 100, but the mutagenic activity of the oils decreased after they had been repeatedly cooked. However, when the

extracts were pre-incubatcd with the bacteria (TA 100) and microsomes the mutagenic activity of the cooked oils was greater than that of the uncooked oil, suggesting the possible contamination of the cooked oils by N-nitrosamines. Mutagenic activity was also detected in random samples of seven other brands of uncooked oils obtained from local markets. By absorption spectrophotometry, aflatoxin B_1 levels in 3 of these oils were in the range 98-150 µg kg^{-1}. DMSO-extracts of 10 samples of poor-grade market groundnuts were also mutagenic and the groundnuts contained 95-1055 µg kg^{-1} aflatoxin B_1.

812. Giridhar, N., and Krishnamurthy, G.V. 1977. Studies on aflatoxin content of groundnut oil in Andhra Pradesh with reference to climatic conditions and seasonal variations. Journal of Food Science and Technology 14(2): 84-88.

The overall aflatoxin content of groundnut oil samples varied from 0 to 5000 µg kg^{-1}. Samples from one dry region had low aflatoxin levels and those from humid coastal regions showed large variations in aflatoxin content. Samples from another area contained very high levels of aflatoxin due to the use of low grade groundnut seeds for oil extraction after separation by hand picking.

813. Ling, K.H., Tung, C.M., Sheh, I.F., Wang. J.J., and Tung, T.C. 1968. Aflatoxin B_1 in unrefined peanut oil and peanut products in Taiwan. Journal of Formosan Medical Association 67(7): 309- 314.

A survey of unrefined groundnut oil, kernels, and peanut butter for aflatoxin contamination was carried out during 1966-1967. Twenty-seven samples of unrefined oil from oil mills located in the Yunglin county and 23 unrefined oil from the markets in Taipei were analyzed. Few samples of groundnut kernels and cake were obtained from the same oil mills. Various kinds of peanut butter were purchased from markets in Taipei. Both the incidence and levels of aflatoxin contamination were as follows : peanut butler > groundnut cake > kernels > oil. Only very low levels of aflatoxin were found in unrefined oil. High levels of aflatoxin were found in peanut butter.

814. Pal, R., Varma, U.K., and Srivastva, D.D. 1979. Aflatoxin in gtounc'nut oil, groundnut cake and hydrogenated oil in Hapur (Uttar Pradesh, India) market. Journal of Food Science and Technology, India 16(4): 169-170.

The extent of aflatoxin contamination in groundnut oil, groundnut cake and hydrogenated oil sold in the market of Hapur, Uttar Pradesh, India, was investigated. Nearly 66.7 % of the total samples of groundnut oil contained aflatoxin. Of these. 70 % contained as much as 2660 µg kg^{-1} aflatoxin B_1. Of the groundnut cake samples, 70 % were contaminated with 1135-2250 µg kg^{-1}. Refined oil or Vanaspathi did not contain any aflatoxin.

815. Parker, N.A., and Melnick, D. 1966. Absence of aflatoxin from refined vegetable oils. Journal of the American Oil Chemists Society 43: 635-638.

The present investigation is the first definitive study of the fate of aflatoxins in vegetable oils undergoing processing. Crude oils, obtained by solvent extraction or by hydraulic pressing of ground moldy groundnuts (not suitable for human consumption), contained only small fractions of the aflatoxin originally present in the groundnuts; the meals retained the bulk of the aflatoxin. Conventional alkali refining and washing of the oils reduced aflatoxin content to a range of 10 to 14 µg kg^{-1}. The subsequent bleaching operations essentially eliminated aflatoxin from the oils; the concentrations after this process being less than 1 µg kg^{-1}. The previous results were confirmed using corn oils obtained from corn germ deliberately contaminated in the laboratory with *Aspergillus flavus.* The nonfluorescing forms of aflatoxins, capable of being produced during the alkali refining operations, are also absent from the refined vegetable oils; these aflatoxin derivatives are readily converted to their original form on acidification and are thereby measurable by fluorescence, if present.

816. Peers, F.G., and Linsell, C.A. 1975. Aflatoxin contamination and its heat stability in Indian cooking oils. Tropical Science 17(4):229-232.

Sixty-nine groundnut oils and 16 other cooking oils on sale in Indian villages were analyzed lor aflatoxin contamination. Groundnut and groundnut meal samples (24) were also analyzed and three were positive for aflatoxin B_1 at 2-270 µg kg^{-1}. Of the 69 groundnut oils, 15 were contaminated with aflatoxin B_1 at 3-175 µg kg^{-1}, of which seven also contained aflatoxin B_2. Of the 16 other oils, only one. a coconut oil, contained 3 µg kg^{-1} aflatoxin B_1. Destruction of aflatoxin B_1 in artificially contaminated maize oil was only apparent at 250ºC and above. Using naturally contaminated groundnut oil (175 µg kg^{-1} aflatoxin) the results were essentially me same and confirmed that destruction of aflatoxin B_1 is likely to occur at normal frying temperature if cooking is done indoors.

817. Sreenivasamurthy, V. 1975. Mvcotoxins in foods. Proceedings of the Nutrition Society of India No.19: 1-6.

Causes of the high aflatoxin levels in foods, in particular in groundnuts and groundnut oil, are discussed. A survey of 22 market samples of unrefined groundnut oil showed aflatoxin levels ranging from 0 to 2600 µg kg^{-1}. With the increase in export of handpicked seeds, the quantity of infected seeds crushed for edible oil seems to have increased considerably in recent years.

5.2.4 Groundnut cake

818. Akano, D.A., and Atanda, O.O. 1990. The present level of aflatoxin in Nigerian groundnut cake ('Kulikuli'). Letters in Applied Microbiology 10(4): 187-189.

Groundnut cake (Kulikuli) purchased from four major markets in Ibadan, Oyo State, Nigeria, during April-November 1988 were analyzed for aflatoxin B_1 and associated mycoflora. In all but 2 of the samples aflatoxin B, levels were between 20 and 455 µg kg^{-1}. Mold counts were low (1.0 - 4.41 x 100 colonies g^{-1}). Eight mold species were isolated. Of these, *Aspergillus niger, Paccilomyccs vartotii, A. flavus* and *Fusarium moniliforme* dominated. It is concluded that groundnut cake on sale in Ibadan markets is unacceptable for animal feed rations and human consumption and there is a need for some form of quality control and decontamination before usage.

819. Balasubramanian, T. 1985. Incidence of aflatoxin B_1 in animal feeds. Indian Veterinary Journal 62(11):982-988.

Aflatoxin B_1 was detected in 66 % of 101 samples of feeds and feed ingredients at up to 2670 µg kg^{-1}. Of the 13 feed ingredients analyzed, only groundnut oilcake contained aflatoxin B_1 (330-2670 µg kg^{-1}).

820. Bartos, J., and Matyas, Z. 1977. |Screening for the presence of aflatoxin B_1 in feeds. |. Screening na pritomnost aflatoxinu B_1 v krmivech. Veterinarni Medicina 22(12):729-735.

The presence of aflatoxin B_1 was examined in 67 samples of feeds (18 kinds), 8 of protein concentrates (3 kinds), 22 of cattle feed mixtures (11 kinds), 16 of pig feed mixtures (8 kinds), 10 of poultry feed mixtures (8 kinds), 4 of sheep feed mixtures (2 kinds), 2 of feed mixtures for game and herbivorous animals in zoological gardens (2 kinds), 15 of fish feed mixtures (4 kinds), 2 of biofactor supplements (2 kinds) and 7 of non-traditional feeds containing varying amounts of pig excrement. Four kinds of currently-used foodstuffs were also tested (semi-fine flour, hulled rice, groundnuts, walnuts). Aflatoxin B_1 was detected in 26 samples of groundnut cake imported from India, 2 of laying hen protein concentrate, 4 of breeding pig concentrate, 2 of supplement feed mixture for lamb fattening, 1 of meat-bone meal, 1 of supplement feed mixture for lactating sows, 2 of protein supplement feed mixture for dairy cows, 1 of concentrate for cattle fattening mixture, 1 of protein concentrate for mixture with fat for broiler fattening, 1 of rape groats. 1 of cocoa shells and 1 of glycine supplement feed mixture for cattle fattening.

821. Bassler, R. 1980. (Estimation of feeds and some oil seeds for aflatoxin.]. Erfahningen bei der untersuchung von futtermittein und einigen olsaaten auf aflatoxin. Qualitas Plantarum Plant Foods for Human Nutrition 30(3/4): 271-282.

Aflatoxins exceeding the maximum permitted limits were detected in groundnut, cottonseed, cocos and palm kernel cakes. Soya products, however, were practically free of aflatoxin. Nearly all the tested samples of mixed feeds for ruminants, which contained a large proportion of oil-seed cakes, contained aflatoxin.

822. Blaha, J., Tamchynova, J., and Reisnerova, H. 1990. The occurrence of moulds and aflatoxin B_1 in Vietnamese feeds. Tropical Science 30: 21-31.

During 1986 and 1987, mold infestation and aflatoxin B_1 occurrence were examined in 52 samples of Vietnamese feeds. *Aspergillus, Rhizopus* and *Penicillium* spp. were the most prevalent fungi in the feeds, with *Aspergillus* spp. predominating. *Aspergillus flavus* was isolated from 78 % of samples. The range of aflatoxin B_1 in the feeds was between 2 and 640 µg kg^{-1} in 1986 and between 5 and 41 µg kg^{-1} in 1987. The highest incidence of aflatoxin B_1 in the 1986 feed samples was found in maize, groundnut oil cake and feed mixtures for chicks. In 1987, the levels of aflatoxin B_1 in the feeds were lower, but the frequency of positive samples was higher than in 1986. However, in 1987, only 14 samples were analyzed for aflatoxin in comparison with 38 samples in 1986.

823. Bryden, W.L., Lloyd, A.B., and Cumming, R.B. 1980. Aflatoxin contamination of Australian animal feeds and suspected cases of mycotoxicosis. Australian Veterinary Journal 56(4): 176-180.

Aflatoxin B_1 was detected in 23 of 55 feedstuffs known to be either water damaged or visibly moldy. The highest level of aflatoxin was 700 µg kg^{-1} of feed and the mean concentration was 140 µg kg^{-1}. Of 36 feedstuffs purchased from local manufacturers, only groundnut meal contained aflatoxin B_1 (500 µg kg^{-1}). Eleven feedstuffs were associated with field outbreaks of animal disease and seven of these contained aflatoxin, but not in sufficient quantity to account for the described disease symptoms.

824. Chelkowski, J., Godlewska, B., and Radomyska, W. 1978. (Occurrence of mycotoxins in food and feed.|. Wystepowanie mykotoksyn w zywnosci i paszach. Przemysl Spozywczy 32(8):285-286.

Suspected samples of Polish grain and imported seed meals obtained from 1970-1976 were analyzed for aflatoxin. Of 21 samples of wheat I contained aflatoxin B, (8.4 µg kg^{-1}). Of 24 samples of barley 1 contained 3 µg kg^{-1} aflatoxin B_1. All 14 samples of groundnut meal had aflatoxin B_1 (about 1000 µg kg^{-1}). Ochratoxin was detected in 2 of 11 samples of rye and in 1 of 5 samples of wheat

with levels 25 and 5 μg kg^{-1}, respectively. Of 5 samples of sorghum from Argentine 2 had ochratoxin (25 μg kg^{-1}).

825. Choudhary, P.G., and Manjrekar, S.L. 1967. Preliminary observations on biological activity of aflatoxin B1-pure. Indian Veterinary Journal 44: 543-548.

Of 150 samples of groundnut cake awaiting shipment in Bombay, 11 harboured *Aspergillus flavus* and *20 A.flavus* var. *oryzae, A. fumigatus, A. niger, Penicillium* sp., *Rhizopus* sp. and *Mucor* sp. Of stored groundnuts, 5 % of the undamaged white and 57 % of the damaged and discolored ones were contaminated; none of the white nuts yielded *A. flavus,* compared with 26 % of discolored nuts. Thin-layer chromatography of extracts of isolates and of cake showed that only two strains of *A.flavus* were fluorescent and hence toxic. Groundnut cake medium was suitable for the growth of *A. flavus* and production of aflatoxin.

826. Choudary, C, and Rao, M.R.K.M. 1982. An outbreak of aflatoxicosis in commercial poultry farms. Poultry Adviser, Bangalore, India 16(6): 75-76.

Cyanotic combs, loss of appetite and listlessncss. with a fall in egg production from 80 to 20 % and 100 % mortality, occurred in poultry farms in and around Chittoor (Andhra Pradesh), India. Post-mortem examination revealed hydropericardium, ascites and large, friable haemorrhagic livers, some of which had ruptured. Aflatoxins (1400-3600 μg kg^{-1}) were found in samples of maize and groundnut cake fed to the birds during the outbreak. The crops used for the feed had been harvested in heavy rain.

827. Connote, M.D., Blaney, B.J., and McEwan, T. 1981. Mycotoxins in animal feeds and toxic fungi in Queensland 1971-80. Australian Veterinary Journal 57(7): 314-318.

Anatoxins were detected in 67 feed samples. 45 of which were groundnut meals and by-products. Aflatoxins were produced by 49 % of 200 isolates *of Aspergillus flavus* cultured on maize meal. Ochratoxin A was detected in 1 of 25 feed samples analyzed. Ochratoxin A was produced by 28 % of 47 *A. ochraceus* isolates and zearalenone by 2 *Fusarium equiseti* isolates.

828. Dietrich, H., and Hoffmann, G. 1978. |A contribution of the study of the aflatoxin content of oilseed residues and the reliability of its identification.]. Ein beitrag zum gehalt von aflatoxin in olsaatruckstanden und zur zuverlassigkeit seiner identifizierung. Landwirtschaftliche Forschung 31(1): 19-25.

Analysis of 93 samples of extracted oilseed residues showed mean aflatoxin concentration (max. in parentheses) of 33 μg kg^{-1} (80) in coconut, 15 (30) in palm kernel, 63 (1 sample only) in Babassu, 26 (75) in cottonseed, < 5 (5) in sunflower, < 5 (<5) in linseed and 5 (20) in maize germ; no detectable aflatoxin was found in sesame, rapeseed and wheat germ residues. These results contrast with a mean 530 MB kg^{-1} in groundnuts. Inferences with the determination by fluorescent compounds with similar R_1 values on TLC as listed by Gedek & Kablau, only rarely cause errors during routine analysis due to improvements in the method.

829. Drabek, J., and Piskac, A. 1979. (Occurrence of aflatoxin in some feed components and complete feed mixtures for swine.). Vyskyt aflatoxinu v nekterych komponentech a kompletnich krmnych smesich pro prasata. Vcterinarni Medicina 24(10): 597-602.

Of 104 feed samples examined chromatographically, 14 contained aflatoxins, the highest level (1920 μg kg^{-1}) being in a sample of groundnut from India. Aflatoxin was also found in samples of feed mixtures for pig fattening, pregnant and lactating sows and boars, levels ranging from 50-350 μg kg^{-1} of feed. Almost all samples of organoleptically altered feed, taken from metal containers outside the animal houses, contained aflatoxin.

830. Fonseca, H. 1973a. (Occurrence of aflatoxin in groundnut *(Arachis hypogaea* L.) flour in the northwest region of the State of Sao Paulo.]. Ocorrencia de anatoxina em farelos de amendoim (*Arachis hypogaea* L.) na regiao noroeste, do Estado de Sao Paulo. Anais da Escola Superior de Agricultura "Luiz de Queiroz" 30: 387-397.

All 48 samples of groundnut flour from crops in two seasons contained 100-20000 μg kg^{-1} aflatoxin. the level being higher (average 4340 μg kg^{-1}) in the March-May in the rainy season than in July-September in the dry season (average 1830 μg kg^{-1}). Only 8.33 % of the material tested could be used as animal fodder. A weak, non-significant correlation was found between aflatoxins B and G.

831. Fonseca, H. 1973b. (Occurrence of aflatoxin in groundnut *{Arachis hypogaea* L.) flour in the Paulista Nova region in the State of Sao Paulo.). Ocorrencia de aflatoxina cm farelos de amendoim *{Arachis hypogaea* L.) na regiao Paulista Nova, do Estado de Sao Paulo. Anais de Escola Superior de Agricultura "Luiz de Queiroz" 30: 403-422.

All 116 samples of groundnut flour from crops in two seasons contained 100-20000 μg kg^{-1} aflatoxin, the level being higher (5500 μg kg^{-1}) in the rainy season than in the dry season (1760 μg kg^{-1}). Only 10.35 % of the material was fit for animal consumption. A weak, statistically significant correlation was found between the production of aflatoxins B and G.

832. Fonseca, H. 1973c. [Occurrence of aflatoxin in groundnut *(Arachis hypogaea* L.) Hour in the Sorocabana region in the State of Sao Paulo.|. Ocorrencia de aflatoxina cm farelos de amcndoim *(Arachis hypogaea* L.) na regiao Sorocabana do Fstado de Sao Paulo. Anais de Eseola Superior de Agricultura "Luiz de Quciroz" 30: 423-433.

All 44 samples of groundnut flour from crops in two seasons contained 100-10000 µg kg^{-1} aflatoxin, the level being higher (average 3780 µg kg^{-1}) in the rainy season than in the dry season (average 1740 µg kg^{-1}). Only 4.54 % of the material tested could be used as animal fodder. There was practically no correlation between the production of aflatoxins B and G.

833. Fonseca, H. 1975. [Occurrence of aflatoxin in groundnut *(Arachis hypogaea* L.) flour in the Araraquarense region of Sao Paulo State.]. Ocorrencia de aflatoxina em farelos de amendoim, *(Arachis hypogaea* L.) na regiao Araraquarense. de listado de Sao Paulo. Anais de Escola Superior de Agricultura "Luiz de Queiroz" 32: 7-19.

All 56 samples of groundnuts from 9 mills produced from crops in the rainy season (March-May) and the dry season (July-Sep) contained aflatoxin (100-20000 µg kg^{-1}), levels being higher in the rainy season (average 4550 µg kg^{-1}) than in the dry season (2400 µg kg^{-1}). Only 16.07 % of the material could be used for admixturing in feedstuffs. In six samples the level of aflatoxin G was higher than that of aflatoxin B. A statistically significant positive correlation was found between the production of aflatoxin B and G.

834. Froeslie, A., and Waasjoe, E. 1974. (Contents of aflatoxin in groundnut meal imported into Norway 1968-1973.]. Aflatoksininnhold i jordnoettmel importert til Norge 1968-1973. Nordisk Veterinaermedicin 26(12): 713-719.

Of 86 samples of groundnut meal, imported into Norway during 1968 to 1973, 19.8 % had < 100, 41.8 % had 100 to 500. 26.8 % had 500 to 1000 and 11.6 % had > 1000 µg aflatoxins B_1 and B_2 kg^{-1}. There were marked differences between countries of origin but small variations between years.

835. Fulsoundar, A.B., and Shukla, P.C. 1978. Correlative studies between quantitative and biological estimations of aflatoxins extracted from cattle feeds and their ingredients of Gujarat State. Gujarat Agricultural University Research Journal 3(2): 78-81.

Of 133 feed samples (groundnuts, cottonseed, kardi, salseed, soybean, sunflower, sheria, ambadi and rubbcrseed) analyzed. 42 % of samples contained aflatoxins and aflatoxin-like substances. Each class of feed, e.g. cakes, cereal grains, cereal by-products and similar samples together with compounded samples contained aflatoxins. Aflatoxin B_1 was present in practically all contaminated samples while aflatoxins B_2 and G_1 were observed in some of them; aflatoxin G_2 was absent from all samples except soybean cake.

836. Gajek, O. 1982. l Aflatoxin in protein food for animals and milk.]. Wystepowanie aflatoksyn w paszach trsciwych i w mleku. Roczinki Panstwowego Zakladu Higieny 33(5-6): 415-420.

Aflatoxin B_1 was detected at 80-2000 µg kg^{-1} (mean 798 µg kg^{-1}) in imported groundnut meal and at 10-175 µg kg^{-1} in 56 % of animal feed mixtures examined. Of 22 samples of milk from state-owned farms, 11 were contaminated with aflatoxin M_1 at 0.01-0.25 µg L^{-1}. Aflatoxins were not found in market milk samples, probably because of mixing samples from various purveyors.

837. Ghewande, M.P., Nagaraj, G., and Rcddy, P.S. 1989. Aflatoxin research at the National Research Centre for Groundnut. Pages 237-243 in Aflatoxin contamination of groundnut : Proceedings of the International Workshop, 6-9 October 1987, ICRISAT Center. India. Patancheru, A.P. 502 324. India r International Crops Research Institute for the Semi-Arid Tropics.

The potential of *Aspergillus flavus* isolates to produce aflatoxins, and the toxicity of their culture filtrates to germinating seeds and seedlings of groundnut were studied. Isolates NRRL 3000 and V3734/10 produced high levels of aflatoxins in culture. Culture filtrates from the isolates and from NRCGAFA 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. S230, derivatives of S230 x PI 337394F. Latur 33 x PI 337394F, and the wild species, *Arachis 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 by NRRL 3000, but only trace levels were produced in *A. cardenasii* and *A. duranensis.* Aflatoxins were found (range of 27-146 µg kg^{-1}) in commercial groundnut cake and in de-oiled 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 colonization by *A. flavus* and of aflatoxin production in *in vitro* inoculation tests.

838. Halliday, D., and Kazaure, I. 1967. The aflatoxin content of Nigerian groundnut cake. Technical Report 8, Annual Report, Nigerian Stored Products Research Institute, 1967 : 73-78.

Work carried out during 1962-67 at the Nigerian Stored Products Research Institute on aflatoxin levels in groundnut cake is reported. A total of 895 samples of groundnut cake from oil mills in Kano were analysed. Most samples had aflatoxin contents of less than 50 or 50-250 μg kg^{-1}, and that only 2 samples had aflatoxin contents in excess of 1000 μg kg^{-1}. The number of samples containing 250-1000 μg kg^{-1} aflatoxin was substantial in the 1963-64 and 1964-65 seasons, almost negligible in the 1965-66 season and nil in the 1966-67 season.

839. Harvey, G. 1980. Poison threat lurks in imported groundnut meal. Farmers Weekly 92(18): 54-55.

There is concern among farmers' leaders and consumers in the UK about high levels of aflatoxin in some imported groundnut oilmeal. The 1976 Fertilizer and Feedingstuffs Regulations specify maximum aflatoxin concentration in groundnut oilmeal sold for animal feed as 50 μg kg^{-1}, and in 'whole feedingstuffs' for dairy cattle and calves as 10 μg kg^{-1}. However, groundnut oilmeal sold as a feedstuff has been found with aflatoxin levels as high as 900 μg kg^{-1}. Farmers are recommended to ascertain whether compound feeds contain groundnut, and to get a certificate showing aflatoxin content of groundnut for inclusion in home-mixed feed. There are no UK recommendations for maximum aflatoxin in milk, but Milk Marketing Board officials are satisfied that current levels are safe. A feed with the statutory maximum aflatoxin B_1 content could result in about 100 μg kg^{-1} of aflatoxin in milk, and an average concentration of 0.25 μg kg^{-1} in cheese or dried milk. A supplementary article reports the experience of one dairy herd where moldy compound feed with aflatoxin content of 600 μg kg^{-1} was associated with loss of condition and coordination, fall in milk yield and increased incidence of mastitis. Pound-sterling 6700 has been received by the fanners as compensation from the feed firm involved.

840. Hertrampf, J. 1978. [Groundnut meal and its problems. J. Erdnussschrot und seine probleme. Muhle + Mischfuttertechnik 115(3): 36.

Problems arising from the contamination of groundnut meal with aflatoxin are reviewed. Progress in the detection of related compounds and knowledge of their toxic properties are summarized, with details of acute toxicity on monkeys, and indications of the chronic effects of very small doses on liver and kidneys of children. Transmission of the toxic constituents from animal feeds to cow's milk and pig liver and kidneys is also reported, although there are no apparent indications of progressive accumulation. Aflatoxin in imported groundnut meal is discussed and the limits imposed by the EEC on feeds of different kinds are tabulated. The practical implications of stringent limits on aflatoxin contents of meal imported into European and other countries are discussed, with particular reference to the danger that more heavily contaminated batches may be retained in the developing countries where the population relies heavily on groundnut meal as a source of protein. The possibility of detoxification with ammonia is briefly discussed, and it is noted that tests with rats indicate that the protein quality is thereby decreased by about 10 %.

841. Horvath, E., Biro, Z., Andrassy, k., und Horvath, 1. 1982. | Aflatoxin in foods of plant and animal origin.]. Aflatoxin novenyi es allati elelmiszerekben. Orvosi Hetilap 123(20): 1235- 1239.

Aflatoxin at up to 200 μg kg^{-1} was detected in 16-20 % of imported groundnut samples analyzed during 1976-1978. In 1979 a highly contaminated Egyptian groundnut lot raised the positivity to > 80 %. with a maximum contamination of 1000 μg kg^{-1}. The efficiency of recovery was 75-90 % with Pons' method and 60-71 % by the Beljaars method. Aflatoxins were not detected in samples of Hungarian flour and vegetables for soup. In an examination of samples of milk from large-scale dairy farms, aflatoxin M_1 at 0.06-0.08 μg L^{-1} was detected in 2 of 20 samples in 1977 and 2 of 30 samples in 1978. The aflatoxin B_1 content of groundnut-base feed at a farm was 60 μg kg^{-1}. The 30 milk samples analyzed in 1979 did not contain aflatoxin. In 1977 one of 20 powdered milk samples examined contained aflatoxin M_1 at 200 μg kg^{-1}. Egg samples examined during 1978-79 contained no aflatoxin. The methods used in milk and egg analyses had extraction efficiencies of 80-90% and 75-80 %, respectively.

842. Johri, T.S., Agarwal, R., and Sadagopan, V.R. 1987. Aflatoxin B_1 contamination of poultry feedstuffs of northeastern region of Uttar Pradesh. Indian Veterinary Journal 64(5): 433-435.

The aflatoxin B_1 content of samples of various feedstuffs, including 52 samples of yellow maize, 50 of groundnut cake. 39 of starter feed, 34 of layer teed, 22 of grower feed, 22 of fish meal, 20 of rice bran, 16 of wheal bran, 5 of mustard oilcake, 4 of guar meal and 2 of meat meal, collected during 1985, was estimated with the Pons' method. Feed samples were found to be contaminated with aflatoxin B_1 as follows : maize (50 % of samples contaminated at 0-1000 μg kg^{-1}; average 200 μg kg^{-1}), groundnut cake (62 %, average 300 μg kg^{-1}), starter feed (38 %, average 140 μg kg^{-1}). layer feed (44 %, average 160 μg kg^{-1}). grower feed (36 %. average 120 μg kg^{-1}). All other samples were free from aflatoxin.

843. Jones, M.G.S., and Ewart, J.M. 1979. Effects on milk production associated with consumption of decorticated extracted groundnut meal contaminated with aflatoxin. Veterinary Record 105(21): 492-493.

Two samples of groundnut meal were collected from a dairy farm where milk production had fallen to 76 % of normal, and many of the cows were in poor condition, with reduced appetites and scouring. Aflatoxins were demonstrated at a level which could give about 50 μg kg^{-1} (dry matter) in the complete diet. In 2

samples of groundnut meal the aflatoxin B_1 levels were 750 and 620, of B_2 40 and 30, of G_1 60 and 50, and of G_2 up to 10 µg kg^{-1}. Within 3-4 days of withdrawal of the meal, scouring ceased and appetite improved. Milk production rose again, though not to its predicted level.

844. Karmacharya, S. 1984. Aflatoxin contamination in foods & feeds of Nepal. Pages 135-144 in First National Seminar on Food Industry and Food Technology, 23-26 April 1984, Dharan Campus, Institute of Science and Technology, Nepal : Tribhuvan University.

Four hundred and sixty-five samples of various food commodities and 94 samples of different feeds and feed ingredients were analyzed for aflatoxin contamination. These samples were collected from different parts of Nepal. Mostly maize and groundnut samples were contaminated with aflatoxins B_1 and B_2. Of 101 samples, 10 samples of maize used as food were contaminated with ailatoxin at levels > 30 µg kg^{-1}, while only 2 of 67 samples of groundnuts were highly contaminated. Among feed samples, 25 of 58 poultry feed samples were highly contaminated. Maize and groundnut cake were mostly contaminated used as feed ingredients. The other commodities used as foods and feeds had aflatoxin levels well within the safe limit (30 µg kg^{-1}).

845. Ketterer, P.J., Blaney, B J., Moore, C.J., McInnes, I.S., and Cook, P.W. 1982. Field cases of aflatoxicosis in pigs. Australian Veterinary Journal 59. 113-117.

Five cases of aflatoxicosis in pigs in Southern Queensland are described. One peracute case where aflatoxin concentration of up to 5000 µg aflatoxin B_1 kg^{-1} was demonstrated in stomach contents was presumed to be caused by consumption of moldy bread. High levels of toxins were also present in the livers. Two cases of acute toxicity were caused by feeding moldy groundnut screenings containing 22000 |ig aflatoxin B_1 kg^{-1}. One case of subacute, and one of chronic toxicity were caused by sorghum grain based rations with lower aflatoxin levels (4640 and 255 µg kg^{-1}). Peracute toxicity caused collapse and deaths within a few hours, acute toxicity caused deaths within 12 h, and subacute toxicity caused deaths after 3 weeks on the toxic rations. Anorexia and ill thrift affecting only growing animals were seen with chronic toxicity. Extensive centrilobular liver necrosis and haemorrhage occurred with peracute toxicity, and in cases of acute poisoning there was hepatic centrilobular cellular infiltration, hepatocyte swelling and bile stasis. With subacute toxicity hepatocyte vacuolation together with bile stasis and bile ductule hyperplasia were seen.

846. Menezes, T.J.B., Tango, J.S., Coelho, F.A.S., and Teixeira, C.G. 1965. (The occurrence of *Aspergillus flavus* and aflatoxin in groundnut seeds, meals and cakes.J. Ocorrencia do *Aspergillus flavus* e da aflatoxina em sementes tortas e farelos de amendoim. Coletanea do Instituto de Tecnologia de Alimentos (Brazil) 1(2): 559-566.

A survey of 43 oil factories in the State of Sao Paulo, Brazil, revealed that due to the high aflatoxin levels only 3.2 % of the groundnut meal samples and 3 % of the cake samples could be utilized as animal feed.

847. Mori, M., Takahashi, T., Onoue, Y., and Takahashi, T. 1977. Contamination of single-component feeds with aflatoxin and the ability of *Aspergillus flavus* to produce this toxin. Journal of the Japan Veterinary Medical Association 30(2): 84-88.

Of 87 samples (maize meal, groundnut meal, palm meal, and cottonseed meal) analyzed, 12 were contaminated with aflatoxins. The most heavily contaminated sample was maize which contained > 1000 µg kg^{-1} aflatoxin B_1. Of 56 isolates of *Aspergillus* spp. isolated from the substrates, 32 were *of A. flavus,* 11 strains of which produced aflatoxin B_1 or B_1 and B_2. One strain isolated from rice bran produced 880 µg g^{-1} aflatoxin B_1.

848. Nahdi, S., and Nusrath, M. 1985. Aflatoxins and other mycotoxins in mixed feeds and its constituents. Indian Journal of Botany 8(1): 16-24.

Of 29 different feedstuffs tested, aflatoxin B, was detected in mixed feed No. 1 and 2 with incidence of 46 % and 36 %, respectively. In the constituents of mixed feed No. 1. aflatoxin B_1 incidence ranged from 20 to 100 % and from 40 to 100 % in those of mixed feed No.2. Groundnut cake showed the maximum percentage of incidence. Among other simple feeds, higher levels of aflatoxin B_1 were detected in cottonseed-cake followed by coconut-cake, green gram, black gram, chickpeas and wheat. No contamination of rice bran was found. Ailatoxin G_1 was found in trace amounts in Murukool (constituent of mixed feed No. 2) and in groundnut cake (simple feeds). Zearalenone was detected in mixed feeds No. 1 and 2 and also in maize meal, maize-oil cake, sunflower cake and maize of feed No. 2. In simple feeds, low levels of zearalenone were detected in chickpeas and wheat. Neosolaniol was detected in mixed feed No. 1 and groundnut cake (simple feed) with a low incidence of 10 % in each case. Low levels of penicillic acid and patulin were detected in legume mixture of feed No. 2 and maize meal of feed No. 1, respectively.

849. Natarajan, K.R., Rhee, K.C., Cater, C.M., and Mattil, K.F. 1975. Distribution of aflatoxins in various fractions separated from raw peanuts and defatted peanut meal. Journal of the American Oil Chemists' Society 52: 44-47.

The present investigation is the first definitive study of the distribution of anatoxins in a wet-milling process of raw groundnuts. The results show that the majority of aflatoxins originally present in the groundnuts remained in the solid fractions, particularly the protein fractions, during wet-milling. In the protein concentrate preparation, the concentrates carried 81-89 % of the total aflatoxin; crude oil, 5-8 %; and whey fraction, 3-14 %. In the case of protein isolate preparation, 51-56 % of the total toxin remained with the isolates, 22-26 % with the residue, 11-17 % with the whey, and 7-8 % with the crude oil. Distribution of aflatoxins in the preparation of protein isolates from defatted groundnut meal showed that 55-65 % of the total toxin originally present in the meal remained with the protein isolates, 20-28 % with the residue, and 10-20 % with the whey fraction. Changes in extraction pHs for the preparation of protein isolates either from raw groundnuts or defatted meal did no(alter the distribution pattern. A new approach based upon the charge- transfer (electron acceptor-donor) complex formation is suggested to shift this aflatoxin distribution from protein products to disposable whey or residue fraction from the processing of raw groundnuts and defatted meal lor protein products.

850. Nusrath, ML, and Nahdi, S. 1983. Occurrence of aflatoxins and other mycotoxins in catde-feeds from Hyderabad. Indian Phytopathology 36(1): 106-109.

Aflatoxin B_1 was detected in all simple and mixed feeds and from most of the ingredients tested. The maximum amount of aflatoxin B_1 was found in groundnut cake and cottonseed cake. Aflatoxin B_2 was found in oil cakes of sunflower and maize, and also in husk. Considerable quantities of ochratoxin A and sterigmatocystin were observed in samples of sunflower cake and green gram, respectively.

851. Pal, R., Varma, B.K., and Srivastva, D.D. 1979. Aflatoxin in groundnut oil, groundnut cake and hydrogenated oil in Hapur (Uttar Pradesh, India) market. Journal of Food Science and Technology, India 16(4): 169-170.

The extent of aflatoxin contamination in groundnut oil, groundnut cake and hydrogenated oil sold in the market of Hapur, Uttar Pradesh, India, was investigated. Nearly 66.7 % of the total samples of groundnut oil contained aflatoxin. Of these, 70 % contained as much as 2660 µg kg^{-1} aflatoxin B_1. Of the groundnut cake samples, 70 % were contaminated with 1135-2250 µg kg^{-1}. Refined oil or Vanaspathi did not contain any aflatoxin.

852. Patcl, P.M., Netke, S.P., Gupta, B.S., and Dabadghao, A.K. 1981. Survey of oilcakes and some feeds for the presence of aflatoxin. Indian Journal of Animal Sciences 51(4): 402-407.

Aflatoxins were estimated in samples of 19 different types of oilcake, 4 cereals, 2 compounded feeds and three other products. 80 % of the samples of cottonseed cake and 64 % of groundnut cake contained 200-520 µg kg^{-1} allatoxin B_1. Anatoxins B_2 and G, were also found in some samples, but in lower concentrations than B_1. More than half of the cereals tested contained aflatoxin B_1 as did 14 of 20 samples of poultry feed and all of 4 samples of cattle feed. None of the samples contained more than the permissible limit (taken as 1000 µg kg^{-1}) of the toxins.

853. Patterson, D.S.P., Roberts, B.A., Shreevc, B J., Wrathall, A.E., and Gitter, M. 1977. Allatoxin. ochratoxin. and zearalenone in animal feedstuffs : some clinical and experimental observations. Veterinary Record 101 : 241-245.

Only 10 % of food samples examined during 1973-1975 contained mycotoxins, viz. aflatoxins B, and G_1, ochratoxin A, sterigmatocystin and zearalenone. Anatoxin B_1 (and somtimes G_1) was present up to 2000 µg kg^{-1} in groundnut meal obtained from farms with production disease in cattle. Ill thrift occurred in calves and milk production was lower. Ochratoxin A was occasionally found in stored barley and it has been suggested that it may cause foetal resorption in farm animals, but experimental studies in pregnant sows did not confirm this when they were fed ochratoxin A or B. Ochratoxin A accumulated in the body tissues of the dam. Zearalenone has been implicated in the aetiology of sprayleg piglets, but only 1 such case was seen among 63 piglets from 7 gilts fed naturally contaminated wheat. However, when such wheat was fed to laying hens, some newly- hatched chicks suffered leg weakness.

854. Patterson, D.S.P., and Roberts, B.A. 1980. Aflatoxin B_1 in dairy concentrates and other animal feedstuffs. Veterinary Record 107(11): 249-252.

Over 13 years, 740 samples of animal feeds were analyzed, about 500 of which were suspected of causing disease in farm animals. Aflatoxin B_1 was detected in only 13.6 % of the samples. Groundnut meal samples nearly always contained the toxin. Where mycotoxicosis was suspected in dairy cattle, 27 % of the samples proved positive, as compared with 9 % of other dairy feed samples.

855. Peers, F.G. 1965. Summary of the work done at Vom (Northern Nigeria) on aflatoxin levels in groundnut flour and Arlac. Nutrition Document : Aflatoxin/8. PAG. (WHO/FAO/UNICEF). July 1965 Meeting - Rome. 15 pp.

Aflatoxin levels in groundnut cake, in groundnut flour, and in Arlac are reported. The mean aflatoxin content of groundnut cake available in Nigeria for animal consumption was established as 340 µg kg^{-1} (range 100 µg - > 800 µg kg^{-1} in 148 samples) by taking periodic samples from the consignments supplied to Vom and to the Piggeries at Minna. Aflatoxin levels in groundnut flour (59 analytical

samples) ranged from < 80 pg to > 250 µg kg^{-1}. Aflatoxin levels in groundnut flour and Arlac were lowered to about 20 µg kg^{-1} by following improved practices of hygiene, storage conditions, initial hand-sorting of nuts, and by utilization of better grade nuts for production of groundnut flour and Arlac.

856. Phutela, R.P., and Kabra, M.S. 1979. A note on incidence of anatoxins in peanut and cottonseed cakes in Ludhiana. Indian Journal of Agricultural Research 13(4): 255-256.

Twenty-six groundnut cake and 13 cottonseed cake samples from Ludhiana, Punjab, India, were analyzed for aflatoxin contamination. Fifty per cent of the groundnut cake samples and 30.7 % of cottonseed cake samples were contaminated with aflatoxin. Aflatoxin B, was present in all the contaminated samples whereas aflatoxins B_2 and G_1 were found in 2 and 10, respectively. Aflatoxin G_2 was not present in any of the samples. The levels of aflatoxin B_1 ranged from 16-2000 and 40-500 µg kg^{-1} in groundnut and cottonseed cake samples, respectively, while the levels of the other aflatoxins were < 1 µg kg^{-1}.

857. Reddy, P.S., Reddy, C.V., Reddy, V.R., and Rao, P.V. 1984. Occurrence of aflatoxin in some feed ingredients in three geographical regions of Andhra Pradesh. Indian Journal of Animal Sciences 54(3): 235-238.

A survey on aflatoxin contamination of some poultry feed ingredients, conducted in Andhra Pradesh during winter of 1981- 82, indicated that groundnut cake (GNC), in general, contained more total aflatoxin (587 pg kg"') than did maize (71 µg kg^{-1}), pearl millet (38 pg kg^{-1}), broken rice (43 µg kg^{-1}) and rice polish (23 µg kg^{-1}). The GNC samples collected from the coastal region had more aflatoxin (974 µg kg^{-1}) than had those from Rayalaseema region (573 µg kg^{-1}), and Telengana region (215 µg kg^{-1}). Maize from Rayalaseema, the coast and Telengana had aflatoxin 99, 62 and 53 µg kg^{-1}, respectively. No consistent trend was observed in the other feedingstuffs. The storage of samples for 2 months significantly increased aflatoxin content in GNC and maize but not in other ingredients.

858. Reddy, P.S., Reddy, C.V., Reddy, V.R., and Rao, P.V. 1986. Incidence of fungal infestation in some feed ingredients in three geographical regions of Andhra Pradesh (India). Indian Journal of Animal Sciences 56(7): 789-792.

Samples of groundnut cake, maize, pearl millet, broken rice and rice polish were collected from two representative districts for each of the 3 regions of Andhra Pradesh, India, over a 30-day period. The standard blotter method was used to detect fungal infestation in maize, pearl millet and broken rice. Fungal contaminants from groundnut cake and rice polish were detected by the Czapek's agar plate method. Samples of feed ingredients were analyzed for aflatoxin soon after collection. The predominant fungi infesting these feeds were *Aspergillus flavus, A. niger, Fusarium, Penicillium, Rhizopus, Curvularia, Drechslera* and *Alternaria.* The percentage incidence of colonies of *A.flavus* ranged from 9 to 12 in maize, 14.7 to 22 in pearl millet and 11 to 12 in broken rice. Most positive samples of *A.flavus* infestation were groundnut cake. The levels of aflatoxin in maize were higher than those found in other feed samples, and it suggested that this might be due to the greater surface area of the maize grain.

859. Sanders, T.H., McMeans, J.L., and Davidson, J.I.Jr. 1984. Aflatoxin content of peanut hulls. Journal of the American Oil Chemists' Society 61(12): 1839-1841.

The degree of aflatoxin contamination in groundnut hulls (shells) used in cattle feed was determined by analyzing inoculated hand- shelled hulls and hulls from groundnuts known to contain aflatoxin. Hulls adjusted to 20 % moisture, inoculated with *Aspergillus flavus* and incubated for 7 days at 25°C supported growth of the fungus but not aflatoxin production. Groundnuts from 20 selected lots with visible *A.flavus* contamination contained 13-353 µg kg^{-1} aflatoxin. The machine-shelled hulls from these lots were analyzed and 3 lots contained no detectable aflatoxin, 13 lots contained 4-88 µg kg^{-1} and 4 lots contained 116 µg kg^{-1}. Aflatoxin concentrations of 53-87 µg kg-1 were detected in hulls when groundnuts containing relatively high levels of aflatoxin (up to 26800 µg kg^{-1} in damaged kern«ls) were carefully machine-shelled. Hulls from the same samples obtained by hand-shelling contained no detectable aflatoxin. Separating hulls over a 4.76 mm screen appeared to provide a mean of removal of most aflatoxin-containing particles in groundnut hulls.

860. Schweighardt, H., and Leibetseder, J. 1981. [Determination of mycotoxins by high pressure liquid chromatography (HPLC).]. Nachweis von Mykotoxinen mittels Hochruckflussigkeitschromatograplue (HPLC). Wiener Tierarztliche Monatsschrift 68: 302-305.

A high pressure liquid chromatography (HPLC) technique is described for detection of mycotoxins (aflatoxins, zearalenone, vomitoxin. and ochratoxin A). The detection limit for toxins was : aflatoxins (B_1, B_2, G_1,. and G_2) 2 µg kg^{-1}, zearalenone 1 µg kg^{-1}, vomitoxin 25 µg kg^{-1}, and ochratoxin A 5 µg kg^{-1}. Of groundnut products, mainly groundnut meal, 87 % were contaminated (maximum 11620 µg kg^{-1} total aflatoxin). In Southeast Styria a high rate of vomitoxin contamination was found in maize (80 %. average 761 µg kg^{-1}), zearalenone was found in 47 % of samples tested (average concentration 145 µg kg^{-1}), 23.6 % of 83 stored grain samples contained ochratoxin A at low concentration.

861. Singh, S. 1987. Incidence of aflatoxin in poultry feeds. Poultry Adviser 20(7): 25-29.

A total of 234 samples of poultry feed from 54 poultry farms in Andhra Pradesh and their ingredients were examined for the presence of aflatoxins. Among the feed ingredients analyzed, groundnut cake was found to be the only major feed component affected by aflatoxin. The overall incidence of aflatoxin was 30.76 % in feedstuff's. The predominant fungi contaminating these feeds were *Aspergillus flavus, A. niger, Fusarium* spp., *Penicillium* spp., *Rhizopus* spp., and *Curvularia* spp.

862. Singh, T., Tyagi, R.P.S., and Ram, B. 1984. Occurrence of aflatoxin B_1 in animal and poultry feeds. Indian Journal of Dairy Science 37(2): 167-170.

A total of 127 samples of animal and poultry feeds were collected from 15 sources in India, ground, sieved and dried. Aflatoxin B_1 was estimated by chromatography. About 35 % of samples were contaminated with aflatoxin B_1 and concentrations were particularly high in groundnut cake and cottonseed cake samples.

863. Sinha, R.R.P., and Arora, S.P. 1985. Survey of common feedstuffs for aflatoxins. Indian Journal of Animal Nutrition 2(4) :194-196.

Of 14 groundnut cake samples collected before the rainy season 8 contained aflatoxin Bl (mean 350 µg kg^{-1}), 17 of 19 samples collected during and after the rainy season had 470 µg kg^{-1}, 3 in each of 4 samples of cattle feed, poultry mash and pig grower mash had 140, 240 and 170 µg kg^{-1}, 2 of 4 samples of linseed meal had 50 µg kg^{-1} and 2 of 10 maize samples 260 µg kg^{-1} Aflatoxin B_2 or G_1 was also detected in some of the groundnut cake samples. Four samples of fish meal, 4 of wheat bran and one of berscem hay did not contain any aflatoxin.

864. Sokolowski, M., and Jurkiewicz, G. 1977. [Aflatoxin B_1 contamination of groundnut meal and feed mixtures.|. Skazcnie aflatoksyna B_1 srut arachidowych i mieszanek paszowych. Medycyna Weterynaryjna 33(6): 346-347.

In 130 samples of groundnut meal and 680 samples of feed mixtures examined during 1974-1976, aflatoxin B_1 levels varied from 0 to 1280 µg kg^{-1} in groundnut meal and from 0 to 200 µg kg^{-1} in feed mixtures. Annual fluctuations in aflatoxin B_1 levels might be influenced by weather.

865. Strselecki, E.L., and Cader-Strzelecka, B. 1988. Correlation of *Aspergillus flavus* and aflatoxin contamination in peanut meals. Proceedings of the Japanese Association of Mycotoxicology Supplement 1: 31-32.

Of 366 samples of groundnut oilmeal from India and Brazil. 90 % of samples contained < 50 colonies g^{-1} of *Aspergillus flavus* and 78 % of samples contained < 100 µg kg^{-1} aflatoxin B_1. It is concluded that there is a correlation between contamination of groundnut oilmeal by *A. flavus* and aflatoxin,

866. Sugimoto, T. 1983. Handling of imported groundnut extraction meal in Japan. |aflatoxins|. Proceedings of the Japanese Association of Mycotoxicology (No. 17): 32-38.

The Japanese Government ordinance specifies groundnut meal as the specific feed which should not contain > 1000 µg kg^{-1} aflatoxin. Aflatoxin levels in imported groundnut meals from India are given. Levels of aflatoxins B_1 ranged from 69 to > 26000 µg kg^{-1} in different groundnut meals. It is assumed that only a small number of highly contaminated consignments (bags) would be the cause of disqualification of the lot.

867. Zilkova, J. 1973. (Aflatoxin levels in imported groundnut oil cakes.]. Hladiny aflatoxinu v arasidovych pokrutinach z dovozu. Veterinarstvi 23(12): 547-548.

Of 36 samples of groundnut oil cake examined in 1971, 17 (47.2 %) contained aflatoxin. Eleven of these 17 contaminated samples contained up to 100 µg aflatoxin kg^{-1} (mainly aflatoxin B_1) and 6 > 100 *µg* kg^{-1}. Of 74 samples examined in 1972, 66 (89.2 %) were contaminated; 13 contained up to 100 µg kg^{-1} and 53 > 100 µg kg^{-1} aflatoxin.

5.2.5 Peanut butter

868. Besrat, A., and Gebre, P. 1981. A preliminary study on the aflatoxin content of selected Ethiopian foods. Ethiopian Medical Journal 19(2): 47-52.

Injera, a fermented, pancake-shaped food prepared from *Eragrostis tef,* showed no aflatoxin contamination as commonly prepared and handled, but fermentation of the dough or storage of injera for prolonged periods did not increase aflatoxin B_1 contamination. Preparations of red pepper powder and its paste showed some aflatoxin contamination (mean 32 µg kg^{-1} for powder. 1 paste sample had 102.2 µg kg^{-1} aflatoxin B_1). Samples of groundnuts and peanut butter had aflatoxin B, at mean values of 34.7 and 105 µg kg^{-1}, respectively.

869. Chang-Yen, I., and Fclmine, J. 1987. Aflatoxin levels in selected bulk foods and feeds in Trinidad. Tropical Agriculture 64(4): 283-286.

Aflatoxins B_1, B_2, G_1 and G_2 were determined in bulk samples of raw groundnuts, peanut butter, wheat flour, and chicken, pig and dairy feeds. Samples were taken

in accordance with an established sampling plan, and dry samples were subsampled with the Dickens Subsampling Mill. A minicolumn procedure was used to screen 64 samples, eight of which were further analysed by thin-layer and high-performance liquid chromatography. Of the eight samples, only one contained aflatoxin G_1 (2 µg kg^{-1}).

870. Eadie, T., and O'Rear, C.E. 1967. The occurrence of aflatoxin in Virginia-North Carolina peanuts and peanut products. Virginia Journal of Science 18: 140-141.

Aflatoxin levels in groundnut samples from the Virginia-North Carolina crop were determined in a survey carried out in the 3- year period 1964-1967. About 6% of samples from the 1964-1965 crop contained aflatoxin, and 23 of 51 samples of peanut butter were contaminated with aflatoxin. Contaminated lots of groundnuts were reduced to 5.3% in 1965-1966 and to 4.4% in 1966-1967. No peanut butler sample was contaminated in 1965-1966.

871. Fonseca, H., Nogueira, J.N., Graner, M., Olivcira, A.J., Caruso, J.G.B., Boralli, C., Calori, M.A., and Khatounian, C.A. 1983. Natural occurrence of mycotoxins in some Brazilian foods. II. Pages 53-54 in Proceedings of the Sixth International Congress of Food Science and Technology. Vol. 3 (McLoughlin, J.V., and McKeenna, B.M., eds.). Boole Press Ltd., Dublin : Irish Republic.

Surveys were carried out for two years to determine the natural occurrence of mycotoxins (aflatoxin, ochratoxin and zearalenone) in some common Brazilian foods such as raw and salted roasted groundnuts, pacoca (a very popular groundnut candy), peanut butter, maize, soybean, cured cheese, salami and copa (cured and dried pork). Eight characteristic regions. Vale do Paraiba. Mogiana. Paulista Velha, Araraquarense, Noroeste, Paulista Nova. Sorocabana and Vale do Ribeira/Litoral Sul, were surveyed and 742 samples were analyzed. Estimation of toxins was made by thin-layer chromatography (IXC) by comparison with standards. Six samples of corn (4.7 %) were contaminated with aflatoxin, ranging from 41 to 2000 µg kg^{-1} of aflatoxin B_1; 81 samples of pacoca (63.3 %). ranging from < 20 to 1187 µg kg^{-1}; 80 samples of peanut butter (62.5 %), from < 20 to 275 µg kg^{-1}; 17 samples of raw groundnuts (17.7 %), from 19 to 3125 fig kg^{-1}; and 9 samples of roasted salted groundnuts (9.4 %) from < 30 to 4250 µg kg^{-1}. From the first to the second year, the percentage of contaminated samples increased slightly for raw groundnuts (from 15.6 to 18.8 %) and decreased for pacoca (67.2 to 59.4 %) for roasted salted groundnuts (12.5 to 7.8 %), corn (6.2 to 3.1 %), and for peanut butter (84.4 to 40.6 %). The average content of aflatoxin of the contaminated samples increased for raw groundnuts (from 166.7 to 641.7 µg kg^{-1}) and roasted sailed groundnuts (410 to 980 µg kg^{-1}) and decreased for pacoca (165.6 to 108.8 µg kg^{-1}), peanut butter (91.3 to 50.4 µg kg^{-1}), and corn (853.3 to 700 µg kg^{-1}).

872. Gelda, C.S., and Luyt, L.J. 1976. Survey of total aflatoxin content in peanuts, peanut bulter and other foodstuffs. Page 2 in Abstracts of the Third International I.U.P.A.C. Sponsored Symposium on Mycotoxins in Foodstuffs, 16-18 September 1976, Paris, France.

A study employing the Best Foods (BF) method was carried out for a period of six years to determine the aflatoxin content in groundnuts, peanut butter and other foodstuffs. The study was divided into three groups. In Group I a total of 3,928 samples were analyzed; 1,679 samples of raw groundnuts, 2,092 samples of peanut butter, and 157 samples of processed groundnuts. 5.8 %, 0.1 %, and 1.3 % of the samples analyzed in these groups contained a total aflatoxin content over 25 µg kg^{-1}, respectively. Group II consisted of other nuts : cashew, almonds, walnuts, pecans, filberts, Brazil nuts, and pistachios, of which 641 samples were analyzed. Two per cent contained aflatoxin over 25 µg kg^{-1}. Group III, other foodstuffs, consisted of sunflower seeds, cocoa beans, beans, corn, mushrooms, and coffee beans. The total number of samples analyzed were 244 and only 0.4 % contained aflatoxin at over 25 µg kg^{-1}. On the basis of the samples analyzed it is evident that in the past six years the incidence of aflatoxin occurring at levels of less than 5 µg kg^{-1} in groundnuts and groundnut products has been 90.1 % of the samples analyzed. In the other nuts tested, 91.7 % of the samples contained less than 5 fig kg^{*1} of aflatoxin. The test data of samples of foodstufs other than nuts showed that 95.9 % of the samples contained aflatoxin at a level of less than 5 µg kg^{-1}.

873. Gelda, C.S., and Luyt, L.J. 1977. Survey of total aflatoxin content in peanuts, peanut butter and other foodstuffs. Annates de la Nutrition et de l'Alimentation 31(4/5/6): 477-483.

A study using the 'Best Foods' (BF) method was done over 6 years to determine aflatoxin contents in groundnuts, peanut butter and other foods. Results showed that of 1679 samples of raw groundnuts, 98 (5.8%) contained > 25 µg kg^{-1} total anatoxins. 89.8 % < 15 µg kg^{-1}. Of 157 samples of processed nuts. 2 (1.3%) contained > 25 µg kg^{-1}, and the rest < 15 µg kg^{-1}. Of 2092 samples of peanut butter, 3 (0.14%) contained > 25 µg kg^{-1} and 12 (0.57%) contained 15-25 µg kg^{-1}. Of 120 samples of cashews, 10 (8.3%) contained > 15 µg kg^{-1} as well as 1 of 78 samples of almonds, 2 (1.3%) of 156 samples of walnuts, 10 (5.7 %) of 175 samples of pistachios. None of the samples of pecans, filberts and Brazilnuts tested contained aflatoxin. Of 244 samples of other foods, only one sample each of sunllower seeds and corn contained > 15 µg kg^{-1}. None of the samples of cocoa beans, beans, mushrooms tested had aflatoxin contamination. Test data of foods other than nuts had aflatoxin levels of < 5 µg kg^{-1} in 95.9% of samples.

874. Gilbert, J., and Shepherd, M.J. 1985. A survey of anatoxins in peanut

butters, nuts and nut confectionery products by HPLC with fluorescence detection. Food Additives and Contaminants 2(3): 171-183.

A preliminary survey of peanut butter in 1982 indicated that 31 of 32 samples of major national brand-named products contained aflatoxin B_1 at up to 10 ng kg ', and that 59 % of those were below the limit of detection (2 µg kg^{-1}). Of 25 peanut butters from specialist 'Health Food' outlets, 64 % contained up to 10 *µg* kg^{-1}, and the remainder contained 16-318 µg kg^{-1}, with one sample having 345 µg kg^{-1} aflatoxin. Surveys of 'Health Food' products in 1983 and 1984 confirmed that manufacturers were still experiencing some difficulty in complying with the 30 *µg* kg^{-1} voluntary guideline limit for total aflatoxin. In 1984, 228 retail samples of nuts and nut confectionery products comprising groundnuts (shelled, unshelled, roasted and salted), mixed nuts, almonds (both unblanched and ground), Brazil nuts (in-shell), hazelnuts (in shell), chocolate-coated groundnuts, peanut brittle and coconut ice were examined. Results showed that 74 % of the samples contained aflatoxin B_1 at up to 0.5 µg kg^{-1}, and 3.1 %, mainly groundnuts and Brazil nuts, exceeded the guideline tolerance for total aflatoxin. Total aflatoxin was greatest in unshelled groundnuts, 4920 µg kg^{-1}, and in a composite sample of visibly molded Brazil nuts, 17926 *µg* kg^{-1}.

875. Karmacharya, S. 1988. Studies on the occurrence of atlatoxins in various food and feed commodities of Nepal. Pages 426-433 in Proceedings of the National Conference on Science and Technology, 24-29 April 1988, Kathmandu, Nepal.

Studies on aflatoxin contamination in various food samples of Nepal were carried out during the period 1980-1986. About 764 samples of various food commodities from hills and Terai region were analyzed for aflatoxin contamination. One hundred and forty-eight samples of different feed ingredients from different Feed Industries of Kathmandu were also analyzed. Of the total number of respective samples analyzed, 19 % of maize, 29.5 % of maize flour, 17.9 % to 33.8 % of groundnuts, 31.7 % of peanut butter and 9.4 % of wheat flour were contaminated with aflatoxin, but tolerance limit (30 µg kg^{-1}) exceeded in 4.5 % to 15.9 % of these samples. Rainy season is the most prone period for aflatoxin contamination. In case of feed, about 50 % of poultry feed, 26.7 % of cattle feed and 20 % of pig feed samples were contaminated with aflatoxins. Twenty % of the poultry feed samples had aflatoxins above the tolerance limit (10 to 50 µg kg^{-1} depending upon the age of the animals).

876. Kuiper-Goodman, T., Kirkpatrick, D.C., and Krewski, D. 1979. The aflatoxin intake of various age groups of Canadians. Toxicology and Applied Pharmacology 48 (1 part 2): A17.

Between 1974 and 1977 the average level of aflatoxins in peanut butter was 4 ng g^{-1}. The average intake of these foods was 0.4 g kg^{-1} bodyweight for children up to 10 years and declined progressively thereafter to < 0.1 g kg^{-1} bodyweight.

877. Lohiya, G., Nichols, L., Hsieh, D., Lohiya, S., and Nguyen, H. 1987. Aflatoxin content of foods served to a population with a high incidence of hepatocellular carcinoma. Hepatology, Baltimore 7(4): 750-752.

A total of 36 samples of foods collected during August 1985, December 1985 and March 1986 and served to mentally retarded clients with a high incidence of hepatocellular carcinoma, were analyzed for aflatoxin. Aflatoxin was not detected (< 5 µg kg^{-1}) by thin-layer chromatography in 35 food samples containing groundnuts, maize, wheat or milk. One peanut butter sample contained 20 µg kg^{-1} aflatoxin. Aflatoxin content of these foods was at or below the level permitted by the Food and Drug Administration. It is concluded that aflatoxin is probably not responsible for liver disease in this population.

878. Lotter, L.H., and Krohm, H.J. 1988. Occurrence of aflatoxins in human foodstuffs in South Africa. Bulletin of Environmental Contamination and Toxicology 40(2): 240-243.

During 1985 and 1986, samples of sorghum beer, sorghum cereal, groundnuts, peanut butter and maize meal, purchased from supermarkets, were analyzed for aflatoxins using HPLC and fluorescence detection methods. Of the samples analyzed during 1985, approximately one-third were contaminated with aflatoxins, although none were above the legal limit of 10 µg kg^{-1} aflatoxins. In 1986, the numbers of contaminated samples increased, but the level of contamination remained low, with only one sample exceeding the legal maximum.

879. Mat, Isa Awang, and Nazarifah, 1. 1986. Aflatoxin contamination in agricultural commodities. Teknologi Makanan, Malaysia 5(1): 54-58.

Aflatoxin contamination in five agricultural commodities namely groundnut, dried cocoa beans, copra, pepper (black and white) and paddy and rice was determined. Shelled groundnuts and their products such as peanut butter and satay gravy were highly susceptible to aflatoxin contamination, but the "menglembu" type groundnut was free of aflatoxin. Copra, dried cocoa beans and pepper (black and white) were the other highly susceptible commodities, but stored paddy and rice were safe but a few samples contained aflatoxin. The extent of contamination in several other commodities is also mentioned and general control methods are discussed.

880. Mortimer, D.N., Shepherd, M.J., Gilbert, J., and Morgan, M.R.A. 1987. A survey of the occurrence of aflatoxin B_1 in peanut butters by enzyme-linked immunosorbent assay. Food Additives and Contaminants 5(2): 127-132.

A survey was carried out in 19X6 for the occurrence of aflatoxin B_1 in peanut butters (129 samples) obtained from specialist Health Food outlets. The results showed that 6.2 % of the samples contained > 10 µg kg^{-1} of aflatoxin, 8 % contained between 2.5 and 10 µg kg^{-1}, and in the remainder (86 %) aflatoxin could not be detected at a limit of 2.5 µg kg^{-1}. These results show a lower contamination by aflatoxin than found in these products in previous surveys (1982-1984). An aflatoxin B,-specific enzyme-linked immunosorbent assay (ELISA) was employed for the first time in these analyses; and to make an assessment of its performance positive aflatoxin results, together with a random selection of those below the ELISA limit of detection, were additionally analyzed by conventional extraction and clean-up followed by HPLC. The ELISA technique offered a significant improvement in speed of analysis over conventional approaches, enabling a six-fold increase in sample throughput as compared with that required for conventional analysis.

881. Ram, B.P., Hart, L.P., Cole, R.J., and Pestka, J.J. 1986. Application of ELISA to retail survey of aflatoxin B_1 in peanut butter. Journal of Food Protection 49(10):792-795.

A simple procedure was devised for the routine screening of aflatoxin B_1 in peanut butter using enzyme-linked immunosorbent assay (ELISA). Peanut butter samples (5g) were spiked with aflatoxin B_1 and extracted by blending with 25 ml of 55 % methanol and 10 ml of hexane. The extract was filtered and aqueous filtrate analyzed by a indirect competitive ELISA. Recovery of aflatoxin B, added to peanut butter samples ranged from 85 to 112 %, with an average coefficient of variation of 18.4 %. Using this procedure, only three of 63 samples of peanut butter had detectable levels (>5.0 µg kg^{-1}) of anatoxin B_1.

882. Ram, B.P., Hart, L.P., Pestka, J.J., Cole, R.J., and Miller, B.M. 1986. Rapid analysis of peanuts and peanut products by enzyme immunoassay for allatoxin. Proceedings of the American Peanut Research and Education Society, Inc. 18: 62.

A simple procedure was devised for the routine screening of aflatoxin B_1 in groundnuts and peanut butter using enzyme-linked immunosorbent assay (ELISA). Samples of peanut butter were spiked with allatoxin B_1 and extracted by blending with 25 ml of 55 % methanol and 10 ml hexane. The extract was filtered and aqueous filtrate analyzed by a direct competitive ELISA. Recovery of aflatoxin B_1 added to peanut butter samples ranged from 85-112 %, with a mean recovery of 97 %. Coefficient of variation between test wells in the assay was 18.4 %. Only 3 of 63 commercial samples of peanut butter showed detectable aflatoxin B_1 (>5 ng g^{-1}) with this procedure.

883. Sellschop, J.P.F., Kriek, N.P.J., and Du Preez, J.C.G. 1965. Distribution and degree of occurrence of aflatoxin in groundnuts and groundnut products. South African Medical Journal 39: 771- 774.

Distribution and degree of occurrence of anatoxins in various samples of groundnuts and groundnut products in the Republic of South Africa are described. Over 1000 samples of groundnuts and groundnut products were examined during 1963 and another 1000 samples of producers' grades were investigated during 1964. The samples obtained from the North-Western Transvaal, or so-called Bushveld areas, were most seriously contaminated with aflatoxin; all of the 75 samples containing > 2000 µg kg^{-1} aflatoxin were from the receiving depots in the North-Western Transvaal. The samples from the Northern Cape Province, Orange Free State and Natal, except for 5 samples with 500-2000 µg kg^{-1} and 11 samples with 100-500 µg kg^{-1}, were free from aflatoxin. Similar results were found in the 1964 survey for aflatoxin contamination; however, only 23 samples from the North-Western Transvaal areas contained > 2000 µg kg^{-1}. High degree of aflatoxin contamination in the groundnut samples from the North-Western Transvaal was ascribed mainly to (1) the damage caused to the maturing groundnut pods and kernels by termites, and (2) protracted droughts followed by late rains. Lower or inferior grade groundnuts contained higher levels of aflatoxins than the higher or superior ones. No aflatoxins were detected in any of the samples of hand-picked selected (HPS) kernels examined in 1963 and 1964. Groundnut cake samples implicated in livestock deaths were also examined for aflatoxin contamination. Eleven of the 16 samples of this groundnut cake contained > 2000 ng kg^{-1} aflatoxin. Of 101 peanut butter samples tested, 17 were contaminated with aflatoxin. Of these 17 samples, 12 had an aflatoxin contents of 100-500 µg kg^{-1} *and* 5 had up to 25 µg kg^{-1}.

884. Sripathomswat, N., and Thasnakorn, P. 1981. Survey of aflatoxin-producing fungi in certain fermented foods and beverages in Thailand. Mycopathologia 73(2): 83-88.

Aflatoxin-producing fungi were found in fermented foods and beverages: fermented rice (kaomak), soybean sauce (taotjo), peanut butter, soy sauce (shoyu), Thai red and white wine, and rice sugar wine. These foods were extracted directly and tested for aflatoxins by thin-layer chromatography (TLC) and high pressure liquid chromatography (HPLC). Four strains of aflatoxin-producing fungi were isolated from peanut butter, taotjo, and shoyu. Direct extracts of 10 % of the peanut butter samples tested and 5 % of the kaomak tested contained high levels of aflatoxins.

885. Tabata, S., and Kamimura, H. 1988. A survey of aflatoxin contamination in Tokyo's commercial foods and foodstuffs. Proceedings of the Japanese Association of Mycotoxicology Supplement No.1: 63-64.

The aflatoxin contamination in Tokyo's commercial foods and foodstuffs during 1982-1986 was investigated. A high incidence of contamination was found in buckwheat (23 of 134 samples positive for aflatoxin) and coix seed (38 of 159 positive), although the aflatoxin levels were relatively low. Of 464 samples of nuts and their products tested, aflatoxin was detected in 3 samples of peanut butter and one of sesame. One sample of butter bean was found to be heavily contaminated with aflatoxins (aflatoxin B_1 : 254 μg kg^{-1}; aflatoxin B_2 : 8.5 μg kg^{-1}). When spices were analyzed, a high incidence (60 %) and level of aflatoxin was found in nutmeg from Indonesia. A high incidence of aflatoxin was also found in white pepper, but not in black pepper.

886. Yndestad, M., and Underdal, B. 1975. [Aflatoxin in foods on the Norwegian market.]. Aflatoksin i naeringsmidler pa det norske marked. Nordisk Verterinaermedicin 27(1): 42-48.

A survey of the aflatoxin content in some food products available on the market in Norway is reported. During autumn 1973, samples of Brazil nuts, groundnuts, peanut butter, hazelnuts, walnuts, mixed nuts, cocoa, cocoa products and dried milk were bought from stores in the Oslo area. In addition, samples of Brazil nuts and groundnuts were taken from import stores. Aflatoxins were detected in 6 of 34 samples of bought groundnuts, 1 of 3 of sorted and pooled groundnuts, 1 of 16 mixed nuts, 1 of 14 walnuts, 1 of 3 cashew nuts. 18 of 27 Brazil nuts and 1 of 40 cocoa products, levels of aflatoxins B_1. B_2, G_1 and G_2 were 4200, 1600, 3100 and 600 μg kg^{-1}, respectively, for one sample of Brazil nuts and 400. 50. 350 and 30 μg kg^{-1} for a sample of groundnuts. Other aflatoxin levels were generally much lower. No aflatoxin was detected in peanut butter, hazelnuts and dried milk.

5.2.6 Groundnut protein concentrate

887. Akano, D.A., and Atanda, O.O. 1990. The present level of aflatoxin in Nigerian groundnut cake ('Kulikuli'). Letters in Applied Microbiology 10: 187-189.

Groundnut cake ('Kulikuli') samples purchased from four major markets in Ibadan. Oyo State, Nigeria, during April-November 1988 were analyzed for aflatoxin B_1 and associated mycoflora. In all but two of the samples aflatoxin B_1 levels were between 20 and 455 μg kg^{-1}. Mold counts were low (1.0×10^2 - 4.40×10^2 colonies g^{-1}). Eight mold species were isolated. Of these Aspergillus niger. Paecilomyces varioti, Aspergillus flavus and Fusarium moniliforme dominated in decreasing sequential order. The results show that groundnut cake on sale in Ibadan markets is unacceptable for animal feed rations and human consumption and there is a need for some form of quality control and decontamination before usage.

888. Amla, 1., Kamala, C.S., Gopalakrishna, G.S., Jayaraj, A.P., Sreenivasamurthy, V., and Parpia, H.A.B. 1971. Cirrhosis in children from peanut meal contaminated by aflatoxin. American Journal of Clinical Nutrition 24: 609-614.

Children suffering from varying degrees of protein-calorie malnutrition had accidentally consumed aflatoxin-contaminated, low fat. commercially produced groundnut protein flour for periods ranging from 5 days to 4 weeks. The hepatic lesions showed a gradual transition from an increase in central and periportal fat to fibrosis and cirrhosis, which does not usually occur in treated Kwashiorkor. The lesions were identical to those of Indian childhood cirrhosis.

889. Amla, I., Farpia, H.A.B., and Jayaraj, A.P. 1971. Cirrhosis in children after consumption of aflatoxin-contaminated peanut meal. Journal of Pathology 103(2): XIX.

Children suffering from varying degrees of protein calorie malnutrition were accidentally fed with aflatoxin (300 μg kg^{-1}) - contaminated groundnut protein flour for periods of 10 days to 4 weeks. Liver biopsies showed a gradual transition from an increase in central and periportal fat of the liver, to formation of fatty cysts, to fibrosis and cirrhosis.

890. Natarajan, K.R., Rhee, K.C., Cater, C.M., and Mattil, K.F. 1975. Distribution of aflatoxins in various fractions separated from raw peanuts and defatted peanut meal. Journal of the American Oil Chemists' Society 52: 44r47.

The present investigation is the first definitive study of the distribution of aflatoxins in a wet-milling process of raw groundnuts. The results show that the majority of aflatoxins originally present in the groundnuts remained in the solid fractions, particularly the protein fractions, during wet-milling. In the protein concentrate preparation, the concentrates carried 81-89 % of the total aflatoxin; crude oil, 5-8 %; and whey fraction, 3-14 %. In the case of protein isolate preparation, 51-56 % of the total toxin remained with the isolates, 22-26 % with the residue, 11-17 % with the whey, and 7-8 % with the crude oil. Distribution of aflatoxins in the preparation of protein isolates from defatted groundnut meal showed that 55-65 % of the total toxin originally present in the meal remained with the protein isolates, 20 28 % with the residue, and 10-20 % with the whey fraction. Changes in extraction pHs for the preparation of protein isolates either from raw groundnuts or defatted meal did not alter the distribution pattern. A new approach based upon the charge- transfer (electron acceptor-donor) complex formation is suggested to shift this aflatoxin distribution from protein products to disposable whey or residue fraction from the processing of raw groundnuts and defatted meal for protein products.

891. Peers, F.G. 1965. Summary of the work done at Vom (Northern Nigeria) on aflatoxin levels in groundnut flour and Arlac. Nutrition Document : Aflatoxin/8. P.A.G. (WHO/FAO/UNICEF). July 1965 Meeting - Rome. 15 pp.

Aflatoxin levels in groundnut cake, in groundnut Hour, and in Arlac are reported. The mean aflatoxin content of groundnut cake available in Nigeria for animal consumption was established as 340 µg kg^{-1} (range 100 µg - > 800 µg kg^{-1} in 148 samples) by taking periodic samples from the consignments supplied to Vom and to the Piggeries at Minna. Aflatoxin levels in groundnut flour (59 analytical samples) ranged from < 80 µg to > 250 µg kg^{-1}. Aflatoxin levels in groundnut flour and Arlac were lowered to about 20 µg kg^{-1} by following improved practices of hygiene, storage conditions, initial hand-sorting of nuts, and by utilization of better grade nuts for production of groundnut flour and Arlac.

892. Stoloff, L., Trucksess, M., and Martinez, W. 1976. The fate *of* aflatoxins in the preparation of protein concentrates and isolates from contaminated peanut and cottonseed flours. Journal of Food Science 41(5): 1251-1253.

With the assistance of the laboratories of six major soybean protein producers, simulated commercial protein concentrate and isolate processes were applied to aflatoxin-contaminated groundnut and cottonseed flours to determine the fate of the aflatoxins. Processes for producing concentrate by washing the flour at the protein isoelectric point (pH 4.5) were ineffective in separating the aflatoxin from products, but a process using an aqueous alcohol wash accomplished in 90% reduction in the aflatoxin level of the concentrate compared to the flour. Processes for producing isolate by protein dissolution in alkali and its recovering by precipitation at the isoelectric point resulted in some aflatoxin loss but an increase in the aflatoxin concentration associated with the protein isolate compared to the original flour. However, a process for applying carbon adsorbent to the alkaline solution accomplished a 90% reduction in the aflatoxin level of the isolate.

5.2.7 Groundnut Hay

893. Mckenzie, R.A., Blaney, B.J., Connole, M.D., and Fitzpatrick, L.A. 1981. Acute aflatoxicosis in calves fed peanut hay. Australian Veterinary Journal 57(6): 284-286.

Acute aflatoxicosis was believed to be the cause of death in 12 of 90 Hereford calves, fed groundnut hay during drought in Queensland. They developed jaundice, photosensitization, diarrhoea, anorexia, and depression. Increase in serum levels of enzymes of hepatic origin and bilirubin content were higher than normal. Haemorrhage, hepatocyte damage and bile duct proliferation were seen in groundnut hay-fed dead calves. The groundnut hay contained up to 2230 µg kg^{-1} aflatoxin, with most toxin concentrated in the nut-in-shell.

894. Ray, A.C., Abbitt, B., Cotter, S.R., Murphy, M.J., Reagor, J.C., Robinson, R.M., West, J.E., and Whitford, H.W. 1986. Bovine abortion and death associated with consumption of aflatoxin- contaminated peanuts. Journal of the American Veterinary Medical Association 188(10): 1187-1188.

When an estimated 10-14 pregnant cows in a 68-cow herd were given moldy groundnuts as a supplementary feed, constituting a large proportion of their diet for 4 days, most of them aborted on or soon after the fifth day. The cows were recumbent and unable to rise; in one, there was a low rectal temperature (99°C) and a tremor of the head. All of the cows that aborted were in the third trimester of pregnancy, and all died within 8 days. Biochemical analyses performed on 3 cows revealed hepatic dysfunction, indicated by high values for lactate dehydrogenase, aspartate transaminase and total bilirubin; there was also evidence of mild dehydration; no fetuses were examined. The groundnuts contianed 77 µg g^{-1} of aflatoxin B_1, which was also found (5 µg g^{-1}) when a liver extract of one cow was analyzed for mycotoxins. Cows fed groundnut hay from the same field as the groundnuts were not affected; the nuts had been kept in the open at freezing temperatures. Abortion preceding fatal mycotoxicosis, as occurred in at least 8 of these cows, is an unusual feature of acute aflatoxicosis.

895. Sanders, T.H., McMeans, J.L., and Davidson, J.I.Jr. 1984. Aflatoxin content of peanut hulls. Journal of the American Oil Chemists' Society 61(12): 1839-1841.

The degree of aflatoxin contamination in groundnut hulls used as cattle feed was determined by analyzing inoculated hand-shelled hulls and hulls from groundnuts known to contain aflatoxin. Hulls adjusted to 20 % moisture, inoculated with *Aspergillus flavus* and incubated for 7 days at 25°C supported growth of the fungus but not aflatoxin production. Groundnuts from 20 selected lots with visible *A.flavus* contamination contained 13-353 *µg* kg^{-1} aflatoxin. The machine-shelled hulls from these lots were analyzed and 3 lots contained no detectable aflatoxin, 13 lots contained 4-88 µg kg^{-1} and 4 lots contained 116 µg kg^{-1}. Aflatoxin concentrations of 53-87 µg kg^{-1} were detected in hulls when groundnuts containing relatively high levels of aflatoxin (up to 26800 fig kg ' in damaged kernels) were carefully machine-shelled. Hulls from the same samples obtained by hand-shelling contained no detectable aflatoxin. Separating hulls over a 4.76 mm screen appeared to provide a means of removal of most aflatoxin-containing particles in groundnut hulls.

6. LIMITS AND REGULATIONS

6.1 REVIEW

It is imperative that food contaminated with a hazardous substance is considered unsafe for human consumption. Therefore all food laws, which prohibit trade in adulterated food, consider food contaminated with a hazardous substance as adulterated food. In many countries, this general legislation has been translated into specific regulations that impose tolerable limits on specific mycotoxins (e.g., aflatoxins) in foods.

After the discovery of the acute and chronic toxicity of aflatoxins in the early 1960s, many countries decided to introduce regulations to control the levels of the toxins in foods for human and animals. In the 1970s, some 18 countries had regulations or guidelines which prescribed maximum permissible levels of aflatoxins in foods and/or feeds (Stoloff 1977). Because of increasing awareness of the risk of aflatoxin contamination of foods and feeds, there has been increased enforcement of regulations and legislations on permissible levels of aflatoxins. Aflatoxin regulations have been changed, expanded or created in various countries. At present some 50 countries impose aflatoxin regulations and most countries have a separate maximum acceptable level for food and feed (van Egmond 1989). Regulated commodities vary from country to country, ranging from all foods and feeds to an individual commodity. Groundnuts and groundnut cake are among the most frequently regulated commodities.

The permitted level of aflatoxin in a commodity varies widely depending on the country and the foodstuffs and also whether the foodstuff is destined for human or animal consumption. For example, the maximum permitted level of aflatoxin in foods for human consumption varies from zero to 50 μg kg^{-1} in those countries with existing regulations (van Egmond 1989). There has been a tendency for regulations to become increasingly stringent as methods of aflatoxin detection have improved (Stoloff 1977, van Egmond 1989).

Aflatoxin Limits in Groundnuts and Groundnut Products for Humans

Many groundnut importing countries have placed limits on the levels of aflatoxins permissible in groundnuts and groundnut products (Table 4). A few countries including Cuba, Dominican Republic, Malaysia, and Portugal have a zero tolerance limit. Several countries including Belgium, Luxembourg, The Netherlands, Norway, Surinam. Sweden. Switzerland, and the USSR have set a reasonable limit of 5 μg aflatoxin(s) kg^{-1}, while most other countries have set "practical limits" of 10 to 30 μg kg^{-1} (either aflatoxin B_1 or aflatoxins B_1, B_2, G_1, and G_2). Some countries consider only aflatoxin B_1. while others use the total of aflatoxins B_1, B_2. G_1. and G_2. A tolerance for aflatoxin B_1 of 5 μg kg^{-1} is commonly applied by many countries. In countries that apply limits for the total aflatoxins, such a uniformity in tolerance levels is not evident. It is not clear whether a tolerance for total aflatoxins contributes to better protection of public health than a tolerance for aflatoxin B_1 alone. Aflatoxin B_1 is the most common toxic and carcinogenic aflatoxin.

The U.K. has a guideline limit of 30 μg kg^{-1} (total aflatoxins) for imported groundnuts and groundnut products (Jewers 1982). The USA has a guideline limit of 25 μg kg^{-1} (total aflatoxins) in raw groundnuts, and 20 μg kg^{-1} in edible nuts and nut products (Stoloff 1977).

In 1966 the WHO/FAO/UNICEF Protein Advisory Group (PAG) set a limit of 30 μg kg^{-1} for groundnut protein supplements for children India initially adopted the PAG recommendation and set a guideline limit of 30 μg kg^{-1} for edible groundnut flour. Later it was impossible to meet this standard; therefore a limit of 60 μg kg^{-1} (aflatoxin B_1) was set (Anonymous 1979).

Countries dependent on export of aflatoxin-susceptible commodities e.g., groundnuts, are obliged to establish export limits that meet importers' requirements. This leads to economic loss if the requirements are unnecessarily strict. Where a local food is also an export item, exportation of the most wholesome food may lead to local consumption of more contaminated food part, thereby increasing the risk of toxic effects in the indegenous population.

Aflatoxin Limits in Animal Feeds

Currently, some 35 countries regulate aflatoxins in animal feedstuffs (van Egmond 1989). The European Economic Community (EEC) has given directives on the maximum permissible levels of aflatoxin in groundnuts and groundnut cake imported into the EEC member countries. The first directive 74/63 became effective 11 February 1974 (EEC's official Journal no. L-38, page 31). The maximum permissible levels ranged from 20 μg kg^{-1} (in whole feedingstuffs for pigs and poultry except piglets and chicks) to 50 μg kg^{-1} (in straight feedingstuffs and whole feedingstuffs for cattle, sheep and goats except dairy cattle, calves, lambs). The EEC limits on aflatoxins in animal feeds were set after the monitoring program of milk in the U.K. showed that many milk samples contained aflatoxin M_1. Groundnuts and cottonseed (raw materials used in dairy feeds) were regarded as the sources of aflatoxin contamination (Jewers 1982, 1988). Consequently, in 1982, the U.K. Government introduced a ban on imports of groundnuts and cottonseed. However, this was soon changed to a ban on imports of groundnuts/meal when contaminated

with aflatoxin exceeding 50 μg kg^{-1} (Cappuccio 1989). The EEC regulations varied in the actual limits on aflatoxins in animal feeds. Some countries e.g., France allowed the importation of groundnut meal containing 700 μg kg^{-1} of aflatoxin B_1 on the condition that it went immediately to feed manufacturers who incorporate the contaminated feedstuff's into animal feeds at an amount that does not exceed the legal limit. The U.K. has a 50 μg kg^{-1} limit for imported groundnut cake.

The 1974 directive was tightened in 1984 when the tolerance for aflatoxin B_1 in complementary feedstuffs for dairy cattle was reduced from 20 to 10 μg kg^{-1} (Cappuccio 1989, U.K. Ministry of Agriculture, Fisheries and Food 1989). This was in accordance with the trend in Western European countries to establish tolerances for aflatoxin M_1 at a level of 0.05 μg L^{-1} milk. A recent development in EEC legislation is the introduction of a tolerance for aflatoxin B_1 in feedstuffs ingredients at 200 μg kg^{-1} (van Egmond 1989). Some EC countries and countries in other parts of the world have already specified tolerances for some feedstuff ingredients (Table 4). It is possible that EC regulations for aflatoxins in animal feedstuffs will be more stringent in the near future.

Concluding Remarks

Differences in maximum permissible levels of aflatoxin(s) in groundnuts and groundnut products vary widely among countries with regulations on aflatoxins. It is imperative to follow reasonably uniform regulations and limits on aflatoxins in groundnuts and groundnut products in order to prevent any unnecessary damage to the exporting countries/agencies and to freedom of international trade in oilseeds. Efforts for uniformity of aflatoxin regulations should be supported by knowledge about the rationale for the decisions that resulted in the enforcement of current regulations in various countries. International organizations including FAO, WHO, IUPAC. and EEC are currently attempting to develop uniform aflatoxin regulations for various foods and feeds. The permissible levels of aflatoxin(s) in foods and feeds must be based on the current knowledge on the toxicity of the toxins to humans and animals and on the methods available for their detection.

Table 4. Maximum permissible aflatoxin levels in foods and feeds in various countries'

Country	Commodity	Aflatoxin limit (μg kg^{-1})	Remarks
Australia	groundnuts and groundnut products	15	
Austria	all feeds	50	
Belgium	all foods	5[a]	
	all feeds	see EEC	EEC regulation
Brazil	groundnut meal	50[a]	Export control
Canada	nuts and nut products	15[b]	Control under hazard to health
Colombia	groundnuts	10	
Cuba	groundnuts	0	
Czechoslovakia	groundnuts	5	
Denmark	groundnuts and groundnut products	10	
	feeds	see EEC	EEC regulation
Dominican Republic	groundnuts	0	
EEC countries	straight feedingstuffs	50[a]	EEC includes
	complete feedingstuffs (not for dairy cattle, (calves, lambs)	50[a]	Belgium, Denmark, France. Germany,
	complete feedingstuffs (for pigs and poultry)	20[a]	Greece. Ireland, Italy.
	other complete feeding-stuffs	10[a]	Luxembourg, The Netherlands.
	complementary feeding-	20[a]	United Kingdom

Country	Commodity	Aflatoxin limit (μg kg^{-1})	Remarks
	stuffs (for dairy cattle)		no more than 2% in feed for dairy cows; no more than 4% in feed for other livestock.
Finland	nuts and nut products	5	
France	all foods	10	
	baby foods	5	
Germany	groundnuts and groundnut products	5* or 10[b]	
	all feeds	see EEC	EEC regulation
Greece	all feeds	see EEC	EEC regulation
Jordan	groundnuts, other foods/feeds	15[a] or 30[b]	
Hong kong	all foods	15	
	groundnuts and groundnut products	20	
India	groundnut meal (food)	60	
	groundnut meal (feed)	1000	
Ireland	all feeds	see EEC	EEC regulation
Israel	all feeds	20	Based on EEC recommendation
Italy	groundnuts and groundnut products	50	
	all feeds	see EEC	EEC regulation
Japan	groundnuts and groundnut products	10[a]	
	groundnut meal (feed imports)	1000[a]	not for use in feed for chickens, calves, or pigs;
Kenya	groundnuts and groundnut products	20	
Luxembourg	groundnuts and groundnut products	5[a]	
	all feeds	see EEC	EEC regulation
Malawi	groundnuts	5[a]	Export regulation
Malaysia	all foods	0	
The Netherlands	groundnuts and groundnut products	5[a]	
	feeds	see EEC	EEC regulation
New Zealand	all foods	15	
Nigeria	foods		Export regulation
Norway	groundnuts	5* or 20"	
	all feeds	50	
Philippines	groundnuts and groundnut products	20	Based on WHO recommendation
Poland	all foods	5[a]	
	all mixed feeds	0-200[a]	Dependent on animal, and regulated limit of groundnut meal in feed

Country	Commodity	Aflatoxin limit (μg kg^{-1})	Remarks
Portugal	groundnuts	0	
Republic of China	groundnuts	50[a]	
Singapore	groundnuts and groundnut products, edible oils	10-15	
South Africa	all foods	5[a] or 10[b]	
Surinam	groundnuts and groundnut products	5*	
Sweden	all foods, groundnuts	5[b]	
	groundnut meal	600[a]	Dairy feed limit 15%
Switzerland	nuts and nut products	1[a] or 5[b]	
Thailand	edible oils	20	
Union of Soviet Socialist Republics	all foods	5	
United Kingdom	nuts and nut products	5[a]	
	feeds	see EEC	EEC regulation
United States	all foods and feeds	20[b]	
	consumer groundnut products	15[b]	
	raw shelled groundnuts	25[b]	
	fluid milk	0.5[c]	
Yugoslavia	groundnuts	10	
	other nuts and nut products	5	
	other foods	1	
Zimbabwe	groundnuts	25[a]	

*Based on Stoloff (1977), Schuller et al. (1982), van Egmond (1989).
[a]Aflatoxin B_1 [b]Total Aflatoxins [c]Aflatoxin M_1

EEC = European Economic Community

6.2 BIBLIOIGRAPHY

6.2.1 LIMITS AND REGULATIONS

896. Anonymous. 1966. Alarm about aflatoxin. Nature 212: 1512.

Alarm was expressed at a recent meeting in Rome of the Food and Agriculture Organization of the United Nations about the amount of aflatoxin in groundnuts and other protein supplements. The maximum concentration of aflatoxin which is permitted was laid down in August 1965 by a joint advisory group from the Food and Agriculture Organization and the World Health Organization which took into account the urgent need to provide extra protein in some parts of the world, and established a level of 30 µg kg^{-1} of foodstuff. Clearly the group would have preferred a lower figure, but felt that the danger of malnutrition was greater than the danger that aflatoxin would produce liver cancer in man.

897. Anonymous. 1989. Aflatoxins. Food Australia 41(2): 598-599.

Aflatoxins. their occurrence, chemistry and toxicology, and regulatory standards for aflatoxin levels in Australian foods, particularly groundnuts, are discussed.

898. Banes, D. 1966. hood toxins of fungal origin : methodology and regulatory aspects. Food Technology 20(6): 51-52.

This paper very briefly reviews some analytical methods and bioassays for aflatoxiris in groundnuts and groundnut products. Regulatory aspects are also discussed, and the actions of the Food and Drug Administration (FDA) of the US are highlighted.

899. Cappuccio, M. 1989. Effects of new rules on EEC trade. Journal of the Association of Oil Chemists' Society 66(10): 1410-1413.

New European legislation on aflatoxin content of six raw materials for animal feeds will increase the cost of using those materials as quality control costs rise. The legislation is too recent to determine yet if usage of the regulated materials has been affected or if feed manufacturers arc looking for alternate nonregulated materials. There are data indicating the level of aflatoxin in imported raw materials is lower, perhaps indicating closer control at the point of origin. The trade is hoping an approved method can be developed to detoxify aflatoxin contaminated materials.

900. Coulibaly, B. 1989. The problem of aflatoxin contamination of groundnut and groundnut products as seen by the African Groundnut Council. Pages 47-55 in Aflatoxin contamination of groundnut : Proceedings of the International Workshop, 6-9 Oct 1987. ICRISAT Center, India. Patancheru. A.P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

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. Aflatoxin is a serious constraint to exports particularly in groundnut cake and meal destined for the traditional western European markets. The AGC launched an aflatoxin control program in 1975. Phases 1 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). Staff have been trained to survey and monitor aflatoxin in fields and laboratories, and laboratories for aflatoxin analysis have been established and equipped. Two pilot detoxification plants have been constructed to supplement cultural control measures. The AGC monitors contacts with RFC representatives and exporters of groundnut products on relevant legislation and standards and their application.

901. Dichter, C.R., and Weinstein, M.C. 1984. Cost-effectiveness of lowering the aflatoxin tolerance level. Food and Chemical Toxicology 22(6): 439-445.

The cost-effectiveness of adopting aflatoxin tolerance levels of 15, 10 and 5 µg kg^{-1} for groundnuts and groundnut products was assessed. Estimates of the annual cost to manufacturers of monitoring and controlling aflatoxin levels at the current 20 *µg* kg^{-1} action level, and estimates of the projected increase in costs of establishing lower tolerances were elicited from producers by a questionnaire. Exposures to groundnut products were derived from the HANES I survey and from groundnut production statistics. The risk of liver cancer at each tolerance level was estimated using both epidemiological and extrapolated experimental data assuming that exposure would be reduced in direct proportion to the decrease in the tolerance. It was found that the 15 µg kg^{-1} tolerance would cost $ 60 000 per cancer death averted (range $ 20000 - $ 700000) and is therefore relatively cost-effective. The marginal costs per life saved for both the 10 µg kg^{-1} and 5 µg kg^{-1} levels were found to be $ 1.7 million (range $ 0.6 million - $ 11.4 million) and $ 1.6 million (range $ 0.6 million - $ 31.1 million), respectively. Conclusions on the optimal regulatory approach should be guided by comparisons of these figures with corresponding cost-effectiveness ratios for alternative regulatory uses of national resources in the interests of public health.

902. Goto, T. 1990. Mycotoxins : current situation. Food Rev. Int. 6: 265-290.

This review dicusses the major groups of mycotoxins found in food and feeds, methods for their analysis and the regulatory action taken by some countries for maximum permissible levels of mycotoxins in food and feeds.

903. Harvey, G. 1980. Poison threat lurks in imported groundnut meal. Farmers Weekly 92(18): 54-55.

There is concern among farmers' leaders and consumers in the UK about high levels of anatoxin in some imported groundnut oilmeal. The 1976 Fertilizer and Feedingstuffs Regulations specify maximum aflatoxin concentration in groundnut oilmeal sold for animal feed as 50 μg kg^{-1}, and in 'whole feedingstuffs' for dairy cattle and calves as 10 μg kg^{-1}. However, groundnut oilmeal sold as a feedstuff has been found with aflatoxin levels as high as 900 μg kg^{-1}. Farmers are recommended to ascertain whether compound feeds contain groundnut, and to get a certificate showing aflatoxin content of groundnut for inclusion in home-mixed feed. There are no UK recommendations for maximum aflatoxin in milk, but Milk Marketing Board officials are satisfied that current levels arc safe. A feed with the statutory maximum anatoxin B_1 content could result in about 100 μg kg^{-1} of aflatoxin in milk, and an average concentration of 0.25 μg kg^{-1} in cheese or dried milk. A supplementary article reports the experience of one dairy herd where moldy compound feed with aflatoxin content of 600 μg kg^{-1} was associated with loss of condition and coordination, fall in milk yield and increased incidence of mastitis. Pound-sterling 6700 has been received by the farmers as compensation from the feed firm involved.

904. Hertrainpf, J. 1978. |Groundnut meal and its problems.]. Erdnussschrot und seine probleme. Mtlhle + Mischfuttertcchnik 115(3): 36.

Problems arising from the contamination of groundnut meal with aflatoxin are reviewed Progress in the detection of related compounds and knowledge of their toxic properties are summarized, with details of acute toxicity on monkeys, and indications of the chronic effects of very small doses on liver and kidneys of children. Transmission of the toxic constituents from animal feeds to cow's milk and pig liver and kidneys is also reported, although there are no apparent indications of progressive accumulation. Aflatoxin in imported groundnut meal is discussed and the limits imposed by the EEC on feeds of different kinds are tabulated. The practical implications of stringent limits on aflatoxin contents of meal imported into European and other countries are discussed, with particular reference to the danger that more heavily contaminated batches may be retained in the developing countries where the population relies heavily on groundnut meal as a source of protein. The possibility of detoxification with ammonia is briefly discussed, and it is noted that tests with rats indicate that the protein quality is thereby decreased by about 10%.

905. Intergovernmental Group on Oilseeds, Oils and Fats, FAO. 1985. Recent developments concerning aflatoxins. Intergovernmental Group on Oilseeds. Oils and Fats, FAO. Rome, Italy 19(2): 13 p.

Many agricultural commodities can be contaminated with aflatoxins. Increasing concern about the effects of atlatoxins on the health of humans and animals has resulted in worldwide regulatory measures limiting the level of aflatoxin contamination that is acceptable in foods and feeds. International agreement on maximum limits for aflatoxin contamination in internationally traded products, and on methods of sampling and analysis, would better protect the consumer and facilitate international trade. It would obviate arbitrary regulatory measures. Improved agricultural practices, quick postharvest drying, proper storage and transport should help to reduce the incidence of aflatoxin contamination, especially if this could be coupled with marketing practices that offer a premium for quality.

906. Jewers, K. 1982. Mycotoxins in food - the application of survey and quality control. Royal Society of Health Journal 102(3): 114-118.

Details are given of the methodology to be followed in conducting a survey of mycotoxin contamination of foods and feedstuff's, including sampling, analysis and treatment of data obtained. Results arc given of a survey of the aflatoxin content of a small proportion of groundnuts from the 1977/78 season imported into the U.K. Thirty-one of the 159 samples exceeded the maximum permitted level of aflatoxin in raw groundnuts (30 *μg* kg^{-1}). The highest level of aflatoxin contamination was in samples from the Gambia and India (> 400 *μg* kg^{-1} in 1 sample each). Results are also given of a survey of the aflatoxin M, content of samples of producer-retailer milk in the U.K., commissioned in 1977 by the Ministry of Agriculture, Fishery and Food. The survey showed that 11.9% of the milk samples tested contained > 0.1 fig L^{-1} aflatoxin M_1 (the limit permitted by European Community Regulations). A parallel study of random samples of dairy feed indicated that 8% contained > 30 μg kg^{-1} aflatoxin B_1. In another study the average level of aflatoxin in a 12 ton batch of groundnut cake obtained from a farmer in the U.K. was 1540 μg kg^{-1}, which would result in feedstuffs containing aflatoxin well in excess of the permitted level.

907. Jewers, K. 1988. [Mycotoxins and their effects on poultry production.]. Mikotoksini i njihovi efekti na zivinarsku proizvodnju. Peredarstvo 23(11-12): 69-73.

To prevent the losses resulting from the presence of mycotoxins in poultry feed the EC has introduced regulations limiting the level of aflatoxin in poultry feeds, and has set maximum levels for aflatoxin in six major raw materials used in animal feed production : babassu, copra, cottonseed, groundnut, maize, and palm kernels. However, the sampling, sample preparation and analytical methodologies required lor the monitoring of aflatoxin in these commodities are not available at present, and further work is required to ensure that the heterogenous distribution of aflatoxin in a commodity is taken into account when new quality control procedures are developed. At present no limits have been set for the other mycotoxins known to produce adverse effects in poultry. Care must be taken by animal feeds processors

and poultry producers to ensure mycotoxins do not enter the food chain, and this may necessitate them introducing quality control procedures even though a legal framework for such testing is not in place. Utilization of highly contaminated raw materials presents a major problem. Blending of highly contaminated and noncontaminated raw materials is not advisable, as most mixing techniques involving unground material are likely to lead to pockets of highly contaminated material which could have a disastrous effect on poultry production. An alternative strategy would be to decontaminate the highly contaminated raw material or the feed prior to the addition of vitamins and other additives. This approach is being investigated in a project in Pakistan.

908. Lenncrts, L. 1989. [Groundnut meal and groundnut cake/- expeller.|. Erdnusscxtraktionsschrot und erdnusskuchcn/-expeller. Muhle + Mischfuttertechnik 126(20): 311-312,315.

Nutrient, mineral, trace elements and vitamin contents in groundnuts are discussed. Particular emphasis is given to storage problems connected with fungal contamination by *Aspergillus flavus* which is widespread in consignments of groundnuts, necessitating strict legislation on aflatoxin B_1 levels, and limiting its use in concentrates for lactating cows, calves, piglets and young chickens. Shelled groundnuts with low aflatoxin levels are used at 10-20% in mixed cattle feeds, 3-5% in pig and poultry feeds, due to low lysine content. Partially shelled groundnuts can only be used in mixed cattle feeds up to 5 or 10%. Unshelled nuts should not be used in any quality mixed feeds.

909. Logten, MJ.Van 1977. IMethods for determining permissible levels of intentional and unintentional additives in foodstuffs.]. Wijze van vaststellen van de toelaatbare gehaltes aan gewenste of ongewenste toevoegingen in voedingsmiddelen. Tijdschrift voor Diergeneeskunde 102(3): 164-172.

Problems relating to the determination of acceptable levels of additives and contaminants in foodstuffs are discussed with examples, including the occurrence of aflatoxins in milk. Since the use of DDT in cattle farming has been prohibited in the Netherlands, the DDT content of milk has been greatly reduced, e.g., in the Alkmaar region only 2 out of a total of 1153 milk samples analysed during 1972-1975 contained > 1.25 ppm DDT/DDE in fat, vs. 29 out of 555 samples in 1970. As aflatoxin M, has been shown to be carcinogenic, efforts are being made to prevent its occurrence in milk by minimizing the amount of aflatoxins in mixed fodder. Dutch legislation specifies that aflatoxins must not be present in detectable amounts in groundnuts and their products.

910. Maselli, J.A. 1977. Controlling aflatoxin in your plant. Manufacturing Confectioner 57(2): 35-38,40-41.

The clinical effects of aflatoxin exposure are briefly described, and the current US FDA regulations and sampling procedure for control of aflatoxins in groundnuts are discussed. Some quality control procedures to ensure that only minimal quantities of toxin persist into the finished product are outlined.

911. Oser, B.L. 1969. Regulatory aspects of control of mycotoxins in foods and feeds. Pages 393-400 in Aflatoxin. Scientific background, control, and implications (Goldblatt, L.A., ed.). Academic Press : New York, USA.

The United States Federal laws and regulations concerned with mold/aflatoxin contamination of foods and feeds arc described. International regulations and tolerance levels for aflatoxins in groundnuts and groundnut products are briefly reviewed.

912. Schmidt, F.R., and Esser, K. 1985. Aflatoxins : medical, economic impact, and prospects for control. Process Biochemistry 20(6): 167-174.

Current knowledge about mode of action of aflatoxins, their medical and economic importance and prospects for their control are reviewed, considering the following aspects : structure, nomenclature and biosynthesis; mode of action; health risks and legislative regulation; postharvest control (segregation, decontamination, storage); and preharvest control (pesticides, and biological control by breeding or microbial interactions, particularly repression of aflatoxin synthesis by *Aspergillus flavus* following infection with a virus from *Penicillium chrysogenum).*

913. Schuller, P.L., Stoloff, L., and van Egmond, H.P. 1982. Limits and regulations. Pages 107-116 in Environmental Carcinogens - Selected Methods of Analysis Vol. 5 - Some Mycotoxins (Egan, H., Stoloff, L., Scott, P., Castegnaro, M.. O'Neill, I.K., and Bartsch, H., eds.). Lyon, International Agency for Research on Cancer (IARC). Scientific Publications No. 44, 459 pp.

Limits and regulations on aflatoxins in agricultural commodities including groundnuts and groundnut products in various countries are described. Problems in enacting regulations on aflatoxins in foods and feeds are highlighted.

914. Scussel, V.M., and Rodrigue/.-Amaya, D.B. 1985. [Aflatoxin levels in peanut and peanut products commercialized in Campinas in the period 1980-1982.]. Teores de aflatoxinas em amendoim e seus produtos comercializados em Campinas em 1980-1982. Boletim da Sociedade Brasileira de Ciencia e Tecnologia de Alimentos Campinas 19(2): 109-119.

Samples of groundnuts and groundnut products (raw shelled and unshelled groundnuts, fried salted groundnuts with or without skin, soygroundnut, sugar-coated

colored groundnuts, chocolate- coated groundnuts, peanut butter, ground groundnut bar and candied groundnut bar), purchased at random from different supermarkets and smaller stores during the period from the second semester of 1980 to the first sememster of 1982, were analyzed for aflatoxin using the Romer method. Of the 241 samples analyzed, 128 had aflatoxins, and 92 of these contained levels above the tolerance limit permitted by Brazilian legislation (30 µg kg^{-1} aflatoxins B_1+G_1). Ground groundnut bar, raw shelled groundnuts, soygroundnut and fried salted groundnuts with the skin had greater contamination levels with 61%, 55% and 42% respectively of their samples having aflatoxin levels above the limit. The highest levels of aflatoxins B_1 and G_1 detected were 1 282 and 476 µg kg^{-1} for the ground groundnut bar, 1 904 and 69 µg kg^{-1} for raw shelled groundnuts and 1 026 and 366 µg kg^{-1} for soygroundnut, respectively.

915. Seibold, R., and Ruch, W, 1977. [Aflatoxin content of mixed feed of dairy cows.J. Der aflatoxingehalt im milchviehmischfutter. Kraftfutter 60(5): 182,184-185.

Of 60 samples of groundnut-free feed compounds for dairy cows, only one contained more than 20 µg kg^{-1} aflatoxin, the maximum level permitted under new Federal German regulations. In feed compounds including groundnut products, however, average aflatoxin levels far exceeded the maximum permitted level. The new regulations are intended to prevent aflatoxin contamination of milk and thus possible hazards to human health.

916. Stoloff, L. 1977. Aflatoxins - An overview. Pages 7-28 in Mycotoxins in Human and Animal Health (Rodricks, J.V., Hesseltine, C.W., and Mehlman, M.A., eds.). Pathotox Publishers, Inc. : Illinois, USA.

In this review the occurrence of aflatoxins in food crops, toxicity of aflatoxins to animals, analytical methods, and regulations for aflatoxins in foods and feeds are briefly described.

917. Stoloff, L. 1986. A rationale for the control of aflatoxin in human foods. Pages 457-471 in Mycotoxins and Phycotoxins (Steyn, P.S., and Vleggar, R., eds.) : A Collection of Invited Papers Presented at the Sixth International IUPAC Symposium on Mycotoxins and Phycotoxins, 22-25 July 1985, Pretoria, Republic of South Africa. Elsevier Science Publishers B.V., Amsterdam.

The regulation of aflatoxin in foods is part of the risk management process that in its initial stages in the period 1964-1969 required considerable speculation to supplement the meager knowledge that formed the basis of the risk assessment. The regulatory stance of most governments is still based on that original speculation, even though a risk assessment using current knowledge does not support the assumptions that were used. The original assumption that aflatoxin might be a potent hepatocarcinogen for humans can not be supported by me considerable data on aflatoxin that has since evolved. But there is clear evidence mat humans can suffer acute liver damage from aflatoxin ingestion. By considering the regulation between the dose levels at which acute effects are manifest, and the frequency distribution of the contamination levels in the ingested foods, a maximum tolerated level can be established that best protects the consumers' health, pocketbook, and food choices.

918. U.K. Ministry of Agriculture, Fisheries and Food. 1989. European perspective on aflatoxin. Journal of the Association of Oil Chemists' Society 66(10): 1408-1409.

This article explains the background and previous regulatory steps that led to current legislation on aflatoxin. The European Economic Community's regulations on aflatoxins in groundnuts and groundnut meal and other feeds are described.

919. Van Egmond, H.P. 1989. Current situation on regulations for mycotoxins. Overview of tolerances and status of standard methods of sampling and analysis. Food Additives and Contaminants 6(2): 139-188.

A worldwide enquiry was undertaken in 1986-87 to obtain up-to- date information about mycotoxins legislation in as many countries of me world as possible. Together with some additional data collected in 1981, information is now available about planned, proposed, existing legislation, or the absence of legislation, in 66 countries. Details of tolerances, legal bases, responsible authorities, prescribed methods of sampling and analysis, and disposition of commodities containing inadmissible amounts of mycotoxins are given. The information concerns aflatoxins in foodstuffs, aflatoxin M, in dairy products, aflatoxins in animal feedstuffs, and other mycotoxins in food- and feedstuffs. In comparison with the situation in 1981, limits and regulations for mycotoxins have been expanded in 1987 with more countries having legislation (proposed or passed) on the subject, more products, and more mycotoxins covered by this legislation. The differences between tolerances in various countries are sometimes quite large, which make harmonization of mycotoxin regulations highly desirable.

920. World Health Organization. 1978. Health laws and regulations - Benelux Economic Union. International Digest of Health Legislation 29(1): 5.

The following regulation to food safety is presented : Decision M (77) 5 of 2 June 1977 of the Committee of Ministers of the Benelux Economic Union concerning the application of Benelux reference methods of analysis with respect to aflatoxin in groundnuts and products derived therefrom. The Government of each of me three Benelux countries is to submit a report to the Committee of Ministers on the measures taken for the enforcement of this decision.

921. World Health Organization. 1978. Health laws and regulations - Belgium. International Digest of Health Legislation 29(4): 721-726.

A selection of Belgian health laws and regulations is presented including the following which relate to food hygiene : Crown Order of 29 July 1977 amending Annexes 2 (Regenerated cellulose film) and 3 (Plastics materials and articles) of the Crown Order of 12 September 1972 concerning the tnanufacture and use of. and trade in, articles and materials intended to come into contact with foodstuffs and substances used as food; and Crown Order of 16 September 1977 establishing the reference method of analysis applicable to aflatoxin in groundnuts and products derived therefrom, is made in pursuance of the Law of 24 January 1977 on the health of consumers with regard to foodstuffs and other products and Decision M (77) 5 of 2 June 1977 of the Committee of Ministers of the Benelux Economic Union.

7. AFLATOXIN ANALYSIS IN GROUNDNUTS AND GROUNDNUT PRODUCTS

7.1 REVIEW

7.1.1 Sampling and Sample Preparation Procedures

Sampling is a very important step in the analysis for aflatoxin in groundnuts, and contributes significantly to its variability. This fact stems from the highly skewed nature of the distribution of aflatoxin in groundnuts (Cucullu et al. 1966, Whitaker and Wiser 1969, Coker 1984). A major problem in sampling is that the toxicity often resides in only a few contaminated kernels, and there is extreme variation in the level of aflatoxin among contaminated kernels (Whitaker et al. 1972, Coker 1989). The problem of sampling shelled groundnuts for aflatoxin has been extensively investigated in the USA and the U.K. (Whitaker and Wiser 1969, Whitaker et al. 1972, Whitaker et al. 1974, Coker 1989). These studies have shown that the highly localized aflatoxin contamination of groundnuts can be represented by the negative binomial distribution. The negative binomial probability function describes a situation with high probabilities of zero level counts and low probabilities of very high level counts. The negative binomial distribution pattern can be obtained if individual kernels in a given lot are analyzed for aflatoxin. A similar pattern can be obtained if large numbers of random sub-samples are analyzed. Thus, sampling accuracy can be maximized by taking large representative samples composed of numerous sub-samples (Whitakcr et al. 1972, Coker 1984).

Several statistical models have been suggested for describing the distribution of aflatoxin in groundnuts. Whitaker et al. (1974) used the negative binomial distribution to develop a method to evaluate sampling plans used to estimate the levels of aflatoxin in shelled groundnuts. Waibel (1977) suggested an alternative statistical model, the compound Poission-gamma distribution. This model and the negative binomial distribution have been shown to differ markedly from each other for large samples as a consequence of the statistical law known as the Central Limit Theorem (Knutti and Schlatter 1982). Brown (1984) examined the suitability of the negative binomial distribution in describing aflatoxin levels in 15 batches of Virginia Bunch and seven batches of Spanish groundnuts. He considered that an appealing aspect of this model is the property that enables distribution results to be inferred for different sample sizes. However, the substantial variability due to sub-sampling and actual analysis was thought to undermine the value of this property. In addition, the negative binomial model did not treat the large number of zero counts in a satisfactory manner. Consequently, Brown employed an alternative model which assumed a normal (Gaussian) distribution for the log of the non-zero aflatoxin levels. Jewers et al. (1986) and Jewers (1987) consider the Weibull distribution to be a suitable model for describing the distribution of aflatoxin in groundnuts.

Sampling Plans

Considerable efforts have been expended on the development of sampling plans for groundnut quality control programs in the USA and U.K. Whitaker et al. (1974) summarized the procedure for developing sampling plans, and evaluated four sampling plans used in the USA during the period 1969-1972 to control aflatoxin content in shelled groundnuts. Two statistically sound sampling plans have been developed based on the negative binomial distribution of aflatoxin in groundnuts and the aflatoxin tolerance limits established (Whitaker and Dickens 1979, Coker 1984). These are the USDA sampling plan (Whitaker and Dickens 1979) currently in use in the USA, and the so called TPI Plan (Coker 1984) used in the U.K. These plans are used to monitor aflatoxin levels in raw groundnut kernels. Both plans require the collection of a representative large sample, composed of at least 100 incremental samples, which is then divided into three portions of equal weight. The main difference between the plans is the weight of the original sample; the TPI plan requires collection of a 10.5 kg sample, whereas the USDA plan utilizes a much larger sample of 66 kg. The objective of the USDA plan is to accept all batches of groundnuts that contain less than 25 $\mu g\ kg^{-1}$ of aflatoxin, and reject all batches in which this level is exceeded. The TPI plan is designed to detect those batches of groundnuts that contain more than 30 $\mu g\ kg^{-1}$. this being the maximum level of aflatoxin permitted in edible groundnuts imported into the U.K.

Development and application of sampling plans for quality control purposes require guidelines by Government authorities or Codex authorities in any country, or an agreement between countries/agencies regarding the maximum level of aflatoxin permitted, definitions of good (acceptable) and of bad (rejectable) lots, and a statement as to acceptable consumer and producer risks (Schuller et al. 1976). In the absence of this information, selection of any sampling plan will be arbitrary. Several countries, e.g., Canada and Australia, have adopted the modified USDA sampling plans for quality control purposes. Other sampling plans include those suggested by Brown (1984) for groundnuts, and Waltking (1980) for peanut butter.

The quality control of in-shell groundnuts is especially difficult. Samples cannot be collected from sacks by simple spear sampling, and the collected samples must either be decorticated prior to analysis, or the analysis result adjusted for the mass of the shells (Coker 1989). Limited work has been done on the distribution of aflatoxin in in-shell groundnuts, groundnut cake, groundnut meal, and peanut butter (Coker 1989, Waltking 1980). Sampling protocols based on statistical grounds need to be devised for these products.

Assessment of the efficiency of a sampling plan is best achieved through determining the operating characteristics of the plan. This requires a knowledge of the consumer and producer risks associated with the plan. The consumer risk is an assessment of the likelihood of accepting a batch of groundnuts whose aflatoxin content exceeds the maximum level imposed for the toxin, and the producer risk is an assessment of the likelihood of rejecting a batch which contains less toxin than the statutory maximum level. Coker (1984) and Jewers (1987) have discussed in detail these risks associated with the USDA and TP1 sampling plans. An ideal operating characteristic would be one which accepts all batches containing less than the statutory maximum level of the toxin, and rejects all the batches that equal or exceed this level, i.e., the consumer and producer risks would be zero. However, this is unlikely to happen in reality. Attempts to reduce the consumer risk by adopting a more rigorous and expensive sampling can only be achieved by increasing the producer risk (Jewers 1987).

Some importing countries use aflatoxin testing plans with accept levels lower than those of most groundnut exporting countries. For example, the U.S. aflatoxin testing plan for shelled groundnuts (kernels) has been designed with a final accept level of 25 μg kg^{-1} total aflatoxin while the accept level of a testing plan used in The Netherlands (a groundnut-importing country) is 5 μg kg^{-1} aflatoxin B_1 or 10 *μg* kg^{-1} total aflatoxin (Whitaker and Dickens 1989). In such cases, some lots accepted by the US testing plan are likely to be rejected by the testing plan of an importing country allowing a lower accept level than 25 *μg* kg^{-1}. The Netherlands testing plan uses four 2-kg samples and only 50-g sub-sample per sample. Whitaker and Dickens (1989) developed computer models to determine the effects of decreasing the final accept level of the U.S. testing plan on the number of lots accepted and rejected in the United States and me number of exported lots accepted and rejected by The Netherlands testing plan. Decreasing the accept level of the U.S. testing plan from 25 to 5 μg kg^{-1} increased the number of lots rejected in the U.S. by 371 % while reducing the number of export lots rejected by 51 % (Whitaker and Dickens 1989). These results indicate the high cost of applying more stringent testing plans to improve the acceptance of exported groundnuts. We have used the example of the USA as groundnut exporting country, the situation would be even more of a problem for most developing countries that export groundnuts.

Sampling plans developed for quality control/regulatory purposes have little relationship to sampling plans for surveillance purposes, for example, for surveys on farms for determining levels of aflatoxin in groundnuts prior to harvest, and/or during postharvest drying in the field. Insufficient attention has been paid to developing statistically based sampling plans for these objectives. For survey purposes, 1-5 kg samples are usually taken (Schuller et al. 1976).

Sample Preparation

Proper grinding and subdivision of the sample is obviously essential before actual aflatoxin analysis can be initiated. Ideally, a subsampling mill should simultaneously grind and subdivide the sample, and that developed by Dickens and Satterwhite (1969) is suitable for mis purpose. Alternatively, me sample may be ground and subdivided in separate operations. Coker (1984) has described several subsampling devices. Rotary sample dividers such as "spinning riffles" and the "cascade sampler" are capable of producing several representative sub-samples which can be useful in aflatoxin analysis (Coker 1984). The size of the sub-sample may vary, depending upon the method of analysis, but for most cases it ranges from 20 to 100 g. A sub-sample size of 50 g is used in most methods and appears to be the best to obtain both solvent economy and a representative sample. The "slurry technique" proposed by Velasco and Morris (1976) is a useful means of reducing sample size.

An experimental study of the relationships of sampling, subsampling, and analytical variances as a function of the size of sample and sub-sample and number of analyses has been described by Whitaker et al. (1974). For the test procedure used in this study, sampling constituted by far me largest single source of error, followed by subsampling, and then by analysis. Tiemstra (1969) also concluded that sampling was the critical variable in determining the aflatoxin content of a lot. The proportion of error due to sampling becomes greater at lower aflatoxin levels. The model of Whitaker and Wiser (1969) appears to be a valuable tool for selecting and comparing sampling plans.

Methods for Analysis of Anatoxins

Three types of assay have been developed for me detection of aflatoxins - biological, chemical and immunochemical. Early methods (biological assays) for detection of aflatoxins relied upon the reaction of the test animals to ingestion of contaminated products. Biological assays are qualitative or at best semiquantitative, and are often non-specific. They are too time consuming for routine analysis. However, biological assays such as the duckling test arc useful confirmatory tests.

Following isolation, purification and characterization of the aflatoxins, physicochemical assay methods were soon developed. Chemical and immunochemical assays are suitable for routine analyses, e.g.. quality control and survey programs. They are invariably quicker, cheaper, more specific, and more reproducible, than biological assays, and often more sensitive.

Various quantification techniques were developed for the estimation of aflatoxins

including thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), high- performance liquid chromatography (HPLC), and fluorometric methods. Some of the TLC methods developed in the 1960s are still widely used. In the 1970s, various applications of HPLC were developed, and as they were more sensitive than the TLC methods, they became popular for analysis when a high degree of accuracy was required. Rapidity of analysis is sometimes more important than absolute accuracy, and the minicolumn methods were evolved with this in mind. In the late 1970s and 1980s, enzyme- linked immunosorbent assays (ELISA) were developed for aflatoxin analysis and are now replacing the minicolumn methods. From the wide range of aflatoxin analysis methods now available, it should be possible to choose method(s) suitable for specific purposes. In this section we critically review various chemical and immunochemical methods for analysis of aflatoxins in groundnuts and groundnut products. Biological assays are discussed in a separate section.

7.1.2 Analytical Methods

A large number of physicochemical methods have been developed for the determination of aflatoxins in agricultural commodities including groundnuts and groundnut products (Stoloff 1972, Schuller et al. 1976. Coker 1984). Many of them are minor modifications of basic methods, adapted to specific commodities or problems, differing in respect of solvents used to extract the anatoxins, and in techniques for estimating the intensity of fluorescence. However, all analytical methods for aflatoxins basically involve the same steps - sampling, extraction, cleanup, separation, and quantitation. Observations by Sargeant et al. (1963) that, (i) aflatoxins are much more soluble in chloroform than in hydrophilic solvents, and (ii) the isolated toxins fluoresce brightly when exposed to longwave ultraviolet light have provided the basis for most of the extraction/separation and quantification procedures developed for aflatoxins. Early methods of extracting aflatoxins from groundnut products used exhaustive techniques in which the samples were extracted for several hours in a Soxhlet extractor using methanol as the solvent. Defatung and lipid removal were considered necessary before extraction. Problems in these systems were loss of the toxins during prolonged extraction, and occurrence of polar lipids, pigments, and other impurities. The remedy lay in the use of rapidly penetrating solvents, coupled with high shear-rate mixers which would reduce substrate particle size and move the solvent rapidly across each particle. The solvents used were methanol: water (Nesheim et al. 1964), then acetone: water (Pons et al. 1966) and later acetonitrile : water (Yin 1969). The ratio of organic solvent to water was adjusted to achieve the best balance between solvent penetration, extraction of interfering materials and subsequent physical separation from the substrate. In a later development water was used as the extracting solvent (Lee 1965), extraction and liquid/liquid partitioning being performed simultaneously. The trick was to remove the aflatoxins from the water quickly by partitioning to an immiscible solvent, chloroform, in which aflatoxins are more soluble. In a similar development, methanol : water/hexane was used to extract and de-fat in one step (Nesheim et al. 1964). Both the chloroform : water and the methanol: water/hexane systems contain a good fat solvent, making them particularly effective with high-fat substrates, e.g., groundnuts. In almost all the proposed methods, one of the following solvent systems is now used for extracting aflatoxins; methanol : water (55:45, v/v), acetone : water (85:15, v/v). acetonitrile : water (9:1. v/v), chloroform . water (250:25, v/v). These methods were based on the recognition that aqueous solvents were needed to penetrate hydrophilic tissues in order to effectively extract the toxins.

Purification and clean-up of aflatoxin extracts can be accomplished by liquid-liquid partitioning, followed by partitioning of impurities and their removal using column chromatography. Partitioning between solvents can occur during extraction as is the case with the solvent mixture of chloroform and water. When other aqueous solvents such as methanol-water and acetone-water are used, the toxins are partitioned into the chloroform layer after extraction. In these situations some prior concentration of the aqueous phase may be needed. Also, a prior cleanup step involving precipitation of interfering materials using lead acetate may be necessary. Lead acetate precipitation removes plant pigments, lipids, fatty acids and other unknown materials which may cause streaking on TLC plates (Pons et al. 1966). Column chromatography may also be used to effect partitioning of aflatoxins from one solvent to another and thereby purify the extract. Columns packed with silica gel, cellulose, acidic alumina, or Florisil may be used to clean and purify an extract. The sample extract is usually added to the column in chloroform or another appropriate solvent, and then washed with one or more solvents in which the toxins are insoluble or less soluble than the impurities.

After removal of impurities the toxins are eluted from the column using a solvent in which the toxin is soluble. The toxin solution can then be collected, concentrated, and examined for quantity of toxin present.

7.1.3 Thin-Layer Chromatography (TLC) Methods

The aflatoxins were first isolated as fluorescent spots seen under longwave ultraviolet light on paper chromatograms (Coomes and Sanders 1963). At this time TLC was emerging as an effective tool and most of the subsequent methods for aflatoxin analysis depended upon this system. TLC separation of aflatoxins has provided the basis for extremely sensitive analytical methods. The use of silica gel (Kieselgel G) coated TLC plates for the resolution of the four aflatoxins B_1, B_2, G_1, and G_2 was introduced by de longh et al. (1964) who used chloroform: methanol (98:2, v/v) for development. Subsequently, various procedures incorporated the use of TLC plates coated with Kieselgel G and several chloroform: methanol

development solvents (Pons and Goldblatt 1969). Improvement in the resolution of individual aflatoxins on silica gel coaled plates was achieved by using chloroform: acetone mixtures for TLC development (Pons et al. 1966). It should be emphasized that R, values and the degree of aflatoxin resolution are markedly influenced by variations in gel preparations, relative humidity, and other variables. Hence the need to use authentic aflatoxin standards chromatographed under the same conditions as the unknown.

The ability to segregate the aflatoxins from other interfering compounds, on the TLC plate, imparts a reasonable level of selectivity and sensitivity to TLC quantification methods. Two-dimensional TLC provides an especially powerful clean-up, but it is very time-consuming as only one sample can be accommodated on a single plate. The use of alumina-backed TLC plates has facilitated the simultaneous 'two-dimensional' clean- up of multiple samples (Coker 1984). Some confirmatory tests also involve the use of TLC. The major disadvantages of TLC are the slow speed and the lack of precision (variation between plates, expressed as coefficient of variation). Investigations on the precision and accuracy of fluorodensitometric estimations of aflatoxins suggest that the technique is a precise and accurate analytical tool, and superior to visual estimation (Pons et al. 1966, Coker 1984). However, fluorodensitometry, while a good research tool, is expensive, time-consuming, and not always practical for routine sample analysis. Several publications describe different types of fluorodensitometers (Pons et al. 1966. Beljaars and Fabry 1972, Pons 1971, Schuller et al. 1976). The application of TLC methods to the quantification of aflatoxins has been extensively reviewed by Schuller et al. (1976) and Coker (1984).

Several collaborative studies have been organized by the AOAC and AOCS to monitor the efficiency of TLC-based procedures developed for aflatoxin analysis in groundnuts and groundnut products (Eppley et al. 1968, Waltking 1970, Stack 1974, Jemmali 1973). The AOAC (1984) recommends the Contamination Branch (CB) and the Best Foods (BF) methods for aflatoxin analysis in groundnuts and groundnut products. The CB method is the standard by which other methods are judged. It is an excellent TLC method, but it has two major disadvantages: (i) it is expensive since it uses large amounts of expensive solvents, and these also create a disposal problem, and (ii) the major solvent used is chloroform and this can be a hazard to workers. The CB method simultaneously extracts the sample with water and chloroform, transfers the lipids and aflatoxins to a silica gel column, selectively elutes the fat with hexane and the pigments and other impurities with absolute ether, and finally elutes aflatoxins from the column with 3% methanol in chloroform. The BF method is faster and more economical of solvents but provides a poorer cleanup. It simultaneously extracts and defats the sample with a 2-phase system, aqueous methanol-hexane, and then partitions the aflatoxins from the aqueous phase into chloroform. After concentration of the aflatoxins in the chloroform by evaporation, both methods separate the aflatoxins by TLC and quantitate them by fluorometry on a TLC plate by visual or densitometry comparison with aflatoxin standards.

Several check sample programs have been organized to monitor the efficiency of TLC-based procedures (CB. BF, Pons and EEC) used for aflatoxin analysis in raw groundnut meal, de-oiled groundnut meal, and peanut butter (Coon et al. 1972, 1973, Friesen et al. 1980, Friesen and Garren 1982). High CVs obtained reflected lack of precision in most cases. It must be emphasized that since these studies did not specify the method of analysis to be used, they are not comparable with collaborative studies of the methods. They reflect the efficiency of the numerous participating laboratories (of widely different levels of expertise) rather than the efficiency of the methods. These programs provide laboratories around the world with a basis for judging their analytical competence and need for quality control.

The International Mycotoxin Check Sample Program, organized by the International Agency for Research on Cancer, has provided interesting statistical comparisons of the CB, BF, and EEC methods used for the analysis of naturally contaminated samples of raw groundnut meal and de-oiled groundnut meal (Friesen and Garren 1982). Over 30 laboratories participated in the program using the CB and BF methods while 10 laboratories used the EEC method. The CV varied with the method, the sample and the aflatoxin concentration. No significant differences were found among means for laboratories using these three methods for the analysis of raw groundnut meal (containing approximately 250 μg kg^{-1} aflatoxin) for both aflatoxins B_1 and B_2. The laboratories which used the CB method found consistently higher levels of aflatoxins B_1 and B_2 than did laboratories using the BF and EEC methods. The BF method gave higher CV values (53 and 66% for B_1 and B_2, respectively) compared to the CB (48 and 49%) and EEC (37 and 39%) methods. For the assay of deoiled groundnut meal (containing approximately 70 µg kg^{-1} total aflatoxin), the laboratories which used the BF method consistently reported significantly lower levels of aflatoxins B_1, B_2, G_1, and G_2 (approximately half of the levels) than did laboratories using the CB and EEC methods. The BF method was associated with CVs of 98, 52, 108 and 136% for B_1, B_2, G_1, and G_2. respectively. The CB method gave CVs of 60, 51, 74 and 79% for B_1, B_2, G_1, and G_2, respectively, while the EEC method afforded CVs of 38, 33, 76 and 50% for B_1, B_2, G_1, and G_2, respectively.

The errors associated with the assay of aflatoxin in raw groundnuts by the BF method have been discussed by Whitaker and Dickens (1981). The TLC quantification step has been reported to be the major source of error (Coker 1984). Interestingly, the Smalley Check Sample Program includes the analysis of standard solutions of the aflatoxins thus facilitating the assessment of the precision of the quantification step alone. The apparent lack of precision (reported CVs of 40-81%) demonstrates the important contribution made by the quantification step to the overall efficiency of the assay. More collaborative studies involving only the

quantification step need to be organized. This approach will enable the efficiency of the various quantification methods to be compared and will facilitate the identification of the most efficient procedure(s) (Coker 1984).

The determination of aflatoxins in vegetable oils, including groundnut oil, is usually based on partition between two immiscible solvents. Aflatoxin is extracted from the oil into a polar solvent and subsequently partitioned into chloroform (Parker and Melnick 1966, Robertson et al. 1965). Although the recoveries are acceptable, these methods are time consuming, require large quantities of solvents, and are subject to occurrence of troublesome emulsions. Miller et al. (1985) developed a simple method for determination of alfatoxins in vegetable oils which is suitable for TLC and LC quantitation. The oil sample, dissolved in hexane, is applied to a silica column and washed with ether, toluene, and chloroform; aflatoxins are then eluted from the column with chloroform: methanol (97:3, v/v). Aflatoxins in chloroform are quantitated using the two-dimensional TLC.

Several multi-mycotoxin screening methods have been developed that are capable of detecting two or more toxins in agricultural commodities including groundnuts and animal feedingstuffs (Vorster 1969, Eppley 1968, Wilson et al. 1976, Roberts and Patterson 1975, Roberts et al. 1981, Howell and Taylor 1981). Although these methods follow the basic pattern described above they vary considerably in solvents or mixtures of solvents used, in clean-up steps, and in the number and nature of toxins that can be detected. TLC has been the most widely used method for the determination of mycotoxins in the multi-mycoioxin screening methods.

Confirmatory Tests

Despite the use of clean-up techniques, some compounds which behave like and so may be confused with aflatoxins may still be present on TLC plates. In order to eliminate false-positives, the identity of the mycotoxin in positive samples has to be confirmed.

Confirmatory tests can be carried out directly on a TLC plate. They are generally based on the formation of a derivative which has different properties, e.g., color of fluorescence and polarity from the presumptive mycotoxin. Both mycotoxin standard and suspected sample are subjected to the same derivatization reaction. Consequently, in positive samples a derivative from the mycotoxin should appear, identical to the derivative from the mycotoxin standard. The procedures for the confirmation of aflatoxin B_1 were originally developed by Przybylski (1975) and Verhulsdonk (1977), and they have been adopted as official methods by AOAC. In both methods, aflatoxin B_1 is derivatized under acid conditions on the TLC plate into its hemiacetal aflatoxin B_2a, which has a blue fluorescence at a lower Rf than B_1. In the method of Przybylski this is achieved by superimposing trifluoroacetic-acid (TFA) directly onto the extract spot before developement. After reaction, the plate is developed and examined under UV light for the presence of the blue fluorescent spot of B_2a, which can be recognized with the help of the B, standard, spotted on the same plate, which underwent the same procedure. As an additional confirmation, sulphuric acid (50%) is sprayed on another part of the plate where unreacted aliquots of extract and B, standard were developed. The sulphuric acid spray changes the fluorescence of aflatoxin from blue to yellow. This test only confirms the absence of aflatoxin, i.e., spots which do not turn yellow are definitely not aflatoxin, whereas many materials other than aflatoxin may give a yellow spot with sulphuric acid.

In the case of very "dirty" sample extracts it may be difficult to observe the hcmiacetal of B_1 (B_2a) due to heavy background fluorescence, and the two-dimensional method of Verhulsdonk in which the so-called separation-reaction-separation technique is carried out should then be the method of choice. Separation is first made in one direction and the plate sprayed with hydrochloric acid. After reaction, separation is carried out in the second direction, under exactly identical conditions. The reaction of hydrochloric acid with aflatoxin B_1 leads to the formation of a hcmiacetal B_2a, which has a specific Rf value, lower than that of B_1. This is recognized after subsequent chromatography in the second direction. Both methods are effective for confirmation of the identity of aflatoxin G_1.

7.1.4 High Performance Thin-Layer Chromatography (HPTLC) Methods

The application of HPTLC to the quantification of aflatoxins has been reviewed by Coker (1984). The lack of precision associated with TLC procedures arises from the introduction of possible errors during the sample application, plate development and plate interpretation steps. HPTLC procedures improve the precision by automating the sample application and plate interpretation steps, by improving the uniformity of the adsorbent layer on the TLC plate and by developing the plate under carefully controlled conditions. A fully automated sample applicator is commercially available. It is important that the spots are accurately positioned if an automated densitometer/scanner is used for quantification of aflatoxins. This technique can be used successfully in conjunction with a computing integrator to complete the automation of the plate interpretation step. Concentrations of aflatoxin as low as 5 pg (B_1, G_1 and M_1) can be delected using HPTLC procedures. Researchers at the Overseas Development Natural Resources Institute (ODNRI) have been able to detect 30 pg of aflatoxin B_1 in groundnuts using HPTLC procedures (Coker 1984); the reproducibility of the quantification was represented by the CV of 3% at an aflatoxin level of 5 μg kg^{-1}. Kamimura et al. (1985) described a simple HPTLC method which compared favorably with the CB method.

The advent of HPTLC procedures may result in renewed interest in TLC as an efficient quantification technique for aflatoxins, ideally suited to the analyses of large numbers of samples.

7.1.5 High Performance Liquid Chromatography (HPLC) Methods

The development of highly automated HPLC systems has afforded very precise, selective and sensitive quantification techniques for aflatoxin analysis. HPLC methods have been developed using both normal and reverse phase systems in conjunction with UV absorption and fluorescence detection techniques. Most normal phase separations have been performed on 5-10 µm silica gel columns using several multicomponent elution solvents. Some researchers e.g.. Hurst and Toomey (1978), have experienced difficulties in reproducing the separation of the aflatoxins. It is difficult to maintain the water saturation of some organic solvents such as chloroform and methylene chloride, and the silica gel column may also absorb excessive water over a period of time. Consequently, several reverse phase methods have been developed where the composition of the elution solvent is more easily reproduced. Most reverse phase separations have been performed on 5 and 10 urn octadecyl (C_{18}) columns using water, methanol and acetonitrile as elution solvents; the normal elution order is G_2, G_1, B_2, and B_1.

Reverse-phase HPLC separations of aflatoxins are more widely used than normal-phase separations. However, the fluorescence intensities of aflatoxins B_1 and G_1 are diminished in reverse- phase solvent mixtures so the derivatives of B_1 and G_1 are generally prepared before injection. The derivatives of B_1 and G_1 are not stable in methanol, which should be used with caution, especially in injection solvent. Acetonitrile-water mixtures do not degrade B_1 and G_1 rapidly and are preferred to the methanol: water mobile phases. The methods developed by DeVries and Chang (1982) and Tarter et al. (1984) use trifluoroacetic acid (TFA) derivatization and compare favorably with other methods. The hemiacetal derivatives of B_1 and G_1 are highly fluorescent and may be produced by pre-column treatment of the parent aflatoxins with TFA. Reverse phase HPLC with derivative formation facilitates the detection down to 5 pg of aflatoxins B_1, B_2, G_1 and G_2. Davis and Diener (1980) developed a reverse-phase method with fluorescence detection of an iodine derivaiive of aflatoxin B_1, and this led to the development of post-column derivatization methods which utilize the interactions between the aflatoxins and iodine. Thiel et al. (1986) have used post-column iodination to determine aflatoxins in peanut butter in conjunction with reverse-phase HPLC. A 3 *µm* C_{18} column was used with 0.01 M potassium dihydrogen phosphate:acetonitrile:methanol (39:9:7, v/v/v) and a 10670 x 0.34 µm reaction coil. The optimum mobile phase and reagent flow rates were 0.6 and 0.3 mL min^{-1} respectively. Shepherd and Gilbert (1984) have investigated the conditions needed for the postcolumn iodination reaction to enhance fluorescence of aflatoxins B, and G_1. They have successfully applied this technique to a survey of aflatoxin in edible nuts, including groundnuts. This method has considerable potential (Coker and Jones 1988).

The sensitivity of fluorescence detection methods can be further enhanced by packing the detector flow cell with silica gel. using normal phase solvents. Panalaks and Scott (1977) developed a silica-gel-packcd flow cell for fluorometric detection of aflatoxins in peanut butter. Sub-nanogram quantities of the aflatoxins could be delected using samples of spiked peanut butter. Other workers have also applied this technique to the assay of peanut butter by normal phase HPLC (Francis et al. 1982, Tarter et al. 1984). The major disadvantage of the packed cell appears to be lack of stability. The cell needs to be repacked often as the detection signal weakens with time. The recent emphasis on the reverse phase separations of the aflatoxins, coupled with the efficiency and convenience of post-column derivatization with iodine, reflects diminished interest in the silica-gel-packed flow-cell technique (Coker and Jones 1988).

Gilbert and Shepherd (1985) have used both normal and reverse phase HPLC in a survey of aflatoxin in groundnuts and their products. Sample extraction and clean-up was performed using a modified version of the AOAC CB method which involved extraction with chloroform-water, separation of the crude extract by centrifugation, and clean-up on a silica gel column. The final peanut butter extracts were dissolved in benzene- acetonitrile (98:2, V/V), while the groundnut and the groundnut- confectionery extracts were dissolved in water-acetonitrile (1:1, V/V). Normal phase HPLC was used for the assay of peanut butter using a 5 urn silica gel column and a non-quenching mobile phase of benzene-acetonitrile-formic acid, 90% (83:12:15, V/V/V). The excitation and emission wavelengths of the fluorescence detector were 360 and 425 nm respectively. Detection limits of 2 µg kg^{-1} were reported for the individual aflatoxins. Edible nuts and nut-confectionery products were assayed by the reverse phase HPLC, using post-column iodination. A detection limit of 0.5 µg kg^{-1} for each aflatoxin was reported.

Tarter et al. (1984) found that the application of a modified CB clean-up method to peanut butter resulted in oily extracts. Hence, they extracted their samples with aqueous methanol and sequentially partitioned the crude extract against hexane and dichloromethane. The final organic layer was dried with anhydrous sodium sulphate and subjected to a silica gel column clean-up. The resultant extract was treated with TFA to convert the aflatoxins B_1 and G_1 to their hemiacetal derivatives. Reverse phase HPLC was employed using a 10 µm C_{18} column with water-acetonitrile-methanol (70:17:17, V/V/V) as the mobile phase in conjunction with fluorescence detection. A detection limit of 0.3 µg kg^{-1} was reported for each

of the aflatoxins. Hurst et al. (1984) have used reverse phase HPLC in combination with TFA derealization to determine the aflatoxins in raw groundnuts. The samples were extracted with aqueous acetone in the presence of cupric carbonate. The resultant filtrate was defatted by partitioning against hexane, and the aflatoxins were then extracted into dichloromethane. The clean-up step was completed using a silica gel cartridge.

No collaborative studies of these HPLC methods have been reported. A few check sample programs have involved the use of HPLC methods for the estimation of aflatoxins in peanut butter and de-oiled groundnut meal (Friesen et al. 1980, Friesen and Garren 1982). High CVs (49-66%) obtained reflected poor interlaboratory precision of the HPLC determination of aflatoxins.

These HPLC methods involve the use of complex extraction and clean-up steps. It is emphasized that the development of assay methods which exhibit a high interlaboratory precision will only occur when the extraction and clean-up procedures have been considerably simplified. Two recent developments - (i) the development of bonded-phase clean-up cartridges, and (ii) the development of automated liquid-handling equipment should assist in attaining these objectives (Coker and Jones 1988). A comprehensive review of HPLC-based methods for the determination of mycotoxins has been given by Coker and Jones (1988).

7.1.6 Minicolumn Methods

For convenience in large scale screening for the detection of aflatoxins, minicolumn methods have considerable advantages. Compared with TLC methods the minicolumn methods are rapid, less expensive, and simpler to use. They are qualitative in their capacity to detect aflatoxins, but cannot accurately distinguish and quantitate the individual aflatoxins (B_1, B_2, G_1, and G_2).

The first rapid screening procedure using a minicolumn for aflatoxin detection in groundnuts was reported in 1968 (Holaday 1968); since then, improved procedures have been widely used to detect aflatoxin in groundnuts (Davis et al. 1980. Holaday 1976. Holaday and Barnes 1973. Holaday and Lansden 1975, Romer 1975). Minicolumns are typically 20 cm long glass tubes with an internal diameter of approximately 6 mm. They may be packed with either a single adsorbent or a combination of several adsorbents. The toxin, after adsorption onto the minicolumn, is detected by observing its natural fluorescence under UV light. The state of the art for minicolumn chromatography has been comprehensively reviewed by Holaday (1981).

The methods developed by Davis et al. (1980). Holaday (1976). and Holaday and Lansden (1975) are rapid screening procedures for detecting aflatoxin in groundnuts. Only a few collaborative studies have been performed to evaluate the efficiency of these methods in detecting aflatoxins in groundnuts (Shotwell and Holaday 1981). The Holaday-Velasco method has been adopted official first action for groundnuts (Official Methods of Analysis, 1980). This method combines the speed and simplicity of the Holaday extraction and clean-up with the sensitivity of the minicolumn originally described by Velasco. The detection limits by these methods range from 5 to 10 μg kg^{-1} total aflatoxins.

The minicolumn method of Romer (1975) has also been adopted by the AOAC for aflatoxin in groundnuts and groundnut products, cottonseed products, corn, edible nuts, and mixed seeds (Stoloff 1980). This method is widely used as a screening test for aflatoxin in groundnuts. Briefly, aflatoxins are extracted with acetone : water (85:15, V/V), and interferences are removed by adding cupric carbonate and ferric chloride gel. The aflatoxins are then extracted from the aqueous phase with chloroform and the chloroform extract is washed with a basic aqueous solution to effect further purification. The chloroform extract is applied to the top of a minicolumn containing successive layers of neutral alumina (top), silica gel, and Florisil (bottom), will) calcium sulfate drier at both ends. The column is developed with chloroform : acetone (9.1, V/V), trapping the aflatoxins as a tight band al the top of the Florisil layer where they can be delected by their blue fluorescence under UV light. The fluorescence of the band under UV light can be measured directly by inserting the developed minicolumn into the fluorotoxinmeter which may be calibrated to give a direct read-out of the total aflatoxins (in μg kg^{-1}) in the sample. The fluorotoxinmeter is a rapid, reasonably priced quantification system that merits greater use.

Reference columns are prepared using chloroform extracts from a non-contaminated sample spiked with a suitable ratio and level of aflatoxins. Packed sample and reference minicolumns are commercially available (source : Myco-lab Co., P.O. Box 321, St. Louis, MO 63017. USA).

An advantage of this screening method is that the remaining chloroform extract is sufficiently clean to be used for a TLC presumptive lest should the screening test prove to be positive. Groundnut oil can also be analyzed by the Romer method using a simple modification of the extraction procedure. The Romer's method has been found to extract considerably lower amounts of aflatoxin B_1 from groundnuts compared with the BF and CB procedures (Mehan et al. 1985). The two alkali treatment steps using NaOH and KOH during clean-up procedures probably result in low extraction efficiency of Romer's method.

The Romer screening method has been subjected to a collaborative study (Romer and Campbell 1976). Considering only the four groundnut and groundnut product samples used, 94% of the participating laboratories correctly identified the negative samples, 60% the 5 μg kg^{-1} samples, 95% the 10 μg kg^{-1}, and 100% the 20 μg kg^{-1}.

Coker (1984) has briefly discussed factors that can affect the chromatographic behaviour of the aflatoxin band in the minicolumn, especially when analyzing groundnuts for aflatoxins. The use of acetone-water and methanol-water extraction solvents can result in the presence of significant levels of acetone/methanol in the final 'chloroform' extract and these can interfere with the reproducibility of the aflatoxin band. Although the aflatoxin appears as a discrete band on the minicolumn, a certain proportion is invariably washed from the column during the elution step. The degree of this leaching of the aflatoxin depends on the variety of groundnut and/or its source, presumably because of variations in the composition of the groundnut constituents (Coker 1984).

Madhyastha and Bhat (1984) developed a minicolumn confirmation method for aflatoxins. They confirmed the identity of aflatoxins on the developed minicolumn by applying 20% H_2SO_4. 20% HCl. or TFA in 20% HNO_3. All acids changed the fluorescence from blue to yellow, with the TFA in 20% HNO_3 having the lowest detection limit.

7.1.7 Immunochemical Methods

In recent years, several simple, sensitive and specific radioimmunoassay (RIA) and enzyme-linked immunosorbent assays (F.LISA) of aflatoxins have been developed, using polyclonal and monoclonal antibodies against the mycotoxins. ELISA in particular has emerged as one of the major immunoassays for aflatoxins in groundnuts and groundnut products (El-Nakib et al. 1981, Chu 1984, Morgan et al. 1986, Ram ct al. 1986). ELISA technique is well suited to large scale screening for the detection of aflatoxins; it is more rapid, more sensitive, and simpler to use than conventional analytical procedures. ELISA does not require extensive extraction and clean-up steps. Several commercial ELISA-applied aflatoxin assay kits, using highly specific monoclonal antibodies, are currently being marketed. Such kits are easily adaptable for analyzing large numbers of samples, and are directly applicable to assaying methanol-water extracts of a wide range of agricultural commodities. The recent commercial success of ELISA kits shows that the value of this technology is being increasingly recognized.

Antibody Production

Aflatoxins are not antigenic and must be conjugated to a protein (this is referred to as hapten) before immunization. Bovine serum albumin (BSA) is the most commonly employed carrier protein. Conjugation of a mycotoxin to a protein is complicated by the functional groups present in the molecule. Aflatoxins lack a reactive group, and a reactive carboxyl or other group must be introduced to the toxin molecule. Methods for preparation of mycotoxin conjugates have been developed, and specific antibodies against aflatoxin B, and B_2a have been produced (Chu et al. 1977, Chu and Ueno 1977, Gaur et al. 1981, Pestka and Chu 1984, Morgan et al. 1986). For instance, the aflatoxins B_2a or B_1 dihydrodiol are converted to their dialdehydic phenolatc ion forms (Gaur et al. 1981, Pestka and Chu 1984) and B_1 to the putative 8,9-epoxide (Groopman et al. 1988) so they can react directly with a carrier protein. Alternatively, functional moieties such as carboxymethyloxime or hemisuccinate groups can be introduced, and the derivative can be conjugated to a protein by carbodiimide, mixed anhydride, or hydroxysuccinimide methods (Chu and Ueno 1977).

The most frequently used method for production of polyclonal antibodies is multiple-site immunization of rabbits with the hapten, using a mixture of hapten and complete Freund's adjuvant (Chu and Ueno 1977). Antibodies having sufficient titer are generally obtained 5 to 7 wk after the initial immunization. Booster injections are essential if the titers are low (Gaur et al. 1981). Polyclonal antisera are less expensive than monoclonals and often contain a subpopulation of high affinity antibodies (Pestka 1988).

The specificity of an antibody is determined primarily by the antigens used in its production. Before performing any immunoassay, the specificity of an antibody preparation must be tested thoroughly by a competitive binding assay utilizing an appropriate antigen. When rabbits are immunized with aflatoxin, conjugated to a protein utilizing cyclopentane portion of the molecule, such as the carboxymethyloxime of aflatoxin B_1 (Chu and Ueno 1977), the antibodies generally recognize the dihydrofuran portion of the molecule. When conjugates are prepared utilizing the dihydrofuran portion of the aflatoxin molecule, such as aflatoxin B_2a (Gaur et al. 1981), the antibody recognizes the cyclopentane ring. Since such diversity in antibody specificity exists, different antibody preparations should be used for analysis of different aflatoxin metabolites (Chu 1984). An alternative approach to polyclonals is the development of stable hybridoma cell lines which secrete reagent-quality monoclonal antibodies to aflatoxins (Pestka 1988). This is achieved by isolating splenic lymphocytes from an immunized mouse with hapten and fusing these with a myeloma cell line. Following a series of selection and screening steps, an "immortalized" clone that consistently produces antibodies of desired affinity, specificity, and performance characteristics can be isolated. Monoclonal antibodies give more reproducible results than polyclonal antibodies. Nevertheless their production is very costly, time-consuming, and requires sophisticated facilities.

In earlier studies polyclonal antibodies were used but more recently monoclonal antibodies have been used in ELISA for the assay of aflatoxins (Groopman et al. 1988. Sun et al. 1983).

The effectiveness of the antibodies used in different immmunological tests is dependent upon their specificity and titers. Specificity provides information on cross-reactivity of antibody with different toxin analogs (quality). High titered antibodies are economical and are likely to give more reproducible results. The antibody specificity is measured by a competition binding assay with different structurally related analogs, while the titers are measured by the amount of a specific marker ligand bound to the antibody. Chu (1989) has discussed in detail the specificity of antibodies for aflatoxins prepared by different approaches.

Immunoassays

Three immunoassays - RIA, ELISA, and affinity binding assay, have been developed for the analysis of mycotoxins in various foods and feeds. These have been used for the analysis of aflatoxin in groundnuts and groundnut products. Both RIA and ELISA techniques are based on the competition between the free aflatoxin in the sample extract and the labelled aflatoxin in the assay system for the specific sites of antibody molecules. In the RIA a radioactive aflatoxin is used, whereas a toxin-enzyme conjugate is used in the ELISA. The affinity binding assay involves the use of an antibody column that specifically traps the aflatoxin in the column (Sun and Chu 1977). The toxin can then be eluted from the column for subsequent analysis.

Radioimmunoassays (RIA)

The RIA procedure involves simultaneous incubation of the sample extract or known standard with a specific antibody, and a constant amount of labelled toxin. Free toxin and bound toxin are then separated by an appropriate technique, and the radioactivity in these fractions is then determined (Chu 1984). The toxin concentration of the unknown sample is determined by comparing the results with a standard curve established by plotting the ratio of radioactivities in the bound fraction and free fraction vs $\log_{10}$ concentration of non-labeled standard toxin. Analysis of aflatoxin B_1 has been achieved by the double antibody technique (Langone and Van Vunakis 1976). RIA can detect 0.25-0.50 ng of purified aflatoxin per assay in a standard preparation. The lower limit for aflatoxin detection in food or feed samples is from 2 to 5 µg kg^{-1}.

Radioimmunoassays have the disadvantages that (i) RIA requires labeling of the toxins with tritium, which is difficult and expensive, (ii) radioactive waste disposal is a problem, and a scintillation counter is expensive, and (iii) only a small number of samples can be screened in a short period of time. For these reasons interest in RIA has diminished.

Enzyme-Linked Immunosorbent Assays (ELISA)

Two types of ELISA have been used for the analysis of aflatoxins - (i) direct ELISA, and (ii) indirect ELISA. Both are heterogeneous competitive assays which involve the separation of free (unreacted) toxin in liquid phase from the bound toxin in solid-phase. Direct competitive ELISA involves the use of aflatoxin-enzymc conjugate, whereas indirect ELISA involves the use of a protein-aflatoxin conjugate and secondary antibody such as goat anti-rabbit IgG to which an enzyme has been conjugated. Although horeradish peroxidase (HRP) is the most commonly used enzyme for conjugation, other enzymes such as alkaline phosphatase have been used (Chu 1984, Anjaiah et al. 1989). Of several methods used in the conjugation of mycotoxin to HRP, water soluble carbodiimide appears to be the least likely to denature proteins.

Direct Competitive ELISA

In this assay, specific antibody is first coated to a solid phase such as a microliter plate (Chu 1984). The sample extract or standard toxin is generally incubated simultaneously with enzyme conjugate or separately incubated in two steps. After appropriate washings, the amount of enzyme bound to the plate is determined by incubation with a specific substrate solution. The resulting color is then measured spectrophotometrically or by visual comparison with standards. Because this assay is based on competition for antibody binding sites, free toxin concentration is inversely related to antibody-bound enzyme conjugate.

Several direct competitive ELISA methods have been reported for the analysis of aflatoxin in groundnuts and groundnut products (El-Nakib et al. 1981. Chu et al. 1987, Ram et al. 1986, Ueno 1985). In earlier studies these assays took rather a long time to complete, and had large CVs for each sample (Chu 1986). Recently, Chu et al. (1987) have developed a new ELISA protocol that takes approximately an hour to complete quantitative analysis of aflatoxin B_1 in groundnuts and maize. In this assay, samples are extracted with 70% methanol in water containing 1% dimethylformamide (1 g of groundnuts in 5 mL of extraction solvent), diluted with assay buffer (sodium-phosphate buffer, pH 7.2, 0.01 mol L^{-1} with 0.15 mol L^{-1} of NaCl) to a final concentration of 3.5% methanol and then directly subjected to the following ELISA procedure : (a) 50 µl of diluted sample or aflaioxin standard is added to each well of a ELISA plate followed by 50 |il of aflatoxin-HRP conjugate, and the plate is then incubated at room temperature (25-28°C) for 30 min, (b) the wells are washed with 300 µL of PBS-tween 3 times, (c) 100 µL of enzyme substrate is added, followed by incubation at room temperature for 10 min; and (d) 100 µL of stopping reagent is added and absorbance measured at 490 nm. High analytical recovery (95.4%) of aflatoxin B_1 added to peanut butter has been reported by this method (Chu et al. 1987). The interwell and interassay CV has been

reported to be 10% or less at the 20 µg kg^{-1} level and above. This protocol has been used in the analysis of naturally occurring aflatoxin in groundnuts. Excellent agreement for aflatoxin levels in over 30 samples of naturally contaminated maize, mixed feed and groundnuts have been found between the ELISA results, and the results obtained from different laboratories using TLC or other analytical methods (Chu 1989). Improvement in precision has been reported when an additional extraction step with hexane was incorporated into the protocol before ELISA (Chu 1989). A similar protocol with longer incubation time for the analysis of aflatoxin B_1 in groundnut products was described by Ram et al. (1986) who included a defatting procedure for the peanut butter samples before HLISA. Amounts of aflatoxin B_1 as low as µg kg^{-1} in peanut butter could be determined (Ueno 1985). The use of various forms of ELISA as screening methods for anatoxins has increased rapidly (Chu et al. 1987, Mortimer et al. 1987). One method using microtiter plates was collaboratively studied (Park et al. 1989a, Park et al. 1989b). and was adopted official first action by AOAC for screening for aflatoxin B_1 in cotton seed and mixed feed (Changes in Official Methods of Analysis 1989). This method has also received interim official first action approval for aflatoxin B, in corn and groundnut products (Park et al. 1989). A similar method has been adopted official first action for screening for aflatoxins (B_1, B_2, G_1, and G_2) at ≥ 20 ng g^{-1} in corn and raw groundnuts (Trucksess et al. 1989).

Indirect ELISA

In the indirect ELISA, an aflatoxin-protein conjugate is coated to the microplate. The plate is then incubated with specific rabbit antibody in the presence or absence of the homologous mycotoxin. The amount of antibody bound to the plate coated with mycotoxin-protein conjugate is then determined by reaction with commercially available goat anti-rabbit IgG- conjugated to an enzyme followed by addition of a suitable substrate. In this assay, toxin in the sample and toxin in the solid-phase compete for the same binding site with the specific antibody in the solution. Indirect ELISA has been used for the analysis of aflatoxins in groundnut products, using different proteins as carriers for the conjugation of aflatoxin (Fan and Chu 1984, Morgan ct al. 1986). Morgan et al. (1986) used an indirect ELISA for the analysis of 18 peanut butter samples obtained from retail stores and found that the aflatoxin levels ranged from less than 1 µg kg^{-1} in eight samples to as high as 775 µg kg^{-1} in one sample. In this procedure, aflatoxin-protein conjugate (Keyhole limpet haemocyanin (KLH)- aflatoxin B_1 conjugate) is coated onto the microliter plate (Nunc Immunoplate 1). Sample or standard aflatoxin B_1 is added to the wells followed by an aliquot of anti-aflatoxin antibody. The amount of antibody bound to the solid phase is detected by the addition of goat anti-rabbit IgG-conjugated to alkaline phosphatase followed by reaction with p-nitrophenyl phosphate to give a colored product. The aflatoxin B_1 content of the sample is determined by reference to a standard curve.

The sensitivity of the indirect ELISA is comparable to that of the direct ELISA. Because only small amounts of antibody are required for the indirect ELISA, this method has been used frequently for monitoring the antibody titers of hybridoma culture fluids for the screening of monoclonal antibody-producing cells (Groopman et al. 1988) in addition to toxin analysis.

Of the two types of ELISA, direct ELISA is usually preferred for analytical purposes because it utilizes a single conjugated protein, requires one less incubation step and one less washing step, and exhibits substantially less variability than the indirect ELISA (Pestka 1988). The improvement and extent of use of ELISA procedures for detecting aflatoxins depend on the availability of specific antibodies. It is also important to develop simple, effective extraction procedures specially suitable for ELISA.

Although several solid phases, such as polystyrene, polyvinyl, irradiated ELISA plates, tubes, and Terasaki plates have been used for ELISA, the microtiter plates are preferred because of the availability of automated throughput systems.

Commercial ELISA-Kits

Several companies in the UK. France, Japan, and USA have produced ELISA-applied aflatoxin assay kits on a commercial basis for routine use as a food safety and quality control tool in analytical laboratories. The commercially available ELISA kits for quantitative analysis of aflatoxin(s) in groundnuts are described in Table 5. Only the "Biokits" uses indirect ELISA procedure, while all other kits involve direct competitive ELISA. Commercial ELISA kits for aflatoxin B_1 have been reported to perform favorably in routine groundnut analysis in some comparative studies (Wilson et al. 1987, Cole et al. 1987). In another study, the sensitivity of the kits used was reported to be higher than that of all other methods (Goto and Manabe 1988). Patey et al. (1989) evaluated three commercial ELISA kits (Quantitox, Aflasure, and Biokits) for analysis of aflatoxin in peanut butter samples in a collaborative study involving 16 United Kingdom analytical laboratories. The Biokits and Aflasure kits were reported to give higher mean values of aflatoxins present than the levels obtained by liquid chromatography analysis. The Biokits appeared more precise than the other two kits. The relative amounts of the different aflatoxins must be taken into consideration when the performance of one kit is compared with that of another since the aflatoxin specificity of each kit is different. For instance, the Biokits assays concentrations of aflatoxins B_1, B_2, G_1, and G_2, the Aflasure kit assays aflatoxins B_1 and B_2, and the Quantitox kit assays aflatoxin B_1 alone.

Rapid Immunoscreen Tests

Some of the ELISA procedures have been designed as screening methods with the objective of determining the aflatoxin level ≥ 20 µg kg^{-1} (e.g., Agri-Screen test for aflatoxin B_1 - ELISA kit from the Neogen Corporation, 620 Lesher Place, Lansing, Michigan 48912-1509, USA). These methods are designed for use in situations that require short analysis time, simple operation, and relatively low cost.

Another approach is to immobilize the antibody on a paper disk mounted in a plastic card (Immunoassay Quick-card Test) (Cole 1987, Goto and Manabe 1988). The principle of the reaction in this assay format is similar to that of direct ELISA. Experimentally, sample extracts (a few drops) are first applied to the test spot (paper disk) in the plastic card, then aflatoxin-enzyme conjugate is applied, and finally the substrate solution is added. The absence of color, at the spot indicates the sample contains aflatoxin as compared with the negative control spot where a bright blue color appears. Cole et al. (1987) and Dorner and Cole (1989) have found this method a convenient way to screen for aflatoxin in groundnuts. Nevertheless this method is not suitable for quantitative estimation of aflatoxins.

Affinity Column Immunoassay

The application of affinity column chromatography for immunoassay of aflatoxins was first reported by Sun and Chu (1977). and recent advances in instrumentation have led to its increasing adoption (Chu 1989). In practice, aflatoxin extracted from the sample is subjected to a disposable affinity column containing anti-aflatoxin Sepharose$^{(R)}$ gel. After washing, aflatoxin is removed from the column with methanol and its fluorescence determined. The affinity column merely serves as a separation and concentration tool for the analysis. A company in the U.K. (May and Baker Diagnostics Ltd.) is currently marketing an affinity column-based ELISA kit (Aflatest P) for determining aflatoxins in groundnuts.

The high cost of commercially available ELISA kits may limit their use in analytical laboratories in developing countries. The major application for ELISA procedures at present appears to be screening for aflatoxin below a predetermined level. More development is required before ELISA techniques can be generally adopted for critical quantification. Though some studies have shown that results obtained from ELISA are comparable to TLC and HPLC methods, more comparative studies are required. Methods need to be developed that will determine B_1, B_2, G_1, and G_2 individually or collectively.

Table 5. Commercially available ELISA kits for analysis of aflatoxin(s) in groundnuts

Test/ character	Kit				
	Quantitox (U.K.)1	Afla-check Kit (Japan)2	Transia Kit (France)3	Aflasure B Kit (U.K.)4	Biokits (U.K.)[5]
Antibody	Monoclonal	Monoclonal	Monoclonal	Monoclonal	Monoclonal
Specificity	Aflatoxin B_1	Ailatoxin B_1	Allatoxins B_1, B_2, G_1, and G_2	Ailatoxin B_1 and B_2	Anatoxins B_1, B_2, G_1 and G_2
Detection	450 nm absorption	492 nm absorption	410 tun absorption	450 nm absorption	414 nm absorption
Quantifi-cation	2-30 µg kg^{-1}	2-40 µg kg^{-1}	1-30 µg kg^{-1}	2-200 µg kg^{-1}	2-200 µg kg^{-1}
Extraction solvent	Methanol : water (55:45, V/V)	Methanol : water (55:45, V/V)	Methanol : water (80:20. V/V)	acetonitrile water (60:40, V/V)	aceto-nitrile water (50:50,V/V)
System	microtitre plate	microti tre plate	microtitre plate	microtitre plate	microtitre plate

1. May & Baker Diagnostics Ltd., 187 George Street. Glasgow G1 1YT, U.K.
2. UBE Industries Ltd.. 12-32. Akasaka 1-Chome, Minato-Ku, Tokyo. 107 Japan.
3. TRANS1A, 8 rue Saint Jean de Dieu - 69007 Lyon. France.
4. Cambridge Life Sciences ple, Milton Raod. Cambridge CB4 4GN, U.K.
5. Thames Genelink Ltd., Deeside, Clwyd CH5 2NT. U.K.

7.1.8 Biological Methods

Tests developed for the bioassay of aflatoxins have utilized bacteria, brine shrimp, chick embryos, day-old ducklings, and albinism in several crop plants. Bioassays for aflatoxins are frequently carried out as confirmatory tests following chemical identification.

1. The Day-old Duckling Bioassay

Toxicity to day-old ducklings is the most widely utilized bioassay technique for aflatoxins in various commodities. It has been used since the outbreak of "Turkey X" disease first highlighted the aflatoxin problem (Asplin and Camaghan 1961). In standardizing this test, solutions of aflatoxins of known concentration may be administered as single doses directly into the stomach of the duckling, or they can be added to the diet in single doses or spread over time, usually for 5 days. This assay is unique in that the specific response of bile duct proliferation occurs within a few days of administering a single dose of aflatoxin (Butler 1964). The minimum concentration of aflatoxin B_1 required to produce bile duct proliferation is 0.04 mg Kg^{-1} (Wogan 1966). Aflatoxins are usually administered in propylene glycol, but can also be given in dimethyl formamide, methanol or in wheat germ oil.

2. The Chick Embryo Bioassay

The development of the chick embryo technique as a definitive bioassay for aflatoxin was reported by Verrett et al. (1964). Fertile eggs from in-bred White Leghorn hens are injected with the toxin prior to incubation. The air cell location is marked and the surface is sterilized by swabbing with alcohol prior to drilling a hole (about 5 mm in diameter) into the shell covering the center of the air cell. The toxin solution is injected into the air cell and deposited onto the inner egg membrane. The hole is then sealed with a small piece of adhesive cellophane tape. The eggs are kept in a vertical position (air cell up) for an hour to let the material disperse, and are then placed in incubator trays at 37.4° C and a relative humidity of 60%. The eggs are candled daily from the fourth day of incubation; all non-viable embryos are removed. Death of the embryo is counted as a positive test. The evaluation is based on mortality at 21 days, although mortality in eggs receiving higher levels of aflatoxin can be seen as early as the fourth day after incubation.

The chick embryo bioassay has been studied collaboratively (Verrett et al. 1973). In general, a linear dose-response relationship was exhibited and good agreement was obtained with the LD_{50}, slope, and associated standard errors. It is interesting to note that the LD_{50} for pure aflatoxin B_1 was 0.02 µg egg^{-1} and that for aflatoxin B_1 isolated form peanut butter it was 0.04 µg. This assay has been approved by the AOAC for aflatoxin B_1.

The chick embryo test is simple to perform, is relatively inexpensive, and is very sensitive. The assay is reproducible and typical lesions are observed in the embroys with subacute levels of aflatoxin B_1 (less than 0.1 µg egg^{-1}. The major disadvantage of this test is that the embryos, while being very sensitive to aflatoxins, are not specific in their response and may be killed by several other compounds.

3. Guinea pig Bioassay

The guinea pig is highly susceptible to aflatoxin toxicity. Butler (1966) reported an LD_{50} of aflatoxin B_1 at a concentration of 1.4 mg Kg^{-1} body weight with a 95% confidence limit of 1.05-1.8 mg Kg^{-1}. This sensitivity is very similar to that of ducklings. The main hepatic lesion reported in guinea pig is also similar to liver lesions in the duck, with biliary proliferation 3-4 days after injection of the toxin.

4. The Brine Shrimp Bioassay

Several workers have reported the brine shrimp bioassay as a convenient test for aflatoxin (Brown et al. 1968, Tanaka et al. 1975, 1979, 1982). In the brine shrimp bioassay, a suspension of newly-hatched brine shrimps is placed in a small petri plate and the total number of shrimps present is counted. A solution of the toxin is then added, and the plate incubated at high humidity for 24-48 h at 30° C. The percentage of shrimps killed is then determined. The test is non-specific and is a good general bioassay for toxic plant constituents.

Microbiological Assay Techniques

Bacillus megaterium and B. stearothermophilus have been used for detecting and assaying aflatoxins (Clements 1968, Reiss 1975). In the B. megaterium assay, the inoculum of the bacterium is uniformly distributed in the tryptone-yeast-glucose (TYG) agar medium. Paper discs containing toxin solutions are placed on the surface of the medium and plates are incubated at 37° C and then checked at intervals for production of inhibition zones around the discs. The inhibition zones indicate the presence of aflatoxin.

B. stearothermophilus is used in a rapid confirmatory test for aflatoxin B,. In this bioassay, the bacterial culture is placed in tubes containing toxin solutions. The cultures are then transferred to tubes of dextrose tryptone broth containing bromocresol purple as pH indicator and incubated at 55" C in the dark for 24-48 h. Change in color of the medium from purple to yellow indicates the absence of alfatoxin. No color change in the nutrient medium means that the toxin is present. Aflatoxin levels as low as 0.01 µg can be detected (Reiss 1975).

Plant Bioassays

Aflatoxins elicit biochemical responses in plant tissues similar to those produced in animal systems. Several plant bioassays have been developed for aflatoxins (Schoental and White 1965. Kang 1970. Mchan and Chohan 1974). Schoental and White (1965) reported a bioassay of aflatoxin based on 'Virescence' or 'albinism' in seedlings of water cress (Lepidium sativum L.). Kang developed a bioassay based on inhibition of germination of okra seeds (Abelmoschus esculentum L.) or chrorophyll inhibition in the cotyledonary leaves depending on the concentration of aflatoxin. This bioassay is very simple and can be used for screening A. flavus isolates for ability to produce aflatoxins, and also for detecting aflatoxins in various commodities (Kang 1970. Mehan and Chohan 1973). Two other bioassays for aflatoxin based on chlorosis and albinism in cotyledonary and primary leaves of Raphanus sativus and Sorghum vulgare seedlings have been reported (Mchan and Chohan 1974).

The role of bioassays in routine laboratory screening of alfatoxins in agricultural commodities appears to have been under-valued. This may have been due to the rapid development of the physicochemical and immunochemical tests which tend to give more specific information as to the particular toxin present.

7.2 BIBLIOGRAPHY

7.2.1 AFLATOXIN ANALYSIS IN GROUNDNUTS AND GROUNDNUT PRODUCTS

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The importance of sampling, sample preparation and assay techniques in the analysis of mycotoxins in various agricultural commodities is discussed, with emphasis on the required efficiency of the quantification step. Thin-layer chromatography (TLC), high performance thin-layer chromatography (HPTLC), high performance liquid chromatography (HPLC), gas-liquid chromatography (GLC), immunoassay, fluorotoxin meter and other methods are described and compared. Future trends in the analysis of mycotoxins are outlined.

923. Coker, R.D., and Jones, B.D. 1988. Determination of mycotoxins. Pages 335-375 in HPLC in Food Analysis (Macrae. R., ed.). 2nd edition. London, UK : Academic Press.

This paper gives a brief background of the occurrence and chemistry of mycotoxins (aflatoxins, ochratoxin A, citrinin, patulin, zearalenone, and trichothecenes) in foods and feeds. The importance of sampling, sample preparation and assay techniques in the analysis of the mycotoxins in various agricultural commodities is discussed. High performance liquid chromatography (HPLC) methods for the analysis of mycotoxins are discussed. Future developments in the HPLC analysis of mycotoxins are anticipated.

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Procedures are discussed for sampling groundnuts and groundnut products and preparing those samples for aflatoxin analysis. Analytical methods available for the analysis of foods and feedingstuffs for aflatoxin are given.

925. Goto, T. 1990. Mycotoxins : current situation. Food Rev. Int. 6: 265-290.

This review dicusses the major groups of mycotoxins found in food and feeds.

methods for their analysis and the regulatory action taken by some countries for maximum permissible levels of mycotoxins in food and feeds.

926. Jones, B.D. 1972. Methods of aflatoxin analysis. Report G 70, T.P.I, London, UK, 58 pp.

This report reviews some of the analytical methods available for the analysis of foods and feedingstuffs for aflatoxin, particularly those which are suitable for use in laboratories in developing countries. Full details of procedures for sampling, extraction, separation and estimation of aflatoxins are given. Methods for the determination of the concentration and purity of aflatoxin standards and the recommendations for the bulk sampling of commodities for aflatoxin analysis are also given.

927. Schuller, P.L., Horwitz, W., and Stoloff, L. 1976. A review of sampling plans and collaboratively studied methods of analysis for aflatoxin. Journal of the Association of Official Analytical Chemists 59: 1315-1343.

This paper reviews the sampling plans and collaboratively studied methods of analysis for aflatoxins in foods and feeds. Aflatoxins are the only food contaminants being monitored routinely on an international scale with methods operating at the order of a magnitude of 10 μg kg^{-1}. At this level, methods of analysis which can achieve coefficients of variation of 30-40 % with recoveries of 70 % or greater in interlaboratory collaborative studies can be considered eligible for reference status. In most cases, sample reproducibility is the variable limiting the reliability of methods of analysis. The inherent uncertainity of the identity of chromatographically separated entities requires the application of confirmatory tests to verify that the characteristics measured result from the presence of aflatoxin. The methods are also inoperable without a verification of the identity, purity, and concentration of the reference standards used. Screening methods which reliably eliminate negative samples from further consideration are indispensible for the practical operation of monitoring programs.

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Developments in methodology for analysis of mycotoxins in foods are reviewed, covering aflatoxins, Alternaria toxins, citrinin, cyclopiazonic acid, ergot alkaloids, ochratoxins, penicillic acid, sterigmatocystin, trichothecenes, and zearalenone. As well as papers on methodology, oilier recent papers of interest on health significance and occurrence of individual mycotoxins are covered.

7.2.2 Sampling and Sample Preparation Procedures

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Fungal species that produce aflatoxins *(Aspergillus flavus* and *Aspergillus parasiticus)* are common in field soils, and invasion of groundnut kernels by these fungi frequently takes place during pod development. In evironments where conditions are suboptimal, entire kernels may not become invaded by these fungi, however, some colonization of testa and outer layers of cotyledons may occur. If mycotoxin-producing fungi become established in these external kernel layers, aflatoxin is most likely to be concentrated in the same areas. A technique was developed for sampling only the testa and outer cotyledonary tissue of raw groundnuts. This involved abrading of kernels up to 4 % of their external tissue. Fungi established in this tissue could be assessed by plating groundnut particles on selective media plates. Aflatoxin levels were measured in abraded particles and chopped whole kernels from the same groundnut samples. Toxin levels in whole kernels samples were 9.2 % of levels measured in panicles abraded from external kernel tissue. When aflatoxin concentration was less than 5 μg kg^{-1} in whole kernels, it was often found to be as high as 50 μg kg^{-1} in external tissue of the kernels. This technique allows measurement of low aflatoxin levels in groundnut lots.

930. Brown, G.H. 1984. The distribution of total aflatoxin levels in composited samples of peanuts. Food Technology in Australia. 36(3): 128-130.

The suitability of the negative binomial distribution in describing aflatoxin levels in composited samples of groundnuts was studied for two varieties of groundnut grown in Kingaroy, Queensland, Australia during the 1981-1982 season. The model was inadequate and an alternative is suggested : the parameteres of this model were fitted to the current data and to previously published data for comparisons. Results obtained have implications in the choice of practical acceptance of sampling plans which are discussed in terms of the current Australian testing level.

931. Campbell, A.D. 1977. Food mycotoxins survey and monitoring programmes. Annales de la Nutrition et de l'Alimentation 31: 403-410.

Effective mycotoxin survey and monitoring programmes are discussed. The US aflatoxin monitoring plan for groundnut processing includes import, export and manufacturing quality control points of testing, sampling, sample preparation, and types of test, e.g., screening and multidetection chemical and confirmation tests, are discussed.

932. Campbell, A.D. 1979. Sampling foodstuffs for mycotoxin analysis. Pure and Applied Chemistry 52: 205-211.

The obvious reason for sampling any given lot of material is to obtain a portion for determination or observation of attributes of the particular lot. It is also obvious that the sample must be representative of the lot to obtain meaningful results. Traditional means of sampling and sample preparation of agricultural crops and foodstuffs are usually not adequate for mycotoxin analyses. The main reason for this is that mycotoxin contamination is usually of a heterogenous nature and this presents problems in the preparation of a homogenous sample for analysis. Sampling plans, sampling equipment, and sample preparation are discussed.

933. Campbell, A.D., Whitaker, T.B., Pohland, A.E., Dickens, J.W., and Park, D.L. 1986. Sampling, sample preparation, and sampling plans for foodstuffs for mycotoxin analysis. Pure and Applied Chemistry 58(2): 305-314.

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The importance of sampling, sample preparation and assay techniques in the analysis of mycotoxins in various agricultural commodities is discussed, with emphasis on the required efficiency of the quantification step. Thin-layer chromatography (TLC), high performance thin-layer chromatography (HPTLC), high performance liquid chromatography (HPLC), gas-liquid chromatography (GLC), immunoassay, fluorotoxin meter and other methods are described and compared. Future trends in the analysis of mycotoxins are outlined.

935. Coker, R.D. 1989. Control of aflatoxin in groundnut products with empasis on sampling, analysis, and detoxification. Pages 123-132 in Aflatoxin contamination of groundnut : Proceedings of the International Workshop, 6-9 Oct 1987, ICRISAT Center, India. Patancheru A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

This paper reviews the work carried out at the Overseas Development Natural Resources Institute (ODNRI), London, on the development of efficient sampling, sample preparation, aflatoxin analysis, and chemical detoxification procedures. The control of aflatoxin in groundnut products requires a combination of quality control and decontamination/detoxification procedures. The use of selected mathematical models to describe the distribution of aflatoxin in groundnut kernels, roasted groundnuts, peanut butter, and groundnut cake has been investigated for developing 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 developed for the accurate analysis of the aflatoxin content of groundnut products using bonded-phase clean- up 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 aflatoxin in 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.

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This paper gives a brief background of the occurrence and chemistry of mycotoxins (aflatoxins, ochratoxin A, citrinin, patulin, zearalenone, and trichothecenes) in foods and feeds. The importance of sampling, sample preparation and assay techniques in the analysis of the mycotoxins in various agricultural commodities is discussed. High performance liquid chromatography (HPLC) methods for the analysis of mycotoxins are discussed. Future developments in the HPLC analysis of mycotoxins are anticipated.

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A micro method, adapted from the aqueous acetone procedure proposed by Pons and Goldblatt for the determination of aflatoxins in cottonseed products, was developed to permit accurate determination of aflatoxins in individual groundnut kernels and kernel sections. Use of this procedure permitted the topographic distribution of aflatoxins within individual kernels to be mapped and indicated that the toxins were not uniformily distributed within contaminated kernels, even when the kernel contained a high level of aflatoxins. Although wrinkling or discoloration sometimes indicated that a kernel was contaminated, this type of physical damage was not found to be a reliable indication of aflatoxin content. Also it was noted that a few apparently sound and mature kernels contained high levels of aflatoxins.

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This report examines and summarizes current knowledge regarding mycotoxins surveys, sampling techniques, conditions conducive to post-collection production of mycotoxins in grain samples, and analytical methods for mycotoxin analysis. Priority attention is given to samples of com suspected of containing aflatoxin. The report includes recommendations where deemed appropriate by the AdHoc Work Group.

939. Dickens, J.W., and Satterwhite, J.B. 1969. Subsampling mill for peanut kernels. Food Technology 23: 90-92.

A subsampling mill designed to simultaneously comminute and subsample large samples of granular material is described for use by the groundnut industry to prepare groundnut samples for aflatoxin analyses. During operation, the cylindrical screen is fastened to a circular platform beneath the blades. The central blades hammer the groundnut kernels against the cylindrical screen until the fragments are small enough to penetrate the 1/8 inch mesh. Most of the comminuted material then falls to waste but a small portion (5 % by weight) passes through each of the two openings and into the sub-sample collection chute.

940. Dickens, J.W., and Whitaker, T.B. 1982. Sampling and sample preparation. Pages 17-32 in Environmental Carcinogens - Selected Methods of Analysis. Vol. 5 - Some Mycotoxins (Egan, H.. Stoloff, L., Scott, P., Castegnaro. M., O'Neill, I.K., and Bartsch, H. eds.). Lyon International Agency for Research on Cancer (IARC), Scientific Publications No. 44, 459 pp.

This review paper provides general guidelines for sampling and subsampling foods for quantitative mycotoxin analyses. Aflatoxin testing program used for groundnuts in the USA is also described.

941. DiProssimo, V.P. 1976. Distribution of aflatoxins in some samples of peanuts. Journal of the Association of Official Analytical Chemists 59: 941-944.

Naturally occurring G aflatoxins were found at approximately 20 times the level of B aflatoxins in one lot of roasted, blanched groundnuts. Official methods, as well as high pressure liquid chromatography, were used to confirm this finding. Additional routine sample analysis data of raw and roasted groundnuts revealed that this finding is not so unusual as formerly thought. It was found that 9.8 % of the raw groundnut samples contained higher levels of G than B aflatoxin; 4.9 % containing 2-8 times more G than B aflatoxins. In a smaller sampling of roasted groundnuts, 28 % of the contaminated groundnut contained more G than B aflatoxins.

942. Dowell, F.E., Dorner, J.W., Davidson, J.I.Jr., Cole, R.J., and Ferguson, W.G. 1988. Detection of aflatoxin in various components of farmers stock peanuts. Proceedings of the American Peanut Research and Education Society 20: 25.

Ten 50 lb pneumatic samples from 17 loads of farmers stock groundnuts were collected during the 1987 harvest season in Georgia. Grade samples were run over a 24/64 belt screen. Loose- shelled kernels (LSK) and small pods that fell through the belt screen were separated, pods were shelled, and the kernels were screened over a 16/64 and 14/64 slotted screen. Pods that rode the 24/64 belt screen were similarly shelled and screened, and in addition, the damaged kernels were removed The resulting components were analyzed separately for aflatoxin by high performance liquid chromatography (HPLC). From the grade samples, the LSK, other kernels (OK), and damaged kernels were combined and the sound mature kernels (SMK) and sound splits (SS) were combined. These two grade components were analyzed separately for aflatoxin by UPLC. Results showed the distribution *of* aflatoxin within each component and provided variance data to determine sample sizes needed to detect specified levels of aflatoxin in each respective component. Results also provided additional information needed for finalizing the design of a pilot study to determine the performance of the belt screen separator in removing foreign material and poor quality groundnuts prior to marketing of farmers stock groundnuts.

943. Food and Agriculture Organization of the United Nations/World Health Organization/United Nations Environment Programme. 1975. Working paper on methods of analysis and sampling for aflatoxins. EC/MAS/FC/75/2, Rome Italy.

Procedures are discussed for sampling groundnuts and groundnut products and preparing those samples for aflatoxin analysis. Analytical methods available for the analysis of foods and feedingstuffs for aflatoxin are given.

944. Francis, O.J.Jr. 1979. Sample preparation of some shelled treenuts and peanuts in a vertical cutter-mixer for mycotoxin analysis. Journal of the Association of Official Analytical Chemists 62(5): 1182-1185.

A procedure has been devised for more rapid preparation of large samples of nutmeals (10-80 lb) for mycotoxin analysis without the use of grindning aids, by combining the grinding and mixing steps into one operation. Ease of cleaning equipment facilitates the preparation of several samples in a short time. The procedure was tested using shelled raw and roasted groundnuts, and raw almonds, walnuts, and pecans. The reduction of particle size to pass a 20 mesh screen was

attained using 25 qt Hobart vertical cutter-mixer (VCM) equipped with standard blade or a smooth-edge blade modified with sharp-edge notches to increase its cutting ability. Portions of ground composite were removed at various time intervals and particle sizes were measured. Original and check analyses of 12 naturally contaminated samples over a 3-year period indicate that this procedure is practical and reproducible.

945. Garfield, F.M. 1989. Sampling in the analytical scheme. Journal of the Association of Official Analytical Chemists 72(3): 405- 411.

The general principles of sampling in the analytical scheme, sampling definitions, and sampling planning arc considered. Statistical considerations are stressed with attention to sampling of attributes and variables and the use of sampling control charts. Sampling techniques, records and chain-of-custody procedures, sample handling, laboratory sampling, and sample preparation for analysis, as well as reasons and causes of sampling errors, are discussed.

946. Hanssen, E., and Waibel, J. 1978. [Sampling for the determination of aflatoxin in groundnuts and other nuts mentioned in the aflatoxin law, from cases and prepared packs.]. Zur probenahme fur die ermittlung des aflatoxin-gehaltes bei erdnuss und anderen in der aflatoxin vo [16] genannten samenkernen aus gebinden und fertigpackungen. Alimenta 17(5): 139-143.

The frequency of aflatoxin contamination in some nuts is discussed, i.e., groundnuts 1 in 4000 (as lifted), reduced to 1 in 10000 after first sorting, 1 in 33000 after second sorting (manual or electronic); hazelnuts 1 in 30000; almonds 1 in 26000-45000; and walnuts 1 in 29000. As these nuts cannot be harvested without some aflatoxin contamination, the Swiss Health Act accepts contamination of 1 in 10000 as standard. The following control method was devised : samples of 2.5 kg, as 10 sub-samples of 250 g, with maximum per sub-sample of < 25 µg aflatoxin B_1, < 125 *µg* $B_2 + G_1 + G_2$, Sample size of 2.5 kg (5000-8000 groundnut kernels) gives a 50 % probability of detecting contaminated kernels and divides the chance of detection equally between the processors and health authorities. Analysis of single packages is worthless.

947. Horwitz, W. 1988. Sampling and preparation of sample for chemical examination. Journal of the Association of Official Analytical Chemists 71(2): 241-245.

Sampling and methods for reducing a laboratory sample to a test sample are discussed, with particular emphasis on sampling groundnuts for aflatoxin analysis as a practical example. The only way to control the total error in the analysis of this heterogeneous product is to take and to analyze many and large samples.

948. Jewers, K. 1982. Mycotoxins in food - the application of survey and quality control. Royal Society of Health Journal 102(3): 114-118.

The methodology for conducting a survey of mycotoxin contamination of foods and feedstuffs, including sampling, analysis and treatment of data obtained is presented. Results are given of a survey of the aflatoxin content of a small proportion of groundnuts from the 1977/78 season imported into the U.K. are given. Thirty-one of the 159 samples exceeded the maximum permitted level of aflatoxin in raw groundnuts (30 µg kg^{-1}). The highest level of aflatoxin contamination was in samples from the Gambia and India (> 400 µg kg^{-1} in 1 sample each). Results are also given of a survey of the aflatoxin M_1 content of samples of producer-retailer milk in the U.K., commissioned in 1977 by the Ministry of Agriculture, Fisheries and Food are presented. The survey showed that 11.9 % of the milk samples tested contained > 0.1 µg L^{-1} aflatoxin M_1 (the limit permitted by European Community Regulations). A parallel study of random samples of dairy feed indicated that 8 % contained > 30 µg kg^{-1} aflatoxin B_1. In another study the average level of aflatoxin in a 12 ton batch of groundnut cake obtained from a farmer in the U.K. was 1540 µg kg^{-1}, which would result in feedstuffs containing aflatoxin well in excess of the permitted level.

949. Jewers, K. 1987. Problems in relation to sampling of consignments for mycotoxin determination and interpretation of results. Presented at the Joint FAO/WHO/UNEP Second International Conference on Mycotoxins, 28 September - 3 October 1987, Bangkok. Thailand, pp. 1-16.

This paper critically reviews sampling procedures and statistical models for describing the distribution of aflatoxins in agricultural commodities including groundnuts. Sampling plans for groundnuts are also discussed.

950. Jewers, K. 1988. Aflatoxin in foods and feeds. Food Laboratory Newsletter (Sweden) 11: 30-34.

This paper reviews sampling and sampling plans for aflatoxin analysis in agricultural commodities including groundnuts. It is suggested that large samples should be collected when conducting surveillance programmes on aflatoxins in commodities or when determining the effect of processes on aflatoxin levels in order to minimize errors.

951. Jewers, K., Coker, R.D., Blunden, G., Jazwinski, J.M., and Sharkey, A.J. 1986. Problems involved in the determination of aflatoxin levels in bulk commodities. Pages 83-88 in Spoilage and Mycotoxins of Cereals and other Stored Products (Flannigan, B., ed.). C.A.B. International, Farnham Royal, Slough SL2 3BN. UK.

The distribution of aflatoxins in groundnuts has been extensively studied and plans have been developed in the USA and the UK for monitoring the levels of aflatoxin in this commodity. Attempts have been made to assess the sampling plans using various statistical models. In determining the aflatoxin levels in bulk commodities errors can arise at several stages, for example, in sampling, sample preparation, and analysis. These errors and the attempts which are being made to limit their effects are discussed. Earlier workers have applied the negative binomial and compound poisson-gamma distribution to the distribution of anatoxins in edible groundnuts, but with limited success. Data for aflatoxin levels in different batches of groundnuts and groundnut cake were found to give a better fit over a wider range using the Weibull distribution than the other distributions.

952. Jones, B.D. 1972. Methods of aflatoxin analysis. Report G 70. T.P.I, London, UK, 58 pp.

This report reviews some of the analytical methods available for the analysis of foods and feedingstuffs for aflatoxin, particularly those which are suitable for use in laboratories in developing countries. Full details of procedures for sampling, extraction, separation and estimation of aflatoxins are given. Methods for the determination of the concentration and purity of aflatoxin standards and the recommendations for the bulk sampling of commodities for aflatoxin analysis are also given.

953. Knutti, R., and Schlatter, C. 1978. [Problems of determining aflatoxin in groundnuts - proposal for a sampling and analysis scheme for import control.]. Probleme der Bestimmung von aflatoxin in Erdnussen - Vorschlag fur einen probenahme - und analysenplan fur die importkontrolle. Mitteilungen aus dem Gebiete der Lebensmittel - untersuchung und Hygiene 69(2): 264- 274.

Problems of analysis of whole groundnuts for aflatoxin are discussed. A proposed samplign plan is based on a limit for rejection of 1 µg kg^{-1} with a producer risk of 1 % and a consumer risk of 1 % for accepting a lot with 5 µg kg^{-1}. Three samples of 10000 kernels are analysed for aflatoxin in the first step. If no decision can be reached another sample of 10000 kernels has to be analysed.

954. Knutti, R., and Schlatter, C. 1982. Distribution of aflatoxin in whole groundnut kernels : Sampling plans for small samples. Zeitschrift fur Lebensmittel-Untersuchung und-Forschung 174(2): 122-128.

Statistical distribution models are considered for investigation into the effect of the number of groundnut kernels sampled on the effectiveness of the detection of aflatoxin contamination. It is shown by the analysis of 368 samples of 1 to 10000 kernels, from the same lot of nuts, that the negative binomial distribution represents a good statistical model. Different sampling plans are considered, and it is concluded that samples of many kilograms must be analyzed to ensure a low risk of a wrong decision both to the consumer and to the producer.

955. Koe, W.J.de., and Defize, P.R. 1990. Reaction to a paper by Whitaker and Dickens on aflatoxin testing plans for shelled peanuts in the U.S. and the export market. Journal of the Official Analytical Chemists 73(5): 809-811.

A technical paper by Whitaker and Dickens [Journal of the Association of Official Analytical Chemists (1989) 72 : 644-648] on aflatoxin testing plans that discusses (without a literature reference) a testing plan used in the Netherlands is examined. It is noted that this testing plan has never been in operation. The current situation in the Netherlands with respect to legislation and sampling plans on aflatoxin, which has fairly important consequences for the results of the simulation study of Whitaker and Dickens, is presented. It is shown that the percentage of rejected US-exported lots in the Netherlands would increase from 16 to 27 % based on the actual testing plan in the Netherlands. The need for international harmonization of testing and the role of Codex Alimentarius is also emphasized.

956. Maselli, J.A. 1977. Controlling aflatoxin in your plant. Manufacturing Confectioner 57: 35-38,40-41.

The clinical effects of aflatoxin exposure are briefly described, and the current US FDA regulations and sampling procedure for control of aflatoxins in groundnuts arc discussed. Some quality control procedures to ensure that only minimal quantities of toxin persist into the finished product are outlined.

957. Park, D.L., and Pohland, A.E. 1989. Sampling and sample preparation for detection and quantitation of natural toxicants in food and feed. Journal of the Association of Official Analytical Chemists 72(3):
399-404.

The primary goal of a sampling plan for natural toxins, i.e., mycotoxins and seafood toxins, is to obtain a sample that accurately represents the conentrations of individual components of a given lot. Factors affecting the ability of the sampling plan to accomplish this goal include : (1) nature of the analyte of interest, (2) distribution of the analyte throughout the lot, (3) physical characteristics of the product, (4) accessibility of the product to random representative sampling, (5) sampling procedure, and (6) size of the sample. Sampling plans are composed of three distinct components : (a) sampling, (b) sample preparation, and (c) analysis. Normally, sampling contributes the largest relative error while analysis comprises the least. Automatic, continuous stream samples provide the most representative samples for commodities such as nuts, cottonseed, and cereal grains. Good sample

preparation equipment is currently available for these commoditis ; the use of this equipment to obtain a representative test sample is discussed.

958. Quesenberry, C.P., Whitaker, T.B., and Dickens, J.W. 1976. On testing normality using several samples : an analysis of peanut aflatoxin data. Biometrics 32(4): 753-759.

A sampling plan for groundnuts must provide a high level of protection for the consumer and reasonable assurance to the processor that lots of good groundnuts are not rejected by the testing program. Samples consisting of replicate detection in groundnut sub-samples were considered statistically. Results of statistical analysis indicated that the normal model fitted the data quite well and definitely better than the log normal model.

959. Schuller, P.L., Horwitz, W., and Stoloff, L. 1976. A review of sampling plans and collaboratively studied methods of analysis for aflatoxin. Journal of the Association of Official Analytical Chemists 59: 1315-1343.

This paper reviews the sampling plans and collaboratively studied methods of analysis for aflatoxins in foods and feeds. Anatoxins are the only food contaminants being monitored routinely on an international scale with methods operating at the order of a magnitude of 10 µg kg^{-1}. At this level, methods of analysis which can achieve coefficients of variation of 30-40 % with recoveries of 70 % or greater in interlaboratory collaborative studies can be considered eligible for reference status. In most cases, sample reproducibility is the variable limiting the reliability of methods ol analysis. The inherent uncertainty of the identity of chromatographically separated entities requires the application of confirmatory tests to verify that the characteristics measured result from the presence of aflatoxin. The methods arc also inoperable without a verification of the identity, purity, and concentration of the reference standards used. Screening methods which reliably eliminate negative samples from further consideration are indispensible for the practical operation of monitoring programs.

960. Stoloff, L., Campbell, A.D., Beckwith, A.C., Nesheim, S., Winbush, J.S., and Fordham, O.M.Jr. 1969. Sample preparation for aflatoxin assay : The nature of the problem and approaches to a solution. Journal of the American Oil Chemists' Society 46: 678- 684.

Cases have been reported of individual groundnuts, cottonseeds or Brazil nuts so highly contaminated with aflatoxin that, for a 50 g portion to be representative of the whole, the sample preparation procedures should grind each unit to a large number of particles and distribute them uniformly throughout the sample. Assuming uniform contamination of the individual kernel, each 50 g sample should contain 1/100 of that kernel. Even though these extreme cases may be encountered only infrequently, the more usual situation still presents difficulties because of great variability in individual kernel contamination. However, if the extreme can be handled, one can expect to handle the more usual situation. Equipement and procedures to achieve this distribution goal are described. The equipement studied includes a food chopper (Hobart), a nut mill (Thomas Mills), a disc mill (Bauer), a hammer mill (Fitzpatrick Model D comminuting machine), a hammer mill designed specifically for groundnut samples (Dicken's subsampling mill), a Polytron homogenizer (Bronwill Scientific), a vertical cutter-mixer (Hobart), and a sample splitter (Jones riffle). Commodities examined were shelled groundnuts and in- shell Brazil nuts, walnuts, pecans and almonds. Comminution and mixing effectiveness were determined by particle size analysis, by distribution of kernels made radioactive by neutron activation and by aflatoxin analysis ol naturally contaminated products. From the results it is concluded that the ultimate in sample uniformity can be achieved with a disc mill, solvent addition to obtain a fluid system and mixing and grinding of the fluid with a dispersion mixer-grinder. A practical uniformity can be achieved in a vertical cutter-mixer with less expenditure of time and effort for the commodities studied.

961. Stoloff, L., Dantzman, J., and Wegener, J. 1972. Preparation of lot samples of nut meats for mycotoxin assay. Journal of the American Oil Chemists' Society 49: 264-266.

A procedure was devised for preparing lot samples of mycotoxin- contaminated nut meats so that a representative analytical sample may be removed. The sample is rapidly reduced to coarse size. A relatively large portion (about 1/10 of total sample) of subsample is then split out and further comminuted to a fine particle size with the aid of a fat solvent (meat solvent, w/v, 3:2). The analytical sample is removed from this mixture. The procedure was tested with shelled almonds and shelled walnuts using radioactive nuts to simulate me mycotoxin contamination and provide a simple, precise measure of the contaminated nut meat distribution. The pooled coefficient of variation was 18 % for the subsamples and 4.4 % for the analytical samples. Considering the dilution factors used (1.50 and 2.14 contaminated nuts 10^4 nuts) and the low degree of reliability of the lot sample, the sample preparation methods tested appear to be practical and reliable.

962. Tiemstra, P.J. 1969. A study of the variability associated with sampling peanuts for aflatoxin. Journal of the American Oil Chemists' Society 46: 667-672.

One lot of groundnuts known to contain aflatoxin was extensively sampled to study the sources of variability. A nested design was used where sections (50 bag units), subsamples and analytical variation were the variables studied. Sample size was the most critical factor in characterizing this lot. Variability from section to section was not significant indicating random distribution of the toxin.

Three 20 lb samples were taken on a number of lots from the 1968 crop, each of which was subdivided into two equivalent subsamples. The aflatoxin was determined in each of these subsamples. The results indicated that all the significant variation came from the subsamples, further enforcing the thesis that sample size is the critical factor in variability, and not lot inhomogeneity. Analyses of 550 Jots from the 1967 crop where triple samples and analyses were available indicated that the magnitude of the variability was a little greater than was found on the experimental lot. Using the pooled standard deviation of the 1967 crop data, operating characteristic curves were plotted to demonstrate the improvement that can be expected by increasing the sample size.

963. Velasco, J., and Morris, S.L. 1976. Use of water slurries in aflatoxin analysis. Journal of Agricultural and Food Chemistry 24(1): 97-103.

A method based on the use of water slurries was developed for aflatoxin analysis. This procedure enables aliquots, from kilogram size samples, to be analyzed without an increase in solvent or reagent costs. Groundnuts, peanut butter, groundnut meal, cottonseed, cottonseed meal, copra and maize were successfully analyzed by this slurry technique. Slurries were prepared in a 1-gal blender and sample weight to water volume ratios were 1:1.5 for peanut butter, 1:2.25 for whole seeds, 1:4 for commercial meals and 1:5 for copra. Extraction of 100-g aliquots of slurry with 200 mL of acetone resulted in aflatoxin yields comparable to those obtained by extraction of the dry products by the standard method. The precision of slurry analysis for the products, expressed as % coefficient of variation, ranged from 2.6 to 7.8 compared to 5.7 to 20.8 for analyses by the standard procedure. Determinations were made by fluorometric measurements of aflatoxin absorbed on the florisil layer in a minicolumn.

964. Waibel, J. 1977. [Sample size for estimation of aflatoxin in groundnuts.]. Stichprobengrosse fur die bestimmung von aflatoxin in erdnussen. Deutsche Lebensmittel - Rundschau 73(11): 353-357.

Problems of sampling of groundnuts for aflatoxin determination are discussed, with reference to the 1976 legislation setting tolerances for aflatoxins in foods. Problems include the relative rarity of contaminated nuts (e.g., 1 in 10000), and the considerable variation in aflatoxin concentration in contaminated nuts. A statistical study on optimization of sample size is described. Tables of data are given showing the likelihood of negative results with sample of 60 g-60 kg with samples of groundnuts with contaminated : sound nut ratios of 1:4000, 1:10000, and 1:30000, and the probability of various aflatoxin concentrations in various sample sizes, as a proportion of the aflatoxin concentration in the total batch. A sample size > 5 kg is recommended for random checks by groundnut processors. Random sampling of commercially available groundnuts by official quality control organizations is also discussed.

965. Waltking, A.E. 1980. Sampling and preparation of samples of peanut butter for aflatoxin analysis. Journal of the Association of Official Analytical Chemists 63(1): 103-106.

Procedures are discussed for sampling peanut butter and preparing those samples for aflatoxin analysis. Special emphasis is placed on sampling the product from shipping pallets and comminuting chunk style peanut butter in order to reduce the variability in the analysis associated with the nonuniform distribution of aflatoxin in the product. The slurry method of preparation is a convenient means of obtaining a sample which is representative of a non-homogeneous product.

966. Whitaker, T.B. 1969. Formulation of sampling plans for the determination of aflatoxin in shelled peanuts. Proceedings of the American Peanut Research and Education Association 1: 101.

A multiple sampling plan, called attribute sampling, is discussed as a method to determine if the mean level of aflatoxin in a lot of shelled groundnuts exceeded a predetermined critical level. Assuming that the distribution of aflatoxin in a lot of groundnuts may be approximated by the negative binomial distribution, the method of computing an operating characteristic curve for a multiple sampling plan is described. From the operating characteristic curve, a measure of both the consumer's and processor's risk can be evaluated. The operating characteristic curve for the sampling plan to be used by the groundnut industry starting in fall 1969 is described.

967. Whitaker, T.B. 1976. Sampling foodstuffs for aflatoxin. Page 4 in Abstracts of the Third International I.U.P.A.C. Sponsored Symposium on Mycotoxins in Foodstuffs, 16-18 September, Paris, France.

Methodology for use in evaluation of aflatoxin testing programs to estimate the aflatoxin concentration in lots of granular material is presented. A computer model is used to simulate sampling, subsampling, and analytical errors in aflatoxin testing programs. The negative binomial function is used to estimate the distribution of sample concentrations taken from contaminated lots and the distribution of subsample concentrations taken from contaminated samples. The normal distribution is used to predict the distribution of analytical results about the subsample concentration. Monte Carlo solution techniques are employed to account for conditional probabilities that arise from use of multiple samples, subsamples, or analyses in testing programs. Operating characteristic curves, computed by the

model, arc used to evaluate the processors' risk, the consumers' risk, and the costs associated with aflatoxin testing programs. The aflatoxin testing programs used for shelled groundnuts in the United States are described and evaluated.

968. Whitaker, T.B. 1977. Sampling granular foodstuffs for aflatoxin. Pure and Applied Chemistry 49: 1709-1717.

Methodology is described for the design and evaluation of testing programs to estimate aflatoxin concentrations in lots of granular foodstuffs. Use of operating characteristic curves and of the prior distribution of lot concentrations for comparing and evaluating processor and consumer risks related to testing programs are demonstrated. Operating characteristic curves, computed from a system of equations that accounts for errors in sampling, subsampling, and analysis are developed for the 1976 groundnut aflatoxin testing program in the United States. Estimated are given of aflatoxin concentration in lots accepted and rejected by the testing program.

969. Whitaker, T.B. 1990. Reply to "reaction to a paper by Whitaker and Dickens on aflatoxin testing plans for shelled peanuts in the U.S. and the export market". Journal of the Association of Official Analytical Chemists 73(5): 812-813.

In reply to a previous paper [Koe, W.J.de, and Defize, P.R. - Journal of the Association of Official Analytical Chemists (1990) 73 : 809-811]. methods used to evaluate aflatoxin testing plans for shelled groundnuts are discussed.

970. Whitaker, T.B., and Dickens, J.W. 1979. Estimation of the distribution of lots of shelled peanuts according to aflatoxin concentrations. Peanut Science 6(2): 124-126.

The distribution of shelled groundnut lots according to their true aflatoxin concentrations is different from the distribution of aflatoxin assays made on those lots. This difference is due to assay errors associated with sampling, subsampling and analysis. A method was developed to estimate the distribution of lots according to their true aflatoxin concentration based on aflatoxin assays. Estimates of the distribution of the 1973. 1974, and 1975 groundnut lots were computed from their aflatoxin assays which were recorded by the Peanut Administrative Committee (PAC). Fifty-five % of the PAC assays lor the three crop years were less than 3 µg kg^{-1} aflatoxin, whereas the estimated lot distribution indicated that only 48 % of the lots were less than 3 µg kg^{-1}. On the other hand, only 94 % of the assays compared to an estimated 96 % of the lots were less than 25 µg kg^{-1}.

971. Whitaker, T.B., and Dickens, J.W. 1979. Evaluation of the Peanut Administration Committee testing program for aflatoxin in shelled peanuts. Peanut Science 6. 7-9.

Under provisions of a USDA Marketing Agreement, an aflatoxin control program for groundnuts produced in the United States is administered by the Peanut Administrative Committee (PAC) composed of groundnut growers and shellers. The PAC requires aflatoxin tests on all commercial lots of shelled groundnuts. The present PAC testing program initiated in 1975 was evaluated for Crop A which averaged 9.5 µg kg^{-1} aflatoxin and for Crop B which averaged 5.2 µg kg^{-1} aflatoxin. For Crop A and B, respectively, 7.3 % and 2.0 % of the lots were rejected. The accepted lots contained an average of 7.5 µg kg^{-1} for Crop A and 4.7 µg kg^{-1} for Crop B. A correct decision (accept a lot with < 25 µg kg^{-1} aflatoxin and reject a lot with > 25 µg kg^{-1} aflatoxin) was made 95 % of the time for Crop A and 98 % of the time for Crop B.

972. Whitaker, T.B., and Dickens, J.W. 1988. Simulating the testing of peanut lots in the export market for aflatoxin. Proceedings of the American Peanut Research and Education Society 20: 26.

The present aflatoxin testing plan used in the United States for shelled groundnuts was designed with a final accept level of 25 µ.g kg^{-1} total aflatoxin. Some of the importers of US groundnuts use aflatoxin testing plans with accept levels lower than the 25 µg kg^{-1} used in the US. For example, the accept level of a testing plan used in The Netherlands is 5 µg kg^{-1} B_1 or 10 µg kg^{-1} total aflatoxin. Computer models were developed to simulate the testing of groundnut lots with the US and The Netherlands testing plans. The model was used to determine the effects of decreasing the final accept level of the US testing program on the number of lots accepted and rejected in the US and the number of exported lots accepted and rejected when using The Netherlands testing plan. Decreasing the final accept level of the US testing program from 25 to 5 µg kg^{-1} increased the number of lots rejected in the US by 371 % while reducing the number of exported lots rejected by 51 %. For every additional 8.3 lots rejected in the US, one less export lot will be rejected.

973. Whitaker, T.B., and Dickens, J.W. 1989. Simulation of aflatoxin testing plans for shelled peanuts in the United States and in the export market. Journal of the Association of Official Analytical Chemists 72(4): 644-648.

The 1987 United States aflatoxin testing plan for shelled groundnuts was designed with a final accept level of 25 µg kg^{-1} total aflatoxin. Some of the importers of U.S. groundnuts use aflatoxin testing plans with accept levels lower than 25 µg kg^{-1}. For example, the accept level of a testing plan used in The Netherlands is 5 µg kg^{-1} B_1 or 10 µg kg^{-1} total aflatoxin. Whenever export lots are re-tested for aflatoxin by an importing country, some lots accepted in the United States will be rejected by the importing country's aflatoxin testing plan. Computer models were developed to determine the effects of decreasing the final accept level of the U.S. testing plan on the number of lots accepted and rejected in the United States and the number of

exported lots accepted and rejected by the Netherlands testing plan. Decreasing the final accept level of the U.S. testing plan from 25 to 5 μg kg^{-1} increased the number of lots rejected in the United States by 371 % while reducing the number of exported lots rejected by 51 %. For every additional 8.3 lots rejected in the United States, one less export lot will be rejected.

974. Whitaker, T.B., Dickens, J.W., and Chew, V. 1985. Development of statistical models to simulate the testing of farmers stock peanuts for aflatoxin using visual, thin layer chromatography, and minicolumn methods. Peanut Science 12: 94-98.

The negative binomial probability function was used to model the distribution of sample aflatoxin test results when replicated grade samples from fanners stock groundnuts were analysed by thin-layer chromatography and minicolumn methods. The Poisson probability function was used to model the distribution of the number of kernels with visible Aspergillus flavus growth found in replicated grade samples of farmers stock groundnuts when the visible A. flavus method was used. The probabilities of accepting a lot of farmers stock groundnuts with given aflatoxin concentrations when using a 465-g grade sample and two different accept/reject levels were predicted with the models and compared to observed acceptance probabilities computed from previously published data for each of the three methods. The comparisons showed good agreement between the predicted acceptance probabilities and the observed acceptance probabilities.

975. Whitaker, T.B., Dickens, J.W., Davidson, J.I.Jr., and Chew, V. 1984. Development of statistical models to simulate the testing of farmers stock peanuts for aflatoxin using visual, TLC, and minicolumn methods. Proceedings of the American Peanut Research and Education Society 16: 46.

The negative binomial equation was used to simulate the distribution of sample aflatoxin test results when replicated grade samples from farmers stock groundnuts were analyzed by TLC and minicolumn methods. The Poisson equation was used to simulate the distribution of samples according to the number of kernels with visible Aspergillus flavus growth found in replicated grade samples from farmers stock groundnuts when the visible A. flavus method was used. The probability of accepting a lot of fanners stock groundnuts with a given aflatoxin concentration when using a 465-g grade sample and four different accept/reject levels were predicted with the models and compared to observed acceptance probabilities for each of the three methods. Comparisons between predicted acceptance probabilities and observed acceptance probabilities from a previous study were good for each method at each accept/reject level.

976. Whitaker, T.B., Dickens, J.W., and Monroe, R.J. 1970. Comparing the observed distribution of aflatoxin in shelled peanuts to the negative binomial distribution. Proceedings of the American Peanut Research and Education Association 2: 142.

Suitability of the negative binomial distribution for use in estimating the probabilities associated with sampling lots of shelled groundnuts for aflatoxin analysis was studied. Large samples, called "mini-lots", were drawn from 164 lots of shelled groundnuts contaminated with aflatoxin. These mini-lots were subdivided into 10 lb samples which were analyzed for aflatoxin. Variance of the sample means about the mean, M, of the mini-lots from which the samples were taken was determined. These variances were then used to compute the % non-contaminated groundnuts, F(o), in the mini-lots by means of the equation for the negative binomial distribution. The relationship between F(o) and lot mean M was found to be described by the regression equation : F(o) = 99.983 - 0.003 M. The observed distribution of 10 sample means from each of 11 mini-lots were compared to the negative binomial distribution by means of the Kolmogorov-Smirnov test. The null hypothesis that each of the 11 observed distributions were negative binomial was not rejected at the 95 % confidence level.

977. Whitaker, T.B., Dickens, J.W., and Monroe, R.J. 1974. Variability of aflatoxin test results. Proceedings of the American Peanut Research and Education Association 6: 52.

Using 12-pound samples, 280-g subsamplcs. the Waltking method of analysis, the densitometric procedures, the sampling, subsampling, and analytical variances associated with aflatoxin test procedures were estimated. Regression analysis indicated that each of the above variance components is a function of the concentration of aflatoxin in the population being tested. Results, for the test procedures given above, showed that sampling constitutes the greatest single source of error, followed by subsampling and analysis. Functional relationships are presented to detennine the sampling, subsampling, and analytical variance for any size sample, subsample, and number of analyses.

978. Whitaker, T.B., Dickens, J.W., and Monroe, R.J. 1974. Variability of aflatoxin test results. Journal of the American Oil Chemists' Society 51(5): 214-218.

Using 12 lb samples, 280 g subsamples, the Waltking method of analysis, and densitometric procedures, the sampling, subsampling, and analytical variances associated with aflatoxin test procedures were estimated. Regression analysis indicated that each of the above variance components is function of me concentration of aflatoxin in the population being tested. Results, for the test procedures given above, showed that sampling constitutes the greatest single source

of error, followed by subsampling and analysis. Functional relationships are presented to determine the sampling, subsampling, and analytical variance for any size sample, subsample, and number of analyses.

979. Whitaker, T.B., Dickens, J.W., Monroe, R.J., and Wiser, E.H. 1972. Comparison of the observed distribution of aflatoxin in shelled peanuts to the negative binomial distribution. Journal of the American Oil Chemists' Society 49: 590-593.

Suitability of the negative binomial distribution for use in estimating the probabilities associated with sampling lots of shelled groundnuts for aflatoxin analysis was studied. Large samples, called "mini-lots", were drawn from 29 lots of shelled groundnuts contaminated with aflatoxin. These minilots were subdivided into 12 lb samples which were analyzed for aflatoxin. The mean and variance of these aflatoxin determinations for each minilot were determined. The shape parameter K and the mean aflatoxin concentration m were estimated for each minilot. A regression analysis indicated the functional relationship between K and m to be $K=(2.0866+2.3898m) \times 10^{-6}$. The observed distribution of sample concentrations from each of the 29 minilots was compared to the negative binomial distribution by means of the Kolmogorov-Smirnov test. The null hypothesis that each of the true unknown distribution functions was negative binomial was not rejected at the 5 significance level for all 29 comparisons.

980. Whitaker, T.B., Dickens, J.W., and Wiser, E.H. 1970. Design and analysis of sampling plans to estimate aflatoxin concentrations in shelled peanuts. Journal of the American Oil Chemists' Society 47: 501-504.

Methodology for use in the design and evaluation of sampling plans to estimate aflatoxin concentrations in lots of shelled groundnuts is presented. Use of the operating characteristic curve for comparing and evaluating processor and consumer risks related to various sampling plans and application of the negative binomial distribution to estimate probabilities associated with sampling lots of shelled groundnuts for aflatoxin concentration are discussed. Operating characteristic curves are developed for two different single-sample plans, an attribute multiple sample plan, and the plan presently used by the groundnut industry to estimate aflatoxin concentrations in commercial lots of shelled grounduts. An estimated prior distribution of lots according to aflatoxin concentration is used to predict, among others, such values as the % of all lots tested that will be accepted by the sampling plans and the average aflatoxin concentration in the accepted lots. All four of the sampling plans described in the paper are compared on the basis of values such as these. Other factors to be considered in critical evaluation and selection of sampling plans for estimating aflatoxin concentrations in commercial lots of shelled groundnuts are discussed.

981. Whitaker, T.B., Dickens, J.W., and Wiser, E.H. 1976. Monte Carlo technique to simulate aflatoxin testing programs for peanuts. Journal of the American Oil Chemists' Society 53(8): 545-547.

A computer model that accounts for sampling, subsampling, and analytical variability was developed to simulate aflatoxin testing programs. Monte Carlo solution techniques were employed to account for conditional probabilities that arise from multiple samples, subsamples, and/or analyses being used in testing programs. The aflatoxin testing program to be used on the 1974 groundnut crop was evaluated by the use of the described model.

982. Whitaker, T.B., Dickens, J.W., Wiser, E.H., and Monroe, R.J. 1974. Development of a method to evaluate sampling plans used to estimate aflatoxin concentrations in lots of shelled peanuts. Technical Report No. 10, pp. 16. International Union of Pure and Applied Chemistry, Cowley Centre, Oxford OX 3YF, U.K.

The design and evaluation of sampling plans for estimating aflatoxin contamination in groundnuts are discussed. A method to predict the number of good lots that will be rejected and the number of bad lots that will be accepted by a given testing program is presented. Four sampling plans used by the groundnut industry in the United States arc evaluated using the described methodology.

983. Whitaker, T.B., and Wiser, E.H. 1969. Theoretical investigations into the accuracy of sampling shelled peanuts for aflatoxin. Journal of the American Oil Chemists' Society 46(7): 377-379.

Effect of sample size on sampling accuracy was studied in relation to estimate the aflatoxin levels in groundnuts. The negative binomial distribution was used to simulate the actual distribution of aflatoxin since it allowed for a high probability of zero counts along with small probabilities of large counts. Using both the Monte Carlo technique and a direct computation method, the effect of sample size on sampling accuracy is quantitatively described.

984. Yamumoto, K. 1983. A study on small scale sampling for aflatoxin test of peanut. Proceedings of the Japanese Association of Mycotoxicology No. 17 :22-25.

A sampling method according to the binomial distribution rule was intended to be established as a simple system for the qualitative evaluation of aflatoxin contamination in groundnuts. A sampling method is described.

7.2.3 Thin-Layer Chromatography (TLC) Methods

985. Alisauskas, V.A. 1974. Determination of aflatoxin in grain and feedstuffs. Food Technology in Australia 26(6): 233-237.

Ninety-two samples of grain and common feed ingredients were analyzed in Australia using thin-layer chromatography (TLC) and a rapid minicolumn screening method. Three samples contained aflatoxins; a sorghum grain sample containing 30 µg kg^{-1} aflatoxin B_1 and two samples of moldy groundnuts containing 2500 µg kg^{-1} aflatoxin B_1 and 400 µg kg^{-1} aflatoxin B_2, and 200 µg kg^{-1} B_1 and 38 µg kg^{-1} B_2, respectively. A non-destructive detection technique involving scanning the grain with a UV lamp and examination of fluorescence is reported.

986. Altenkirk, B.A., Purchase, I.F.H., and Rensburg, S.J.Van. 1974. Estimation of aflatoxin in peanut butter. South African Medical Journal 48(54): 2256-2258.

Collaborative studies with various laboratories were organized to test the efficiency of various methods, but the results revealed a disturbing frequency of underestimation. The 'best food' (BF) method in conjunction with a personnel training program is recommended.

987. Andreilos, P.J., Beckwith, A.C., and Eppley, R.M. 1967. Photochemical changes of aflatoxin B,. Journal of the Association of Official Analytical Chemists 50(2): 346-350.

Irradiation of aflatoxins B_1 and G_1 with UV light (principal wavelength 365 mµ) converts both compounds to new fluorescent photoproducts which have much lower Rf values than aflatoxin B_1 and G_1 when chromatographed on silica gel thin layer plates. Photoproducts of aflatoxin B_1 form much faster on a silica gel surface than in methanol solution. Photoconversion of aflatoxin B_1 is shown to alter fluorescence comparison assays and identification tests. The principal photoproduct developed from aflatoxin B_1 is significantly less toxic than the parent aflatoxin.

988. Andreilos, P.J., and Reid, G.R. 1964. Confirmatory tests for aflatoxin B_1. Journal of the Association of Official Analytical Chemists 47(5): 801-803.

Three confirmatory tests have been devised to identify aflatoxin B_1. Portions of the isolated toxin are treated with formic acid-thionyl chloride, acetic acid-thionyl chloride, and trifluoroacetic acid, respectively, and aliquots of the three fluorescent reaction products are spotted on thin-layer chromatography (TLC) plates. Standards treated with each of three reagents, plus an untreated standard, are spotted on the same plate, and after development the spots are compared under ultraviolet light.

989. Association of Official Analytical Chemists. 1980. Mycotoxins Methodology, Natural Poisons, in Official Methods of Analysis, Association of Official Analytical Chemists, Chapter 26, Washington, D.C.

Analytical methods based on thin-layer chromatography are described for detection and estimation of aflatoxins in agricultural commodities including groundnuts and groundnut products. Full details of official procedures for sampling, extration, separation and estimation of aflatoxins are given.

990. Banes, D. 1966. Food toxins of fungal origin : methodology and regulatory aspects. Food Technology 20(6): 51-52.

This paper very briefly reviews some analytical methods and bioassays for aflatoxins in groundnuts and groundnut products. Regulatory aspects are also discussed, and the actions of the Food and Drug Administration (FDA) of the US are highlighted.

991. Bartos, J., and Matyas, Z. 1977. [Selection of screening methods for the detection of aflatoxins in feeds.1. Vyber screeningovych metod pro zjistovani aflatoxinu v krinivech. Veterinarni Medicina 22(12): 723-728.

The suitability of five screening methods for the detection of aflatoxin B_1 in 10 different feeds, feed concentrates and complete feed mixtures was compared. Aflatoxin B_1 was found in samples of groundnut cake and protein concentrates for laying hens and breeding pigs. No aflatoxins were detected in soya, sunflower, maize, fish meal, table trout iced, carp stock feed, table carp feed and duckling fattening mixture.

992. Basappa, S.C., Sreenivasa Murthy, V., and Rajalakshmi, D. 1977. Analysis of aflatoxin in groundnut and its products. Indian Journal of Technology 15: 311-313.

A rapid method for aflatoxin analysis in groundnut and its products is described. Aflatoxin-containing food samples in 80 % methanol were dialysed against 20 % aqueous methanol as dialysing solvent. The toxin in the dialysate was estimated by direct extraction with chloroform and quantified by a visual fluorescence extinction method using a long wave UV light. The percentage of aflatoxin dialysed was linear up to 60 min and then decreased gradually. Temperature did not influence the toxin yield. Advantages of the method are : least interference with fluorescence; clean chromatograms; less changes of thermal degradation of compounds; elimination of usual cleanup procedures; and selective separation of the toxin from common interfering substances.

993. Beckwith, A.C., and Stoloff, L. 1968. Fluorodensitometric measurement of aflatoxin thin layer chromatograms. Journal of the Association of Official Analytical Chemists 51(3): 602-608.

The precision limit of visual comparison procedures used in aflatoxin assay methods can be no better than + 20 % for a single observation and, under operating conditions, it is probably close to ± 28 %. Fluorodensitometric procedures described in the literature can improve this precision to ± 9 % for the average of multiple observations. A possible source of inaccuracy in the published densitometric procedures is pointed out and a method using internal standards to avoid the inaccuracy is presented. Tested with spiked extracts of "clean" peanut butter, the procedure gave an average 101 ± 3 % recovery of added allatoxin B_1 and 89 ± 6 % recovery of allatoxin B_2 with a precision for individual assays equal to the precisions reported for the other procedures. The major source of error was shown by elimination to reside in the thin-layer chromatography and aflatoxin instability.

994. Beljaars, P.R., and Fabry, F.H.M. 1972. Quantitative fluorodensitometric measurements of aflatoxin B_1 with a flying-spot densitometer. I. Fluorodensitometric study of the behaviour of aflatoxin B_1 standard spots on different types of silica gel. Journal of the Association of Official Analytical Chemists 55: 775-780.

The procedure for measurements of standard aflatoxin B, spots on thin-layer chromatographic (TLC) plates by flying-spot densitometer is described. Three different types of silica gel layers were spotted with standard aliquots of aflatoxin B_1 covering a range of 0.4-20 ng. Silica gel H plates were developed with a mixture of chloroform-trichloroethylene-n-amyl alcohol-formic acid (80+15+4+1), while silica gel G and MN-G-HR plates were developed with a mixture of chloroform-acetone (90+10). TLC plates were then scanned with a reflectance flying-spot densitometer. Sharp and defined separation patterns for aflatoxins B_1, B_2, G_1, and G_2 were recorded on H and MN-G-HR plates, while unsatisfactory separation was found on G plates. The relationship between peak area and concentration was linear for H and MN-G-HR plates, but not for G plates. Variations of 15-20 % were calculated among the fluorescence of aflatoxin B_1 spots between plates with same sorbent layers. Coefficients of variation (CVs) of 5-7 % for single densitometric measurements on one plate under normal conditions were calculated for a series of 3 or 4 aflatoxin B_1 spots ranging from 1 to 4 ng on H and MN-G-HR plates, whereas CVs of 11-12 % were recorded for G plates. Results are compared with those of previous investigators.

995. Beljaars, P.R., Fabry, F.H.M., Pickott, M.M.A., and Peeters, M J. 1972. Quantitative fluorodensitometric measurement of aflatoxin B_1 with a flying-spot densitometer. II. Comparative study of B, measurements in spiked and naturally contaminated peanut products. Journal of the Association of Official Analytical Chemists 55(6): 1310-1315.

Peanut butter extracts and samples spiked with 5-40 µg aflatoxin B_1 kg^{-1} were analyzed, together with naturally contaminated groundnut products, by three extraction procedures : the official Dutch method (KB), the Liem et al. method (methanol) and the IUPAC method. The latter procedure was selected as a reference method since it has international application. KB extracts were separated on silica gel G plates with a mixture of chloroform-acetone (90+10), whereas IUPAC extracts were separated similarly on MN-G-HR plates. Methanol extracts were resolved on silica gel H plates, using chloroform-trichloroethylene-n-amyl alcohol- formic acid (80+15+4+1) as the developing solvent. After development, plates were scanned with a reflectance flying-spot densitometer. With such techniques, average recoveries for spiked peanut butter extracts ranged from 99 to 105 %, with variation values of 11-12%. Recovery values of 69 % (KB method) and 84 % (methanol and IUPAC methods) were obtained for spiked peanut butter samples. Coefficients of variation ranged from 13 to 15 % for fluorodensitometric measurements. In naturally contaminated groundnuts and groundnut products, precision values were 13.6 % for fluorodensitometric measurements compared to 36 % for visual estimations. Both the methanol and IUPAC methods yield extracts suitable for densitometric analysis after spotting on TLC plates; the analytical results are comparable. Extracts from the KB method contained more interfering fluorescent material than the other two methods.

996. Beljaars, P.R., Verhulsdonk, C.A.H., Paulsen, W.E., and Liem, D.H. 1973. Collaborative study of two-dimensional thin layer chromatographic analysis of aflatoxin B_1 in peanut butter extracts, using the antidiagonal spot application technique. Journal of the Association of Official Analytical Chemists 56(6): 1444-1451.

A collaborative study was carried out by 20 laboratories in The Netherlands, representing governmental and industrial institutes, on the determination of aflatoxin B_1 in peanut butter extracts. Blank peanut butter extracts prepared according to the proposed official Dutch method were spiked with aflatoxin B_1, representing contamination levels of 0, 3, 6, and 12 µg kg^{-1}. Sample extracts and standards were spotted on silica gel G thin-layer chromatographic (TLC) plates by the anitdiagonal spot application technique. Spotted plates were developed by two-dimensional TLC with diethyl ether:methanol:water (94:4.5:1.5) in a lined tank in the first direction and with chloroform:acetone (90:10) in an unlined tank in the second direction. Separated aflatoxin B_1 spots from sample and standard developed in both directions were free from background interference. The quantities of aflatoxin B_1 present in the sample were determined by visual comparison of the fluorescent intensities of samples and standard B_1 spots. For this procedure the variability of measurements

within and between laboratories was statistically investigated : 80-90 % of the complete results given by the participants were correct for the blank and spiked samples (contamination level of 12 µg kg^{-1}). For contamination levels of 3 and 6 µg kg^{-1}, an approximate coefficient of variation of 35 % was calculated from within- and between- laboratory results. Results obtained in this investigation were compared with those found by other investigators who used the one-dimensional TLC technique. It is concluded that, with the antidiagonal procedure, small amounts of aflatoxin B_1 (3 µg kg^{-1}) may be detected.

997. Bottalico, A., Lerario, P., and Ricci, V. 1979. Use of the Velasco method for analysis of mycotoxins in various products of plant origin. Revista della Societa Italiana di Scienza dell' Alimentazione 8: 299-302.

The method of Velasco based on separation of aflatoxins on a Florisil/silica gel/alumina minicolumn and UV fluorometric detection, using a Velasco Fluorotoxin Meter, was compared with visual detection after separation on the minicolumns, and thin-layer chromatographic (TLC) determination. Trials were conducted on spiked samples of groundnuts, soybean and sunflower presscakes, chickpeas, beans, almonds, wheat, maize, barley, cassava, silage, and a feed mixture. Severe interference by other constituents of most of the food or feed samples studied was observed with Velasco's method. The filter fluorometer system did not allow differentiation of interfering impurity bands having maximum fluorescence wavelengths close to those of aflatoxins but differing in colour ; visual evaluation of the column permitted differentiation of these. The Velasco minicolumn/UV fluorometry method gives a total aflatoxin concentration rather than values for individual aflatoxins, whereas TLC gives data for concentration of individual aflatoxins. It is concluded that TLC techniques are likely to give better results than Velasco's method.

998. Broadbent, J.H., Cornelius, J.A., and Shone, G. 1963. The detection and estimation of aflatoxin in groundnuts and groundnut materials. Part II. Thin-layer chromatographic method. Analyst 88: 214-216.

A semi-quantitative method capable of detecting 0.006 µg of aflatoxin B_1 in groundnuts and groundnut products, especially groundnut meals, by thin-layer chromatography (TLC), is described.

999. Campbell, A.D., and Funkhouser, J.T. 1966. Collaborative study on the analysis of aflatoxins in peanut butter. Journal of the Association of Official Analytical Chemists 49(4): 730-739.

A second collaborative study was carried out on the determination of aflatoxin in peanut butter under the sponsorship of the Aflatoxin Methodology Working Group. Thirteen collaborators, representing industrial, independent, and government laboratories, analyzed 12 peanut butter samples containing known amounts of aflatoxin at levels of 10 and 110 µg kg^{-1} of peanut butter. Two samples of naturally contaminated peanut butter containing aflatoxins B_1 and B_2 were also analyzed. The study was designed to estimate the accuracy and precision of the method, both within laboratories and between laboratories. Some samples and collaborators were common to both this study and an international study sponsored by the IUPAC. Results of the two studies have been compared. The method was recommended for adoption as official, first action.

1000. Cauderay, P. 1979. Rapid chemical confirmation method for aflatoxins B_1 and G_1 by direct acetylation on a thin layer plate before chromatography. Journal of the Association of Official Analytical Chemists 62(1): 197.

A method of acetylation of aflatoxins B_1 and G_1 before identification is described, Extracts containing aflatoxin B_1 and/or G_1 are spotted on a silica gel TLC plate and overspotted with acetic anhydride and HCl. After reaction, the plate is developed and the Rf values of reacted aflatoxin B, and/or G_1 compared with those of authentic standards treated similarly. The procedure has been successfully applied to a variety of samples such as groundnuts, pistachios, hazelnuts and almonds.

1001. Chang, H.H.L., DeVries, J.W., and Hobbs, W.E. 1979. Comparative study of two methods for extraction of aflatoxin from peanut meal and peanut butter. Journal of the Association of Official Analytical Chemists 62(6): 1281-1284.

The difference between the CB and BF methods in extracting aflatoxins from groundnut products was studied. The CB method gave 60, 121, 35, and 22 % higher results for aflatoxins B_1, B_2, G_1, and G_2, respectively for four samples of groundnut meal and six samples of peanut butter tested. Both reverse-phase liquid chromatography and thin-layer chromatography were used to quantitate the extracted aflatoxins.

1002. Chang, C M., and Lynd, J.Q. 1968. UV aflatoxin quantitation with polaroid recordings. Agronomy Journal 60: 582-584.

Polaroid color print recordings were made of aflatoxins separated by thin-layer chromatography (TLC) and revealed by fluorescence in ultraviolet light.

1003. Chang-Yen, I., Stoute, V.A., and Felmine, J.B. 1984. Effect of solvent composition on aflatoxin fluorescence. Journal of the Association of Official Analytical Chemists 67(2): 306-308.

The influence of the chloroform-methanol solvent system on the fluorescence of

aflatoxins B_1, B_2, G_1, and G_2 was investigated. Fluorescence intensity is markedly affected by solvent composition. The formation of excited state dimers and ionization are proposed to explain the effects observed.

1004. Chen, Shui-Chin, and Friedman, L. 1966. Anatoxin determination in seed meal. Journal of the Association of Official Analytical Chemists 49(1): 28-33.

An assay method for aflatoxin B_1 in cottonseed meal and groundnut seed meal with a sensitivity limit of < 0.02 µg kg^{-1} is described. The sample is extracted with aqueous methanol in a Waring blendor. The extract is purified by lead acetate precipitation, followed by partition chromatography on Celite, and assayed by comparison of fluorescence intensity with aflatoxin B_1 standards on silica gel G-HR thin-layer chromatograms. This method eliminated potential interference from a "new blue spot" found in cottonseed meal extracts.

1005. Chu, F.S., Lee, R.C., Trucksess, M.W., and Park, DX. 1988. Evaluation by enzyme-linked immunosorbent assay of cleanup for thin-layer chromatography of aflatoxin B_1 in corn, peanuts, and peanut butter. Journal of the Association of Official Analytical Chemists 71(5): 953-956.

A simple, rapid enzyme-linked immnosorbent assay (ELISA) was used to evaluate the performance of each step (extraction, filtration, solvent partition and silica gel column chromatography) of a solvent-efficient thin-layer chromatography (TLC) method undergoing interlaboratory collaborative study for the determination of aflatoxin B_1 in maize, raw groundnuts and peanut butter. The apparent mean recoveries using the ELISA method were approximately 30-50 % higher than those using the TLC method if only the amount of aflatoxin B, added to the samples was used in the calculations. After the cross reaction of the antibody with other aflatoxins added to the samples was considered, the amounts recovered approached the levels of the aflatoxins added in all the three commodities tested. With no cleanup treatment, ELISA recoveries at aflatoxin B_1 levels >7.5 ng g^{-1} were 84, 79 and 103 % for maize, raw groundnuts, and peanut butter, respectively. With each cleanup step in the TLC method, ELISA detected a progressive decrease in recovery from 150.5 to 105.3 % (before correction for the presence of other aflatoxins) or from 93.5 to 65.4 % (after correction for other aflatoxins) of aflatoxin B_1 added to samples. It is concluded that cleanup treatments are not necessary in the ELISA and that when large amounts of other aflatoxins are present, an understanding of the cross-reactivity of antibody with other aflatoxins in the ELISA is essential for final interpretation of the data.

1006. Coker, R.D. 1984. High performance liquid chromatography and other chemical quantification methods used in analysis of mycotoxins in foods. Pages 207-263 in Analysis of Food Contaminants (Gilbert, J., ed.), Elsevier Science Publishers, London, UK.

The importance of sampling, sample preparation and assay techniques in the analysis of mycotoxins in various agricultural commodities is discussed, with emphasis on the required efficiency of the quantification step. Thin-layer chromatography (TLC), high performance dun-layer chromatography (HPTLC), high performance liquid chromatography (HPLC), gas-liquid chromatography (GLC), immunoassay, fluorotoxin meter and other methods are described and compared. Future trends in the analysis of mycotoxins are oudined.

1007. Coomes, T.J., Crowther, P.C., Francis, B.J., and Shone, (I. 1964. The detection and estimation of aflatoxin in groundnuts and groundnut materials. Part III. Classification of aflatoxin B_1 levels. Analyst 89: 436-437.

Paper-chromatography and thin-layer chromatography methods were compared for detection and estimation of aflatoxin in groundnuts and groundnut meals. The lowest weight of pure aflatoxin B_1 that gave an observable fluorescence on Whatman No. 1 filter-paper after development in the solvent system described by Coomes and Sanders (1963) was determined as 0.1 µg while it was 0.003 µg on alumina chromatographic plates (740 ±10 µ thick), under the same excitation conditions (Y - 365 mµ). Work on extracts of groundnuts, using Kieselgel G chromatographic plates, indicated that more satisfactory estimates of known aflatoxin levels were feasible by using the technique of diluting the extract until no fluorescence was observed on the plates. However, over-estimates of the toxin are possible with certain samples.

1008. Coomes, T.J., Crowther, P.C., Francis, B.J., and Stevens, L. 1965. The detection and estimation of aflatoxin in groundnuts and groundnut materials. Part IV. Routine assessment of toxicity due to aflatoxin B_1. Analyst 90: 492-496.

A sensitive, high-resolution thin-layer chromatographic (TLC) method is described for determining aflatoxin B1 in groundnut kernels, groundnut meals and peanut butter by fluorescence. Kieselgel G is used as adsorbent and is shown to offer advantages over alumina. Aflatoxin levels are determined by a dilution technique.

1009. Coomes, T.J., and Feuell, A.J. 1965. Recommended procedures for the detection and estimation of afaltoxin B_1 in groundnuts and groundnut materials. TPI Report No. G13, 24 pp.

This report reviews some of the analytical methods available for the analysis of groundnuts and groundnut products for aflatoxin B_1. Full details of recommended procedures for sampling, extraction, separation and estimation of aflatoxin B_1 are given.

1010. Coomes, TJ., and Sanders, J.C. 1963. The detection and estimation of aflatoxin in groundnuts and groundnut materials. Part 1. Paper-chromatographic procedure. Analyst 88: 209-213.

A method based on paper-chromatography for the detection and semi-quantitative determination of aflatoxin B_1 is described. The correlation between the analytical results by this method and the biological response in ducklings based on the ten groundnut samples tested appeared satisfactory.

1011. Coon, F.B., Baur, F.J., and Symmes, L.R.L. 1972. International aflatoxin check sample program : 1971 study. Journal of the Association of Official Analytical Chemists 55(2): 315-327.

An International Aflatoxin Check Sample Committee was formed in 1971 to establish a check sample program of commodities or materials with aflatoxin contamination, which should be available to laboratories throughout the world. A set of four aflatoxin- contaminated groundnut samples was prepared and sent to 150 participating laboratories. Sufficient data were obtained from this study to permit a statistical comparison of three of the four AOAC methods for analysis of aflatoxins in groundnuts and groundnut products. The analysis showed significantly higher means for the CB method than for the BF or Pons method for two of the samples examined.

1012. Coon, F.B., Baur, F.J., and Symmes, L.R.L. 1973. International aflatoxin check sample program : 1972 study. Journal of the Association of Official Analytical Chemists 56(2): 322-327.

In the second International aflatoxin check sample series, a single peanut butter sample was submitted to 152 laboratories throughout the world. Sufficient data were obtained from 117 responding laboratories to permit a statistical comparison of the BF, CB, and Pons methods for aflatoxin determinations. No significant differences in mean values were observed. One quarter of the participating laboratories were from countries other than Canada and the United States.

1013. Crisan, E.V. 1968. A 2,4-dinitrophenylhydrazine spray for the identification of aflatoxin B, on thin-layer chromatoplates. Contributions from Boyce Thompson Institute 24(2): 37-38.

Aflatoxin B_1 reacts with 2,4-dinitrophenylhydrazine (DNPH) to form a colored aflatoxin dinitrophenylhydrazone. This derivative can be prepared in situ on thin-layer chromatograms of crude extracts by spraying them with a DNPH reagent solution. The use of this reagent spray provides a simple method whereby the identity of a fluorescent chromatographic spot suspected of being aflatoxin B_1 can be confirmed rapidly in situ.

1014. Crisan, E.V., and Grefig, A.T. 1967. The formation of aflatoxin derivatives. Contributions from Boyce Thompson Institute 24(1): 3-8.

Aflatoxins B_1 and B_2 contain a reactive carbonyl group in their cyclopentenone rings, which can take part in several classical carbonyl reactions. Procedures are given for forming the oximes and 2,4-dinitrophenylhydrazones of the B toxins. Lacking the carbonyl group, aflatoxins G_1 and G_2 are nonreactive. The formation of the aflatoxin derivatives is proposed as confirmatory tests for aflatoxins B_1 and B_2. These procedures can also be used to remove traces of the B toxins from preparations of the G toxins.

1015. Crisan, E.V., and Mazzucca, E. 1967. Separation of aflatoxin on selectively deactivated silicic acid. Contributions from Boyce Thompson Institute 23(11): 361-365.

A reliable method for separation of individual aflatoxins from crude extracts is described, based on the use of selectively deactivated adsorbents. Activated Silic AR, Mallinckrodt type CC- 4, permitted recovery of 87 % of the aflatoxin B_1 contained in a crude extract. Methods of purifying the remaining aflatoxin G are also described. Using these methods, working quantities of the major aflatoxins, B_1 and G,, can be obtained.

1016. Crosby, N.T. 1984. Review of current and future analytical methods for the determination of mycotoxins. Food Additives and Contaminants 1(1): 39-44.

Chemical methods of analysis for the extraction, cleanup and determination of aflatoxins from animal feedingstuffs and foods arc described. The advantages and disadvantages of thin-layer chromatography (TLC) and high pressure liquid chromatography (HPLC) arc discussed. Immunoassays are briefly discussed. Methods for other mycotoxins such as ochratoxin, patulin, and the trichothecenes are also outlined.

1017. Cucullu, A.F., Lee, L.S., Mayne, R.Y., and Goldblatt, L.A. 1966. Determination of aflatoxins in individual peanuts and peanut sections. Journal of the American Oil Chemists' Society 43(2): 89-92.

A micro method, adapted from the aqueous acetone procedure proposed by Pons and Goldblatt for the determination of aflatoxins in cottonseed products, was developed to permit accurate determination of aflatoxins in individual groundnut kernels and kernel sections. Use of this procedure permitted the topographic distribution of

aflatoxins within individual kernels to be mapped and indicated that the toxins were not uniformly distributed within contaminated kernels, even when the kernel contained a high level of aflatoxins. Although wrinkling or discoloration sometimes indicated that a kernel was contaminated, this type of physical damage was not found to be a reliable indication of aflatoxin content. Also it was noted that a few apparently sound and mature kernels contained high levels of aflatoxins.

1018. Cucullu, A.F., Lee, L.S., Pons, W.A.Jr., and Goldblatt, L.A. 1970. Determination of aflatoxins in peanut and cottonseed soapstocks. Journal of the American Oil Chemists' Society 47(6): 226-230.

An accurate and sensitive procedure is proposed for estimating aflatoxins in both alkaline and acidualated soapstocks. Sample suspensions in aqueous acetone are adjusted to pH 3 with hydrochloric acid, extracted in a high speed blender, treated with lead acetate and partitioned into chloroform. After silica gel cleanup, aflatoxins in purified extracts are estimated by thin-layer chromatography. The use of acetone and lead acetate together apparently catalyzes the relactonization of aflatoxins B_1 and G_1 and leads to essentially quantitative recovery of aflatoxin B_1 and somewhat lower recovery of G_1 added to alkaline or acidulated soapstock.

1019. Czerwiecki, L. 1976. fSpectrofluorometric method for determination of aflatoxins in groundnuts.]. Spektrofluorymetryczna metoda oznaczania zawartosci allatoksyn w orzechach arachidowych. Roczniki Panstwowego Zakladu Higieny 27(6): 621-627.

A spectrofluorometric method is described for quantitative determination of aflatoxins B_1, B_2, G_1 and G_2 in groundnuts. Aflatoxins were extracted with an 80:20 mixture of acetone and water. The extracts were purified with a 20 % solution of lead acetate and then on an aluminium oxide column. After chromatographic separation of the aflatoxins on a thin-layer plate, the gel areas fluorescing in UV light were eluted with ethanol. The intensity of the fluorescence was measured with an Aminco-Bowman spectrofluorimeter. The content of aflatoxins was determined on the basis of standard curves in ethanol. The detectability of aflatoxins B_1, B_2, G_1, and G_2 was respectively 5.0, 2.0, 3.0, and 0.5 µg kg^{-1}.

1020. Davidson, J.I.. Dickens, J.W., Chew, V., Sanders, T.H., Holaday, C.E., Cole, R.J., and Whitaker, T.B. 1984. Performance of the visual, minicolumn and TLC methods in detecting aflatoxin in 20 contaminated lots of farmers stock peanuts. Peanut Science 11(2): 77-83.

Standard grade samples (16) from each of 20 selected minilots were used to evaluate three methods for detecting minilots of farmers stock groundnuts with unacceptable levels of aflatoxin. A visual, a minicolumn and a modified thin-layer chromatography (TLC) method were used to compare analytical results, variation, and probability of acceptance for minilots having mean aflatoxin levels ranging from 8 to 255 µg kg^{-1}. Mean values obtained by each of the three methods increased linearly with mean aflatoxin levels of the minilots and variation for each method as determined by the variance and coefficient of variation (CV) was very large. The CV for all three methods decreased as aflatoxin level increased. Overall performances of the methods were similar in accepting and rejecting these minilots on the basis of the 1.8 kg grade samples. The greatest difference in the three methods occurred at the zero acceptance level where the modified TLC, minicolumn and visual methods rejected 97, 98 and 88 %, respectively, of the minilots with more than 60 µg kg^{-1} aflatoxin. At this acceptance level the TLC, minicolumn and visual methods also rejected 55, 50 and 30 %, respectively, of the minilots with < 30 µg kg^{-1} aflatoxin.

1021. Davis, N.D., Guy, M.L., and Diener, U.L. 1980. A fluorometric rapid screen method for aflatoxin in peanuts. Journal of the American Oil Chemists' Society 57(3): 109-110.

Groundnuts were screened for aflatoxin using a rapid, inexpensive fluorometric method. Groundnuts were ground and extracted with methanol, and the extract was treated with acidified zinc- acetate-sodium chloride solution, filtered and diluted with water. Fluorescence of the extracts was compared with that from aflatoxin-free control groundnuts. Test samples (160) of several varieties and grades of groundnuts, obtained from storage and several commercial sources, were screened for the presence of aflatoxin. One hundred thirty-five samples (84 %) were identified by this method as aflatoxin positive (> 15 µg kg^{-1}) or aflatoxin negative (< 15 µg kg^{-1}). Although 22 samples (13.6 %) were incorrectly labeled as aflatoxin positive, most of these showed evidence of the presence of mold metabolites other than aflatoxin. Three samples (1.8 %) were incorrectly labeled as aflatoxin negative when they actually contained 20, 33 and 34 µg kg^{-1} aflatoxin.

1022. Davis, N.D., Hayes, A.W., Eldridge, D.W., and Diener, U.L. 1966. Note on the isolation and purification of aqueous solutions of aflatoxins from fermentation medium. Journal of the Association of Official Analytical Chemists 49(6): 1224-1226.

A method is described for the separation and preparation of aqueous solutions of aflatoxins pure enough for experimentation with most biological systems. It is simple and rapid and has been used successfully for preparing solutions of aflatoxins G_2 and C^{14}-labeled B_1. The toxicity of aqueous solutions of aflatoxin B_1, prepared as described, was confirmed by a bioassay using fertile chicken eggs. The method has the advantage over other procedures in that the final product is dissolved in water and may be directly utilized in experiments involving most biological systems.

1023. delongh, H., Van Pelt, J.G., Ord, W.O., and Barrett, C.B. 1964. A semi-quantitative determination of aflatoxin B_1 in groundnut meal, groundnuts, and peanut butter. Veterinary Record 76: 901- 903.

A method for the semi-quantitative determination of aflatoxin B_1 in groundnut meal, groundnuts and peanut butter is described. A specially purified extract of the groundnut product is examined by thin-layer chromatography (TLC), making use of the intensive blue-violet fluorescence of the toxin, the intensity of the aflatoxin B_1 spot is visually compared with that of a known concentration of pure aflatoxin B_1.

1024. DeVries, J.W., and Chang, H.L. 1982. Comparison of rapid high pressure liquid chromatographic and CB methods for determination of aflatoxins in corn and peanuts. Journal of the Association of Official Analytical Chemists 65(2): 206-209.

A method is described for the rapid determination of aflatoxins in corn and groundnut samples by high pressure liquid chromatography. The method was compared with the current CB method. For seven samples of corn and 14 samples of groundnut meal and peanut butter, the correlation between methods was 0.991, and no significant differences were noted between methods for aflatoxins, using the students' t-test at 15.7 % x-risk.

1025. Dickens, J.W., McClure, W.F., and Whitakcr, T.B. 1980. Densitometric equipment for rapid quantitation of aflatoxins on thin layer chromatograms. Journal of the American Oil Chemists' Society 57: 205-208.

A densitometric instrument (Spotmeter) has been designed for rapid measurement of aflatoxin on thin-layer chromatographic plates. The Spotmeter measures the total amount of fluorescence of the aflatoxin spot and this eliminates the need for scanning mechanisms, plotters and integrators incorporated into most commercially available densitometers used for aflatoxin analyses. In comparison with a Schoeffel SD 3000 Spectrodensitometer, the Spotmeter was more accurate in one test and at least equally accurate in another test. The Spotmeter made measurements in 1/4 the time required for the Spectrodensitometer and would cost about 1/6 as much as commercially available densitometers suitable for aflatoxin analyses.

1026. Dickens, J.W., and Whitaker, T.B. 1983. Dilution errors in aflatoxin determinations caused by compounds extracted from peanuts. Journal of the Association of Official Analytical Chemists 66(5): 1059-1062.

Several methods have been developed to analyze groundnuts for aflatoxin by using thin-layer chromatography (TLC); solvent solutions used to extract aflatoxin from groundnuts also extract measurable quantities of other compounds such as oils, fats, sugars, and protein. The volume of these extracted compounds causes error in measuring the proportion of the solvent solution analyzed for aflatoxin. Also, because the cleanup procedures for some methods are inadequate, the volume of some of these extracted compounds also causes error in measuring the proportion of the extracted aflatoxin placed on TLC plates. These two errors cause underestimation of aflatoxin concentrations by approximately 11, 14, and 5 % for the CB method, the modified version of the BF method generally used for raw groundnuts, and a water slurry method, respectively. The correction specified by the CB method for fats in the extraction solvent reduces the approximate error for the CB method from 11 to 1 %.

1027. Dimitrov, M., Doncheva, I., Bonchev, N., and Dikova, 1. 1986. The problem of aflatoxins in foods, and the need for precise methods for their detection. Khigiena i Zdraveopazvane 29(3): 39-43.

Reference samples of dried milk from WHO's International Agency for Research on Cancer (1ARC) were analysed for aflatoxin M_1 by a mycotoxicology laboratory in Bulgaria and by laboratories in other countries, using one-dimensional or two-dimensional thin-layer chromatography (TLC). Modification of the method specified in the relevant Bulgarian standard for detection of aflatoxin in milk and milk products is recommended in the light of the results. Similar collaborative studies on detection of aflatoxins in maize and groundnut meal are also reported.

1028. DiProssimo, V.P. 1974. Scale-up of AOAC Method III for quantitation and isolation of aflatoxins in nuts and nut products for chemical and biological confirmation. Journal of the Association of Official Analytical Chemists 57(2): 349-352.

The official AOAC Method III for aflatoxins in groundnuts and groundnut products (Best Foods or BF method) has been adapted to a larger sample size (500 g), approaching the sample handling capacity of the 1 kg official AOAC Method I (FDA Contaminants Branch or CB method). The modified procedure (NY method) was used in place of the 1 kg CB method for obtaining quantitative assay results and for isolation of pure aflatoxin B_1 for derealization and bioassay. Two samples of naturally contaminated unshelled pistachio nuts and four groundnut product samples were analyzed using the modified NY method and the results were compared with quantitative assays using official methods. Results were in excellent agreement with the official methods. The thin-layer chromatographically pure aflatoxin B_1 recovered using the modified procedure on various products was 39 to 57 % of that calculated to be present in the samples, a favorable recovery compared to the 1 kg CB method (usually about 50 %). All derealizations carried out were successful (3 samples) and all biological confirmations were successful (2 samples were tested by the chick embryo bioassay for aflatoxin B_1 toxicity).

1029. Egmond, H.P.van., and Wagstaffe, P.J. 1989. Aflatoxin B_1 in peanut meal reference materials : tntercomparisons of methods. Food Additives and Contaminants 6(3): 307-319.

The results of two intercomparison exercises involving some 20 European laboratories who applied a wide variety of analytical methods to determine aflatoxin B_1 levels in groundnut meal are presented. It is shown that the major source of error and discrepancy is connected with incomplete extraction and/or losses during cleanup and that, provided correction for recovery/background interference is made, many methods can achieve acceptable accuracy. Sources of error and their control are discussed, and essential details of the methods used are presented. It is concluded that analytical quality assurance is more important than the use of standarized methods when a high degree of accuracy and comparability are required.

1030. Eller, K.I., Maximenko, L.V., and Tutelyan, V.A. 1982. An improved method for aflatoxin content determination in different foods of plant origin. Voprosy Pitaniya 6: 62-66.

The method described involves extract purification by two- dimensional thin-layer chromatography and quantitative determination by fluorescence. Using this technique, aflatoxin B_1 was delected in 24 of 145 food samples examined. Maximum aflatoxin content was in maize and groundnuts. Maize samples also contained aflatoxins B_2, G_1 and G_2,

1031. Eppley, R.M. 1966. A versatile procedure for assay and preparatory separation of aflatoxins from peanut products. Journal of the Association of Official Analytical Chemists 49(6): 1218-1223.

A procedure in which the direct extraction of a water-wetted sample by chloroform is combined with the use of a silica gel column for defatting and cleanup has been devised for obtaining clean quantitative extracts of aflatoxins from 50-g and 1-kg groundnut product samples. The low level of interference permits the detection and estimation of as little as 1 µg aflatoxins kg^{-1} of groundnut product sample.

1032. Eppley, R.M. 1968. Screening method for zearalenone, aflatoxin, and ochratoxin. Journal of the Association of Official Analytical Chemists 51(1): 74-78.

A rapid and sensitive procedure has been developed for detecting aflatoxin, ochratoxin, and zearalenone. This procedure uses a water-chloroform extraction combined with sequential elution of the mycotoxins from a silica gel column. Applicability to a wide variety of products was demonstrated by analysis of 31 different commodities. Three commodities (cottonseed, green coffee, and capsicum peppers) contained serious interferences for all three toxins.

1033. Eppley, R.M., Stoloff, L., and Campbell, A.D. 1968. Collaborative study of "A versatile procedure for assay of aflatoxins in peanut products", including preparatory separation and confirmation of identity. Journal of the Association of Official Analytical Chemists 51(1): 67-73.

A collaborative study of the CB procedure for aflatoxin in groundnut products was carried out on naturally contaminated peanut butter and groundnut meal and on peanut butter spiked with aflatoxins B_1 and G_1. Part of the study included preparatory isolation of the aflatoxin B_1 found in the naturally contaminated samples and confirmation of the identity by both chemical and biological tests. The results from 13 collaborators demonstrated both between- and within- laboratory precision and accuracy equal to the official, first action procedure ; advantages in speed and convenience were noted. The preparatory separation was easily accomplished with clear chemical and biological proof of satisfactory isolation by seven of the nine participants in this phase of the study.

1034. Fischbach, H. 1969. Report of the Joint AOAC-AOCS Aflatoxin Committee. Journal of the Association of Official Analytical Chemists 52(5): 970-975.

The Joint AOAC-AOCS Aflatoxin Committee, first proposed in 1964 and an active committee in 1965, coordinates methods development in the two societies. The history and accomplishments of this committee are outlined and the areas of interest of the AOAC and the AOCS arc defined. Since the committee's inception the following actions have been taken : AOAC has adopted two methods for groundnuts and groundnut products and a chemical derivative confirmation test as official final action and a method for cottonseed as official first action ; the AOCS has adopted one of the above methods for groundnuts and groundnut products as a tentative method. The AOAC Associate Referee topics for aflatoxins are listed and the current status of research under these topics is described.

1035. Friesen, M.D., and Garren, L. 1982. International Mycotoxin Check Sample Program : Part 1. Report on laboratory performance for determination of aflatoxins B_1, B_2, G_1, and G_2 in raw peanut meal, deoiled peanut meal, and yellow corn meal. Journal of the Association of Official Analytical Chemists 65(4): 855-863.

Three aflatoxin-contaminated samples (raw groundnut meal, deoiled groundnut meal, and yellow corn meal) were analyzed by 121 laboratories in 31 countries. Sufficient data were obtained to permit a statistical comparison of the performance of laboratories using the BF, CB, and EEC methods and those using high performance liquid chromatography (HPLC) for quantitation. No significant differences were found between means for laboratories using these four methods for the analysis of raw groundnut meal or yellow corn meal. However, for deoiled groundnut meal,

means were significantly different for laboratories using BF method compared with the CB or EEC methods for B_1 and B_2, and for laboratories using the CB method compared with HPLC methods for G_2.

1036. Friesen, M.D., Walker, E.A., and Castegnaro, M. 1980. International check sample program. Part I. Report on the performance of participating laboratories. Journal of the Association of Official Analytical Chemists 63(5): 1057-1066.

Three aflatoxin-contaminated samples, raw groundnut meal, finished peanut butter, and white corn meal, were analyzed by 139 laboratories in 34 countries. Sufficient data were obtained to permit a statistical comparison of the performance of laboratories using the BF, CB, and Pons methods and those using high performance liquid chromatography for quantification. A raw groundnut meal sample showed no significant differences among means for laboratories using the four methods, and a white corn meal sample showed only one such significant differences; however, a finished peanut butter sample containing less than 10 (ig total aflatoxins kg^{-1} showed 10 significant differences among means for laboratories using the four methods considered.

1037. Fritz, W., Engst, R., and Krug, G. 1977. Problems concerning mycotoxin determination. Zeszyty Problemowe Postepow Nauk Rolniczych 189: 285-290.

Methods used in the German Democractic Republic for detecting specific aflatoxins in routine inspection of foods are discussed. Some moldy foods (cherries, strawberry jam, grapes and baby foods) contained fluorescent compounds which were not aflatoxins, but absence of aflatoxins was only demonstrated by UV or fluorescence spectroscopy. The reagent 'Echtblausalz-B' was found useful for spraying TLC plates, as specific color compounds are formed with aflatoxins. Elution of fluorescent spots from TLC plates followed by UV spectroscopy can detect 0.01 µg aflatoxin kg^{-1}. Fluorescence spectrophotometers can record spectra directly from TLC plates, and direct fluorodensitometric quantification is possible. Recovery of aflatoxin B_1 is 89 %, standard deviation 6.4 % with a variation coefficient of 7.3 %. Similar methods can be applied to ochratoxins. Aflatoxin contents of oranges and groundnuts determined during routine investigation of spontaneously molded foods are given.

1038. Genest, C., and Smith, D.M. 1963. A note on the detection of aflatoxins in peanut butter. Journal of the Association of Official Agricultural Chemists 46: 817-818.

A modified method for analysis of aflatoxins in peanut butter is described. Samples are first treated with a mixture of pentane:hexane (4:1) to remove oil, and aflatoxins are then extracted with methanol in a Soxhlet extraction apparatus. The methanolic solution (yellow solution) is filtered through glass wool and washed with the pentane:hexane mixture to remove residual oil. The precipitate is filtered off and the methanolic solution is evaporated to dryness, the residue is dispersed in distilled water and then saturated with sodium chloride. After deflating, aflatoxins are partitioned into chloroform for cleanup with column chromatography. Aflatoxins are eluted with chloroform:methanol (95:5, v/v), the eluate is evaporated to dryness, and the residue is then dissolved in chloroform. Aflatoxins are determined by thin-layer chromatography.

1039. Goto, T., and Manabe, M. 1989. Methods for the analysis of aflatoxins in groundnut and other agricultural commodities. Pages 173-182 in Aflatoxin contamination of groundnut : Proceedings of the International Workshop, 6-9 October 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

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. Recently several enzyme-linked immunosorbent assay (ELISA) system kits for aflatoxin analysis have been developed and some of them found suitable for the kind of tesing 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.

1040. Hagan, S.N., and Tietjen, W.H. 1975. A convenient thin layer chromatographic cleanup procedure for screening several mycotoxins in oils. Journal of the Association of Official Analytical Chemists 58(3): 620-621.

A thin-layer chromatography (TLC) cleanup development with benzene : hexane (3:1) effectively removed lipids and some contaminants from mixtures of mycotoxins in com oil, olive oil, groundnut oil, soybean oil, and seed extracts. A second development in the same direction as the first, using toluene : ethyl acetate : formic acid (6:3:1) or benzene : acetic acid (9:1), separated the mycotoxins. Satisfactory separation was achieved for commercial oils spiked with sterigmatocystin, zearalenone, ochratoxins A, B, and C, and aflatoxins B_1, B_2, G_1, and G_2. This technique permits detection of 5 µg kg^{-1} aflatoxin B_1 in corn.

1041. Hartley, R.D., Nesbitt, B.F., and O'Kelly, J. 1963. Toxic metabolites of Aspergillus flavus. Nature 198: 1056-1058.

Four closely related aflatoxins B_1, B_2, G_1, and G_2 were isolated and characterized and their inter-relationships studied. The molecular formulae of the aflatoxins were established from elementary analyses and mass spectrometric determinations. The infra-red and ultraviolet absorption spectra of the four aflatoxins were very similar and indicated that all four compounds were closely related. Aflatoxins B_2 and G_2 were far less toxic than B_1 and G_1.

1042. Heusinkveld, MR., Shcra, C.C., and Baur, F.J. 1965. Note on aflatoxin analysis in peanuts, peanut meals, and peanut products. Journal of the Association of Official Agricultural Chemists 48(2): 448-449.

A rapid analytical method for aflatoxin in groundnuts and groundnut products is described. Aflatoxins are extracted with an acetone:hexane:water solvent mixture (60:35:5. v/v/v). The extract is filtered and the solvent evaporated, the residue is dissolved in tetrahydrofuran, and then transferred to a Florisil-packed chromatographic column. Lipids and other extraneous materials are eluted together with tetrahydrofuran, and the aflatoxins are eluted with acetone. After evaporation of acetone, the aflatoxin residue is taken up in chloroform. Aflatoxins are then determined by thin-layer chromatography.

1043. Howell, M.V., and Taylor, P.W. 1981. Determination of aflatoxins, ochratoxin A and zearalenone in mixed feeds, with detection by thin-layer chromatography or high performance liquid chromatography. Journal of the Association of Official Analytical Chemists 64(6): 1356-1363.

A sensitive, reliable, and economical method for the determination of six mycotoxins in mixed feeds is described. The feed is extracted with chloroform-water and the extract is cleaned up using a disposable Sep-Pak silica cartridge. The procedure requires less time (15 min from sample extraction to extract preparation) and less solvent (approximately one-tenth) compared with conventional methods and is suitable for a fast, economical screen. Additional cleanup procedures, involving dialysis or extraction into base, are described for samples containing high levels of interfering compounds. Thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC) with fluorescence detection are described for identification and estimation of mycotoxins. The method has been applied to a wide range of mixed feeds, including laboratory animal diets and raw materials. The limit of detection is 1 μg kg^{-1} for all mycotoxins measured by 11PLC.

1044. Jemmali, M. 1973. Collaborative studies on the determination of aflatoxins in peanut products in France. Pure and Applied Chemistry 35(3): 267-270.

In order to develop a method for the determination of aflatoxins in groundnuts, groundnut products, cereals and cereal products, a comparative study was made of some existing procedures (CB, TPI, BF, Cclite, Pons). Collaborative studies using these procedures were undertaken. The CB procedure was selected on account of the quality of the extracts obtained, the low level of background interference and the relative precision and simplicity of the required equipment. Taking into account the frequently high levels of aflatoxin B, (around 1 ppm) in groundnuts and the quality of silica gel and chloroform available in France, some minor changes have been incorporated into the CB procedure : the chloroform used for the column is washed and dried before use, the volume of elution is increased and an ether : methanol : water (96:3:1) mixture is used as a developing system.

1045. Jones, B.D. 1972. Methods of aflatoxin analysis. Report G 70, T.P.I, London, UK, 58 pp.

This report reviews some of the analytical methods available for the analysis of foods and feedingstuffs for aflatoxin, particularly those which are suitable for use in laboratories in developing countries. Full details of procedures for sampling, extraction, separation and estimation of aflatoxins are given. Methods for the determination of the concentration and purity of aflatoxin standards and the recommendations for the bulk sampling of commodities for aflatoxin analysis are also given.

1046. Kamimura, H., Nishijima, M, Yasuda, K., Ushiyama, H., Tabata, S., Matsumoto, S., and Nishima, T. 1985. Simple, rapid cleanup method for analysis of aflatoxins and comparison with various methods. Journal of the Association of Official Analytical Chemists 68(3): 458-461.

A method is described for simple and rapid detection of aflatoxins in corn, buckwheat, groundnuts and cheese. Aflatoxins were extracted with a chloroform-water solvent and were purified by a florisil column chromatographic procedure. Column eluates were concentrated and spotted on a high performance thin-layer chromatography (HPTLC) plate, which was then developed in chloroform acetone (9:1) and/or ether:methanol:water (94:4.5:1.5) or chloroform:isopropanol:acetone (85:5:10). Aflatoxins were quantitated by densitometry. The minimum detectable aflatoxin concentration (μg kg^{-1}) in various test materials were 0.2 B_1. 0.1 B_2, 0.2 G_1, 0.1 G_2, and 0.1 M_1. Recoveries of the aflatoxins added to com, groundnuts, and cheese samples at 10-30 μg kg^{-1} were > 69 % (aflatoxin G_2) and averaged 91 % B_1, 89 % B_2. 91 % G_1, and 78 % G_2, and 92 % M_1. The simple method described was compared with the AOAC CB, AOAC BF, and AOAC milk and cheese methods. These methods were applied to corn, groundnuts, and cheese composites spiked with known amounts of aflatoxins, and

to naturally contaminated buckwheat and cheese. Recoveries were much lower for the BF method compared with the simple method and the CB method.

1047. Kmieciak, S., and Niewiadomski, H. 1972. [Evaluation of methods for quantitative analysis of aflatoxin B_1 in groundnut meals.]. Evaluation des methodes de dosage de l'aflatoxine B_1 dans les tourteaux d'extraction d'arachide. Revue Francaise des Corps Gras 19 (7): 455-460.

Five analytical methods, viz. AOAC "BF" and "Celite". the T.P.I. method, a Dutch method and a Polish method were compared for determination of aflatoxins in groundnut meals. The T.P.I, method was considered optional. The best separation of aflatoxin on thin-layer chromatographic (TLC) plates was achieved with chloroform-acetone (90+10) as the developing solvent.

1048. Koonanuwatchaidet, P., and Fong, L.Y.V. 1980. Determination of aflatoxin B, in groundnut extracts and its mutagenicity. Journal of the Science Society of Thailand 6(3): 143-145.

Aflatoxin B_1 at 560-1550 µg kg^{-1} was determined by thin-layer chromatography and spectrophotometry in all of 10 batches of groundnuts from Hong Kong. There was good correlation between presence of aflatoxin B_1 and mutagenic activity of extracts, demonstrated by using the Ames test.

1049. kostyukovskii, Ya.L., Malamed, D.B., and Nesterin, M.F. 1981. A method for the determination of aflatoxins in plant products. Prikladnaya Biokhimiya i Mikrobiologiya 17(5): 759-765.

A technique is described, involving thin-layer chromatography and fluorescence spectrometry, for the estimation of aflatoxins. Using this technique, aflatoxin B_1 was detected in imported cashewnuts and groundnuts at 2.6-6.8 µg kg^{-1}.

1050. Krug, G., and Kuschc, P. 1973. lAspects of aflatoxin contamination of foodstuffs concerned with preventive measures in health protection.]. Aspekte der aflatoxinkontamination von lebensmitteln als aufgabe des vorbeugenden gesundheitsschutzes. Zeitschrift fur die gesamte Hygiene und ihre Grenzgebiete 19(5): 342-348.

A thin-layer chromatographic (TIX) method for semi-quantitative determination of aflatoxin B_1 in foodstuffs, suitable for routine analysis, is described. The application of the method lo the analysis of groundnut kernels is discussed. Of groundnut samples imported into Germany in 1970-1971, 10 % of samples had aflatoxin. Measures for improving health control of imported groundnuts are discussed.

1051. Lee, W.V. 1965. Quantitative determination of aflatoxin in groundnut products. Analyst 90: 305-307.

A rapid extraction method is described for determining aflaloxin in groundnut meal. It involves the extraction of the toxin from the ground meal by chloroform in the presence of water at room temperatures. A determination can be carried out on a de-fatted meal in less than 1 h. The toxin is determined by using thin- layer chromatography. The method worked equally well with groundnut kernels that had been de-fatted by the normal procedure, i.e., with light petroleum or hexane.

1052. Lemieszek-Chodorowska, K. 1976. [Minicolumn and thin-layer chromatographic methods for the detection and semiquantitative determination of aflatoxin B_1 in groundnuts.]. Wykywanie i polilosciowe oznaczanie aflatoksyny B_1 w orzechach arachidowych metoda chromatografii minikolumnowej i cienkowarstwowej. Roczniki Panstwowego Zakladu Higieny 27(5): 535-542.

A minicolumn method is described for determination of aflatoxin B_1 in groundnuts. The method allowed detection of aflatoxin B_1 at more than or equal to 1 µg kg^{-1}. The thin-layer chromatography (TLC) method was used for semiquantitative visual determination of the toxin; mean recoveries for the values more than or equal to 2.5 µg kg^{-1} were 76-87 %.

1053. Lemke, P.A., Davis, N.D., Iyer, S.K., Creech, G.W., and Diener, U.L. 1988. Fluorometric analysis of iodinated aflatoxin in minicultures of Aspergillus flavus and Aspergillus parasiticus. Journal of Industrial Microbiology 3(2): 119-125.

A convenient miniassay for aflaloxin is described for two strains of Aspergillus flavus (NRRL 6539 and 5565) and one strain of A. parasiticus (NRRL 3240). Cultures were grown for 3-10 days in 10 mL of a coconut extract medium. Sensitivity of the assay as measured by photofluorometry (365 nm max. excitation; 445 nm max. emission) was of the order of 0.01 µM (3.12 ng mL^{-1}) for aflatoxin B_1 dissolved in aqueous iodine (0.26 mM). High pressure liquid chromatography (HPLC), monitored by fluorometric analysis of aflatoxin B_1 standard and selected culture filtrates, was used to confirm the sensitivity of the assay and gave an indication of the specificity for iodine-enhanced fluorescence of aflatoxin in the coconut extract medium. Thin-layer chromatography (TLC) was used to further confirm aflatoxin litres and specificity for enhancement of aflatoxins B_1 and G_1 in culture filtrates. The method provides a semiquantitative assay for aflaloxin in fermentation cultures allowing tor genetic selection and scale-up for critical examination of toxigenic potential of A. flavus and A. parasiticus strains growing in a coconut-based medium. The potential for the development of the method as a

substitute for direct assay of aflatoxin from contaminated field samples or other substrates is considered.

1054. Liem, D.H., and Beljaars, P.R. 1970. Note on a rapid determination of aflatoxins in peanuts and peanut products. Journal of the Association of Official Analytical Chemists 53: 1064-1066.

A simple and rapid aflatoxin extraction procedure, based on a direct extraction introduced by Pons and single liquid-liquid extraction proposed by Waltking, is described for groundnuts and groundnut products. Methanol is used as the extraction solvent. Thin-layer chromatography (TLC), using the system described by Engstrom, gave satisfactory results. The method is suitable for aflatoxin B_1 levels of 8 µg kg^{-1} or higher in peanut butter and roasted groundnut products. Aflatoxin B_1 levels down to 3 µg kg^{-1} can be detected in raw groundnuts and groundnut shavings.

1055. Logten, M.J.Van 1977. [Methods for determining permissible levels of intentional and unintentional additives in foodstuffs.]. Wijze van vaststellen van de toelaatbare gehaltes aan gewenste of ongewenste toevoegingen in voedingsmiddelen. Tijdschrift voor Diergeneeskunde 102(3): 164-172.

Problems relating to the determination of acceptable levels of additives and contaminants in foodstuffs are discussed with examples, including the occurrence of aflatoxins in milk. Since the use of DDT in cattle farming has been prohibited in the Netherlands, the DDT content of milk has been greatly reduced, e.g., in the Alkmaar region only 2 out of a total of 1153 milk samples analysed during 1972-1975 contained > 1.25 ppm DDT/DDE in fat, vs. 29 out of 555 samples in 1970. As aflatoxin M_1 has been shown to be carcinogenic, efforts are being made to prevent its occurrence in milk by minimizing the amount of aflatoxins in mixed fodder. Dutch legislation specifies that aflatoxins must not be present in detectable amounts in groundnuts and their products.

1056. Mayura, K., and Sreenivasamurthy, V. 1969. Quantitative method for estimation of aflatoxins in peanuts and peanut products. Journal of the Association of Official Analytical Chemists 52(1): 77-81.

A quantitative method for the estimation of four aflatoxins, based on chromatographic separations and selective destruction of aflatoxins B_1 and G_1 by nitric acid, has been developed. To enchance the specificity of the method, a sample blank is determined and is used to correct the values for non-specific interfering substances.

1057. McKinney, J.D. 1981. Evaluation of laboratory performance with aflatoxin methods by means of the AOCS Smalley Check Sample Program. Journal of the Association of Official Analytical Chemists 64(4): 939-949.

The American Oil Chemists' Society Smalley Check Sample Program offers ongoing check sample series for determination of aflatoxins in groundnut meal, cottonseed meal, and corn meal. Laboratories participating in this program represent a worldwide cross section of industry, regulatory, and commercial laboratories. Each annual series consists of seven samples including a solution of an unknown mixture of aflatoxins B_1, B_2, G_1, and G_2 for direct spotting and quantitation. Participants analyses of the solution of aflatoxins resulted in coefficients of variation generally higher than those of any sample. Comparison of four years of results for analysis using BF and CB methods of analysis for groundnut meal samples with aflatoxin B_1 levels ranging from 2 to 56 ng g^{-1} resulted in higher aflatoxin B_1 results for the CB method. The number of participants reporting results by HPLC method is small; however, their results compare closely with those using TLC methods.

1058. McKinney, J.D. 1984. Analyst performance with aflatoxin methods as determined from AOCS Smalley Check Sample Program : Short-term and long-term views. Journal of the Association of Official Analytical Chemists 67(1): 25-32.

The International Smalley Aflatoxin Check Sample Program of the American Oil Chemists' Society has offered check sample series for aflatoxins in groundnut meal, cottonseed meal, and corn meal since 1976, and an aflatoxin M in raw milk series since 1980. This paper provides the computed mean of all analysts' results and between-laboratory precision for each of the samples in each of the check sample series distributed in 1980-81 and 1981-82. In addition, a comparison is made of the relative measurement and analytical accuracy of those analysts who have participated in the groundnut meal series for at least four years and in the cottonseed and corn meal series since their inception (six years). For this comparison, each analyst's result for each sample was calculated as a % of the mean for all analysts for that sample; these values were then averaged for each analyst over all the meal samples in all the series for each meal type in which the analyst had participated, to obtain an overall measure of analytical accuracy. A similar calculation was made using the reported results for the defined solution of aflatoxins included in each series, to obtain an overall measure of measurement accuracy. An evaluation of the meal series results for the past two seasons shows an overall within-laboratory precision in the range reported for the collaborative studies by which the methods were validated; the between-laboratory precision, although improved over past years. is still far from the collaborative study range. The precision data for the aflatoxin solution included in each series indicate this bias could be related, in large part, to the reference standards used. The extended period evaluation of analysts' performance shows no apparent correlation between measurement and analytical accuracy except for a general positive trend for those analysts using the BF method

for aflatoxins in groundnut meal. A comparison of the accuracy of the BF and CB methods for aflatoxins in groundnut meal shows no significant difference between results by the two methods on the basis of the extended period evaluation, in contrast to the generally higher results for the CB method in the past two seasons' evaluation. The scatter in the average analysts' analytical accuracy is essentially the same, regardless of the method used.

1059. McKinney, J.D., and Cavanagh, G.C. 1977. The American Oil Chemists' Society's Smalley Mycotoxin Check Sample Program : an evaluation. Annales de la Nutrition et de l'Alimentation 31: 519-529.

Operational and statistical evaluations are presented for two Smalley Subcommittee series check sample programs for mycotoxin-contaminated commodities, i.e. collaborative programs on deoiled aflatoxin-contaminated groundnut meal and cottonseed meal series. Methods of analysis were restricted to the AOAC 'CB' or the 'BF' methods for groundnut products and the Pons procedure for cottonseed products.

1060. Mehan, V.K., Aujla, S.S., and Chohan, J.S. 1973. Comparison of different methods of extraction for detection and estimation of aflatoxin B, in groundnut. Indian Phytopathology 25(3): 467-471.

Six different methods of extraction were compared for estimation of aflatoxin B_1 in groundnuts. Partially ground groundnut kernels were autoclaved and inoculated with an aflatoxigenic isolate of Aspergillus flavus. After 8 days of incubation at 22-25°C, extraction of aflatoxin B_1 from the substrate was done according to different methods used. Aflatoxin B_1 was determined using thin-layer chromatography (TLC). Marked differences were found in the amounts of aflatoxin B_1 extractable by different methods. Of all the methods tested that of Leim and Beljaars (1970) proved the best for extracting maximum amount of aflatoxin in short time.

1061. Mehan, V.K., Bhavanishankar, T.N., and Bedi, J.S. 1985. Comparison of different methods for extraction and estimation of aflatoxin B_1 in groundnut. Journal of Food Science and Technology, India 22(2): 123-125.

Aflatoxin extraction methods namely Best Food (BF), Contamination Branch (CB), Pons' and Romer's and analytical methods namely thin-layer chromatography (TLC), spectrophotometry and minicolumn techniques, in different combinations, were compared for their efficiency for determining aflatoxin B_1 occurring naturally in groundnuts and also for estimating aflatoxin B_1 levels in groundnut meal samples spiked with known quantities of the toxin. The BF and Pons' methods gave better efficiency than the other two methods. The spectophotometric method was more effective than the TLC and minicolumn techniques. The BF method was relatively less expensive and less time consuming than the other extraction methods. The Pons' method was convenient for handling larger numbers of samples, especially in the absence of centrifuge facilities required for the BF method.

1062. Miller, N., Pretorius, H.E., and Trinder, D.W. 1985. Determination of aflatoxins in vegetable oils. Journal of the Association of Official Analytical Chemists 68(1): 136-137.

A simple method for determination of aflatoxins in vegetable oils is described. The method was successfully applied to both crude and degummed oils. The oil sample, dissolved in hexane, was applied to a silica column and washed with ether, toluene and chloroform ; aflatoxins were eluted from the column with chloroform : methanol (97:3). As quantitated by thin-layer chromatography (TLC) and liquid chromatography, the oilr> analyzed contained aflatoxin B_1 at levels of 5-200 µg kg^{-1}. Recoveries of aflatoxin B_1 standards added to aflatoxin-frce oils were between 89.5 and 93.5 %, with coefficient of variation of 6.3-8.0 %.

1063. Miiller, H., and Siepe, V. 1978. |Determination of aflatoxin B_1, B_2, G_1, and G_2 in food products : modified method using methyl chloride instead of chloroform for extraction from groundnuts and cereal grains.]. Quantitative bestimmung der aflatoxine B_1, B_2, G_1, und G_2 in lebensmitteln. Deutsche Lebensmittel-Rundschau 74(4): 133-136.

Chloroform is an extraction agent and solvent which is frequently used for aflatoxin analysis. But this may involve a considerable carcinogenic potential. Therefore, tests were carried out to replace chloroform by a harmless solvent. The modified method of Seitz and Mohr (Seitz, L.M., and Mohr. H.E. 1974. Cereal Chemistry 51 : 487-491) using methylene chloride was used for the determination of aflatoxin levels in food products. The recovery rates and reproducibility of the results were within a range as known from previously applied methods.

1064. Nabney, J., and Nesbitt, B.F. 1965. A spectrophotomelric method for determining the aflatoxins. Analyst 90: 155-160.

A method is described for determining the aflatoxins, particularly aflatoxin B,, based on the intensity of the ultraviolet absorption at 363 mµ, after purification by thin-layer chromatography. This procedure has been applied to groundnut meal and crude aflatoxin isolated from cultures of certain strains of Aspergillus flavus and A. parasiticus. This method has only been applied to meals of high toxicity and is not applicable, in its present form, to meals of low or medium toxicity. The extraction and decomposition of aflatoxin B_1 have also been investigated.

1065. Nesheim, S. 1964. Mycotoxins: Studies of the rapid procedure for aflatoxins

in peanuts, peanut meal, and peanut butter. Journal of the Association of Official Agricultural Chemists 47(6): 1010-1017.

A rapid procedure for aflatoxin (developed by Neshcim et al. 1964) was collaboratively tested in 15 laboratories on samples of groundnuts, groundnut meal, and peanut butter. For a semiquantitative method the results were in good agreement. It is recommended that the method be further studied.

1066. Neshcim, S. 1969. Conditions and techniques for thin layer chromatography of aflatoxins. Journal of the American Oil Chemists' Society 46: 335-338.

Satisfactory resolution of the four common aflatoxins, B_1, B_2, G_1, and G_2. on thin layer chromatograms has been a recurring problem. The most frequently observed cause of poor resolution and tailing of spots in the chromatograms was the variable properties of the commercial silica gel-calcium sulfate adsorbent preparations. Variations in quality were observed even from one container to the next within single lots produced by individual manufacturers. Other variables which affected the chromatography to some degree included adsorbent particle size, concentration and nature of the calcium sulfate binder, silica gel layer thickness and moisture content, vapor phase composition in the developing chamber and the solvent used for development.

1067. Nesheim, S., Banes, D., Stoloff, L., and Campbell, A.D. 1964. Note on aflatoxin analysis in peanuts and peanut products. Journal of the Association of Agricultural Chemists 47(3): 586.

A rapid method for detecting aflatoxins in peanut butter is described. The sample is comminuted in a waring blender for 2 minutes with a mixture of methanol:water:hexane. The resulting slurry is centrifuged or filtered, and an aliquot is mixed with diatomaceous earth and uansferred to a chromatographic column. Lipids and other extraneous materials are eluted with hexane, and the aflatoxins are then eluted with a chloroform:hexane mixture. The solvent is removed, and the residue is dissolved in chloroform and chromatographed on silica gel thin-layer chromatographic (TLC) plates. The ailaioxins are determined on the plates by means of their fluorescent characteristics under ultraviolet light.

1068. Nordic Committee on Food Analysis. 1975. Aflatoxin : Determination in peanuts and peanut products. Nordic Standard No. 90. 3 p.

A method for determination of aflatoxins in groundnut and groundnut products is described. Aflatoxins (B_1, B_2, G_1, and G_2) are extracted from the ground groundnut sample by water and chloroform. After filtration the extract is purified by column chromatography using hexane and anhydrous ether, and the components are eluted with methanol-chloroform (3:97, v/v). Separation and quantification are by thin-layer chromatography (TLC). involving use of internal standards. Detection limit is in the range of 1-5 µg kg^{-1}, with a coefficient of variation of 20-30 % by visual quantification, or approximately 5 % using a densitometry technique.

1069. Park, D.L., Diprossimo, V., Abdel-Malek, E., Trucksess, M.W., Nesheim, S., Brumley, W.C., Sphon, J.A., Barry, T.L., and Petzinger, G. 1985. Negative ion chemical ionization mass spectrometric method for confirmation of identity of aflatoxin B, : Collaborative study. Journal of the Association of Official Analytical Chemists 68(4): 636-640.

An interlaboratory study of a negative ion chemical ionization mass spectrometric (MS) confirmation procedure for aflatoxin B_1 was conducted in laboratories in the USA, UK and Federal Republic of Germany. Twelve partially purified, dry film extracts from naturally and artificially contaminated roasted groundnuts, cottonseed, and ginger root containing various quantities of aflatoxin B_1 were distributed to the participating laboratories. The extracts required additional cleanup before MS analysis, using cither an acidic alumina column and preparative thin-layer chromatography (TLC) or a two-dimensional TLC procedure. Recovery of aflatoxin B_1 was influenced by degree of recovery of the sample from acidic alumina and/or TLC plate and incomplete elution of aflatoxin B_1 from silica gel. Factors affecting MS confirmation included the purity and recovery of aflatoxin and MS instrument sensitivity. Aflatoxin B, identity was confirmed in 19.5, 90.9, and 100 % of samples containing < 5, 5-10, > 10 ng aflatoxin B_1 g^{-1} product, respectively, by solid probe introduction using full mass scans. The MS method has been adopted as official first action.

1070. Parker, N.A., and Melnick, D. 1966. Absence of aflatoxin from refined vegetable oils. Journal of the American Oil Chemists Society 43: 635-638.

The present investigation is the first definitive study of the fate of the aflatoxins in vegetable oils undergoing processing. Crude oils, obtained by solvent extraction or by hydraulic pressing of ground moldy groundnuts (not suitable for human consumption), contained only small fractions of the aflatoxin originally present in the groundnuts; the meals retained the bulk of the aflatoxin. Conventional alkali refining and washing of the oils reduced aflatoxin content to a range of 10 to 14 µg kg^{-1}. The subsequent bleaching operations essentially eliminated aflatoxin from the oils; the concentrations were now less than 1 µg kg^{-1}. The above results were confirmed using corn oils obtained from corn germ deliberately contaminated in the laboratory with Aspergillus flavus. The nonfluorescing forms of aflatoxins, capable of being produced during the alkali refining operations, are also absent from the refined vegetable oils, these aflatoxin derivatives are readily converted to their original form on acidification and thereby measurable by fluorescence, if present.

1071. Peterion, R.E., and Clegter, A. 1967. Separadon of aflatoxins by two-dimensional thin-layer chromatography. Journal of Chromatography 31: 250-251.

Two-dimensional chromatography gave better separation of aflatoxins B_1, B_2, G_1, and G_2 than the routinely used one- dimensional thin-layer chromatography (TLC). By mis method the four aflatoxins were easily distinguishable. In addition, several new fluorescent compounds were noted which are not separated from the aflatoxins by the usual one-dimensional chromatogrpahic system. Failure to separate such compounds can lead to erroneous results in quantitative analyses.

1072. Peterson. R.E., Ciegler, A., and Hall, H.H. 1967. Densitometric measurement of aflatoxin. Journal of Chromatography 27: 304-307.

An inexpensive darkroom densitometer was modified to read fluorescence of aflatoxins on thin-layer chromatographic (TLC) plates. The performance of this simple, low-cost unit proved that ultrasophistication in instrumentation, with its accompanying high-cost, is unnecessary to secure data accurate enough for routine work. Densitometry and visual comparisons were made on extracts of fermentation broths in which aflatoxins B, and G_1 were being produced by Aspergillus flavus. These comparisons indicated the accuracy of the two methods of aflatoxin estimation. The curve from densitomctric measurements was the type to be expected for the fermentation, while the one from visual comparisons appeared anomalous.

1073. Piskorska-Pliszczynska, J. 1973. [Application of the Cucullu's fast method for detection of aflatoxin contamination in peanut meal and animal feeds.]. Przydatnosc szybkiej melody Cucullu do oznaczania aflatoksyn w scrucie arachidowej i mieszankach paszowych. Medycyna Weterynaryjna 29(12): 746-748.

The fast screening method described for cottonseed products by Cucullu et al. was slightly modified and used for detecting aflatoxins in groundnut meal. The method consists of a blender extraction of the sample with acetonitrile-water, partition of aflatoxins into benzene and chromatography in small chromatographic columns filled with zones of acidic alumina and silica gel. Aflatoxins were detected by blue fluorescent ring under longwave ultraviolet light examination. The entire analytical procedure requires 20-25 minutes and sensitivity of the method is 10 µg kg^{-1} total aflatoxins. The Cucullu's procedure may be useful and recommended for preliminary estimation of aflatoxin in groundnut meal and animal feeds.

1074. Pohland, A.E., Yin, L., and Dantzman, J.G. 1970. Rapid chemical confirmatory method for aflatoxin B_1. Development of the metfiod. Journal of the Association of Official Analytical Chemists 53(1): 101-102.

A revised method has been developed for the formation of aflatoxin B_1 derivatives for chemical confirmation. The method involves treating the aflatoxin-coniaining extract with concentrated HCl and water to yield the water adduct and with concentrated HCl and acetic anhydride to yield the epimeric acetates. The method is considered simpler to perform and produces fewer side reactions man the AOAC official final action method.

1075. Pons, W.A..Jr. 1971. Evaluation of reflectance fluorodensitometry for measuring aflatoxins on thin layer plates. Journal of the Association of Official Analytical Chemists 54(4): 870-873.

A reflectance fluorodensitometer employing illumination of chromatograms with longwave UV light at 45 degree angles to the plate surface and measurement of reflected fluorescence at 90 degree was found to be suitable for measuring aflatoxins on silica gel-coated thin-layer plates. The relationship of peak area vs. concentration was linear for 1-20 ng anatoxins B_1 and G_1/spot. Degradation of aflatoxins was slight. Five repetitive scans of the same chromatogram containing 5 ng each of B_1 and G_1 reduced the recorded areas an average of 1 % per scan. Consecutive scans of 8 identical standard chromatograms containing 5 ng each of B_1 and G_1 and 1.5 ng each of B_2 and G_2 showed a reproducibility, as measured by coefficients of variation, of + 4-5 % (B_1 and G_1) and + 5-9 % (B_2 and G_2), representing the combined errors of standard application, TLC development, and scanning. Analysis of aflatoxins in purified sample extracts from six contaminated oilseed meals, 3-500 µg aflatoxins kg^{-1}, in which the same TLC plates were scanned by a transmission densitometer and the reflectance densitometer yielded essentially equivalent values.

1076. Pons, W.A.Jr., Cucullu, A.F., Franz, A.O.Jr., Lee, L.S., and Goldblatt, L.A. 1973. Rapid detection of aflatoxin contamination in agricultural products. Journal of the Association of Official Analytical Chemists 56(4): 803-807.

A rapid and simple method, originally developed for detecting aflatoxin contamination in cottonseed products, was modified for application to a variety of agricultural products. The modified method involves rapid blender extraction of the sample with aqueous acetonitrile, treatment of an aliquot of the filtrate with lead acetate solution to remove interfering pigments, and rapid partition of aflatoxins in the treated extract into benzene to effect an 8-fold concentration of the aflatoxins. A portion of the benzene extract is adsorbed by capillary attraction onto the bottom of a small column (4 mm x 20 cm) filled with zones of acidic alumina and silica gel and the column is allowed to develop 5 min in chloroform-acetonitrile-2-propanol (93+5+2). Aflatoxin is detected as a sharp blue fluorescent band about 1 cm above the alumina zone when the developed column is viewed under longwave UV light. The total analysis time is about 20 min. The

method was successfully applied to cottonseed, groundnut and many other agricultural products. As little as 10 µg aflatoxin kg^{-1} can be detected.

1077. Pons, W.A.Jr., Cucullu, A.F., Lee, L.S., Robertson, J.A., Franz, A.O., and Goldblatt, L.A. 1966. Determination of aflatoxins in agricultural products : Use of aqueous acetone for extraction. Journal of the Association of Official Analytical Chemists 49(3): 554-562.

An analytical procedure originally developed for the determination of aflatoxins in cottonseed products has been modified for application to many agricultural commodities. Aflatoxins are rapidly extracted free of lipid contamination with 70 % acetone. Many interfering pigments are removed from the crude extract by precipitation as insoluble lead derivatives, transfer of aflatoxins into chloroform, and further purification of the chloroform extract with silica gel. The procedure was compared with four recently proposed methods for the analysis of groundnut products. This procedure is capable of detecting as little as 0.3 µg kg^{-1} of aflatoxin B_1 in groundnuts and peanut butter. Adequate recovery of added aflatoxins was obtained when this method was applied to different agricultural products.

1078. Pons, VV.A.Jr., and Goldblatt, L.A. 1968. Instrumental evaluation of aflatoxin resolution on TLC plates. Journal of the Association of Official Analytical Chemists 51(6): 1194-1197.

An objective instrumental evaluation system is suggested for determining the degree of resolution of aflatoxins on thin-layer chromatography (TLC) plates coated with silica gel. The plates are spotted with a standard containing the four major aflatoxins, developed, and scanned with a densitometer equipped for fluorescence measurement. Numerical resolution factors are calculated from the recorder traces and used as an index of degree of separation of the aflatoxins.

1079. Pons, W.AJr., and Goldblatt, L.A. 1969. Physicochemical assay of aflatoxins. Pages 77-105 in Aflatoxin:Scientific background, control, and implications (Goldblatt, L.A., ed.) New York, USA : Academic Press.

This paper critically reviews the physicochemical methods of analysis for aflatoxins in agricultural commodities including groundnuts and groundnut products. Collaboratively studied methods of analysis for aflatoxins in groundnut and cottonseed products are also discussed.

1080. Pons, W.A.Jr., Robertson, J.A., Cucullu, A.F., and Goldblatt, L.A. 1970. Absorptivity and solid state and solution fluorescence of mixed aflatoxin standards stored in solution and as dry films. Journal of the Association of Official Analytical Chemists (2): 293-299.

Mixed aflatoxins B_1, B_2, G_1, and G_2 standards were stored for one year in chloroform and benzene solutions and as dry films at 28 and -18°C. Weighted average molar absorptivity and solution and thin-layer chromatography plate fluorescence were used as indices of deterioration. Changes in all these indices were greater for standards stored at 28°C than for those stored at -18°C. Storage at 28°C resulted in appreciable deterioration of dry film standards. Both solution and plate fluorescence were more sensitive indices than molar absorptivity for detecting changes in the properties of stored standards. A good correlation was obtained between aflatoxin concentration as measured by UV molar absorptivity and the plate fluorescence of the stored standards. An accelerated storage study of aflatoxins B, and G_1 stored 34 days in benzenc-acetonitrile (98+2) at 51°C showed no detectable change in molar absorptivity or plate fluorescence.

1081. Pons, W.AJr., Robertson, J.A., and Goldblatt, L.A. 1966. Objective fluorometric measurement of aflatoxins on TLC plates. Journal of the American Oil Chemists' Society 43(12): 665-669.

Measurements of the solid state fluorescence of aflatoxins on silica gel-coated thin-layer chromatography (TLC) plates on a densitometer equipped for fluorescence measurements showed a linear relationship between peak areas and concentration over a range of at least 2 to 105 x 10^{-4} µg of aflatoxins per spot. Response of individual aflatoxins was in order of $B_2 > G_2 > B_1 > G_1$. Aflatoxins can be measured with a precision of + 2-4 %.

1082. Przybylski, W. 1975. Formation of aflatoxin derivatives on thin layer chromatographic plates. Journal of the Association of Official Analytical Chemists 58(1): 163-164.

Rapid confirmation of the presence of aflatoxins B_1 and G_1 in foods is provided by reaction with trifluoroacetic acid at the origin of a thin-layer chromatographic (TLC) plate. The procedure has been used successfully with various nuts, grains, coffee and cocoa beans, and other foods.

1083. Pusey, M., and Oliver, J. 1979. Aflatoxin in peanuts - a new approach to consumer protection. Manufacturing Confectioner 59: 45-47.

A semi-mechanized method of analysis for aflatoxins in groundnuts is described which enable samples from every ton of nuts used to be analyzed, and increases the accuracy of determinations which is limited at present by sampling errors. The technique is a modification of the AOAC BF method with changes to some of the reagents, elimination of centrifugation, replacement of some of the apparatus with disposable items, and reduction to a semi- micro scale. The complete process is outlined with reference to a schematic diagram. The method, termed 'Aflamatic'

allows an analyst to perform upto 100 analyses in 8 h, has a detection limit of 2-3 µg kg^{-1} aflatoxin B_1 and a precision of + 20 %.

1084. Rati, E.R., Prema, V., and Shantha, T. 1987. Modification of Pons's method of estimating aflatoxin B_1 in corn, groundnut and groundnut cake. Journal of Food Science and Technology, India 24(2): 90-91.

Pons' method for estimation of aflatoxin B, was modified by eliminating the column clean-up step and by introducing additional thin-layer chromatography (TLC) with ether and hexane (1:1). Estimation of aflatoxin B_1 in contaminated groundnut meal by this method compared well with the standard method.

1085. Roberts, B.A., Clancy, E.M., and Patterson, D.S.P. 1981. Rapid, economical method for determination of aflatoxin and ochratoxin in animal feedstuff's. Journal of the Association of Official Analytical Chemists 64(4): 961-963.

A quantitative procedure widely used in European Economic Community (EEC) countries has been successfully scaled down to produce a rapid mediod for determination of aflatoxin B_1 (and other aflatoxins) in animal feeds. Without modification, the method may be used for simultaneous ochratoxin A determination in simple feeds, but a slightly different extraction procedure is required for compound feeds. Validity of the method has been demonstrated by comparison with the full EEC procedure for aflatoxin B_1 and the Neshcim method for ochratoxin A. Analyses may be completed within 2 h and there is a considerable savings in materials over the two reference methods. The procedure is also less hazardous because volumes of toxic extract are small, and the operator is exposed to minimum solvent vapor.

1086. Roberts, B.A., and Patterson, D.S.P. 1975. Detection of twelve mycotoxins in mixed animal feedstuffs, using a novel membrane cleanup procedure. Journal of the Association of Official Analytical Chemists 58(6): 178-1181.

A multimycotoxin thin-layer chromatographic screening method is described which is applicable to most animal feedstuffs. Interference from nonspecific lipid, pigment, and other cotnponenls of simple and mixed feeds is reduced to a minimum by using a membrane cleanup step. Aflatoxins B_1, B_2, G_1, and G_2, citrinin, diacetoxyscirpenol, ochratoxin A. patulin, penitrem A, sterigmatocystin, T-2 toxin, and zearalenone may be reliably detected. The sensitivity of the method is generally low for mixed feeds but even so aflatoxin can be delected at a level of 3 µg kg^{-1} and ochratoxin A at 80 µg kg^{-1}. While the basic method is less sensitive for sterigmatocystin (330 µg kg^{-1}), patulin (600 µg kg^{-1}), zearalenone (1000 µg kg^{-1}), and the trichothecenes (1000-4000 µg kg^{-1}). it may be adapted so as to reduce the above detection limits when the presence of these toxins is suspected. Lower levels may be detected in extracts of simple feeds.

1087. Robertson, J.A.Jr., Lee, L.S., Cucullu, A.F., and Goldblatt, L.A. 1965. Assay of aflatoxin in peanuts and peanut products using acetone-hexane-water for extraction. Journal of the American Oil Chemists' Society 42: 467-471.

A method for analysis of aflatoxins in groundnut products is described. The method involves extraction of aflatoxin from the sample with a homogeneous acetone:hexane:water solvent mixture followed by purification of the extract by phasic extraction of the aflatoxin with aqueous sodium chloride and then with chloroform. The purified chloroform extract is analyzed by thin-layer chromatography by comparision of the intensity of fluorescence of any aflatoxin with the intensity of known standard. The aflatoxin analyses of groundnuts were found very variable due to sampling, and this variability was greatly reduced by finely grinding and thoroughly mixing 2 kg sample before removal of an aliquot for assay. The mediod is sensitive to approximately 2 µg kg^{-1}.

1088. Robertson, J.A.Jr., Pons, W.A.Jr., and Goldblatt, L.A. 1970. Stability of individual aflatoxins B_1, B_2, G_1, and G_2 standards in benzene and chloroform solutions. Journal of the Association of Official Analytical Chemists 53(2): 299-302.

Aflatoxins B_1, B_2, G_1, and G_2 standards were stored in benzene and chloroform solutions for one year at 28 and -18°C. Storage at either temperature produced only minor changes in molar absorptivity ; the decreases ranged from 0.5 to 2.9 % and averaged 1.9 % at 28°C and ranged from 0.3 to 1.4 % and averaged 1 % at -18°C. Solution fluorescence KQ showed a greater decrease ; it ranged from 8 to 22 % and averaged 15 % at 28°C and ranged from 7 to 19 % and averaged 14 % at -18°C. Thin-layer chromatography of standards stored for one year showed a single spot for each aflatoxin and the absence of any other fluorescent derivatives.

1089. Rodricks, J.V. 1969. Note on adsorption of aflatoxin standards to glass. Journal of the Association of Official Analytical Chemists 52 (5): 979-980.

Aflatoxins deposited as dry films in glass vials are adsorbed to the surface and are not completely recovered by the addition of solvent. The concentrations of solutions prepared from dry films must be determined by UV absorptivity measurements.

1090. Rodricks, J.V., and Stoloff, L. 1970. Determination of concentration and purity of aflatoxin standards. Journal of the Association of Official Analytical Chemists 53(1): 92-95.

The concentration of solutions of aflatoxin is determined by ultraviolet absorbance measurement and the purity is determined by examination of developed 50 ng spots of the aflatoxins on thin-layer chromatography (TLC) plates. Results from three collaborative studies demonstrated the effectiveness of the method. This procedure should be used each time solutions of aflatoxins are prepared for use in quantitative analysis. The method is recommended for adoption as official first action.

1091. Rodricks, J.V., and Stoloff, L. 1971. Collaborative study of a method for determination of concentration and purity of aflatoxin standards and use of the method for measuring stability of standards. IUPAC Information Bulletin, Tech. Rep. No. 1, 18 pp.

An analyst should have the capability of determining the concentration and purity of any reference standard before he uses the standard in analysis and should be aware of the period over which he can rely on the concentration and purity of the standards. A method for aflatoxin standard solutions has been developed based on UV absorption measurements and thin-layer chromatography (TLC) examination. The method has been described in detail in a report (1) of the Associate Referee for Mycotoxin standards of the Association of Official Analytical Chemists (AOAC). This report includes a detailed justification for the criteria employed and recommends adoption of the method as official, first action. The recommendation, which was accepted, was based on a history of successful use for over a year by the U.S. Food and Drug Administration (FDA) District Laboratories, good precision and accuracy when used to calibrate standards employed in two separate AOAC collaborative studies of aflatoxin assay procedures, and partial results from this IUPAC sponsored collaborative study. The IUPAC sponsored study has now been completed and is herein reported in detail.

1092. Rodricks, J.V., StolofT, L., Pons, W.A.Jr., Robertson, J.A., and Goldblatt, L.A. 1970. Molar absorptivity values for aflatoxins and justification for their use as criteria of purity of analytical standards. Journal of the Association of Official Analytical Chemists 53(1): 96-101.

Measurements of molar absorptivities in methanol were carried out by two laboratories on samples of aflatoxins B_1 and G_1 prepared and purified independently in four laboratories and on samples of aflatoxins B_2 and G_2 prepared and purified independently in three laboratories. Molar absorptivities of pure aflatoxins B_1, B_2, G_1, and G_2 in benzene-acetonitrile (98+2) were determined in two laboratories. With the exception of aflatoxin G_2, no significant difference between aflatoxin samples could be demonstrated. Molar absorptivity values and absorbance ratios for each aflatoxin, based on these data, are given. Statistical 95 % confidence limits were established for a single determination of molar absorptivity. A statistical study was made of the components of variance which contribute to the accuracy of the determination of molar absorptivity. A comparison of the fluorescence intensities of spots of various aflatoxin preparations, developed on silica-gel coated thin-layer plates, with the amount of aflatoxin in each spotting solution as determined by absorbance measurements, demonstrated the validity of using absorbance and fluorescence comparison measurements for aflatoxin quantitation.

1093. Romer, T.R. 1975. Screening method for the detection of aflatoxins in mixed feeds and other agricultural commodities with subsequent confirmation and quantitative measurement of aflatoxins in positive samples. Journal of the Association of Official Analytical Chemists 58(3): 500-506.

A screening method is described for detecting total aflatoxins ($B_1+B_2+G_1+G_2$) in mixed feeds, grains, nuts, and fruit products in samples containing 5-15 μg kg^{-1} In addition, the presence of aflatoxins in positive samples can be confirmed and the toxins can be quantitatively measured, using the same extract as that used for screening. In the screening method, aflatoxins are extracted with acetone:water (85:15), and interferences arc removed by adding cupric carbonate and ferric chloride gel. The aflatoxins are extracted from the aqueous phase with chloroform and the chloroform extract is washed with a basic aqueous solution. A Velasco-type minicolumn is used to further purify the extract and capture the aflatoxins in a tight band. The screening method has been successfully applied to 24 different agricultural commodities. Quantitative TLC was also performed with extracts of each of these commodities. An average recovery of 94 % B_1, 108 % B_2, 130 % G_1, and 103 % G_2 was obtained compared to the official final action AOAC method for cottonseed products. Within- laboratory coefficients of variation of 10-15 % were obtained for each of the aflatoxins and total aflatoxins in a sample of groundnut meal naturally contaminated with 11 μg B_1 + 3 μg B_2 + 11 μg G_1 + 5 μg G_2 kg^{-1}.

1094. Rosen, R.T., Rosen, J.D., and DiProssimo, V.P. 1984. Confirmation of aflatoxins B_1 and B_2 in peanuts by gas chromatography/mass spectrometry/selected ion monitoring. Journal of Agricultural and Food Chemistry 32(2): 276-278.

A rapid confirmatory method for aflatoxins B_1 and B_2 has been developed. The extract used for thin-layer chromatography quantitation is rapidly cleaned up by elution through a silica gel Sep-PAK cartridge and then analyzed by gas chromatography/mass spectrometry/selected ion monitoring at 3000 resolution using a bonded-phase fused silica capillary column with on-column injection. Limits of detection for aflatoxins B_1 and B_2 in groundnut samples were 0.1 μg kg^{-1}.

1095. Surgeant, K., Sheridan, A., Q'Kelly, J., and Carnaghan, R.B.A. 1961. Toxicity associated with certain samples of groundnuts. Nature 192: 1096-1097.

The toxic extract of Brazilian groundnut meal was further purified and a fluorescent method of identification after chromatographic separation was devised. The toxic

substance was isolated from a fungus, Aspergillus flavus. When the fungus was grown on sterilized groundnuts and fed to ducklings, it resulted in typical liver lesions in ducklings.

1096. Schuller, P.L., Horwitz, W., and Stoloff, L. 1976. A review of sampling plans and collaboratively studied methods of analysis for aflatoxin. Journal of the Association of Official Analytical Chemists 59: 1315-1343.

This paper reviews the sampling plans and collaboratively studied methods of analysis for aflatoxins in foods and feeds. Aflatoxins are the only food contaminants being monitored routinely on an international scale with methods operating at the order of a magnitude of 10 µg kg^{-1}. At this level, methods of analysis which can achieve coefficients of variation of 30-40 % with recoveries of 70 % or greater in interlaboratory collaborative studies can be considered eligible for reference status. In most cases, sample reproducibility is the variable limiting the reliability of methods of analysis. The inherent uncertainity of the identity of chromatographically separated entities requires the application of confirmatory tests to verify that the characteristics measured result from the presence of aflatoxin. The methods are also inoperable without a verification of the identity, purity, and concentration of the reference standards used. Screening methods which reliably eliminate negative samples from further consideration are indispensible for the practical operation of monitoring programs.

1097. Shantha, T., Sreenivasamurthy, V., and Parpia, H.A.B. 1974. An integrated distinguishing test for aflatoxin. Journal of Food Science and Technology (India) 11(4): 194-196.

A procedure is described to distinguish aflatoxin from non- specific fluorescent compounds. The method is based on the poor mobility of aflatoxin on thin-layer chromatography (TLC) plates with diethyl ether as developing solvent and photo-decomposition of aflatoxins by UV light into slow moving and fast moving compounds on TLC. Two tests, namely, preliminary development of the chromatograph with ether, and exposure of the spots to UV light prior to development with the methanol-chloroform solvent can be adopted as tests complementary to each other for distinguishing aflatoxin B_1 from other non-specific fluorescent compounds.

1098. Sobolewski, T., and Kmieciak, S. 1968. [Estimation of aflatoxins in groundnuts and groundnut cakes imported into Poland.|. Oznaczanie aflatoksyn w arachidach i makuchach arachidowych importowanych do Polski. Przem. spoz. 22: 155-156.

The Tropical Products Institute (TPI) method was used to estimate aflatoxins in 45 samples of groundnut. There were 15 non-toxic or slightly toxic, 25 moderately toxic and 5 highly toxic samples. Aflatoxin was estimated by a modification of TPI method. In 230 samples of groundnut cake from India levels of aflatoxin ranged from 5-2000 µg kg^{-1}. The modified method was suitable for analysis of aflatoxin in groundnut cake.

1099. Sreenivasamurthy, V., Jayaraman, A., and Parpia, H.A.B. 1965. Aflatoxin in Indian peanuts : Analysis and extraction. Pages 251-260 in Mycotoxins in Foodstuffs (Wogan, G.N., ed.). The Masssachusetts Institute of Technology : The M.I.T. Press.

Of the nearly 150 isolates of Aspergillus flavus from groundnuts tested, only four produced aflatoxin B_1. These isolates differed from the type strain of A. flavus M001 (obtained from the Tropical Products Institute, London) in certain morphological characteristics. Of the several salt solutions tested, 1 % $CaCl_2$ was found most promising in extracting aflatoxin from the contaminated groundnut cake. In the preparation of the protein isolates, precipitation of the protein in $CaCl_2$ solution helped in removing nearly 80 % of the toxin from the protein. Certain apparently healthy groundnuts showed bright bluish-violet fluorescence under ultraviolet light. Extracts of such groundnuts on thin-layer chromatography (TLC) plates showed the fluorophor at the same Rf as that of aflatoxin B_1. A simple solvent system of benzene : cyclohexane : acetic acid (3:5:2, v/v/v) was found to give on paper chromatogram as good resolutions of the aflatoxin complex as found on alumina TLC with chloroform and methanol as solvent.

1100. Stack, M.E. 1974. Collaborative study of AOAC methods 1 and III for the determination of aflatoxins in peanut butter. Journal of the Association of Official Analytical Chemists 57(4): 871-874.

A collaborative study was designed and carried out to test the accuracy and precision, at low levels of contamination, of AOAC methods I and III, for the determination of aflatoxins in peanut butter. Ten test samples and one practice sample of each peanut butter were analyzed by each method. Two test samples were naturally contaminated with about 2 µg aflatoxin B_1 kg^{-1}. The eight spiked samples (duplicate samples for each level) had 0, 2, 4, and 8 µg B_1, added kg^{-1} and aflatoxin B_2 was added at one-fourth the aflatoxin B_1 level. The seven collaborators were instructed to analyze the samples according to methods I and III except for minor modifications necessary to handle the low levels of aflatoxins in the sample extracts and to quantitate the aflatoxin content both visually and densitometrically. The results of the study indicated that the analyses were able to determine the aflatoxins in the 2-10 µg kg^{-1} range with either method although method I gave better accuracy and precision and had less interferences than method III.

1101. Stack, M.E., and Pohland, A.E. 1975. Collaborative study of a method for chemical confirmation of the identity of aflatoxin. Journal of the Association of Official Analytical Chemists 58(1): 110-113.

The chemical method for confirmation of the identity of aflatoxin by derivative formation directly on the thin-layer chromatographic (TLC) plate was studied collaboratively by eight participants. Aflatoxin B_1 was confirmed in 17 of 17 sample extracts representing 15 µg aflatoxin B_1 kg^{-1} peanut butter, in 13 of 16 extracts representing 5 µg kg^{-1}, and in none of the seven aflatoxin-free extracts. Collaborators commented that the method was easily performed and gave good results. The method has been adopted as official first action.

1102. Stoloff, L. 1967. Collaborative study of a method for the identification of aflatoxin B_1 by derivative formation. Journal of the Association of Official Analytical Chemists 50(2): 354- 360.

A modification of the Andrellos procedure for identification of aflatoxin B_1 was studied collaboratively in 19 laboratories. The procedure, based on the altered chromatographic behaviour of the aflatoxin after reaction with trifluoroacetic acid, formic acid/thionyl chloride, and glacial acetic acid/thionyl chloride, was modified by an improved silica gel column cleanup and a clearer definition of sources of difficulty. Each collaborator examined three extract samples : two naturally contaminated with 5 µg aflatoxin B_1/sample, and one aflatoxin-free extract to which an aflatoxin B_2 artifact was added. No false identifications were made. Sixteen laboratories obtained reasonably good results with the trifluoroacetic acid and formic acid/thionyl chloride reagents. Twelve laboratories obtained reasonably good results with the acetic acid/thionyl chloride reagent but there was general difficulty with a side reaction assumed to be caused by inability to maintain anhydrous conditions. The method was recommended for adoption as official first action.

1103. Stoloff, L. 1970. Rapid chemical confirmatory method for aflatoxin B_1. II. Collaborative study. Journal of the Association of Official Analytical Chemists 53(1): 102-104.

A revised method for the preparation of aflatoxin B_1 derivatives for chemical confirmation has been compared with the AOAC official final action method in collaborative study, using TLC- pure aflatoxin B_1 isolated by the individual collaborators from peanut butter, groundnut meal, and corn. The revised method was simpler, shorter, and more reliable than the official method. It is recommended for adoption as an official first action alternative to the preparation and observation portions of the official final action method.

1104. Stoloff, L. 1972. Analytical methods for mycotoxins. Clinical Toxicology 5(4): 465-494.

This paper critically reviews analytical methods for mycotoxins in agricultural commodities including groundnuts and groundnut products. Procedures for extraction, separation and estimation of various mycotoxins including aflatoxins are reviewed. Procedures for confirmation of mycotoxins are also discussed.

1105. Stoloff, L. 1982. Analytical methods for aflatoxins - An overview. Pages 33-61 in Environmental Carcinogens - Selected Methods of Analysis Vol. 5 - Some Mycotoxins (Egan, H., Stoloff, L., Scott, P., Castegnaro, M.. O'Neill, I.K., and Bartsch, H., eds.). Lyon, International Agency for Research on Cancer (1ARC), Scientific Publications No. 44, 459 pp.

This paper reviews analytical methods for aflatoxins in agricultural commodities including groundnuts and groundnut products with emphasis on thin-layer chromatographic (TLC) and high performance liquid chromatographic (HPLC) based-methods. Procedures for confirmation of aflatoxins are also discussed. Screening methods including minicolumn techniques are briefly reviewed.

1106. Strezleck, S., and Kogan, L. 1966. Note on thin layer chromatography of aflatoxin. Journal of the Association of Official Analytical Chemists 49(1): 33.

A thin-layer chromatography procedure for improved resolution of aflatoxin is described. This procedure eliminates potential interference from a fluorescent material found in extracts of groundnuts with red testa. Improved resolution of aflatoxins was obtained by developing thin-layer chromatography plates at 40 F and using 9 % methanol in chloroform as the developing solvent.

1107. Stubblefleld, R.D., Shannon, G.M., and Shotwell, O.L. 1969. Aflatoxins : Improved resolution by thin layer chromatography. Journal of the Association of Official Analytical Chemists 52(4): 669-672.

Water was added to solvent systems for thin-layer chromatography of aflatoxins to achieve more reproducible results in laboratories where temperature and relative humidity vary. Resolution of aflatoxins also improved. Increments of water were added to solvent systems composed of acetone-chloroform (10+90, 12+88. and 15+85. v/v). As the water concentration was increased, separation of aflatoxins B_2 and G_1 improved. These two aflatoxins are usually the most difficult to resolve in these solvent systems. Separations were the best with water-acetone-chloroform (1.5+12+88. v/v/v). Water added to methanol-chlorolorm (3+97, v/v) improved resolution of this system but not enough for quantitative analysis, or at times, qualitative analysis. The solvent system water-methanol-ether (1+3+96, v/v/v) separated aflatoxins as well as water-acetone-chloroform (1.5+12+88, v/v/v).

1108. Takeda, Y., Isohata, E., Amano, R., Uchiyama, M., Naoi, Y., and Nakao, M. 1976. Studies on analysis of mycotoxins. (V). Systematic analysis of mycotoxins in grains. Journal of the Food Hygienic Society of Japan 17(2): 193-199.

The method used for the extraction and determination of aflatoxins. sterigmatocystin, ochratoxin and citrinin from contaminated grains is described. The mycotoxins were extracted with a 20 % H_2SO_4-4 % KCl-acetonitrile (2+20+178), and transferred to chloroform. By means of silica gel column chromatography, aflatoxins and sterigmatocystin were eluted using a chloroform-methanol mixture (97:3) and the ochratoxin and citrinin were recovered by elution with bcnzene-acetone-acetic acid (75:20:5) after intermediate washing of the column with chloroform-methanol (93:7). Each fraction was concentrated and applied to thin-layer chromatography (TLC) for final determination, in which ochratoxin and citrinin were separated using DC-Fertigplatten Kieselgel for the solid layer and chloroform-methanol-water-90 % formic acid (90:10:1:1) as the developing solvent. The recovery percentage of each mycotoxin from polished and unpolished rice, maize and groundnuts is given.

1109. Takeda, Y., Isohata, E., Amano, K., and Uchiyama, M. 1979. Simultaneous extraction and fractionation and thin-layer chromatographic determination of 14 mycotoxins in grains. Journal of the Association of Official Analytical Chemists 62(3): 573-578.

A simple, systematic analytical method for multiple mycotoxins was developed for detecting 14 mycotoxins : aflatoxins B_1, B_2, G_1, and G_2, sterigmatocystin, T-2 toxin, diacetoxyscirpenol, neosolaniol, fusarenon X, zearalenone, ochratoxin A, citrinin, luteoskyrin, and rugulosin. These mycotoxins were extracted with 20 % H_2SO_4 - 4 % KCl - acetonitrile (2+20+178), defatted with isooctane, and transferred to chloroform. The chloroform extract was cleaned up by silica gel column chromatography ; the first 10 toxins were eluted with chloroform-methanol (97+3) and the remaining four toxins with benzene-acetone-acetic acid (75+20+5). Each fraction was analyzed by thin-layer chromatography for the final determination. The method has been applied to polished rice, rough rice, corn, wheat, and groundnuts as an analytical screening procedure. The detection limits in these commodities ranged from 10 to 800 µg kg^{-1}, depending upon the mycotoxin, but all limits were higher than those obtained for the individual mycotoxins by using other methods.

1110. Thurm, V. 1977. |Thin-layer chromatographic detection of aflatoxin on silica gel prepared plates.]. Zum dunnschicht chromatographischen Nachweis von Aflatoxin auf Kieselgel- Fertigplatten. Nahrung 21: 255-257.

A method is described for estimation of aflatoxins by thin-layer chromatography (TLC) using prepared kieselgel plates coated with starch on to alumina foil (Silufol). Reagents used for separation and development were benzene : ethyl acetate : formic acid (5:4:1), followed by chloroform : methanol 97:3 and ethyl acetate : 10 % ammonia 1:1. Spots were examined by UV light at 380 nm. Clear separation of aflatoxins B_1. G_1. M_1 and M_2 was obtained with both standard solutions and extracts from contaminated milk and groundnuts. Aflatoxin B_1 was just detectable at 0.15 ng and distinctly at 0.3 ng, vs 0.4 and 0.6 ng for conventional glass-coated plates ; development time is also shorter with Silufol, which is recommended for routine food examinations.

1111. Trager, W.T., Stoloff, L., and Campbell, A.D. 1964. A comparison of assay procedures for aflatoxin in peanut products. Journal of the Association of Official Agricultural Chemists 47: 993-1001.

Several analytical procedures for aflatoxin assays, which were used in the autumn of 1963 by the government and industry laboratories, were compared for their efficacy in the detection and quantitative estimation of aflatoxin in groundnut meal and peanut butter. All but one of these procedures employed aluminium oxide thin-layer chromatography (TLC) in the determinative step, but the method employing silica gel TLC was superior to the aluminium oxide technique. When silica gel was used as the determinative step in each of the procedures, all but one were almost equal. The other procedure did not effectively extract the aflatoxin.

1112. Trucksess, M.W., Brumley, W.C., and Nesheim, S. 1984. Rapid quantitation and confirmation of aflatoxins in corn and peanut butter, using a disposable silica gel column, thin layer chromatography, and gas chromatography/mass spectrometry. Journal of the Association of Official Analytical Chemists 67(5): 973-975.

A simple, rapid, and solvent-efficient method for determining aflatoxins in corn and peanut butter is described. Aflatoxins B_1, B_2, G_1, and G_2 were extracted from a 50-g sample with 200 mL methanol-water (85+15). A portion of the extract was diluted with 10 % NaCl solution to a final concentration of 50 % methanol, and then defatted with hexane. The aflatoxins were partitioned into chloroform. The chloroform solution was evaporated, and the residue was placed on a 0.5 g disposable silica gel column. The column was washed with 3 mL each of hexane, ethyl ether, and methylene chloride. Aflatoxins were eluted with 6 mL chloroform-acetone (9+1). The solvent was removed by evaporation on a steam bath, and the aflatoxins were determined using thin-layer chromatography (TLC) with silica gel plates and a chloroform-acetone (9+1) developing solvent. Overall average recovery of aflatoxin B_1 from corn was 82 %, and the limit of detection was 2 ng g^{-1}. For mass spectrometric (MS) confirmation, aflatoxin B_1 in the extract from a 3 g sample (20 ng g^{-1}) was purified by TLC and applied by direct column injection at 40°C into

a 6 m fused silica capillary gas chromatographic column. The column was connected directly to the ion source. After injection, the temperature was rapidly raised to 250°C, and the purified extract was analyzed by negative ion chemical ionization MS.

1113. Trucksess, M.W., Young, K., Donahue, K.F., Morris, O.K., and Lewis, E. 1990. Comparison of two immunochemical methods with thin-layer chromatographic methods for determination of aflatoxins. Journal of the Association of Official Analytical Chemists 73(3): 425-428.

Three different methods were compared for the determination of total aflatoxins in corn and groundnuts naturally contaminated with aflatoxins and in corn, groundnuts, cottonseed, peanut butter, and poultry feed spiked with aflatoxins B_1, B_2, and G_1. The three methods were an enzyme-linked immunosorbent assay (F.LISA) screening test, a monoclonal antibody-affinity column-solid phase separation method, and the AOAC official thin-layer chromatography (TLC) methods for all except poultry feed, for which Shannon's TLC method for mixed feed was used. The ELISA test is designed to provide only positive results for total aflatoxins at > 20 ng g^{-1} or negative results at < 20 ng g^{-1}. The affinity column separation is coupled with either bromination solution fluorometry to estimate total aflatoxins or liquid chromatography (LC) to quantitate individual aflatoxins. Fluorodensitometry was used to determine aflatoxins in commodities analyzed by the TLC methods. The LC and TLC results were in good agreement for all the analyses. The results for the affinity column using bromination solution fluorometry were similar except those for cottonseed, which were about 60 % higher. The ELISA screening method correctly identified naturally contaminated corn and groundnut positive samples. No flase positives were found for controls. The correct response for spiked corn, raw groundnuts, peanut butter, and cottonseed at > 20 ng aflatoxins g^{-1} was about 90 %. The correct response for spiked poultry feed at > 20 ng ailatoxins g^{-1} was about 50 %.

1114. Tsai, Y.J., Davidson, J.I.Jr., Chew, V., Cole, R.J., and Sanders, T.H. 1989. Characteristics of visual, minicolumn and TLC methods in detecting aflatoxin contaminated loads of farmers stock peanuts. Peanut Science 16(1): 1-5.

Results from two 1980 experiments were used to compare the performance of the visual, minicolumn and thin-layer chromatography (TLC) methods in detecting loads of farmers stock groundnuts with aflatoxin. The first experiment was conducted to establish variability of the methods under ideal conditions. The second experiment was conducted to evaluate the performance of the three methods under commercial conditions. Date and empirical models were used to explore potential improvements in detecting low-level contaminated lots. The minicolumn and TLC methods were very sensitive and provided consistent measurements. Operation characteristic curves developed by using empirical models (Logistic and Gompertz) were compared to statistical distribution functions used by Whitaker and coworkers. Assuming a desired probability of acceptance of 15 %, both the minicolumn and TLC methods would accept lots with 60 µg kg^{-1} aflatoxin, while the visual methods would accept lots with 150 µg kg^{-1} aflatoxin. In crop years similar to 1980, the currently used method would not segregate groundnuts to meet the 15 µg kg^{-1} (total) tolerance levels and would provide excessive sheller risk. Because of the objectivity, precision and higher sensitivity, the minicolumn and TLC have potential for improving the detection of contaminated loads of farmers stock groundnuts.

1115. Velasco, J., and Morris, S.L. 1976. Use of water slurries in aflatoxin analysis. Journal of Agricultural and Food Chemistry 24(1): 97-103.

A method based on the use of water slurries was developed for aflatoxin analysis. This procedure enables aliquots, from kilogram size samples, to be analyzed without an increase in solvent or reagent costs. Groundnuts, peanut butter, groundnut meal, cottonseed, cottonseed meal, copra and maize were successfully analyzed by this slurry technique. Slurries were prepared in a 1-gal blender and sample weight to water volume ratios were 1:1.5 for peanut butter, 1:2.25 for whole seeds, 1:4 for commercial meals and 1:5 for copra. Extraction of 100-g aliquots of slurry with 200 mL of acetone resulted in aflatoxin yields comparable to those obtained by extraction of the dry products by the standard method. The precision of slurry analysis for the products, expressed as % coefficient of variation, ranged from 2.6 to 7.8 compared to 5.7 to 20.8 for analyses by the standard procedure. Determinations were made by fluorometric measurements of aflatoxin absorbed on the florisil layer in a minicolumn.

1116. Verhulsdonk, C.A.H., Schuller, P.L., and Paulsen, W.E. 1977. Confirmation reactions on aflatoxin B_1 and G_1. Zeszyty Problcmowe Postepow Nauk Rolniczych 189: 277-283.

Confirmation reactions of aflatoxins B_1 and G_1 are described. The techniques involve the preparation of hemiacetal and epimeric acetates of the toxins. The hemiacetals of B_1 and G_1 have a lower R_1 value in comparison with the epimeric acetates. All the reaction products have lower R_1 value than their original substances. Confirmation tests for aflatoxin directly on thin-layer plates are usefully performed in any laboratory without much experience.

1117. Vorster, L.J. 1969. A method for the analysis of cereals and groundnuts for three mycotoxins. Analyst 94: 136-142.

A method is proposed for the analysis of groundnut samples for three mycotoxins, aflatoxin, ochratoxin and sterigmatocystin, by suitable treatment of a single sample

extract. Based on the objective evaluation of thin-layer chromatography (TLC) of the extract, results can be reproduced with an accuracy of + 20 %. The method is considered to be satisfactory for the purposes of a field survey when the determination of the approximate level of mycotoxin contamination of cereals and groundnuts in the shortest possible time is of prime importance. Problems encountered with samples that have high oil contents or that are darkly pigmented are dealt with by suitable modifications of the method.

1118. Waltking, A.E. 1970. Collaborative study of three methods for determination of aflatoxin in peanuts and peanut products. Journal of the Association of Official Analytical Chemists 53(1): 104113.

A collaborative study directly comparing the propsed BF method with the two AOAC official first action methods for aflatoxin in groundnut products (commonly known as the CB and the Celite methods) was carried out on both naturally contaminated groundnuts and specially prepared peanut butters, as well as commercial peanut butter and groundnut meal to which known amounts of the aflatoxins were added. Included in the study were : comparisons of a proposed variation in the CB method ; a study of silica gels used for the clean-up step of the CB method ; comparison of the visual and instrumental evaluation of the thin-layer chromatography (TLC) plates; and a spectrophotomctric procedure for calibration of the standards. Statistical evaluation of the results indicated that no advantage was obtainable through the proposed revision in the CB method nor in the use of a common silica gel lot for the clean-up column. The standard calibration method was shown to be both accurate and precise and instrumental evaluations of the TLC plates were more precise than visual determinations. In addition to being faster and more convenient and requiring less reagents, the BF method was equal to the official methods with respect to between- and within-laboratory precision and accuracy.

1119. Waltking, A.E., Bleffert, G., and Kiernan, M. 1968. An improved rapid physicochemical assay method for aflatoxin in peanuts and peanut products. Journal of the American Oil Chemists' Society 45: 880-884.

A practical, short cut, sensitive method for more rapidly determining aflatoxin in groundnuts and groundnut products has been described. Through reductions in solvent volumes, utilization of pressure filtration for clarification, and substitution of liquid : liquid extraction for a lengthy column cleanup, equivalent results are possible in less than one half the time required for the current official procedures. Sensitivity, precision and accuracy are comparable to the current methods for raw nuts and peanut butter. Using this method, one can analyze a ground sample of groundnuts within a period of less than 90 min and one analyst can assay more than 16 samples within an 8 h working day.

1120. Whitaker, T.B., and Dickens, J.W. 1979. Evaluation of the Peanut Administration Committee testing program for aflatoxin in shelled peanuts. Peanut Science 6: 7-9.

Under provisions of a USDA Marketing Agreement, an aflatoxin control program for groundnuts produced in the United States is administered by the Peanut Administrative Committee (PAC) composed of groundnut growers and shellers. The PAC requires aflatoxin tests on all commercial lots of shelled groundnuts. The present PAC testing program initiated in 1975 was evaluated for Crop A which averaged 9.5 μg kg^{-1} aflatoxin and for Crop B which averaged 5.2 μg kg^{-1} aflatoxin. For Crop A and B, respectively, 7.3 % and 2.0 % of the lots were rejected. The accepted lots contained an average of 7.5 μg kg^{-1} for Crop A and 4.7 μg kg^{-1} for Crop B. A correct decision (accept a lot with < 25 μg kg^{-1} aflatoxin and reject a lot with > 25 μg kg^{-1} aflatoxin) was made 95 % of the time for Crop A and 98 % of the time for Crop B.

1121. Whitaker, T.B., and Dickens, J.W. 1981. Errors in aflatoxin analyses of raw peanuts by thin layer chromatography. Peanut Science 8(2): 89-92.

Estimates were made of the errors associated with the extraction, cleanup, drying, and quantification steps of the analytical procedure used by the Food Safety and Quality Service to test groundnuts for aflatoxin. An analysis of variance indicated that the errors associated with the extraction, cleanup, and drying steps were each negiligible and that the quantification step was the major source of analytical error. The error associated with the quantification step (coefficient of variation average 18.6 %) probably comes from three sources (1) differences in fluorescence among replicated spots on a TLC plate (spot to spot error), (2) differences in fluorescence among spots on different TLC plates (spot to spot plus plate to plate error), and (3) errors in measuring the fluorescence of the spots (reading error). The fluorescence spots on the TLC plates were read densitometrically.

1122. Whitaker, T.B., and Dickens, J.W. 1983a. Comparing the amount of aflatoxin extracted from raw peanuts using AOAC methods 1 and II. Proceedings of the American Peanut Research and Education Society 15(1): 91. (Abstract).

Four lots of raw shelled groundnuts, naturally contaminated with aflatoxin, were each ground into a paste. Sixty-four 50-g samples were removed from three of the lots and tony 50-g samples were removed from the fourth lot. For each lot, aflatoxin was extracted from half of the samples by the AOAC Method 1 (CB) and from the remaining half by the AOAC Method II (BF). The four lots averaged 52, 115, 215, and 402 μg kg^{-1} total aflatoxin when measured by the CB method. On the average across the four lots, the BF method extracted 26, 25, 22 and 18 % less aflatoxin B_1, B_2, G_1, and G_2, respectively, than the CB method.

1123. Whitaker, T.B., and Dickens, J.W. 1983b. Comparison of the amounts of aflatoxin extracted from raw peanuts using AOAC methods I and II. Peanut Science 10(2): 52-54.

Four lots of raw shelled groundnuts, naturally contaminated with aflatoxin, were each ground into a paste. Sixty-four 50-g samples were removed from three of the lots and forty 50-g samples were removed from the fourth lot. For each lot, aflatoxin was extracted from half of the samples by the AOAC Method I (CB) and from the remaining half by the AOAC Method II (BF). The four lots averaged 57.8. 127.6, 238.5. and 447.1 μg kg^{-1} total aflatoxin when measured by the CB method. On the average across the four lots, the BF method extracted 26, 25, 22, and 18 % less aflatoxin B_1, B_2, G_1, and G_2, respectively, than the CB method.

1124. Whitaker, T.B., and Dickens, J.W. 1986. Efficacy of the visual, minicolumn, and thin layer chromatography methods to test farmers stock peanuts for aflatoxin. Peanut Science 13: 74-77.

This study estimated the efficacy of the visual A. flavus (VAF), minicolumn (MCL), and thin-layer chromatography (TLC) methods to detect farmers' stock groundnuts which contained aflatoxin. Aflatoxin tests on grade samples from each of 2300 lots of farmers' stock groundnuts were used to estimate the distribution of farmers' stock lots according to their aflatoxin concentration (lot distribution). This lot distribution (with an average aflatoxin concentration of 59.5 μg kg^{-1}) was incorporated into each of the 3 computer models that simulate the testing of farmers' stock groundnuts for aflatoxin when the VAF, MCL, and TLC methods are used. The number of lots accepted and the average aflatoxin concentration (AA) in the accepted lots was predicted. Results indicate that when a given percentage of the lots are accepted, lots accepted by the VAF method have less aflatoxin than those lots accepted by either the MCL or TLC methods. When the present visual method was used to test the above lot distribution, 75.8 % of the lots tested were accepted and the AA in the accepted and rejected lots were 4.1 and 232.8 μg kg^{-1}, respectively.

1125. Whitaker, T.B., Dickens, J.W., and Chew, V. 1985. Development of statistical models to simulate the testing of fanners stock peanuts for aflatoxin using visual, thin layer chromatography, and minicolumn methods. Peanut Science 12: 94-98.

The negative binomial probability function was used to model the distribution of sample aflatoxin test results when replicated grade samples from farmers stock groundnuts were analysed by thin-layer chromatography and minicolumn methods. The Poisson probability function was used to model the distribution of the number of kernels with visible Aspergillus flavus growth found in replicated grade samples of farmers stock groundnuts when the visible -4. flavus method was used. The probabilities of accepting a lot of farmers stock groundnuts with given aflatoxin concentrations when using a 465-g grade sample and two different accept/reject levels were predicted with the models and compared to observed acceptance probabilities computed from previously published data for each of the three methods. The comparisons showed good agreement between the predicted acceptance probabilities and the observed acceptance probabilities.

1126. Whitaker, T.B., Dickens, J.W., Davidson, J.I.Jr., and Chew, V. 1984. Development of statistical models to simulate the testing of fanners slock peanuts for aflatoxin using visual, TLC, and minicolumn methods. Proceedings of the American Peanut Research and Education Society 16: 46.

The negative binomial equation was used to simulate the distribution of sample aflatoxin test results when replicated grade samples from farmers stock groundnuts were analyzed by TLC and minicolumn methods. The Poisson equation was used to simulate the distribution of samples according to the number of kernels with visible Aspergillus flavus growth found in replicated grade samples from farmers stock groundnuts when the visible A. flavus method was used. The probability of accepting a lot of fanners stock groundnuts with a given aflatoxin concentration when using a 465-g grade sample and four different accept/reject levels were predicted with the models and compared to observed acceptance probabilities for each of the three methods. Comparisons between predicted acceptance probabilities and observed acceptance probabilities from a previous study were good for each method at each accepty/reject level.

1127. Whitaker, T.B., Dickens, J.W., and Giesbrecht, F.G. 1984. Effects of methanol concentration and solvent : peanut ratio on extraction of aflatoxin from raw peanuts. Journal of the Association of Official Analytical Chemists 67(1): 35-36.

Aflatoxin B, was extracted by a water slurry process using methanol concentrations of 55, 60, 65, and 70 % in water and solvent: groundnut ratios of 3,4, 5, and 6 mL g^{-1}. Results failed to show that methanol concentration had an effect on amount of B, extracted ; however, the amount of B_1 extracted increased with an increase in solvent : groundnut ratio. Aflatoxin B_1 was also extracted by the official AOAC method II, using methanol concentrations of 55, 60, 65, and 70 % in water and solvent : groundnut ratios of 2, 3, 4, and 5 mL g^{-1}. Results showed that the amount of aflatoxin B_1 extracted increased with % methanol at low solvent : groundnut ratios but not at high ratios. Also, the amount of aflatoxin B_1 extracted increased with solvent : groundnut ratios at all methanol concentrations.

1128. Whitaker, T.B., Dickens, J.W., and Giesbrecht, F.G. 1986. Optimum methanol concentration and solvent/peanut ratio for extraction of aflatoxin from raw

peanuts by modified AOAC method II. Journal of the Association of Official Analytical Chemists 69(3): 508-510.

The amount of aflatoxin extracted from raw groundnuts by using the water-slurry modification of AOAC method II was determined for 49 different combinations of methanol concentration and solvent : groundnut ratio. Results indicate that the amount of aflatoxins B_1 and B_2 extracted from raw groundnuts is a function of both methanol concentration and solvent : groundnut ratio. A cubic equation was developed, using regression techniques to describe the combined effects. From the functional relationship, the predicted methanol concentration and solvent:groundnut ratio that extracts the most aflatoxin B_1 was computed to be 60 % and 10.8 mL solvent g^{-1} groundnuts, respectively. This combination extracted 12.1 % more aflatoxin than did AOAC method II.

1129. Whitaker, T.B., Dickens, J.W., and Monroe, R.J. 1980. A water slurry method of extracting aflatoxin from peanuts. Journal of the American Oil Chemists' Society 57(9): 269-272.

A water slurry method in which 1100-g of comminuted groundnuts were blended with 1500 ml of tap water for 3 min in a blender and the aflatoxin in a 130-g portion of the slurry was extracted by solvent according to methods similar to those used in Method II of AOAC was compared to the method presently used by the Food Safety and Quality Service (FSQS), USDA. The proposed water slurry method required only 180 mL and 60 mL per sample, respectively, of methanol and hexane compared to the 1650 and 1000 mL, respectively, required by the FSQS method. Blending comminuted groundnuts with water reduced the average particle size and distributed the contaminated particles throughout the slurry. Ninety-four % of the blended particles passed a sieve with 149-µ openings compared to only 66 % of the unblended product. Variance among analyses with the FSQS method did not differ significantly from the variance among analyses with the slurry method. However, analyses with the slurry method averaged 16 % more aflatoxin than with the FSQS method.

1130. Whitaker, T.B., Dickens, J.W., and Slate, A.B. 1990. Computerized system to quantify aflatoxin using thin layer chromatography. Peanut Science 17(2): 96-100.

A microcomputer was interfaced to an instrument (spotmeter) previosly designed to measure the fluorescent intensity of aflatoxin spots on thin layer chromatography (TLC) plates. Software was developed that uses a cubic regression equation to describe the relationships between the spotmeter readings and the known quantities of aflatoxin in standard spots on TLC plates. The regression technique also provides methods to detect spotting and/or measurement errors. Based on the regression equation and measurements of sample extract spots on the same TLC plate, the system computes and records the amount of aflatoxin in the sample extract spots and the concentration of aflatoxin that was in the extracted sample. The percent error associated with computed amounts of aflatoxin in sample extract spots is affected by the amount of aflatoxin in the sample extract and standard spots on the plate. The average percent error ranged from 14.9 % for a 2.6 ng spot to 4.1 % for a 13 ng spot.

1131. Wiley, M. 1966. Note on the analysis of aflatoxins. Journal of the Association of Official Analytical Chemists 49: 1223-1224.

An improved cleanup procedure for analysis of aflatoxins in various agricultural commodities is described. The cleanup procedure consists of an initial thin-layer chromatography (TLC) development of the sample extract with methyl acetate as the solvent The Rf values of the impurities and aflatoxins vary in this solvent from those in the methanol-chloroform system; thus, the aflatoxins are readily separated from interfering fluorescent compounds. This method has given good results on a wide variety of agricultural materials such as feed, grains, nuts, and vegetables. In a relatively clean material such as wheat, aflatoxins can be detected at concentrations of 10 µg kg^{-1}, while in alfalfa, which contains many interfering fluorescent compounds, the lower limit is about 40 µg kg^{-1}.

1132. Wilson, D.M. 1989. Analytical methods for aflatoxins in com and peanuts. Archives of Environmental Contamination and Toxicology 18(3): 308-314.

This review includes discussion of safety, sampling, standards, presumptive and screening methods, quantitative methods, including thin-layer chromatography (TLC), high-pressure liquid chromatography (HPLC) and immunochemical methods, and selection of analytical approach in relation to the detection and estimation of aflatoxins in maize and groundnuts.

1133. Wilson, D.M., Beaver, R.W., Layton, R.C., Trantham, A.L., and McMillan, D.L. 1987. Comparison of immunochemical methods for aflatoxin determination in raw peanuts. Proceedings of the American Peanut Research and Education Society 19: 36.

Three immunochemical and three chemical methods were compared for aflatoxin analysis, using fifty naturally contaminated lots of raw shelled groundnuts. The USDA/AMS analyzed all lots using the water slurry modification of the Best Foods (BF) method. The lots were chosen so mat aflatoxin levels ranged from 0 to 100 µg kg^{-1}. The Federal-Suites Inspection Service modified Dickens mill was used to collect special 10 lb, subsamples from commercial lots. Each 10 lb sample was divided, and duplicate analytical subsamples for each method were prepared to make

each subsample as representative as possible. The Aflatest-P mycotoxin testing system uses a monoclonal based affinity column and was provided by Cambridge Naremco, Springfiled, MO. The ELISA methods used were Agrichek aflatoxin test provided by the Agri Tech Systems, Inc., Portland, ME, and the Agri-Screen test supplied by Neogen Corporation, Lansing, MI. The chemical methods used were the water-slurry method , CB method and an HPLC method using normal phase separation and a silica gel packed cell with fluorescence detection following the CB extraction step. The overall mean from all analyses and methods was 19 g kg^{-1} with a range of 0 to 100 μg kg^{-1} total anatoxins ; the coefficient of variation (CV) was 36 %. Analysis of variance showed no significant differences between methods. The methods contributed little to variation, but samples contributed to 56 % and subsamples to 35 % of the variation. All methods showed significant relationships ($P< 0.01$) with regression using the water slurry method as the dependent variable. The R values ranged from 0.66 to 0.83. All methods performed well and were comparable. Any one of the immunochemical methods could be routinely used by trained personnel to perform aflatoxin analyses.

1134. Wilson, D.M., Tabor, W.H., and Trucksess, M.W. 1976. Screening method for the detection of aflatoxin, ochratoxin. zearalenone, penicillic acid and citrinin. Journal of the Association of Official Analytical Chemists 59(1): 125-127.

The method uses 0.5 N phosphoric acid-chloroform (1 + 10) in the initial extraction; the extract is divided and eluted from two columns to provide a quantitative thin-layer chromatographic (TLC) method for aflatoxin and ochratoxin in maize and dried beans. Aflatoxin and zearalenone are eluted from 1 column and ochratoxin, penicillic acid and citrinin from the other. Ochratoxin A recoveries were low (50 %) in groundnuts. Zearalenone, penicillic acid and citrinin were quantitatively recovered from maize and beans; zearalenone and penicillic acid were recovered from groundnuts but citrinin was not.

1135. Wiseman, H.G., Jacobson, W.C., and Harmeyer, W.C. 1966. Note on removal of pigments from chloroform extracts of aflatoxin culture with copper carbonate. Journal of the Association of Official Analytical Chemists 50(4): 982-983.

When certain strains of *Aspergillus flavus* are grown on substrates for the production of aflatoxins, many yellow to orange pigments may also be produced which can be extracted along with the aflatoxins. These pigments must be removed before chromatography for several reasons. Thhey absorb light in the spectral region of the aflatoxin fluorescence and can thereby obscure the progress of fluorescent zones on columns; they cause some degree of streaking on thin-layer chromatographic (TLC) plates. The pigments may be easily separated from aflatoxins by briefly swirling the chloroform extracts containing 1 % ethyl alcohol with insoluble basic green copper carbonate which absorbs the pigments or forms complexes with them. Filtration of the copper-treated solution through a thin layer of Celit 545 yields a nearly colorless filtrate. The copper reagent is useful for quantitative analytical procedures within the limits of error imposed by visual comparison of thin-layer spots. It is effective in removing pigments from extracts of *A. flavus* cultures grown on substrates of wheat, corn, groundnuts, rice, straw, and alfalfa.

1136. Yin, L. 1969. Note on acetonitrile as an extracting solvent for aflatoxins. Journal of the Association of Official Analytical Chemists 52(4): 880.

An extraction procedure for extracting aflatoxins from contaminated groundnuts is described which does not require extensive cleanup of the original extract to obtain the desired sensitivity. Aflatoxins are extracted with acetonitrile:water (9:1, v/v) and hexane. The extracts are filtered, acetonitrile-water layer is removed and evaporated to dryness. The residue is dissolved in benzene for spotting on silica gel thin-layer chromatographic (TLC) plates for detection and quantification of aflatoxins.

7.2.4 High Performance Thin-Layer Chromatography (HPTLC) Methods

1137. Coker, R.D. 1984. High performance liquid chromatography and other chemical quantification methods used in analysis of mycotoxins in foods. Pages 207-263 in Analysis of Food Contaminants (Gilbert, J., ed). Elsevier Science Publishers, London, UK.

The importance of sampling, sample preparation and assay techniques in the analysis of mycotoxins in various agricultural commodities is discussed, with emphasis on the required efficiency of the quantification step. Thin-layer chromatography (TLC), high performance thin-layer chromatography (HPTLC), high performance liquid chromatography (HPLC), gas-liquid chromatography (GLC), immunoassay, fluorotoxin meter and other methods are described and compared. Future trends in the analysis of mycotoxins are outlined.

1138. Coker, R.D., Jewers, K., Tomlins, K.I., and Blunden, G. 1988. Development of rapid methods for the analysis of aflatoxin in agricultural produce. I. Evaluation of instrumentation used for high-performance thin-layer chromatography of aflatoxins. Chromatographia 25(10): 875-880.

Initial results arc presented of a study of the determination of aflatoxins B_1, B_2, G_1 and G_2 in foods and feeds by high- performance thin-layer chromatography (HPTLC) on either glass plates (20 cm x 10 cm) or aluminium sheets (20 cm x 20 cm) coated with silica gel 60. Samples were applied as solution in benzene-

acetonitrile (49:1) and development was in the absence of light with a mobile phase of chloroform-xylene-acetone (6:3:1) in two 20-min stages. Spots were quantified by densitometric scanning at 366 nm (monochromator) or 360 nm (filter) vs. an external standard containing the analytes. The more expensive monochromatic densitometer was required for accurate results, with 30-fold increased sensitivity for aflatoxin B,.

1139. Kamimura, H., Nishijima, M., Yasuda, K., Ushiyama, H., Tabata, S., Malsumoto, S., and Nishima, T. 1985. Simple, rapid cleanup method for analysis of aflatoxins and comparison with various methods. Journal of the Association of Official Analytical Chemists 68(3): 458-461.

A method is described for simple and rapid detection of aflatoxins in corn, buckwheat, groundnuts and cheese. Aflatoxins were extracted with a chloroform-water solvent and were purified by a florisil column chromatographic procedure. Column eluates were concentrated and spotted on a high performance thin-layer chromatography (HPTLC) plate, which was then developed in chloroform acetone (9:1) and/or ether:methanol:water (94:4.5:1.5) or chloroform:isopropanol:acetone (85:5:10). Aflatoxins were quantitated by densitometry. The minimum detectable aflatoxin concentration (μg kg^{-1}) in various test materials were 0.2 B_1, 0.1 B_2, 0.2 G_1, 0.1 G_2. and 0.1 MI. Recoveries of the aflatoxins added to com, groundnuts, and cheese samples at 10-30 μg kg^{-1} were > 69 % (aflatoxin G_2) and averaged 91 % B_1, 89 % B_2, 91 % G_1, and 78 % G_2, and 92 % M1. The simple method described was compared with the AOAC CB, AOAC BF, and AOAC milk and cheese methods. These methods were applied to corn, groundnuts, and cheese composites spiked with known amounts of aflatoxins, and to naturally contaminated buckwheat and cheese. Recoveries were much lower for the BF method compared with the simple method and the CB method.

1140. Tosch, D., Waltking, A.E., and Schlesier, J.F. 1984. Comparison of liquid chromatography and high performance thin layer chromatography for determination of aflatoxin in peanut products. Journal of the Association of Official Analytical Chemists 67(2): 337-339.

Liquid chromatography (LC) and high performance thin-layer chromatography (HPTLC) techniques were compared for determination of aflatoxins in peanut butter. With respect to precision, accuracy, sensitivity, recovery, and linearity of response, HPTLC appeared to be equivalent to LC. These points illustrate the viability of HPTLC as an alternative technique in the determination of aflatoxin.

7.2.5 High Pressure Liquid Chromatography (HPLC) Methods

1141. Baker, D.R., Williams, R.C., and Steichen, J.C. 1974. A comparison of photometric detectors for high speed liquid clromatography. Journal of Chromatographic Science 12(9): 499- 505.

The following detectors were compared : a fixed wavelength (254 nm) photometer, a multiwavelength filter photometer, a combination absorbance-fluorescence photometer, and a variable wavelength spectrophotometer. Advantages and disadvantages of each detector are discussed as well as their abilities to function either as all-purpose or specific detectors. The detection of vitamin A alcohol in milk extract using a multi-wavelength filter photometer operating at 254 and 334 nm, and of aflatoxin B_1 in peanut butter extract at 254 and 365 nm are discussed, as well as the detection of riboflavin in a noodles and beef dinner using an absorbance-fluorescence detector. Chromatograms are given.

1142. Beaver, R.W. 1989. Determination of aflatoxins in corn and peanuts using high performance liquid chromatography. Archives of Environmental Contamination and Toxicology 18(3): 315-318.

The various high performance liquid chromatographic techniques for the detection of aflatoxins in foodstuffs were examined. The need for resolution, selectivity, and sensitivity is emphasized and examples from the literature are used to illustrate this need. Examples include both normal and reversed-phase techniques. Sample cleanup, detection methods, chromatographic conditions, and pre- and post-column derivatization methods are discussed.

1143. Beaver, R.W. 1990. Degradation of aflatoxins in common HPLC solvents. Journal of High Resolution Chromatography 13(12): 833- 835.

It was demonstrated that solvent mixtures commonly used for sample and standard dissolution in the reversed-phase HPLC determination of aflatoxins in agricultural products can lead to degradation of the toxins. Exposure to light in non-HAc containing solvents led to the most rapid and extensive degradation. Even when stored at -18°C, MeOH : H_2O resulted in toxin degradation. Since samples and standards may remain in autosamplers for many hours and cooling of autosarnplers is not usually feasible, it is noted that it is important to utilize solvents which result in minimal degradation. If the toxin solutions can be protected from light, either MeCn:H,():HAc (50:50:0.5) or MeOH:H_2O:HAc (50:50:0.5) were shown to result in little degradation of any of the toxins. If light cannot be easily excluled, MeCN:H,0:HAc (50:50:0.5) provided greatest stability. All four of the studied

aflatoxins proved to be stable in crude groundnut extracts (80:20 MeOH:H_2O). However, the preliminary clean-up and preparation of HPLC removed the stability conferring substances and groundnut samples prepared for HPLC were no more stable than the solutions of standard toxins.

1144. Beebe, R.M. 1978. Reverse phase high pressure liquid chromatographic determination of anatoxins in foods. Journal of the Association of Official Analytical Chemists 61(6): 1347-1352.

A method for determining anatoxins by high pressure liquid chromatography (HPLC) with nuorescence detection after CB extraction and cleanup has been applied to various foods. Recoveries at 1-15 µg kg^{-1} levels from green coffee and peanut butter were 72-85 and 74-104 %, respectively. Precision of the method has been tested for peanut butter. Other products to which the method has been successfully applied included tree nuts, seeds, gTains, chocolate-covered-peanut butter candy, and roasted, salted-in-shell groundnuts. High levels of anatoxins found in several samples of nuts by this method have been verified by the official thin-layer chromatographic (TLC) method. The advantages of this HPLC method are speed, precision, sensitivity, selectivity, and immediate chemical confirmation of anatoxins B_1 and G_1. None of the products analyzed required special cleanup procedures.

1145. Bicking, M.K.L., Kniseley, R.N., and Svec, H.J. 1983. Coupled- column system for quantitating low levels of aflatoxins. Journal of the Association of Official Analytical Chemists 66(4): 905- 908.

A technique is described involving a combination of Styragel and Florisil columns for determination of anatoxins. Two switching values between the columns reduce the analysis time and exposure to solvents, Florisil is modified with oxalic acid, allowing recovery of aflatoxins at levels below 500 pg. The method is useful for determining aflatoxins in corn and groundnut meal samples.

1146. Blanc, M., Midler, O., and Karleskind, A. 1976. [Application of high performance liquid chromatography to the quantitative analysis of aflatoxins and mycotoxins in press-cakes and by-products.]. Application de la chromatographic liquide haute performance au dosage des aflatoxines et des mycotoxines dans les tourteaux et les produits derives. Oleagineux 31(11): 495-499.

A simple quick method for estimating aflatoxin in press cakes was developed, using the chloroform extract without purification. The method has a sensitivity of 1 ng with good repeatability. Analysis of aflatoxins by high performance liquid chromatography (HPLC) with spectrophotometric estimation at 362 nm was more sensitive and accurate than current thin-layer chromatography (TLC) and fluorescence methods. The method can also be used for other mycotoxins such as ochratoxin, zearalenone, patulin, aflatoxin M (in milk), and sterigmatocystin.

1147. Campbell, A.D., Francis, O.J.Jr., Beebe, R.A., and StololT, L. 1984. Determination of aflatoxins in peanut butter, using two liquid chromatographic methods : Collaborative study. Journal of the Association of Official Analytical Chemists 67(2): 312-316.

Two methods for determining aflatoxins in peanut butter, one using normal phase and the other reverse phase liquid chromatography (LC). were studied by 8 and 10 collaborators, respectively. Fluorescence detection was used for the determinative step in both methods. For reverse phase LC, aflatoxins B_1 and G_1 were converted to B_2a and G_2a; for normal phase LC, a silica gel-packed flow cell was placed in the irradiating light path of the detector. The samples included spiked and naturally contaminated peanut butter with total aflatoxin levels from about 5 to 20 ng g^{-1} and controls in a balanced pair design. For the normal phase LC method, recoveries of B_1, B_2, G_1, and G_2 from spiked samples averaged 79. 92, 74, and 88 %, respectively; for the reverse phase method, the recoveries were 103. 104, 89, and 163 %. For the normal phase LC method, pooled repeatabilities were 20, 23, 28, and 17 % for B_1, B_2, G_1, and G_2, respectively ; for the reverse phase method, the repeatabilities were 19, 22, 38, and 31 %. For me normal phase method, pooled reproducibilities were 34, 33, 39, and 34 % for B_1, B_2, G_1, and G_2 respectively; for the reverse phase method, the reproducibilities were 32, 46, 51, and 52 %. Both methods show an improved limit of detection and better within-laboratory precision over current AOAC methods; however, between-laboratory precision is no better, and the reverse phase method shows evidence of interferences being measured. For these reasons and because of no benefits of present value, neither method was submitted as official first action.

1148. Candlish, A.A.G., Haynes, C.A., and Stimson, VV.H. 1988. Detection and determination of aflatoxins using affinity chromatography. International Journal of Food Science & Technology 23(5): 479-485.

Aflatoxins B_1, B_2, G_1 and G_2 can be easily and rapidly detected in aqueous solutions using an affinity chromatography column coupled to a monoclonal antibody specific for the toxin molecules. A method for their detection, based on this procedure, is described. Water : memanol extracts of aflatoxin-free food were spiked with aflatoxins, diluted with water and passed through the affinity matrix. The monoclonal antibody is bound to the aflatoxins, which can then be released using a small volume of methanol. This concentrated and separated the aflatoxin present in solutions. As little as 5 ng aflatoxin can be seen if the methanol eluate is passed over a small florisil tip under UV light, while 0.5 ng can be detected if the eluate is analysed by HPLC. This system can be used to analyse aflatoxins in contaminated

samples by spot testing (>5 ng) or as a means of clean-up for quantitative analysis at subnanograin levels. Advantages of this immunological assay in relation to other immunoassays and traditional methods are discussed.

1149. Chamkascm, N., Cobb, W.Y., Latimer, G.W., Salinas, C., and Clement, B.A. 1989. Liquid chromatographic determination of aflatoxins, ochratoxin A, and zearalenone in grains, oilseeds, and animal feeds by post-column derivatization and on-line sample cleanup. Journal of the Association of Official Analytical Chemists 72(2): 336-341.

A liquid chromatographic method using on-line sample cleanup, reverse flow analytical column loading, gradient elution, and post-column derivatization with iodine permits direct, rapid determination of aflatoxins B_1, B_2, G_1, and G_2, as well as ochratoxin A and zearalenone. Limits of quantitation are 5 µg kg^{-1} for the aflatoxins and ochratoxin A and 30 µg kg^{-1} for zearalenone. This procedure performs well as a multimycotoxin screen for cereal grains and oilseeds, with more limited success in complete animal feeds.

1150. Chang, H.H.L., DeVries, J.W., and Hobbs, W.E. 1979. Comparative study of two methods for extraction of aflatoxin from peanut meal and peanut butter. Journal of the Association of Official Analytical Chemists 62(6): 1281-1284.

The difference between the CB and BF methods in extracting aflatoxins from groundnut products was studied. The CB method gave 60, 121, 35, and 22 % higher results for aflatoxins B_1, B_2, G_1, and G_2, respectively for four samples of groundnut meal and six samples of peanut butter tested. Both reverse-phase liquid chromatography and thin-layer chromatography were used to quantitate the extracted aflatoxins.

1151. Coker, R.D. 1984. High performance liquid chromatography and other chemical quantification methods used in analysis of mycotoxins in foods. Pages 207-263 in Analysis of Food Contaminants (Gilbert, J., ed.), Elsevier Science Publishers, London, UK.

The importance of sampling, sample preparation and assay techniques in the analysis of mycotoxins in various agricultural commodities is discussed, with emphasis on the required efficiency of the quantification step. Thin-layer chromatography (TLC), high performance thin-layer chromatography (HPTLC), high performance liquid chromatography (HPLC). gas-liquid chromatography (GLC), immunoassay, fluorotoxin meter and other methods are described and compared. Future trends in the analysis of mycotoxins are outlined.

1152. Coker, K.D., and Jones, B.D. 1988. Determination of mycotoxins. Pages 335-375 in HPLC in Food Analysis (Macrae, R., ed.). 2nd edition. London, UK : Academic Press.

This paper gives a brief background of the occurrence and chemistry of mycotoxins (aflatoxins, ochratoxin A, citrinin, patulin, zearalenone, and trichothecenes) in foods and feeds. The importance of sampling, sample preparation and assay techniques in the analysis of the mycotoxins in various agricultural commodities is discussed. High performance liquid chromatography (HPLC) methods for the analysis of mycotoxins are discussed. Future developments in the HPLC analysis of mycotoxins are anticipated.

1153. Cole, R.J., Dorner, J.W., Kirksey, J.W., and Dowell, F.E. 1988. Comparison of visual, enzyme-linked immunosorbent assay screening and HPLC methods in detecting aflatoxin in farmers stock peanut grade samples. Peanut Science 15(2): 61-63.

Grade samples from 152 lots of farmers stock groundnuts were analysed for aflatoxin by an enzyme-linked immunosorbent assay (ELISA) rapid screening test and high-pressure liquid chromatography (HPLC) and the results were compared with those of the visual inspection method used by the Federal State Inspection Service (FSIS) in Georgia. The results showed 41 % of the grade samples with visible Aspergillus flavus (Segregation 3) contained < 20 µg kg^{-1} aflatoxin when analysed by both ELISA and HPLC methods; 18.7 % of Segregation groundnuts (no visual contamination) contained 26-2542 |i.g kg^{+1} aflatoxin. The results of ELISA and HPLC agreed in 98.6 % of the composite lot analyses with the detection of 20 µg kg^{-1} or greater. However, the ELISA rapid screening test failed to give positive tests 12 of 13 times when the aflatoxin content was 20-43 µg kg^{-1} in the component samples.

1154. Colley, P.J., and Ncal, G.E. 1979. The analysis of aflatoxins by high-performance liquid chromatgraphy. Analytical Biochemistry 93: 409-418.

The potential of high-performance liquid chromatography as a technique for separating aflatoxins B_1, B_2, G_1, G_2, B_2a, Q_1, M_1, P_1, aflatoxicol, and a degradation product of aflatoxin B,, 2,3- dihydrodiol, has been assessed. A microparticulate silica adsorption column used with a 1:1 chloroform-dichloromethane eluant provided good resolution of aflatoxins B_1, B_2, G_1, and G_2 but the addition of 1 % propan-2-ol was necessary for the elution of aflatoxins M_1 and Q_1. By selecting appropriate solvent mixtures, good resolution of all of the aflatoxins studied was obtained using columns containing an octadecyl (C18) reversed- phase bonded to a microparticulate support. Details are given for resolving : (1) aflatoxins B_1, B_2, G_1, and G_2 using a 5 % tetrahydrofuran-15 % dimethylformamide in water eluant and (2) aflatoxins B_1, B_2a, Q_1, M_1, P_1, aflatoxicol, and a product of aflatoxin B_1,

2,3-dihydrodiol treated with Tris-bui'fer, using either 15 % dimethylfonnamide in water or 10 % tetrahydrofuran in water as eluant.

1155. Crosby, N.T. 1984. Review of current and future analytical methods for the determination of mycotoxins. Food Additives and Contaminants 1(1): 39-44.

Chemical methods of analysis for the extraction, cleanup and determination of aflatoxins from animal feedingstuffs and foods are described. The advantages and disadvantages of thin-layer chromatography (TLC) and high pressure liquid chromatography (HPLC) are discussed. Immunoassays are briefly discussed. Methods for other mycotoxins such as ochratoxin, patulin, and the trichothecenes are also outlined.

1156. Davis, N.D., and Diencr, U.L. 1980. Confirmatory test for the high pressure liquid chromatographic determination of aflatoxin B_1. Journal of the Association of Official Analytical Chemists 63: 107-109.

The iodine derivative of aflatoxin B, was determined by reverse phase high pressure liquid chromatography with fluorescence detection. Aqueous solutions of aflatoxin B_1 were chromatographed on a Bondapak C18 column with methanol-water (66:34) before and after treatment with iodine. A 4.5-min shift in retention time and a 25-fold increase in fluorescence resulted from the reaction.

1157. DeVries, J.W., and Chang, H.L. 1982. Comparison of rapid high pressure liquid chromatographic and CB methods for determination of aflatoxins in corn and peanuts. Journal of the Association of Official Analytical Chemists 65(2): 206-209.

A method is described for the rapid determination of aflatoxins in corn and groundnut samples by high pressure liquid chromatography. The method was compared with the current CB method. For seven samples of corn and 14 samples of groundnut meal and peanut butler, the correlation between methods was 0.991, and no significant differences were noted between methods for aflatoxins, using the students' t-test at 15.7 % x-risk.

1158. Diebold, GJ., and Zare, R.N. 1977. Laser fluorimetry : Subpicogram detection of aflatoxins using high-pressure liquid chromatography. Science 196: 1439-1441.

The use of high-pressure liquid chromatographic separation in conjunction with laser-induced fluorescence detection permits the analysis of trace fluorescent species at new limits of sensitivity. This technique was applied to aflatoxins B_1, B_2, G_1, and G_2 which were linearly quantitated to 7.5 x 10" g. The procedure consists of forming more fluorescent aflatoxin derivatives, eluting the 'aflatoxins from a reverse-phase column, focusing the 325-nanometer output of a helium-cadmium ion laser into a suspended droplet of the eluent, arid measuring the resulting fluorescence using phase-sensitive detection.

1159. Dorner, J.W., and Cole, R.J. 1988. Rapid determination of aflatoxins in raw peanuts by liquid chromatography with postcolumn iodination and modified minicolumn cleanup. Journal of the Association of Official Analytical Chemists 71(1): 43-47.

A method is described for rapid cleanup followed by reverse-phase liquid chromatography (LC) quantitation of aflatoxins in raw groundnuts. A modified minicolumn cleanup is used for sample preparation, and preliminary estimation of aflatoxin content by minicolumn can be made so that highly contaminated samples can be diluted before LC analysis. Use of minicolumn cleanup eliminates the need for further column or cartridge cleanup. Sensitive quantitation is achieved using a phenyl column, mobile phase of water/tetrahydrofuran (80+20, v/v), and postcolumn derivatization with water-saturated iodine followed by fluorescence detection. Recoveries of aflatoxins B_1, B_2, G_1, and G_2 from groundnut meal spiked at three levels ranged from 71.7 to 88.3 %(average 80 %), with coefficients of variation from 2.7 to 10.4 %.

1160. Dorner, J.W., and Cole, R.J. 1989. Comparison of two ELISA screening tests with HPLC for the determination of aflatoxins in raw peanuts. Proceedings of the American Peanut Research and Education Society 21 : 38.

Two enzyme-linked immunosorbent assay (ELISA) rapid screening tests were compared to high performance liquid chromatographic (HPLC) analyses of 100 raw groundnut extracts for the determination of aflatoxins. Identical extracts, all contaminated between 0 and 70 μg kg^{-1}, were analyzed in duplicate by the Afla- 10 cup test, the EZ-screen quick card test, and an HPLC method. The screening tests had detection thresholds of 10 μg kg^{-1} and 20 μg kg^{-1}, respectively. Both assays were accurate compared to HPLC when samples were negative for aflatoxins or contained aflatoxins above their respective detection thresholds. Errors that did occur were associated with samples that contained aflatoxins at concentrations just below their detection thresholds. The cup test identified as > 10 μg kg^{-1} 9 % of samples that were in the 5-10 μg kg^{-1} range as determined by HPLC. The card test identified as > 20 μg kg^{-1} 53 % of samples that fell in the 10-20 μg kg^{-1} range by HPLC. Of the samples that were outside the range of 5-10 μg kg^{-1}, the cup test was accurate with both replications compared to HPLC 97 % of the time. Similarly, the card test results (both replications) were comparable to HPLC in 91 % of samples outside the 10-20 μg kg^{-1} range.

1161. Dorner, J.W., and Cole, R J. 1989. Comparison of two ELISA screening tests with liquid chromatography for determination of aflatoxins in raw peanuts. Journal of the Association of Official Analytical Chemists 72(6): 962-964.

A study was conducted to evaluate the performance of two enzyme-linked immunosorbent assays (ELISA) for rapidly screening samples of groundnuts for the presence of aflatoxin. The EZ-Scerren Quick Card Test and Alfa-10 Cup Test were compared with liquid chromatography in duplicate analysis of common extracts of groundnut contaminated in the range of 0-70 ng g^{-1}. Each assay properly identified 95 % of the samples containing no detectable aflatoxin as negative and >97 % of samples containing >10 ng g^{-1} aflatoxin as positive. The card test, which had a 20 µg kg^{-1} detection threshold, identified as positive 32 of 34 samples in the 11-20 µg kg^{-1} range. This indicates that the test card test might actually have a detection threshold closer to 10 ng g^{-1}. Most of the errors associated with the assays occurred on the samples containing <10 ng g^{-1} aflatoxins. The cup and card tests identified 76 and 67 % of the samples, respectively, as negative, in the range of 4-10 ng g^{-1}. For the samples either negative or positive contaminated above their detection thresholds for the assays, the methods are well suited for use as rapid screening tests.

1162. Duhart, B.T., Shaw, S., Wooley, M., Allen, T., and Grimes, C. 1988. Determination of aflatoxins B_1, B_2, G_1 and G_2 by high-performance liquid chromatography with electrochemical detection. Analytica Chimica Acta 208(1-2): 343-346.

A 20 µl sample containing aflatoxins in H20-acetonitrile (9:1) was shaken on a vortex mixer for 1 minute before injection onto the HPLC column (15 cm x 4.6 mm) of Spherisorb ODS-II (5 µm). The mobile phase (1.25 mL min^{-1}) with Britton-Robinson buffer (pH 7) - methanol-acctonitrile (627:179:194). The order of aflatoxin elution was G_2, G_1, B_2 and B_1. Amperometrie detection was carried out at 0.5 µA in differential pulse mode at a dropping-mercury electrode, drop time 1 s, with 100-mV modulation amplitude. A potential of -1.28 V (vs. Ag-AgCl) allowed simultaneous determination of the aflatoxins, with maximum sensitivity at - 1.27 V (G_1 and G_2) or -1.32 V (B_1 and B_2). No derealization was necessary. The detection limit of underivatized aflatoxin standards using the technique described was 5 ng. Average recoveries of aflatoxins from peanut butter were 81 %, 87 %, 77 % and 76 %, for G_2, G_1. B_2 and B_1, respectively. The method was applied to peanut butter.

1163. Francis, O.J.Jr., Lipinski, L.J., Gaul, J.A., and Campbell, A.D. 1982. High pressure liquid chromatographic determination of aflatoxins in peanut butter using a silica gel-packed flowcell for fluorescence detection. Journal of the Association of Official Analytical Chemists 65(3): 672-676.

A high pressure liquid chromatographic method has been developed for determining aflatoxins B_1, B_2, G_1 and G_2 in peanut butter. The method is based on extraction with acidified aqueous methanol, partition of the aflatoxins into methylene chloride, and purification of the extract on a 2 g silica gel column. The extracted aflatoxins are resolved on a microparticulate (10 µm) porous silica gel column in Ca 10 min with a water-washed chloroform-cyclohexane-acetonitrile solvent that contains 2 % isopropanol. The fluorescence detection system determines aflatoxins B_1, B_2, G_1 and G_2 at low levels, i.e., 0.25 µg kg^{-1} B_1, 0.5 µg kg^{-1} G_1. and 0.2 µg kg^{-1} B_2 and G_2. Multiple assays of five samples of naturally contaminated peanut butters containing total aflatoxins (B_1+B_2+G_1+G_2) at levels of 1, 2, 3, 9 and 17 µg kg^{-1} gave intralaboratory coefficients of variation of 7, 4, 4, 11, and 3 %, respectively. Samples spiked at levels of 5, 9, and 17 µg kg^{-1} total aflatoxins showed recoveries of 79, 81. and 81 %. respectively.

1164. Friesen, M.D., and Garren, L. 1982. International Mycotoxin Check Sample Program : Part 1. Report on laboratory performance for determination of aflatoxins B_1, B_2, G_1, and G_2 in raw peanut meal, deoiled peanut meal, and yellow corn meal. Journal of the Association of Official Analytical Chemists 65(4): 855-863.

Three aflatoxin-contaminated samples (raw groundnut meal, deoiled groundnut meal, and yellow corn meal) were analyzed by 121 laboratories in 31 countries. Sufficient data were obtained to permit a statistical comparison of the performance of laboratories using the BF, CB, and EEC methods and those using high performance liquid chromatography (HPLC) for quantitation. No significant differences were found between means for laboratories using these four methods for the analysis of raw groundnut meal or yellow corn meal. However, for deoiled groundnut meal, means were significantly different for laboratories using BF method compared with the CB or EEC methods for B_1 and B_2, and for laboratories using the CB method compared with HPLC methods for G_2.

1165. Friesen, M.D., Walker, E.A., and Castegnaro, M. 1980. International check sample program. Part I. Report on the performance of participating laboratories. Journal of the Association of Official Analytical Chemists 63(5): 1057-1066.

Three aflatoxin-contaminated samples, raw groundnut meal, finished peanut butter, and white corn meal, were analyzed by 139 laboratories in 34 countries. Sufficient data were obtained to permit a statistical comparison of the performance of laboratories using the BF, CB, and Pons methods and those using high performance liquid chromatography for quantification. A raw groundnut meal sample showed no significant differences among means for laboratories using the four methods, and a white corn meal sample showed only one such significant differences; however, a finished peanut butter sample containing less than 10 µg total aflatoxins kg^{-1} showed

10 significant differences among means for laboratories using the four methods considered.

1166. Garner, R.C. 1975. Aflatoxin separation by high-pressure liquid chromatography. Journal of Chromatography 103(1): 186-188.

A method for complete and quantitative separation of the naturally occurring aflatoxins using a commercial high-pressure liquid chromatography, which is nearly as sensitive as TLC methods, is reported. Retention times were extremely reproducible and each run took < 10 min. The method should be applicable to the quantitative assay of anatoxins in foods.

1167. Gilbert, J., and Shepherd, M.J. 1985. A survey of aflatoxins in peanut butters, nuts and nut confectionery products by HPLC with fluorescence detection. Food Additives and Contaminants 2(3): 171-183.

A preliminary survey of peanut butter in 1982 indicated that 31 of 32 samples of major national brand-named products contained aflatoxin B_1 up to 10 *µg* kg^{-1}, and that 59 % of those were below the limit of detection (2 µg kg^{-1}). Of 25 peanut butter samples from specialist 'Health Food' outlets, 64 % contained 10 µg kg^{-1}. and the remainder contained 16-318 µg kg^{-1}, with one sample having 345 µg kg^{-1} aflatoxin. Surveys of 'Health Food' products in 1983 and 1984 confirmed that manufacturers were still experiencing some difficulty in complying with the 30 µg kg^{-1} voluntary guideline limit for total aflatoxin. In 1984, 228 retail samples of nuts and nut confectionery products comprising groundnuts (shelled, unshelled, roasted and salted), mixed nuts, almonds (both unblanched and ground), Brazil nuts (in shell), hazelnuts (in shell), chocolate-coated groundnuts, peanut brittle and coconut ice were examined. Results showed that 74 % of the samples contained aflatoxin B_1 at up to 0.5 µg kg^{-1} , and 3.1 %, mainly groundnuts and Brazil nuts, exceeded the guideline tolerance for total aflatoxin. Total aflatoxin was greatest in unshelled groundnuts, 4920 µg kg^{-1}, and in a composite sample of visibly molded Brazil nuts. 17926 µg kg^{-1}.

1168. Goto, T., and Manabe, M. 1989. Methods for µ« analysis of aflatoxins in groundnut and other agricultural commodities. Pages 173-182 in Aflatoxin contamination of groundnut : Proceedings of the International Workshop, 6-9 October 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

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 rapiditity of analysis are more important than accuracy, and the bright greenish yellow fluorescence (BGYF) and minicolumn methods were evolved with this in mind. Recently several enzyme-linked immunosorbent assay (ELISA) system kits for aflatoxin analysis have been developed and some of them found suitable for the kind of tesing 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.

1169. Goto, T., Matsui, M., and Kitsuwa, T. 1988. Determination of aflatoxins by capillary-column gas chromatography. Journal of Chromatography 447(2): 410-414.

Aflatoxins B_1. B_2, G_1 and G_2 were dissolved in benzene-acetonitrile (49:1) and a portion of the solution was injected on to a column (15 m x 0.25 mm) coated with DB-5 (0.25 µm) and operated with temperature programming from 50 to 300 at 15 or 20 degree min^{-1}, He as carrier gas and FID; confirmation of identities was achieved by 70-eV MS. Calibration graphs were rectilinear from 1 to 50 ng and the coefficients of variation (at the 2 to 4 ng level) for aflatoxins B_1, B_2, G_1, and G_2 were 3.2, 4.3, 6.0, and 3.8 %, respectively (n=7); the detection limit was 1 ng for each.

1170. Howell, M.V., and Taylor, P.W. 1981. Determination of aflatoxins, ochratoxin A and zearalenone in mixed feeds, with detection by thin-layer chromatography or high performance liquid chromatography. Journal of the Association of Official Analytical Chemists 64(6): 1356-1363.

A sensitive, reliable, and economical method for the determination of six mycotoxins in mixed feeds is described. The feed is extracted with chloroform-water and the extract is cleaned up using a disposable Sep-Pak silica cartridge. The procedure requires less time (15 min from sample extraction to extract preparation) and less solvent (approximately one-tenth) compared with conventional methods and is suitable for a fast, economical screen. Additional cleanup procedures, involving dialysis or extraction into base, are described for samples containing high levels of interfering compounds. Thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC) with fluorescence detection are described for identification and estimation of mycotoxins. The method has been applied to a wide range of mixed feeds, including laboratory animal diets and raw materials. The limit of detection is 1 µg kg^{-1} for all mycotoxins measured by HPLC.

1171. Hurst, W.J., Snyder, K.P., and Martin, R.AJr. 1984. The HPLC analysis of aflatoxins in raw peanuts with Sep-pak cleanup. Peanut Science 11: 21-23.

A rapid method, economic in solvent usage, for determination of aflatoxins in raw groundnuts is described. Aflatoxins are extracted with aqueous acetone and interfering compounds precipitated with $CuCO_3$. After defatting, aflatoxins are partitioned into dichloromethane for cleanup with silica Sep-pak which eliminates other interfering compounds. Aflatoxins are then eluted with chloroform : ethanol (95:5). The eluate is dried, derivatized with trifluroacetic acid and analysed by high pressure liquid chromatography (HPLC) on a Spherisorb ODS column with a mobile phase of water/acetonitrile/methano) (62:18.18, v/v/v), using a fluorometer detector. Limits of detection are 2-5 ng g^{-1} total aflatoxin. Coefficients of variation were < 8 % and recoveries of individual aflatoxins were in the range 89-103 %. There is negligible background interference, and detection takes 25-30 min.

1172. Hurst, W.J., Snyder, K.P., and Martin, R.AJr. 1987. Determination of aflatoxins in peanut products using disposable bonded-phase columns and post-column reaction detection. Journal of Chromatography 409: 413-418.

A method for the determination of aflatoxins in groundnut extracts using disposable bonded-phase columns for sample cleanup is described. The only solvents used for extraction and cleanup were methanol and water. Results indicated that the method is accurate and precise with lower limits of detection of 100 pg total aflatoxins and linearity from this limit to 100 times that level with a regression coefficient of 0.99. An additional attribute was that the time to prepare a sample for analysis was about 20 min.

1173. Hurst, W.J., and Toomey, P.B. 1978. Determination of aflatoxins in peanut products using reverse phase HPLC. Journal of Chromatographic Science 16(8): 372-376.

A high pressure liquid chromatogrpahy (HPLC) method is described for determination of the 4 major aflatoxins, B_1, B_2, G_1, and G_2 in groundnut products (sweetened and unsweetened peanut butter, ground groundnuts). The aflatoxins were extracted by adapting a procedure developed by Pons, and quantitated utilizing a new 5 urn reverse-phase column with NaCl/acetonitrile/methanol mobile phase (3+1+1). The 5 µm column achieved baseline resolution of each of the 4 aflatoxins. Retention times and peak heights were reproducible. The procedure was successfully applied to several types of groundnut products, which is a decided advantage over the current CB and BF extraction methods. Additionally, it can be used for sweetened groundnut matrixes with no interferences in the chromatography. The total time required for sample preparation and aflatoxin detection is < 1.5 h.

1174. Jansen, H., Jansen, R., Brinkman, U.A.T., and Frei, R.W. 1987. Fluorescence enhancement for aflatoxins in HPLC by post-column split-flow iodine addition from a solid-phase iodine reservoir. Chromatographia 24: 555-559.

A method for post-column derivatization of aflatoxins with iodine is described. The method was found to be cost effective and yielded reproducible results, a linear response over at least two orders of magnitude and detection limits of about 1 µg kg^{-1}, both for standard solutions and for peanut butter samples.

1175. Kawamura, K., Tsuboi, S., Iwamura, N., Imanaka, Y., Wada, T., Kohno, N., and Cruz, M.L. 1984. Development of a sensitive method for detection of aflatoxin B_1 in foodstuffs. ICMR Annals 4: 153-165.

The aflatoxin B_1 content of various foodstuffs from Indonesia, Philippines and Japan was determined by high-performance liquid chromatography (HPLC) using a micro-Porasil column and a micro- Bondapak column after the conversion of aflatoxin B, to its water adduct aflatoxin B_{2B}. Aflatoxin B_1 levels in foods from Indonesia and Philippines were generally higher than in foods from Japan, especially in groundnuts, cassava and maize; levels were significantly less in raw products than that in processed products.

1176. Kmieciak, S. 1976. Determination of aflatoxins in groundnut meals by high performance liquid chromatography. Zeitschrift fur Lebensmittel - Untersuchung und - Forschung 160(3): 321-324.

A method for determination of aflatoxins in groundnut meals by high performance liquid chromatography (HPLC) was investigated. After extraction and purification according to the AOAC 'CB' method, an extract is injected on a Corasil 11 column and eluted by n-hexanc/chlorofonn/ethanol (50:49.7:0.3). Detection is performed with an UV detector. The method determines aflatoxins B_1, B_2, G_1, and G_2. The detection limit for aflatoxin B_1 is 50 µg kg^{-1} using a 254-mm UV detector, or less with more effective detectors (absorption at 350-360 nm).

1177. Kmieciak, S. 1977a. Application of high performance liquid chromatography to the analysis of aflatoxin B_1 in groundnut meals. Archives de l'Institut Pasteur de Tunis 54: 289-303.

A method of aflatoxin analysis by high performance liquid chromatography (HPLC) was investigated and used for the routine control of imported groundnut meal. The optimal resolution of aflatoxins B_1, B_2, G_1, and G_2 was obtained with a Corasil 11 column and a mobile phase composition : n-hexane:chloroform:ethanol (50:49.5:0.5, v/v/v). The column dimensions were 50 x 0.2 cm; pressure 15-30 arm; flow rate 1.2 mL min^{-1}. Retention volume for aflatoxin B, was about 12 mL, and retention time

10 min. Reproducibility of retention volume was examined and the coefficients of variation for afiatoxins B_1, B_2, G_1, and G_2 were 0.76-0.91 %. Detection was performed by UV-254 detector. The detection limit was about 30 µg kg^{-1}. Linearity of detection was tested and found to be satisfactory in the range of 100-2500 ng aflatoxin B_1 injected onto the column. Recovery of aflatoxin B_1 added to the noncontaminated samples in the range 100-2000 µg kg^{-1} was 95.2-118 % (average 105.4 %). Precision of the method was examined at the levels of 200 and 500 |!g kg^{-1}; coefficients of variation were 4.99 and 2.59 %. respectively. The HPLC method and a standard thin-layer chromatography (TLC) method were tested on 30 commercial samples of naturally contaminated groundnut meal. The application of paired statistical analysis did not show significant differences between the methods for aflatoxin levels.

1178. Kmieciak, S. 1977b. Studies on separation of afiatoxins by liquid chromatography. Zeszyty Problemowe Postepow Nauk Rolniczych 189: 269-276.

Column chromatographic separation of afiatoxins from groundnuts on adsorption columns or an Sephadex was attempted. Chromatography on Sephadex G-25 gave reasonably good resolution of afiatoxins B_1 and B_2, but aflatoxin B_2a was present in some fractions and was absent before chromatography. Large-diameter silica gel columns gave unsatisfactory results, but good resolution of afiatoxins B_1, B_2, G_1, and G_2 was obtained by chromatography on 50 x 0.15 cm silica gel (200/240 mean ASTM) columns using chloroform/carbon tetrachloride (1+2) as eluent, or on Corasil II columns using chloroform/n-hexane/acetonitrile (80+20+0.3, v/v/v) as eluent. High inlet pressure could be used to increase flow rate.

1179. Knutti, R., Balsiger, C., and Sutter, K. 1979. Routine application of HPLC for quantification of afiatoxins in whole peanut kernels. Chromatographia 12(6): 349-353.

High pressure liquid chromatography (HPLC) was applied for separation and quantification of the aflatoxin levels in 500 samples of shelled, unroasted groundnuts. Requirements of the method and reasons for choosing HPLC are discussed, as well as the problems arising from using the technique for this routine application. Sample preparation of a series of 10, plus 2 controls required 4-5 h. Reproducibility of peak area from 2 succeeding injections of the same sample solution was 2 %, overall standard deviation of the method 15 %. At high aflatoxin levels, there were problems of extraction efficiency. Simultaneous detection by UV absorption and fluorescence was crucial for interpretation of the results.

1180. Lansden, J.A. 1977. A clean-up procedure for HPLC analysis of afiatoxins in agricultural commodities. Journal of Agricultural and Food Chemistry 25(4): 969-971.

A minicolumn method was modified as a simple, efficient cleanup procedure for use with high pressure liquid chromatography (HPLC) for detection of afiatoxins in groundnuts, rice and com. The procedure, which utilizes a heavy metal salt precipitation combined with a short alumina filtration under vacuum, can be easily adapted to various sample sizes and provides adequate cleanup for use with both micro-particulate and pellicular solid support columns.

1181. Lazaro, F., Luque de Castro, M.D., and Valcarcel, M. 1988. Fluorimetric determination of afiatoxins in foodstuffs by high- performance liquid chromatography with flow-injection analysis. Journal of Chromatography 448(1): 173-181.

A high-pressure liquid chromatographic (HPLC) technique was used to study the recovery of afiatoxins B_1, B_2, G_1 and G_2 from extracts of maize and groundnuts spiked with 16.4 and 32.8 ng of each toxin. Average recovery of each toxin was 101.7 %, with an average deviation of (+) 2.6 % from 100 %. The technique was improved by addition of a flow injection analysis step. The integrated method combined the advantages of flow injection analysis, namely rapidity, simplicity and economy, with the separation capability of HPLC.

1182. Manabe, M, Goto, T., and Matsuura, S. 1978. High performance liquid chromatography of afiatoxins with fluorescence detection. Agricultural and Biological Chemistry 42: 2003-2007.

Afiatoxins B_1, B_2, G_1, and G_2 were quantitatively detected by high performance liquid chromatography using a 12 ul flow-cell in the fluoremetric detector and as mobile phase, a toluene system instead of a chloroform, dichloromethane or methanol system. Various kinds of columns and mobile phases were tested, and fine mutual separation of all me 4 afiatoxins without quenching their fluorescence was achieved by using a silica gel column and toluene/ethyl acetate/formic acid/methanol (89.0:7.5:2.0:1.5). The relationship between the fluorescence peak area and the amount injected was linear in the range 0.3-120 ng. This method, as applied to food (rice, corn, groundnut meal) and feed extracts, is sensitive at the 10-20 µg kg^{-1} levels of the 4 kinds of afiatoxins.

1183. McKinney, J.D. 1981. Evaluation of laboratory performance with aflatoxin methods by means of the AOCS Smalley Check Sample Program. Journal of the Association of Official Analytical Chemists 64(4): 939-949.

The American Oil Chemists' Society Smalley Check Sample Program offers ongoing check sample series for determination of afiatoxins in groundnut meal, cottonseed meal, and corn meal. Laboratories participating in this program represent a worldwide cross section of industry, regulatory, and commercial laboratories. Each

annual series consists of seven samples including a solution of an unknown mixture of aflatoxins B_1, B_2, G_1, and G_2 for direct spotting and quantitation. Participants analyses of the solution of aflatoxins resulted in coefficients of variation generally higher than those of any sample. Comparison of four years of results for analysis using BF and CB methods of analysis for groundnut meal samples with aflatoxin B_1 levels ranging from 2 to 56 ng g^{-1} resulted in higher aflatoxin B_1 results for the CB method. The number of participants reporting results by HPLC method is small; however, their results compare closely with those using TLC methods.

1184. Murata, H., Fukushima, S., and Kashimoto, T. 1979. High performance liquid chromatography of aflatoxins with fluorescent detection. Proceedings of Osaka Prefectural Institute of Public Health, Food Sanitation 10: 73-75.

Aflatoxins were extracted from groundnuts using methyl cyanide and chloroform, or from milk using chloroform, and estimated by high performance liquid chromatography using chloroform : benzene : methyl cyanide : ethanol (225:75:10:25) on a Lichrosorb SI 60 column using a Shimadzu RF-500 LC detector. The lower limit of detection was 10 µg kg^{-1} for aflatoxin B_2 and M_1 and 0.1 µg kg^{-1} for G_1 and G_2. No further purification of samples from milk was required prior to estimation of aflatoxin M_1.

1185. Panalaks, T., and Scott, P.M. 1977. Sensitive silica gel-packed flowcell for fluorometric detection of aflatoxins by high pressure liquid chromatography. Journal of the Association of Official Analytical Chemists 60: 583-587.

Aflatoxins B_1, B_2, G_1, and G_2 were quantitated by high pressure liquid chromatography (HPLC) on a 5 µm Lichrosorb column, using a silica-gel packed flowcell in the fluorometric detector. The relationship between peak height and the amount injected was linear only up to about 2 ng but showed a linear log-log relationship. Methods for constructing and packing the flowcell are given. A guard column and venting valve were used to minimize deterioration of the analytical column and adsorbent-packed flowcell. The method was applied to a peanut butter extract. Although with the cleanup procedure used, the life expectancy of the flowcell is limited.

1186. Park, D.L., Diprossimo, V., Abdel-Malek, E., Trucksess, M.W., Nesheim, S., Brumley, W.C., Sphon, J.A., Barry, T.L., and Petzinger, G. 1985. Negative ion chemical ionization mass spectrometric method for confirmation of identity of aflatoxin B_1 : Collaborative study. Journal of the Association of Official Analytical Chemists 68(4): 636-640.

An interlaboratory study of a negative ion chemical ionization mass spectrometric (MS) confirmation procedure for aflatoxin B_1 was conducted in laboratories in the USA, UK and Federal Republic of Germany. Twelve partially purified, dry film extracts from naturally and artificially contaminated roasted groundnuts, cottonseed, and ginger root containing various quantities of aflatoxin B_1 were distributed to the participating laboratories. The extracts required additional cleanup before MS analysis, using either an acidic alumina column and preparative thin-layer chromatography (TLC) or a two-dimensional TLC procedure. Recovery of aflatoxin B_1 was influenced by degree of recovery of the sample from acidic alumina and/or TLC plate and incomplete elution of aflatoxin B_1 from silica gel. Factors affecting MS confirmation included the purity and recovery of aflatoxin and MS instrument sensitivity. Aflatoxin B_1 identity was confirmed in 19.5, 90.9, and 100 % of samples containing < 5, 5-10, > 10 ng aflatoxin B_1 g^{-1} product, respectively, by solid probe introduction using full mass scans. The MS method has been adopted as official first action.

1187. Park, D.L., Nesheim, S., Trucksess, M.W., Stack, M.E., and Newell, R.F. 1990. Liquid chromatographic method for determination of aflatoxins B_1, B_2, G_1, and G_2 in corn and peanut products : collaborative study. Journal of the Association of Official Analytical Chemists 73(2): 260-266.

A collaborative study of a liquid chromatographic method for the determination of aflatoxins B_1, B_2, G_1 and G_2 was conducted in laboratories located in the United States, Canada, South Africa and Switzerland. Twenty-one artificially contaminated raw groundnuts, peanut butter and maize samples containing varying amounts of aflatoxins B_1, B_2, G_1 and G_2 were distributed to participating laboratories. The test portion was extracted with methanol-0.1 N HCl (4+1), filtered, defatted with hexane and then partitioned with methylene chloride. The concentrated extract was passed through a silica gel column. Aflatoxins B_1 and G_1 were derivatized with trifluoroacetic acid and the individual aflatoxins were determined by reverse-phase liquid chromatography with fluorescence detection, Statistical analysis of the data was performed to determine or confirm outliers and to compute repeatability and reproducibility of the method. For maize, relative standard deviations for repeatability (RSDr) for aflatoxin B_1 ranged from 27.2 to 8.3 % for contamination levels from 5 to 50 ng g^{-1}. For raw groundnuts and peanut butter, RSDr values for aflatoxin B_1 were 35.0 to 41.2 % and 11.2 to 19.1 %, respectively, for contamination levels from 5 to 25 ng g^{-1}. RSDr values for aflatoxins B_2, G_1 and G_2 were similar. Relative standard deviations for reproducibility (RSDR) for aflatoxin B_1 ranged from 15.8 to 38.4 %, 24.4 to 33.4 % and 43.9 to 54.0 % for maize, peanut butter and raw groundnuts, respectively. It is reported that the method has been adopted official first action for the determination of aflatoxins B_1, B_2, G_1 and G_2 in peanut butter and maize at concentration more or =13 ng total aflatoxins g^{-1}.

1188. Patey, A.L., Sharman, M., and Gilbert, J. 1990. Determination of aflatoxin levels in peanut butter using HPLC and ELISA procedures : inter-laboratory comparison. Mycotoxin Research 6(1): 2-6.

Six laboratories analysed portions of the same aqueous acetonitrile extracts of 3 peanut butters for aflatoxin concentration by an HPLC procedure (using immunoaffinity column clean-up) and an ELISA procedure. The extracts were from a nominal "blank" peanut butter, a peanut butter naturally contaminated with aflatoxins (mostly B1) and from a "blank" peanut butter to which equal amounts of aflatoxins B_1, B_2, G_1 and G_2 standards had been added. Similar results for the HPLC and ELISA procedures were obtained for the blank (means 2.8 and 4.9 μg kg^{-1}, respectively) and naturally contaminated (means 26.0 and 25.9 μg kg^{-1}, respectively) peanut butters. However, the results by ELISA (mean 16.7 μg kg^{-1}) for the spiked peanut butler was much lower than that obtained by HPLC (mean 28.3 μg kg^{-1}).

1189. Pons, W.A.Jr. 1976. Resolution of aflatoxins B_1, B_2, G_1, and G_2 by high-pressure liquid chromatography. Journal of the Association of Official Analytical Chemists (1): 101-105.

Aflatoxins were completely resolved as sharp peaks in the order B_1-B_2-G_1-G_2 by high-pressure liquid chromatography (HPLC) on a small particle (10 μm) porous silica gel column in 7-13 min (B_1 through G_2) by a water-saturated chloroform-cyclohexane- acetonitrile clution solvent (25:7.5:1.0), with detection by UV absorbance at 360 nm. The relationship between peak height and amount injected was linear over a 5-400 ng range for each aflatoxin. Both retection times and peak heights were highly reproducible, multiple injections of mixed standards giving coefficients of variation of 1.0-1.4 % (retention time) and 1.6- 2.8 % (peak height) for the four aflatoxins. Detection was highly sensitive, with mean peak height, mm ng^{-1}. of 7.1 (B_1), 6.4 (B_2), 4.5 (G_1), and 4.1 (G_2), allowing detection of 1-2 ng of each aflatoxin.

1190. Pons, W.A.Jr., and Franz, A.O.Jr. 1978. High pressure liquid chromatographic determination of aflatoxins in peanut products. Journal of the Association of Official Analytical Chemists 61(4): 793-800.

A precise and sensitive high pressure liquid chromatographic method is described for determining aflatoxins B_1, B_2, G_1, and G_2 in all types of groundnut products. The method is based on acidified aqueous methanol extraction, partition of aflatoxins into dichloromethane, and purification of the extract on a 2-g silica gel column. Aflatoxins in the purified extract are completely resolved on a microparticulate (10 μm) porous silica gel column in approximately 10 min with a water-saturated chloroform-cyclohexane-acetoniirile solvent. The preferred detection system. B_1 and B_2 by ultraviolet absorbance at 360-365 nm and G_1 and G_2 by fluorescence, allows accurate and sensitive detection of all four aflatoxins at levels as low as 0.3 - 1.0 μg kg^{-1}. Repetitive assay of three samples of naturally contaminated peanut butter containing total aflatoxins (B_1+B_2+G_1+G_2) at levels of 5, 10, and 15 μg kg^{-1} gave within-laboratory coefficents of variation of 11. 5, and 5 %, respectively.

1191. Rosen, R.T., Rosen, J.D., and DiProssimo, V.P. 1984. Confirmation of aflatoxins B_1 and B_2 in peanuts by gas chromatography/mass spectrometry/selected ion monitoring. Journal of Agricultural and Food Chemistry 32(2): 276-278.

A rapid confirmatory method for aflatoxins B_1 and B_2 has been developed. The extract used for thin-layer chromatography quantitation is rapidly cleaned up by elution through a silica gel Sep-PAK cartridge and then analyzed by gas chromatography/mass spectrometry/selected ion monitoring at 3000 resolution using a bonded-phase fused silica capillary column with on-column injection. Limits of detection for aflatoxins B_1 and B_2 in groundnut samples were 0.1 μg kg^{-1}.

1192. Sabino, M., and Zorzetto, M.A.P. 1987. [Separation and quantification of aflatoxins B_1, B_2, G_1 and G_2 by high resolution liquid chromatography.]. Separacao e quantificacao das aflatoxinas B_1, B_2, G_1 e G_2 por cromatografia liquida de alta resolucao. Revista do Instituto Adolfo Lutz, Brazil 44(2): 101- 108.

The method used for determination of aflatoxins is described. Aflatoxins were extracted, chromatographed on reverse phase column and detected by UV at 350 nm. Recovery was 92-106 %. In 10 samples of groundnut flour tested, the limit of detection was 2 μg kg^{-1}.

1193. Sarr, A.B., and Phillips, T.D. 1989. Rapid detection of aflatoxins in peanut with the SAM assay. Proceedings of the American Peanut Research and Education Society, Inc. 21: 18.

A new method for the rapid detection of aflatoxins (i.e., SAM) was compared with a method of high pressure liquid chromatography (HPLC) method. Fifty groundnut samples were analyzed in this study. For the SAM method, groundnut samples (40 g) were ground and extracted with methanol : water (80:20). Aliquots (5 mL) of the methanolic phases were added to 5 mL of water and then partitioned with 3 mL of toluene. Aliquots (0.5 mL) of the toluene phases were added to the top of SAM detectors. Following elution with toluene : chloroform : acetone (95:20:5, v/v/v) all samples were analyzed for aflatoxins with longwave ultraviolet light (365 nm). Groundnut samples (20 g) were ground for HPLC analysis and extracted with acetone : water (75:25, v/v). Fats were removed with hexanc, and the aflatoxins were extracted with chloroform and analyzed by normal phase HPLC. No differences was observed between the two methods. All samples positive by HPLC were positive by SAM, and all samples negative by HPLC were negative by SAM. The percent recovery and the stability of aflatoxin B_1 in the toluene phase in the SAM assay were determined. Aliquots (5 mL) of the extraction solvent were "spiked" with aflatoxin B_1 at levels of 5500, 550, 220, and 55 ng. Aflatoxin B_1 was partitioned with the toluene phase and analyzed by HPLC. The percent recovery of aflatoxin B_1 was 70 % (a recovery of 90 % is routinely attained when chloroform

is used to extract the methanolic phase). A correction factor was determined for standards of aflatoxin B_1 in toluene. Aflatoxin B, was found stable in the toluene phase of SAM. Simple and rapid screening tests such as SAM for the detection of aflatoxins in groundnuts may greatly facilitate prevention through effective monitoring programs that allow for the diversion of contaminated crops and animal feeds.

1194. Schweighardt, H., and Leibetseder, J. 1981. [Determination of mycotoxins by high pressure liquid chromatography (HPLC).J. Nachweis von Mykotoxinen mittels Hochruckflussigkeitschromatographie (HPLC). Wiener Tierarztliche Monatsschrift 68: 302-305.

A high pressure liquid chromatography (HPLC) technique is described for detection of mycotoxins (aflatoxins, zearalenone, vomitoxin, and ochratoxin A). The detection limit for toxins was : aflatoxins (B_1, B_2, G_1, and G_2) 2 µg kg^{-1}, zearalenone 1 |ig kg^{-1}, vomitoxin 25 µg kg^{-1}, and ochratoxin A5 µg kg^{-1}. Of groundnut products, mainly groundnut meal, 87 % were contaminated (maximum 11620 µg kg^{-1} total aflatoxin). In Southeast Styria a high rate of vomitoxin contamination was found in maize (80 %, average 761 µg kg^{-1}), zearalenone was found in 47 % of samples tested (average concentration 145 µg kg^{-1}), 23.6 % of 83 stored grain samples contained ochratoxin A at low concentration.

1195. Seiber, J.N., and Hseih, D.P.H. 1973. Application of high-speed liquid chromatography to the analysis of aflatoxins. Journal of the Association of Official Analytical Chemists 56(4): 827-830.

Partial resolution of aflatoxins B_1, B_2, G_1, G_2, and P_1 was achieved by high-speed liquid chromatography (HSLC) on a porous layer silica adsorbent, using chloroform-isooctane as the eluting solvent and a 254 nm UV monitor for detection. The resolution was somewhat less than, although comparable with, that obtained by thin-layer chromatography (TLC), using Adsorbosil-1 adsorbent and fluorodensitometric detection. The HSLC response to aflatoxins B_1 and G_1 was linear in the 400-3000 ng range, allowing application of the technique to the quantitative analysis of B_1 and G_1 in crude extracts of Aspergillus parasiticus cultures. The cocfficcnts of variation were 4.2 % for B_1 and 23.2 % for G_1 in a scries of four replicate injections. The advantages and limitations of the technique for quantitative analysis and isolation are compared with those of more conventional chromatographic methods.

1196. Stoloff, L. 1982. Analytical methods for aflatoxins - An overview. Pages 33-61 in Environmental Carcinogens - Selected Methods of Analysis Vol. 5 - Some Mycotoxins (Egan, H., Stoloff, L., Scott, P., Castegnaro, M.. O'Neill, I.K., and Bartsch, H., eds.). Lyon, International Agency for Research on Cancer (IARC), Scientific Publications No. 44, 459 pp.

This paper reviews analytical methods for aflatoxins in agricultural commodities including groundnuts and groundnut products with emphasis on thin-layer chromatographic (TLC) and high performance liquid chromatographic (HPLC) based-methods. Procedures for confirmation of aflatoxins are also discussed. Screening methods including minicolumn techniques are briefly reviewed.

1197. Tarter, E.J., Hanchay, J.P., and Scott, P.M. 1984. Improved liquid chromatographic method for determination of aflatoxins in peanut butler and other commodities. Journal of the Association of Official Analytical Chemists 67(3): 597-600.

A combination of the extraction and cleanup procedures of Pons and Franz with the liquid chromatographic derivatization technique of Beebe is proposed as an accurate, precise, and sensitive method for determination of aflatoxins B_1, B_2, G_1, and G_2 in various foodstuffs. Mean recoveries of total aflatoxins added in the range 15-34 ng g^{-1} to peanut butter were 86-94 % in two laboratories. Recovery of total aflatoxins (30 µg g^{-1}) from shelled groundnuts, shelled Brazil nuts, pistachios, cashews, walnuts, and filberts exceeded 86 % and from pumpkin seeds was 80 %. Coefficients of variation for total aflatoxins were less than 7 % for spiked samples and 15 % for groundnuts naturally contaminated with 16.7 µg g^{-1} of total aflatoxins (B_1+B_2). Analyses of groundnut meal, yellow corn meal, and peanut butter check samples (AOCS Smalley Series 1980/81 and 1981/82, IARC Series 1982/83) by this method compared favorably with reported averages. The detection limit is about 0.3 ng g^{-1} for each aflatoxin.

1198. Thiel, P.G. 1986. HPLC determination of aflatoxins and mammalian aflatoxin metabolites. Pages 329-340 in Mycotoxins and Phycotoxins - A collection of Invited Papers Presented at the Sixth International IUPAC Symposium on Mycotoxins and Phycotoxins, Pretoria, Republic of South Africa, 22-25 July 1985. 1986 Elsevier Science Publishers B.V., Amsterdam.

A technique for the analysis of aflatoxins by high performance liquid chromatography (HPLC) using post-column derivatization with iodine (Thorpe et al. 1982) and fluorescence detection was optimized. The advantages of the procedure to increase the sensitivity of detection for aflatoxins B_1 and G_1 were confirmed as well as the ability to verify the presence of these two toxins. The time required for a chromatographic run was considerably shortened to less than 8 minutes. The successful application of the procedure for the analysis of several agricultural commodities as well as blood samples is illustrated. The analysis of blood samples included the separation and detection of aflatoxin M_1 and Q_1 as well as aflatoxicol

while the presence of aflatoxin Q_1 could be confirmed in a similar manner to aflatoxins B, and G,. The sensitivity of the procedure compares favorably with the most sensitive chromatographic procedures available while the technique is also highly reproducible.

1199. Tosch, D., Waltking, A.E., and Schlesier, J.F. 1984. Comparison of liquid chromatography and high performance thin layer chromatography for determination of aflatoxin in peanut products. Journal of the Association of Official Analytical Chemists 67(2): 337-339.

Liquid chromatography (LC) and high performance thin-layer chromatography (HPTLC) techniques were compared for determination of aflatoxins in peanut butter. With respect to precision, accuracy, sensitivity, recovery, and linearity of response, HPTLC appeared to be equivalent to LC. These points illustrate the viability of HPTLC as an alternative technique in the determination of aflatoxin.

1200. Tutelyan, V.A., Eller, K.I., and Sobolev, V.S. 1989. A survey using normal-phase high-performance liquid chromatography of aflatoxins in domestic and imported foods in the USSR. Food Additives and Contaminants 6(4): 459-465.

A highly sensitive normal-phase HPLC method was developed to study the occurrence of aflatoxins in Soviet imported and domestic foodstuffs. The detection limit was 0.1 μg kg^{-1} and the coefficients of variation were 11 % and 8.5 % at contamination levels of 10 and 100 μg kg^{-1} aflatoxin B_1. respectively. A survey of the occurrence of aflatoxins B_1, B_2, G_1 and G_2, in domestic and imported cereals and nuts (totalling 4532 samples) collected during 1985-87, showed that 26.9 % of imported groundnuts, 2.2 % of maize and 28.3 % of cottonseed were contaminated by aflatoxins at levels exceeding the maximum tolerance level established in the USSR (5 μg kg^{-1} for aflatoxin B_1 in foodstuffs of all types excluding baby foods), maximum concentrations were 3650, 600 and 153 μg kg^{-1}, respectively.

1201. Tutelyan, V.A., Sobolev, V.S., Rybakova, N.V., and Eller, K.I. 1989. A survey using normal phase HPLC of aflatoxins in domestic and imported foods and dairy products in the USSR. Journal of Toxicology, Toxin Reviews 8(1-2): 375-387.

An improved normal phase HPLC method was developed to study the occurrence of aflatoxins B_1, B_2, G_1, G_2 and M, in domestic and imported foods. Ether-methanol-water (95:4:1) mobile phase and fluorometric detector with silica gel packed flow cell were used. The detection limit of the method was 0.1 μg kg^{-1} for aflatoxin B_1, coefficients of variation were 11 and 8.5 % at contamination levels 10 and 10 μg kg^{-1} of aflatoxin B_1, respectively. Recoveries of added aflatoxins B_1, B_2, G_1, and G_2 for maize ranged from 78 to 88 %. This method allowed the determination of aflatoxins B_1, B_2, G_1, G_2 and M_1, B_{2a}, M_2, as well as other aflatoxin metabolites. The method was used in monitoring aflatoxin contamination of foods, the first stage of which is a preliminary screening of samples by TLC (the detection limit is 1 μg kg^{-1} for aflatoxin B,). A survey of the occurrence of aflatoxins B_1, B_2, G_1, G_2 in Soviet domestic and imported cereals, nuts, beans and oilseeds harvested in 1985-87 (> 4300 samples) as well as aflatoxin M_1 in domestic dairy products (> 250 samples) was carried out using HPLC and TLC methods. It was shown that 26.9 % of imported groundnuts, 2.8 % of maize and 6.2 % of barley were contaminated with aflatoxins at levels exceeding the maximum tolerated level established in the USSR (5 μg kg^{-1} for aflatoxin B, in foods of all kinds excluding baby foods). Maximum concentrations were 3600, 155 and 8 μg kg^{-1}, respectively. As much as 28.3 % of domestic cottonseed samples, which were chosen for analysis due to toxic effects on animals, were also contaminated with aflatoxins.

1202. Uyakul, D., Isobe, M, and Goto, T. 1989. Mycotoxin analysis by fast atom bombardment tandem mass spectrometry. Journal of the Association of Official Analytical Chemists 72(3): 491-497.

Positive fast atom bombardment tandem mass spectrometry is demonstrated to be an effective technique for determination of crude aflatoxins and sterigmatocystin-related compounds. The molecular ion was selected by the first system and bombarded to produce characteristic daughter ions that could be used to identify mycotoxins in mixtures and with the same molecular weight.

1203. Wei, D.L., and Wei, R.D. 1980. High pressure liquid chromatographic determination of aflatoxins in peanut and peanut products of Taiwan. Proceedings of the National Science Council, Taiwan 4(2): 152-155.

Analysis was carried out on 401 samples of locally-consumed groundnut and groundnut products including peanut butter, groundnut cake, fried or roasted groundnuts and oil, using a high pressure liquid chromatography (HPLC) method. The incidence of aflatoxin contaminated samples was 23.4 %. Aflatoxin B_1 was found in all positive samples.

1204. Wilson, D.M. 1989. Analytical methods for aflatoxins in com and peanuts. Archives of Environmental Contamination and Toxicology 18(3): 308-314.

This review includes discussion of safety, sampling, standards, presumptive and screening methods, quantitative methods, including thin-layer chromatography (TLC), high-pressure liquid chromatography (HPLC) and immunochemical methods, and selection of analytical approach in relation to the detection and estimation of aflatoxins in maize and groundnuts.

1205. Wilson, D.M., Beaver, R.W., Layton, R.C., Trantham, A.L., and McMillan, D.L. 1987. Comparison of immunochemical methods for aflatoxin determination in raw peanuts. Proceedings of the American Peanut Research and Education Society 19: 36.

Three immunochemical and three chemical methods were compared for aflatoxin analysis, using fifty naturally contaminated lots of raw shelled groundnuts. The USDA/AMS analyzed all lots using the water slurry modification of the Best Foods (BF) method. The lots were chosen so that aflatoxin levels ranged from 0 to 100 µg kg^{-1}. The Federal-States Inspection Service modified Dickens mill was used to collect special 10 lb, subsamples from commercial lots. Each 10 lb sample was divided, and duplicate analytical subsamples for each method were prepared to make each subsample as representative as possible. The Aflatest-P mycotoxin testing system uses a monoclonal based affinity column and was provided by Cambridge Naremco, Springfiled, MO. The ELISA methods used were Agrichek aflatoxin test provided by the Agri Tech Systems, Inc., Portland, ME, and the Agri-Screen test supplied by Neogen Corporation, Lansing, MI. The chemical methods used were the water-slurry method , CB method and an HPLC method using normal phase separation and a silica gel packed cell with fluorescence detection following the CB extraction step. The overall mean from all analyses and methods was 19 µg kg^{-1} with a range of 0 to 100 µg kg^{-1} total aflatoxins ; the coefficient of variation (CV) was 36 %. Analysis of variance showed no significant differences between methods. The methods contributed little to variation, but samples contributed to 56 % and subsamples to 35 % of the variation. All methods showed significant relationships ($P< 0.01$) with regression using the water slurry method as the dependent variable. The R values ranged from 0.66 to 0.83. All methods performed well and were comparable. Any one of the immunochemical methods could be routinely used by trained personnel to perform aflatoxin analyses.

7.2.6 Minicolumn Methods

1206. Alisauskas, V.A. 1974. Determination of aflatoxin in grain and feedstuffs. Food Technology in Australia 26(6): 233-237.

Ninety-two samples of grain and common feed ingredients were analyzed in Australia using thin-layer chromatography (TLC) and a rapid minicolumn screening method. Three samples were found to contain aflatoxins; a sorghum grain sample containing 30 µg kg^{-1} aflatoxin B_1, and two samples of moldy groundnuts containing 2500 µg kg^{-1} aflatoxin B_1 and 400 µg kg^{-1} aflatoxin B_2, and 200 µg kg^{-1} B_1 and 38 µg kg^{-1} B_2, respectively. A non-destructive detection technique involving scanning the grain with a UV lamp and examination of fluorescence is reported.

1207. Bottalico, A., Lerario, P., and Ricci, V. 1979. Use of the Velasco method for analysis of mycotoxins in various products of plant origin. Revista della Societa Italiana di Scienza dell' Alimentazione 8: 299-302.

The method of Velasco based on separation of aflatoxins on a Florisil/silica gel/alumina minicolumn and UV fluorometric detection, using a Velasco Fluorotoxin Meter, was compared with visual detection after separation on the minicolumns, and thin-layer chromatographic (TLC) determination. Trials were conducted on spiked samples of groundnuts, soybean and sunflower presscakes. chickpeas, beans, almonds, wheat, maize, barley, cassava, silage, and a feed mixture. Severe interference by other constituents of most of the food or feed samples studied was observed with Velasco's method. The filter fluorometer system did not allow differentiation of interfering impurity bands having maximum fluorescence wavelengths close to those of aflatoxins but differing in colour; visual evaluation of the column permitted differentiation of these. The Velasco minicolumn/UV fluorometry method gives a total aflatoxin concentration rather than values for individual aflatoxins, whereas TLC gives data for concentration of individual aflatoxins. It is concluded that TLC techniques are likely to give better results than Velasco's method.

1208. Coker, R.D. 1984. High performance liquid chromatography and other chemical quantification methods used in analysis of mycotoxins in foods. Pages 207-263 in Analysis of Food Contaminants (Gilbert, J., ed.), Elsevier Science Publishers, London, UK.

The importance of sampling, sample preparation and assay techniques in the analysis of mycotoxins in various agricultural commodities is discussed, with emphasis on the required efficiency of the quantification step. Thin-layer chromatography (TLC), high performance thin-layer chromatography (HPTLC), high performance liquid chromatography (HPLC), gas-liquid chromatography (GLC), immunoassay, fluorotoxin meter and other methods are described and compared. Future trends in the analysis of mycotoxins are outlined.

1209. Davidson, J.I.Jr., Dickens, J.W., Chew, V., Sanders, T.H., Holaday, C.E., Cole, R.J., and Whitaker, T.B. 1984. Performance of the visual, minicolumn and TLC methods in detecting aflatoxin in 20 contaminated lots of farmers stock peanuts. Peanut Science 11(2): 77-83.

Standard grade samples (16) from each of 20 selected minilots were used to evaluate three methods for detecting minilots of farmers stock groundnuts with unacceptable levels of aflatoxin. A visual, a minicolumn and a modified thin-layer chromatography (TLC) method were used to compare analytical results, variation, and probability of acceptance for minilots having mean aflatoxin levels ranging from

8 to 255 µg kg^{-1}. Mean values obtained by each of the three methods increased linearly with mean aflatoxin levels of the minilots and variation for each method as determined by the variance and coefficient of variation (CV) was very large. The CV for all three methods decreased as aflatoxin level increased. Overall performances of the methods were similar in accepting and rejecting these minilots on the basis of the 1.8 kg grade samples. The greatest difference in the three methods occurred at the zero acceptance level where the modified TLC, minicolumn and visual methods rejected 97, 98 and 88 %. respectively, of the minilots with more than 60 µg kg^{-1} aflatoxin. At this acceptance level the TLC, minicolumn and visual methods also rejected 55, 50 and 30 %, respectively, of the minilots with < 30 µg kg^{-1} aflatoxin.

1210. Davis, N.D., Guy, M.L., and Diener, U.L. 1980. A fluorometric rapid screen method for aflatoxin in peanuts. Journal of the American Oil Chemists' Society 57(3): 109-110.

Groundnuts were screened for aflatoxin using a rapid, inexpensive fluorometric method. Groundnuts were ground and extracted with methanol, and the extract was treated with acidified zinc-acetate-sodium chloride solution, filtered and diluted with water. Fluorescence of the extracts was compared with that from aflatoxin-free control groundnuts. Test samples (160) of several varieties and grades of groundnuts, obtained from storage and several commercial sources, were screened for the presence of aflatoxin. One hundred thirty-five samples (84 %) were identified by this method as aflatoxin positive (> 15 µg kg^{-1}) or aflatoxin negative (< 15 |ig kg^{-1}). Although 22 samples (13.6 %) were incorrectly labeled as aflatoxin positive, most of these showed evidence of the presence of mold metabolites other than aflatoxin. Three samples (1.8 %) were incorrectly labeled as aflatoxin negative when they actually contained 20, 33 and 34 µg kg^{-1} aflatoxin.

1211. Goto, T., and Manabe, M. 1989. Methods for the analysis of aflatoxins in groundnut and other agricultural commodities. Pages 173-182 in Aflatoxin contamination of groundnut : Proceedings of the International Workshop, 6-9 October 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

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. Recently several enzyme-linked immunosorbent assay (ELISA) system kits for aflatoxin analysis have been developed and some of them found suitable for the kind of tesing 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.

1212. Holaday, C.E. 1968. Rapid method for detecting aflatoxins in peanuts. Journal of the American Oil Chemists' Society 45: 680- 682.

A rapid and simple minicolumn procedure for detecting and quantifying aflatoxin in groundnuts is described. Minicolumns are prepared by filling a length of 4-mm diameter glass tubing with silica gel to a depth of about 4.5 cm. The minicolumn is developed in a chloroform-methanol extract of a groundnut sample. If aflatoxin is present, a blue fluorescent band at the lower end of the column is observed when the column is exposed to long-wave ultraviolet light. Sensitivity is in the order of 5 µg kg^{-1} and an assay can be completed in 15-25 min. Some degree of quantification is possible by comparison with columns developed in extracts with known aflatoxin contents.

1213. Holaday, C.E. 1976. A rapid screening method for the aflatoxins and ochratoxin A. Journal of the American Oil Chemists' Society 53: 603-605.

A rapid, economical minicolumn procedure for screening aflatoxins and ochratoxin A in a wide range of products (wheat, barley, sorghum, corn, rice, rye and groundnuts) is presented. The technique includes high speed blending of the sample with aqueous methanol, purification of the extract with a solution of zinc sulphate and phosphotungstic acid, partitioning in benzene, and minicolumn chromatography. Sensitivities of 4 µg kg^{-1} for the aflatoxins and 20 µg kg^{-1} for ochratoxin A could be achieved ; and the simplicity of the method, which includes the use of disposable plastics and glass items, makes it practical for field or in-plant applications.

1214. Holaday, C.E. 1981. Minicolumn chromatography : State of Art. Journal of the American Oil Chemists' Society 58(12): 931A-934A.

It was recognized in the early 1960s that a rapid screening method for aflatoxin was needed. Holaday first proposed the minicolumn chromatography method as a rapid screening method in 1968. Since that time, many improvements have been made in this method. The latest minicolumn method has a limit of detection of 5 µg kg^{-1} and can be completed in 5-7 min. The minicolumn technique has been expanded to include screening commodities other than groundnuts for aflatoxin, as well as for other mycotoxins including ochratoxin, zearalenone and aflatoxin M_1.

1215. Holaday, C.E., and Barnes, P.C.Jr. 1973. Sensitive procedure for aflatoxin detection in peanuts, peanut butter, peanut meal, and other commodities. Journal of Agricultural and Food Chemistry 21(4): 650-652.

An improved minicolumn procedure for detecting aflatoxins in groundnuts and groundnut products is described. The technique has a sensitivity of about 3-5 μg kg^{-1} and can be completed in approximately 15 min. This can be used on a number of commodities. Proximate quantitation of aflatoxins is also possible.

1216. Holaday, C.E., and Iansden, J. 1975. Rapid screening method for aflatoxin in a number of products. Journal of Agricultural and Food Chemistry 23(6): 1134-1136.

An improved qualitative minicolumn procedure for screening a number of products, e.g. peanut butter, groundnut meal, corn, rice, and cottonseed meal for aflatoxin is described. High-speed blending of the sample with aqueous methanol followed by purification with zinc acetate and re-extraction with benzene before subjecting to minicolumn chromatography is a simple, economical, and rapid method for aflatoxin detection. Sensitivities of 2 μg kg^{-1} can be achieved and the use of disposable plastics and glass items makes the method practical for field or in-plant application.

1217. Lansden, J.A., and Holaday, C.E. 1974. An improved millicolumn procedure for detecting aflatoxin in peanut butter and other agricultural commodities. Proceedings of the American Peanut Research and Education Association 6(1): 53.

An improved procedure for detecting aflatoxin in peanut butler and oilier agricultural commodities has been developed. Heretofore, considerable difficulty was encountered using the millicolumn for detecting aflatoxin in peanut butter. This new procedure overcomes the difficulty by using alumina and florisil as packing materials in the column and by salting out highly fluorescent pigments in the extract before it is placed on the column. Time for an analysis is about 8-9 minutes. Sensitivity as low as 2-3 μg kg^{-1} is possible.

1218. Lemieszek-Chodorowska, K. 1976. [Minicolumn and thin-layer chromatographic methods for the detection and semiquantitative determination of aflatoxin B_1 in groundnuts.]. Wykywanie i polilosciowe oznaczanie aflatoksyny B_1 w orzechach arachidowych metoda chromalografii minikolumnowej i cienkowarstwowej. Roczniki Panstwowego Zakladu Higieny 27(5): 535-542.

A minicolumn method is described for determination of aflatoxin B_1 in groundnuts. The method allowed detection of aflatoxin B_1 at more than or equal to 1 μg kg^{-1}. The thin-layer chromatography (TLC) method was used for semiquantitative visual determination of the toxin; mean recoveries for the values more than or equal to 2.5 μg kg^{-1} were 76-87 %.

1219. Madhyastha, M.S., and Bhat, R.V. 1984. Application of TLC chemical confirmatory tests to minicolumn chromatography of aflatoxins. Journal of the American Oil Chemists' Society 61: 907-908.

The minicolumn proposed by Holaday and Lansden was developed with standard aflatoxin solution and also with the extracts of com, rice, wheat, cottoseed, groundnut cake and black pepper; each having different levels of aflatoxins. One-half mL each of 2, 4-dinitrophenylhydrazine, p-anisaldehyde, 20% H_2SO_4, 20% HC1 and trifluoroacetic acid (TFA) with 25% HNO_3, which were used for confirming aflatoxins on TLC, were applied to the developed column. Among these, all the three acid reagents changed the blue fluorescence of aflatoxins to yellow and thus were found to be satisfactory confirmatory tests. The TFA with 25% HNO_3 had the lowest detection limit (5 μg kg^{-1}).

1220. Mehan, V.K., Bhavanishankar, T.N., and Bedi, J.S. 1985. Comparison of different methods for extraction and estimation of aflatoxin B_1 in groundnut Journal of Food Science and Technology, India 22(2): 123-125.

Aflatoxin extraction methods namely Best Food (BF), Contamination Branch (CB), Pons' and Romer's and analytical methods namely thin-layer chromatography (TLC), spectrophotometric and minicolumn techniques, in different combinations, were compared for their efficiency for determining aflatoxin B_1 occurring naturally in groundnuts and also for estimating aflatoxin B_1 levels in groundnut meal samples spiked with known quantities of the toxin. The BF and Pons' methods gave better efficiency than the other two methods. The spectophotometric method was more effective than the TLC and minicolumn techniques. The BF method was relatively less expensive and less time consuming than the other extraction methods. The Pons' method was convenient for handling larger numbers of samples, especially in the absence of centrifuge facilities required for the BF method.

1221. Pettit, R.E., Sarr, B.A., Machen, M.D., and Phillips, T.D. 1990. Detection and detoxification of aflatoxin-contaminated groundnut products in West Africa. Page 97 in Summary Proceedings of the First ICRISAT Regional Groundnut Meeting for West Africa, 13-16 Sep 1988, Niamey, Niger. Patancheru, A.P. 502 324, India. International Crops Research Institute for the Semi-Arid Tropics.

A new mycotoxin detection procedure termed "Selectively Absorbed Mycotoxins (SAM)", has been developed. It is a modification of the Holaday-Velasco Minicolumn procedure for aflatoxin screening. With the SAM assay procedure, mycotoxins are extracted into a methanol-water solvent. Toulene is mixed with the

extract to form a two-phase solution where aflatoxins and zearalenone partition into the upper toulene phase, thus separating them from potential interfering compounds. The toulene sample is passed through a SAM-Aflatoxin Zearalenone (SAM-AZ) tube which removes other interfering compounds in a pre-absroption layer, and selectively absorbs any aflatoxin and/or zearalenone at specific bands in the tip of the tube. Each positive band glows with an obvious blue color under long wave ultraviolet light. The sensitivity of the assay is designed to provide a YES/No screen at designated levels of aflatoxin and zearalenone. Detoxification of aflatoxin-contaminated groundnut products has been accomplished by the addition of a high-affinity sorbent (hydrated sodium calcium aluminosilicate) when added at 0.5 % by weight. Aluminas, silicas, and aluminosilicates were evaluated for their ability to absorb aflatoxin from groundnut oil and aqueous solution. Sorbents such as beohmite alumina, synthetic xeolite and muscovite silica sorbed less than 50 % of the aflatoxin present. Novo Sil (hydrated sodium calcium aluminosilicate, HSCAS), Pyran RG 140 pyrophyllite, and Filtrol (acid-activated bleaching earth), sorbed 85 % or more of the aflatoxin. When HSCAS was added to chicken feed containing 7.5 mg aflatoxin, the toxicity was reduced so that broiler and leghorn chicks appeared normal in their growth and their livers were normal. The safety and effectiveness of the detoxification procedure was measured with the Ames assay.

1222. Romer, T.R. 1975. Screening method for the detection of aflatoxins in mixed feeds and other agricultural commodities with subsequent confirmation and quantitative measurement of aflatoxins in positive samples. Journal of the Association of Official Analytical Chemists 58(3): 500-506.

A screening method is described for detecting total aflatoxins (B_1+B_2+G_1+G_2) in mixed feeds, grains, nuts, and fruit products in samples containing 5-15 µg kg^{-1}. In addition, the presence of aflatoxins in positive samples can be confirmed and the toxins can be quantitatively measured, using the same extract as that used for screening. In the screening method, aflatoxins are extracted with acetone:water (85:15), and interferences are removed by adding cupric carbonate and ferric chloride gel. The aflatoxins are extracted from the aqueous phase with chloroform and the chloroform extract is washed with a basic aqueous solution. A Velasco-type minicolumn is used to further purify the extract and capture the aflatoxins in a tight band. The screening method has been successfully applied to 24 different agricultural commodities. Quantitative TLC was also performed with extracts of each of these commodities. An average recovery of 94 % B_1, 108 % B_2, 130 % G,, and 103 % G_2 was obtained compared to the official final action AOAC method for cottonseed products. Within- laboratory coefficients of variation of 10-15 % were obtained for each of the aflatoxins and total aflatoxins in a sample of groundnut meal naturally contaminated with 11 µg B_1 + 3 µg B_2 + 11 µg G_1 + 5 µg G_2/kg.

1223. Romer, T.R., and Campbell, A.D. 1976. Collaborative study of a screening method for the detection of aflatoxins in mixed feeds, other agricultural products, and foods. Journal of the Association of Official Analytical Chemists 59(1): 110-117.

A screening method for aflatoxins was collaboratively tested on 11 different agricultural and food products : white and yellow corn, groundnuts, peanut butter, pistachio nuts, groundnut meal, cottonseed meal, chicken, pig and turkey starter rations, and dairy cattle feed. The method involves a rapid extraction and cleanup procedure followed by the detection of total aflatoxins (B_1+B_2+G_1+G_2) as a fluorescent band on the florisil layer of a Velasco-type minicolumn. The results of 32 collaborators from 10 countries are presented. Samples containing 0, 5, 10, 15, 20 and 25 µg aflatoxins kg^{-1} were analysed. 84 % of the negative samples and 89 % of the samples containing 10-25 µg total aflatoxins/kg were correctly identified. This method has been adopted as official first action for the detection of aflatoxins in corn, groundnuts, peanut butter, groundnut meal, cottonseed meal, mixed feeds, and pistachio nuts.

1224. Sarr, A.B., and Phillips, T.D. 1989. Rapid detection of aflatoxins in peanut with the SAM assay. Proceedings of the American Peanut Research and Education Society 21: 18.

A new method for the rapid detection of aflatoxins (i.e., SAM) was compared with a method of high pressure liquid chromatography (HPLC) method. Fifty groundnut samples were analyzed in this study. For the SAM method, groundnut samples (40 g) were ground and extracted with methanol: water (80:20). Aliquots (5 mL) of the methanolic phases were added to 5 mL of water and then partitioned with 3 mL of toluene. Aliquots (0.5 mL) of the toluene phases were added to the top of SAM detectors. Following elution with toluene : chloroform : acetone (95:20:5, v/v/v) all samples were analyzed for aflatoxins with longwave ultraviolet light (365 nm). Groundnut samples (20 g) were ground for HPLC analysis and extracted with acetone : water (75:25, v/v). Fats were removed with hexane, and the aflatoxins were extracted with chloroform and analyzed by normal phase HPLC. No differences was observed between the two methods. All samples positive by HPLC were positive by SAM, and all samples negative by HPLC were negative by SAM. The percent recovery and the stability of aflatoxin B_1 in the toluene phase in the SAM assay were determined. Aliquots (5 mL) of the extraction solvent were "spiked" with aflatoxin B_1 at levels of 5500, 550, 220, and 55 ng. Aflatoxin B_1 was partitioned with the toluene phase and analyzed by HPLC. The percent recovery of aflatoxin B_1 was 70 % (a recovery of 90 % is routinely attained when chloroform is used to extract the methanolic phase). A correction factor was determined for standards of aflatoxin B_1 in toluene. Aflatoxin B, was found stable in the toluene phase of SAM. Simple and rapid screening tests such as SAM for the detection of aflatoxins in groundnuts may greatly facilitate prevention through effective

monitoring programs that allow for the diversion of contaminated crops and animal feeds.

1225. Shashidhar, R.B., Ramakrishna, V., and Bhat, V.R. 1988. Rapid detection of aflatoxins by pressure mini-column technique. Anal. Lett. 21(3): 507-518.

Aflatoxins in food and feeds are detected simply, rapidly and reliably by using a 5-mL glass syringe packed (from the bottom) with 3 mm of anhydrous Na_2SO_4, 5 mm of silica gel, 1 mm of Florisil and 4 mm of anhydrous Na_2SO_4. The column is activated at 110 C for 1 to 2 hr. A sample extract (2 mL) is mixed for 1 min with 400 mg of neutral $A1_2O_3$, and 1 mL of extract is applied to the packed syringe, followed by 3 mL of hexane-$CHCl_3$-THF (7:2:1). The solvent is rapidly drained under pressure, and the syringe is examined under 365 nm radiation. A blue fluorescent band at the silica-Florisil interface indicates the presence of aflatoxins; this can be confirmed by standard chemical tests, preferably by reaction with trifluoroacetic acid-H_2SO_4. Semi-quantitative determination of aflatoxins is achieved by comparing the intensity of nuorescence with that for a standard. Depending on which confirmatory test is used, the detection limit is 10 to 50 ug kg^{-1}.

1226. Shotwell, O.L., and Holaday, C.E. 1981. Minicolumn detection methods for aflatoxin in raw peanuts : Collaborative study. Journal of the Association of Official Analytical Chemists 64(3): 674-677.

The Holaday-Velasco method and a modified Holaday method were compared for anatoxin analysis in raw groundnuts. The former method combines the speed and simplicity of the Iloladay extraction and cleanup with the sensitivity of the minicolumn originally described by Velasco. The combination method has been approved by the AOAC and the AACC for determining aflatoxin in com. The Iloladay method was modified by substituting toluene for benzene in the solvent partition, and methylene chloride for chloroform in the minicolumn development to eliminate use of hazardous solvents. The neutral alumina in the Iloladay minicolumn was changed from activity V to activity III to provide a more stable column. At aflatoxin levels in raw groundnuts of 13-20 ng g^{-1}, the presence of aflatoxin was missed by the modified Holaday method in four analyses (3 laboratories) of 42 reported. There were no misses in this contamination range by the Holaday-Velasco method. There were no misses by either method with samples containing > 20 ng total aflatoxins g^{-1}. Analysis of non-contaminated raw groundnuts by the modified Holaday method resulted in two false positives of 14 reports; the Holaday- Velasco method produced no false positive reports from 15 analyses of non-contaminated groundnuts. The Holaday-Velasco method was adopted as official first action for groundnuts.

1227. Sylos, C.M., and Rodriguez-Amaya, D.B. 1989. Inexpensive, rapid screening method for aflatoxins in peanuts and peanut products. Journal of the Science of Food and Agriculture 49(2): 167-172.

The extraction and cleanup steps of the AOAC minicolumn technique were modified to provide an inexpensive, rapid screening procedure for aflatoxins in groundnuts and groundnut products. A total of 52 samples of groundnuts and groundnut products were collected from shops and markets in Campinas, Brazil, and were analysed for aflatoxins by the old and the modified methods. The results by both methods were the same : 28 samples were negative, four contained <20 µg kg^{-1} aflatoxin, 12 contained 20-50 µg kg^{-1}, three had 50-100 µg kg^{-1} and five had >100 µg kg^{-1}. The detecuon limit in both minicolumn methods was 10 µg kg^{-1}.

1228. Tsai, Y.J., Davidson, J.I., Chew, V., Cole, R.J., and Sanders, T.H. 1989. Characteristics of visual, minicolumn and TLC methods in detecting aflatoxin contaminated loads of farmers stock peanuts. Peanut Science 16(1): 1-5.

Results from two 1980 experiments were used to compare the performance of the visual, minicolumn and thin-layer chromatography (TLC) methods in detecting loads of fanners stock groundnuts with aflatoxin. The first experiment was conducted to establish variability of the methods under ideal conditions. The second experiment was conducted to evaluate the performance of the three methods under commercial conditions. Date and empirical models were used to explore potential improvements in detecting low-level contaminated lots. The minicolumn and TLC methods were very sensitive and provided consistent measurements. Operation characteristic curves developed by using empirical models (Logistic and Gompertz) were compared to statistical distribution functions used by Whitaker and coworkers. Assuming a desired probability of acceptance of 15 %, both the minicolumn and TLC methods would accept lots with 60 µg kg^{-1} aflatoxin, while the visual methods would accept lots with 150 µg kg^{-1} aflatoxin. In crop years similar to 1980, the currently used method would not segregate groundnuts to meet the 15 µg kg^{-1} (total) tolerance levels and would provide excessive sheller risk. Because of the objectivity, precision and higher sensitivity, the minicolumn and TLC have potential for improving the detection of contaminated loads of farmers stock groundnuts.

1229. Velasco, J., and Morris, S.L. 1976. Use of water slurries in aflatoxin analysis. Journal of Agricultural and Food Chemistry 24(1): 97-103.

A method based on the use of water slurries was developed for aflatoxin analysis. This procedure enables aliquots, from kilogram size samples, to be analyzed without an increase in solvent or reagent costs. Groundnuts, peanut butter, groundnut meal, cottonseed, cottonseed meal, copra and maize were successfully analyzed by this slurry technique. Slurries were prepared in a 1-gal blender and sample weight to water volume ratios were 1:1.5 for peanut butter, 1:2.25 for whole seeds, 1:4 for

commercial meals and 1:5 for copra. Extraction of 100-g aliquots of slurry with 200 mL of acetone resulted in aflatoxin yields comparable to those obtained by extraction of the dry products by the standard method. The precision of slurry analysis for the products, expressed as % coefficient of variation, ranged from 2.6 to 7.8 compared to 5.7 to 20.8 for analyses by the standard procedure. Determinations were made by fluorometric measurements of aflatoxin absorbed on the florisil layer in a minicolumn.

1230. Whitaker, T.B., and Dickens, J.W. 1986. Efficacy of the visual, minicolumn, and thin layer chromatography methods to test farmers stock peanuts for aflatoxin. Peanut Science 13: 74-77.

This study estimated the efficacy of the visual A. flavus (VAF), minicolumn (MCL), and thin-layer chromatography (TLC) methods to detect farmers' stock groundnuts which contained aflatoxin. Aflatoxin tests on grade samples from each of 2300 lots of fanners' stock groundnuts were used to estimate the distribution of farmers' stock lots according to their aflatoxin concentration (lot distribution). This lot distribution (with an average aflatoxin concentration of 59.5 µg kg^{-1}) was incorporated into each of the 3 computer models that simulate the testing of farmers' stock groundnuts for aflatoxin when the VAF, MCL, and TLC methods are used. The number of lots accepted and the average aflatoxin concentration (AA) in the accepted lots was predicted. Results indicate that when a given percentage of the lots are accepted, lots accepted by the VAF method have less aflatoxin than those lots accepted by either the MCL or TLC methods. When the present visual method was used to test the above lot distribution, 75.8 % of the lots tested were accepted and the AA in the accepted and rejected lots were 4.1 and 232.8 µg kg^{-1}, respectively.

1231. Whitaker, T.B., Dickens, J.W., and Chew, V. 1985. Development of statistical models to simulate the testing of farmers stock peanuts for aflatoxin using visual, thin layer chromatography, and minicolumn methods. Peanut Science 12: 94-98.

The negative binomial probability function was used to model the distribution of sample aflatoxin test results when replicated grade samples from farmers stock groundnuts were analysed by thin-layer chromatography and minicolumn methods. The Poisson probability function was used to model the distribution of the number of kernels with visible Aspergillus flavus growth found in replicated grade samples of farmers stock groundnuts when the visible A. flavus method was used. The probabilities of accepting a lot of farmers stock groundnuts with given aflatoxin concentrations when using a 465-g grade sample and two different accept/reject levels were predicted with the models and compared to observed acceptance probabilities computed from previously published data for each of the three methods. The comparisons showed good agreement between the predicted acceptance probabilities and the observed acceptance probabilities.

7.2.7 Immunochemical Methods

1232. Anjaiah, V., Mehan, V.K., Jayunthi, S., Reddy, D.V.R., and McDonald, D. 1989. Enzyme-linked immunosorbent assay (ELISA) for aflatoxin B_1 estimation in groundnuts. Pages 183-189 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 October 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

The commercially available hapten, aflatoxin-B_1-oxime-bovine serum albumin (hapten-BSA), 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 aqueous 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, standards or groundnut samples 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.

1233. Biermann, A., and Terplan, G. 1982. [Results with micro-ELISA for aflatoxin B_1 determination in food.]. Erfahrungen mit einem Mikro-ELISA zur Aflatoxin B_1- Bestimmung in Lebensmitteln. Archiv fur Lebensmittelygiene 33(1): 17-20.

An enzyme-linked immunosorbent assay (ELISA) technique is described for determining aflatoxin B_1 in contaminated groundnut meal. The technique showed a 100 pg g^{-1} sensitivity and a recovery rate from spiked samples with higher concentrations of c. 90 %.

1234. Candlish, A.A.G., Haynes, C.A., and Stimson, W.H. 1988. Detection and determination of aflatoxins using affinity chromatography. International Journal of Food Science & Technology 23(5): 479-485.

Aflatoxins B_1, B_2, G_1 and G_2 can be easily and rapidly detected in aqueous solutions using an affinity chromatography column coupled to a monoclonal antibody specific for the toxin molecules. A method for their detection, based on this procedure, is described. Water : methanol extracts of aflatoxin-free food were spiked with aflatoxins, diluted with water and passed through the affinity matrix. The monoclonal antibody is bound to the aflatoxins, which can then be released using a small volume of methanol. This concentrated and separated the aflatoxin present in solutions. As little as 5 ng aflatoxin can be seen if the methanol eluate is passed over a small florisil tip under UV light, while 0.5 ng can be detected if the eluate is analysed by HPLC. This system can be used to analyse aflatoxins in contaminated samples by spot testing (>5 ng) or as a means of clean-up for quantitative analysis at subnanogram levels. Advantages of this immunological assay in relation to other immunoassays and traditional methods are discussed.

1235. Candlish, A.A.G., Smith, J.E., and Stimson, VV.H. 1990. Aflatoxin monoclonals : academic development to commercial production. Letters in Applied Microbiology 10: 167-169.

A monoclonal antibody (mAb) has been produced to aflatoxin B_1 after immunization of mice and fusion of sensitised spleen cells with myeloma cancer cells. The mice were immunized with aflatoxin B_1-oxime-protein conjugate. Positive mAbs were screened using an indirect ELISA specific for aflatoxin B,. The selected mAb was then developed in direct competitive ELISA and immunoaffinity column chromatography methods for aflatoxin detection in foods and feeds. Both assays are rapid, sensitive, specific and require only the minimum of sample preparation. Both immunological assays have now been commercialised and are produced in convenient ready-made kit formats.

1236. Candlish, A.A.G., Stimson, W.H., and Smith, J.E. 1987. The detection of aflatoxin B_1 in peanut kernels, peanut butter and maize using a monoclonal antibody based enzyme immunoassay. Food Microbiology 4(2): 147-153.

A simple procedure is described for the routine immunochemical analysis of aflatoxin B_1 in groundnut kernels, peanut butter and maize. The specificity, affinity and sensitivity of the monoclonal antibody employed is such that minimal sample preparation is required. The enzyme immunoassay (EIA) has a sensitivity for standard aflatoxin B_1 of 0.2 ng mL^{-1} with working range up to 30 ng mL^{-1} and is not significantly affected by matrix interference of samples. Essential protocol features are: blending substrate with methanol: water, filtering blended sample, and analysis of filtrate by EIA after dilution with buffer. Average recoveries of aflatoxin B, spiked samples at levels of 6-400 µg kg^{-1} were 90-112.5 %. Using laboratory prepared samples contaminated with Aspergillus flavus there was high positive correlation (r=0.97) when EIA results were compared with thin-layer chromatography (TLC) techniques.

1237. Carvajal, M., Mulholland, F., and Carner, R.C. 1990. Comparison of the EAS1-EXTRACT immunoaffinity concentration procedure with the AOAC CB method for the extraction and quantitation of aflatoxin B_1 in raw ground unskinned peanuts. Journal of Chromatography 511: 379-383.

A commercial immunoaffinity column (EASI-EXTRACT) for the routine assay of aflatoxins in groundnuts was compared with the official first action AOAC CB method. For the CB method, 82.0 and 84.1% of aflatoxin B_1 was recovered from the 10 and 50 µg kg^{-1} spiked groundnut samples, respectively. For the immunoaffinity procedure, the recoveries were 93 and 95.5%, respectively. These values were significantly ($P < 0.05$) higher than the CB method. There was also considerable time-saving using the EASI-EXTRACT method.

1238. Chu, F.S. 1984. Immunoassays for analysis of mycotoxins. Journal of Food Protection 47(7): 562-569.

During the past few years, several laboratories have prepared specific antibodies against aflatoxins B_1, M_2, B_{2a} and Q_1, ochratoxin A, T-2 toxin, and zearalenone. With the avalability of these antibodies, specific, simple and sensitive radioimmunoassay (R1A) and enzyme linked immunosorbent assay (ELISA) procedures for monitoring mycotoxins and their metabolites in foods, feeds and body fluids have been developed. In this review details are given for the preparation of the antibodies and the application of RIA and ELISA to determine aflatoxins B, and M_1, ochratoxin A and T-2 toxin in corn, groundnut, milk and other biological fluids. The sensitivity of ELISA for analysis of these mycotoxins in foods varied from 0.1 µg L^{-1} for aflatoxin M, in milk to 5 µg kg^{-1} of aflatoxin B_1 in groundnuts. The advantages of ELISA for monitoring mycotoxins in foods and feeds are discussed. In addition, a description of recent progress on simplified cleanup procedures which may increase the sensitivity of immunoassays is presented.

1239. Chu, F.S. 1986. Immunoassays for mycotoxins. Pages 207-237 in Modern Methods in the Analysis and Structural Elucidation of Mycotoxins. (Cole, R.J., ed.). Academic Press, Inc., New York.

This paper reviews methods for production of specific antibodies against mycotoxins, and the applications of radioimmunoassay (RIA) and an enzyme-linked immunosorbent assay (ELISA) for analysis of several mycotoxins in various agricultural commodities.

1240. Chu, F.S. 1989. Current immunochemical methods for analysis of aflatoxin in groundnuts and groundnut products. Pages 161-172 in Aflatoxin Contamination of Groundnut : Proceedings of the International Workshop, 6-9 October, 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

With the availability of specific monoclonal and polyclonal antibodies against mycotoxins in recent years, simple, sensitive and specific radioimmunoassay (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 picograms/assay. Simple and quick immunoassay protocols (ELISA) for monitoring aflatoxin B_1 in groundnuts and groundnut products, that require less than 1h to complete, have been developed and successfully tested in naturally contaminated groundnut samples at levels about 5 to 10 µg kg^{-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 the recent progress on the production of the antibodies, antibody specificity, and the advantages and disadvantages of different immunoassays, as well as problems associated with immunochemical research on mycotoxins, with emphasis on anatoxins, are reviewed. Emphasis is centered on the immunoassays of aflatoxin in groundnut products.

1241. Chu, F.S., Fan, T.S.L., Zhang, Guang-Shi, Xu, Yi-Chun, Faust, S., and McMahon, P.L. 1987. Improved enzyme-linked immunosorbent assay for aflatoxin B_1 in agricultural commodities. Journal of the Association of Official Analytical Chemists 70(5): 854-857.

An improved enzyme-linked immunosorbent assay (ELISA) for aflatoxin B_1 in cornmeal and peanut butter was developed. Aflatoxin B_1 in cornmeal and peanut butter samples was extracted with 70 % methanol in water containing dimcthylformamide diluted with assay buffer to a final concentration of 7 % methanol, and directly subjected to an ELISA procedure that took less than lh for quantitative analysis and less than 30 min. for screening tests. Analytical recoveries for 5-100 ng g^{-1} B_1 added to the cornmeal and peanut butter were 91 and 95.4 %, respectively. The interwell and interassay coefficient of variation was 10 % or less at the 20 ng g ' level and above. Agreement for B_1 levels in more than 30 naturally contaminated corn, mixed feed, and peanut butter samples was excellent between the ELISA data and the data obtained from different independent laboratories using thin-layer chromatography (TLC) or other analytical methods.

1242. Chu, F.S., Hsia, M.T.S., and Sun, P. 1977. Preparation and characterization of aflatoxin B_1-1-(O-carboxymethyl) oxime. Journal of the Association of Official Analytical Chemists 60: 791-794.

A method is described for the preparation and purification of aflatoxin B,-1-(O-carboxymethyl) oxime from aflatoxin B_1. The overall yield was about 73-83%. The new aflatoxin B, derivative was characterized by mass, ultraviolet, infrared, and nuclear magnetic resonance spectral analyses, and was nontoxic to 8-day- old chicken embryos when tested at a concentration of 3.48 µg egg^{-1},

1243. Chu, F.S., Lee, R.C., Trucksess, M.W., and Park, D.L. 1988. Evaluation by enzyme-linked immunosorbent assay of cleanup for thin-layer chromatography of aflatoxin B_1 in corn, peanuts, and peanut butter. Journal of the Association of Official Analytical Chemists 71(5): 953-956.

A simple, rapid enzyme-linked immnosorbent assay (ELISA) was used to evaluate the performance of each step (extraction, filtration, solvent partition and silica gel column chromatography) of a solvent-efficient thin-layer chromatography (TLC) method undergoing interlaboratory collaborative study for the determination of aflatoxin B_1 in maize, raw groundnuts and peanut butter. The apparent mean recoveries using the ELISA method were approximately 30-50 % higher than those using the TLC method if only the amount of aflatoxin B_1 added to the samples was used in the calculations. After the cross reaction of the antibody with other aflatoxins added to the samples was considered, the amounts recovered approached the levels of the aflatoxins added in all the three commodities tested. With no cleanup treatment, ELISA recoveries at aflatoxin B_1 levels >7.5 ng g^{-1} were 84, 79 and 103 % for maize, raw groundnuts, and peanut butter, respectively. With each cleanup step in the TLC method, ELISA detected a progressive decrease in recovery from 150.5 to 105.3 % (before correction for the presence of other aflatoxins) or from 93.5 to 65.4 % (after correction for other aflatoxins) of aflatoxin B_1 added to samples. It is concluded that cleanup treatments are not necessary in the ELISA and that when large amounts of other aflatoxins are present, an understanding of the cross-reactivity of antibody with other aflatoxins in the ELISA is essential for final interpretation of the data.

1244. Chu, F.S., Steinert, B.W., and Gaur, P.K. 1985. Production and characterization of antibody against aflatoxin G,. Journal of Food Safety 7(3): 161-170.

Antibody against aflatoxin G_1 (AFG,) was obtained from rabbits after immunizing the animals with AFG, hemiacetal (AFG_{2a}) conjugated to bovine serum albumin. A direct heterogenous ELISA in which AFG_{2a} was conjugated to horseradish peroxidase was used for monitoring the antibody titers and for toxin detection.

Competitive ELISA assay revealed that the antibody was most specific for AFG_{2a} and least for AFB_2. The relative cross-reactivity of this antiserum with aflatoxins G_{2a}, G_2. G_1, M_1, B_1 and B_2 was found to be 1, 7, 13, 47, 48 and 63%, respectively. The lower detection limits for detection of AFG_1 after derivatizing to AFG_2a was around 15-25 pg $assay^{-1}$.

1245. Chu, F.S., and Ueno, I. 1977. Production of antibody against aflatoxin B_1. Applied Environmental Microbiology 33: 1125-1128.

Antibody against aflatoxin B_1 was obtained after one multiple- site injection of bovine serum albumin-aflatoxin B_1 conjugate into rabbits. The antibody had greatest binding efficiency for aflatoxin B_1, less efficiency for B_2, G_1, and Q_1, and least for aflatoxicol, G_2, and M_1. Sterigmatocystin, coumarin, and 4- hydroxycoumarin did not give a cross-reaction with the antibody. The sensitivity of the binding assay for detection of aflatoxin B, is in the range of 0.2 to 2.0 ng 0.5 mL^{-1} sample. Detailed methods for the preparation of the conjugate, production of immune serum, and methods for antibody titer determination are described.

1246. Coker, R.D. 1984. High performance liquid chromatography and other chemical quantification methods used in analysis of mycotoxins in foods. Pages 207-263 in Analysis of Food Contaminants (Gilbert, J., ed.), Elsevier Science Publishers, London, UK.

The importance of sampling, sample preparation and assay techniques in the analysis of mycotoxins in various agricultural commodities is discussed, with emphasis on the required efficiency of the quantification step. Thin-layer chromatography (TLC), high performance thin-layer chromatography (HPTLC). high performance liquid chromatography (HPLC), gas-liquid chromatography (GLC), immunoassay, fluorotoxin meter and other methods are described and compared. Future trends in the analysis of mycotoxins are outlined.

1247. Cole, R.J., Dorner, J.W., Kirksev, J.W., and Dowell, F.E. 1988. Comparison of visual, enzyme-linked immunosorbent assay screening and HPLC methods in detecting aflatoxin in farmers stock peanut grade samples. Peanut Science 15(2): 61-63.

Grade samples from 152 lots of farmers stock groundnuts were analysed for aflatoxin by an enzyme-linked immunosorbent assay (ELISA) rapid screening test and high-pressure liquid chromatography (HPLC) and the results were compared with those of the visual inspection method used by the Federal State Inspection Service (FSIS) in Georgia. The results showed 41 % of the grade samples with visible Aspergillus flavus (Segregation 3) contained < 20 |ig kg^{-1} aflatoxin when analysed by both ELISA and IIPLC methods; 18.7 % of Segregation groundnuts (no visual contamination) contained 26-2542 µg kg^{-1} aflatoxin. The results of ELISA and HPLC agreed in 98.6 % of the composite lot analyses with the detection of 20 µg kg^{-1} or greater. However, the ELISA rapid screening test failed to give positive tests 12 of 13 times when the aflatoxin content was 20-43 µg kg^{-1} in the component samples.

1248. Cole, R.J., Dorner, J.W., Kirksey, J.W., and Henning, R.J. 1987. Critical evaluation of an aflatoxin immunoassay quick-card test in farmers stock peanuts. Proceedings of the American Peanut Research and Education Society 19: 35.

An immunoassay quick-card test was evaluated on farmers' stock groundnuts relative to the criteria of speed, reproducibility, sensitivity, and economic consideration. The test was conducted at a buying point laboratory by two-technical personnel. Fifty-two 25-lb samples of farmers' stock groundnuts were collected by pneumatic probe from 26, 4-5 ton groundnut wagons (2 samples $load^{-1}$) representing live fields that experienced some drought stress. Each sample was processed by the Federal State Inspection Service in the same fashion as regular grade samples resulting in five grade categories for each sample (sound mature kernels (SMK), sound splits, other kernels, loose-shelled kernels and damaged kernels). The results of the immunoassay were compared to high performance liquid chromatography (HPLC) analyses of the same sample extracts. The official thin-layer chromatographic (TLC) analysis was only conducted on the SMK category due to limiting amounts of other sample categories. The immunoassay test was set up to detect 0-20 µg kg^{-1}. 20-100 µg kg^{-1}, and > 100 µg kg^{-1} aflatoxin. Of the 520 analyses, 12 were in wide disagreement between the immunoassay and HPLC. Errors resulting from the immunoassay were possible in six of the 12 discrepancies. The test, using two personnel, one to weigh, extract and filter samples, and the other to actually conduct the test, achieved a rate of 30 analyses h^{-1}. The rate was achieved doing 20 analyses at a time.

1249. Cole, D.L., and Masuka, A.J. 1989. Evaluation of new rapid methods for aflatoxin detection in groundnuts in Zimbabwe. Pages 185-189 in Proceedings of the third Regional Groundnut Workshop for Southern Africa, Lilongwe, Malawi, 13-18 March 1988. Patancheru 502 324, A.P., India : International Crops Research Institute for the Semi-Arid Tropics.

Two new rapid methods that use monoclonal antibodies to detect aflatoxins in groundnuts were tested. The Quantitox is an enzyme immunoassay using microtitre wells and the aflatest is based on monoclonal antibodies absorbed onto an affinity column. It was confirmed that aflatoxin contamination occurs preharvest and that storage under low-moisture conditions prevents additional toxin development. There were differences in susceptibility of local cultivars to aflatoxin development. The aflatest procedure was useful to rapidly screen groundnut samples for aflatoxin

contamination, but there were problems with the Quanritox method. Aflatoxin contamination occurred in the field prior to harvest and was not aggravated by storage conditions at Cleveland Depot during 1987. There was a 19.23 % incidence of aflatoxin contamination in the samples taken in early 1987, but it was not related to the farm management system (communal, small-scale commercial, and large-scale commercial) or any particular pest or disease.

1250. Crosby, N.T. 1984. Review of current and future analytical methods for the determination of mycotoxins. Food Additives and Contaminants 1(1): 39-44.

Chemical methods of analysis for the extraction, cleanup and determination of aflatoxins from animal feedingstuffs and foods are described. The advantages and disadvantages of thin-layer chromatography (TLC) and high pressure liquid chromatography (HPLC) are discussed. Immunoassays are briefly discussed. Methods for other mycotoxins such as ochratoxin, patulin, and the trichothecenes are also outlined.

1251. Dorner, J.W., and Cole, R.J. 1988. Rapid determination of aflatoxins in raw peanuts by liquid chromatography with postcolumn iodination and modified minicolumn cleanup. Journal of the Association of Official Analytical Chemists 71(1): 43-47.

The recoveries of aflatoxins B_1, B_2, G_1, and G_2 from groundnut meal spiked at 3 levels ranged from 71.7 to 88.3 % (average 80 %) with coefficients of variations from 2.7 to 10.4 %, using the technique described.

1252. Dorner, J.W., and Cole, R.J. 1989. Comparison of two ELISA screening tests with HPLC for the determination of anatoxins in raw peanuts. Proceedings of the American Peanut Research and Education Society 21 : 38.

Two enzyme-linked iminunosorbent assay (EL1SA) rapid screening tests were compared to high performance liquid chromatographic (HPLC) analyses of 100 raw groundnut extracts for the determination of aflatoxins. Identical extracts, all contaminated between 0 and 70 µg kg^{-1}, were analyzed in duplicate by the Afla- 10 cup test, the EZ-screen quick card test, and an HPLC method. The screening tests had detection thresholds of 10 µg kg^{-1} and 20 µg kg^{-1}, respectively. Both assays were accurate compared to HPLC when samples were negative for aflatoxins or contained aflatoxins above their respecu ve detection thresholds. Errors that did occur were associated with samples that contained aflatoxins at concentrations just below their detection thresholds. The cup test identified as > 10 µg kg^{-1} 9 % of samples that were in the 5-10 µg kg^{-1} range as determined by HPLC. The card test identified as > 20 µg kg^{-1} 53 % of samples that fell in the 10-20 µg kg^{-1} range by HPLC. Of the samples that were outside the range of 5-10 µg kg^{-1}, the cup test was accurate with both replications compared to HPLC 97 % of the time. Similarly, the card test results (both replicatioas) were comparable to HPLC in 91 % of samples outside the 10-20 µg kg^{-1} range.

1253. Dorner, J.W., and Cole, R.J. 1989. Comparison of two ELISA screening tests with liquid chromatography for determination of aflatoxins in raw peanuts. Journal of the Association of Official Analytical Chemists 72(6): 962-964.

A study was conducted to evaluate the performance of two enzyme- linked immunosorbent assays (ELISA) for rapidly screening samples of groundnuts for the presence of aflatoxin. The EZ-Screen Quick Card Test and Alfa-10 Cup Test were compared with liquid chromatography in duplicate analysis of common extracts of groundnut contaminated in the range of 0-70 ng g^{-1}. Each assay properly identified 95 % of the samples containing no detectable aflatoxin as negative and >97 % of samples containing > 10 ng g^{-1} aflatoxin as positive. The card test, which had a 20 µg kg^{-1} detection threshold, identified as positive 32 of 34 samples in the 11-20 µg kg^{-1} range. This indicates that the test card test might actually have a detection threshold closer to 10 µg g^{-1}. Most of the errors associated with the assays occurred on the samples containing <10 ng g^{-1} aflatoxins. The cup and card tests identified 76 and 67 % of the samples, respectively, as negative, in the range of 4-10 ng g^{-1}. For the samples either negative or positive contaminated above their detection thresholds for the assays, the methods are well suited for use as rapid screening tests.

1254. El-Nakib, O., Pestka, J.J., and Chu, F.S. 1981. Determination of aflatoxin B, in corn, wheat, and peanut butter by enzyme-linked immunosrbent assay and solid phase radio immunoassay. Journal of the Association of Official Analytical Chemists 64: 1077-1082.

Determination of aflatoxin B_1 in corn, wheat, and peanut butter by an enzyme-linked immunoassay (ELISA) and a solid phase radio immunoassay (RIA) were compared. Samples spiked with 2.9-43.2 ng g^{-1} B_1 were subjected to the AOAC extraction procedure. The extracts were concentrated, redissolved in methnol, diluted in phosphate-buffered saline with Tween 20, and directly analysed for B_1 by either ELISA or R1A. At >5.8 ng g^{-1} recoveries for B_1 in corn, wheat, and peanut butter samples were 80.0, 86.6and 94.8 by ELISA and 61.0. 93.3 and 110.0 % by RIA, respectively. Recoveries >120 % were obtained for wheat and peanut butter samples spiked with 2.9 ng g^{-1} aflatoxin B_1 by the RIA method but not by the ELISA. Overall results indicated that ELISA gave more consistent data, lower s.d. and lower coefficients of variation than did RIA. Analysis of three samples naturally contaminated with the aflatoxins revealed that the ELISA data were comparable to those obtained by other established chemical methods.

1255. Fan, T.S.L., and Chu, F.S. 1984. Indirect enzyme-linked immunosorbent assay for detection of aflatoxin B_1 in com and peanut butter. Journal of Food Protection 47(4): 263-266.

The method described involves coating aflatoxin B_1 poiylysine conjugate on microtitre plates as immobilized antigen, followed by incubation with free toxin standard or sample extract and anti-aflatoxin antibody from rabbits. The amount of antibody bound to the solid phase was determined by subsequent incubation with a secondary antibody conjugated with an enzyme (goat anti- rabbit 1gG-horseradish peroxidase conjugate) and reaction with the chromogenic substrate. Anatoxins were extracted from peanut butter and maize meal and dissolved in assay buffer for ELISA. Using the technique 79.5-98.6 % and 68-97 % of aflatoxin B_1 added in the range 5-40 µg kg^{-1} to the maize meal and peanut butter were recovered, respectively. The specificity for aflatoxin B_1 as that obtained from the direct ELISA, with an additional advantage that much less antibody was required for the assay.

1256. Fukal, L., and Reisnerova, H. 1989. Effect of organic solvent concentration on radioimmunoassay of aflatoxin B_1. Journal of Radioanalytical and Nuclear Chemistry 132(2): 315-319.

An essential requirement for the immunoassay of aflatoxin in a food extract is the complete solubilization of the toxin in an aqueous buffer pipetted in the radioimmunoassay procedure. One means of achieving this is by diluting methanol or acetone extract in buffer. However, organic solvent can affect the antigen-antibody interaction. This study determined the effect of acetone and methanol contents in an aqueous casein-buffer solution pipetted with aflatoxin B_1 in the radioimmunoassay procedure on some parameters of radioimmunochemical detection of aflatoxin B_1. These organic solvents lower the antiserum litre and the relative zero specific binding, and at higher concentration worsen the detection limit and the accuracy of radioimmunoassay. However, in radioimmunoassay of food extracts containing very low levels of aflatoxin, it could be advantageous to add the extract volume to an organic solvent concentration of 60 %.

1257. Fukal, L., Reisnerova, H., and Rauch, P. 1988. Applications of radioimmunoassay with 125-iodine for determination of aflatoxin B_1 in foods. Sciences des Aliments 8(3): 397-400.

A simple and sensitive radioimmunoassay [see Journal of Radioanalytical Nuclear Chemistry Letters (1986) 108:259-268 and (1987) 109:383-391] was applied to detection of aflatoxin B, in spiked food samples. Before the radioimmunoassay procedure was performed a considerable amount of interfering substances was removed from crude chloroform extracts using Sep-Pak cartridge clean-up. This made possible the detection of 0.1, 0.5, 0.5, 0.1 and 1.0 µg of aflatoxin B_1/kg in wheal, corn, groundnuts, muscle and liver, respectively. The % recoveries for aflatoxin B_1 were 77-128 % in the range of detection limit to 10.0 µg kg^{-1}. If crude chloroform extracts were not cleaned up on Sep-Pak cartridges, false positive results prevented detection of aflatoxin B, at concentrations of <1.0 µg kg^{-1}.

1258. Garner, R.C., Mulholland, F., Arnold, P.S., Mason, C., Chadha, G., and Martin, C.N. 1988. The use of Oxoid aflatoxin Easi- Extract columns to analyse total aflatoxin contamination in human foods. Proceedings of the Japanese Association of Mycotoxicology Supplement 1: 53-54.

Two methods of aflatoxin analysis are described both of which use monoclonal antibodies for immunoconcentration for aflatoxin detection. The methods were used to detect aflatoxins in spiked milk and peanut butter.

1259. Gaur, P.K., Lau, H.P., Pestka, J.J., and Chu, F.S. 1981. Production and characterization of aflatoxin B_{2a} antiserum. Applied and Environmental Microbiology 41(2): 478-482.

The specificity and sensitivity of antiserum elicited from rabbits against aflatoxin B_{2a}-bovine serum albumin conjugates were characterized with radio immunoassay (RIA) and an enzyme- linked immunosorbent assay (ELISA). Aflatoxin B_1 was first converted to aflatoxin B_{2a} and then conjugated to bovine serum albumin and horseradish peroxidase by the Tedutive alkylation method. The antiserum was developed in New-Zealand While rabbits by mutiple-site injection with aflatoxin B_{2a}-bovine serum albumin conjugate. Antibody titers were determined by both RIA and ELISA. Competitive RIAs with various aflatoxin analogs indicated that the antiserum was most reactive with aflatoxin B_1 and slightly cross-reactive with aflatoxins B_{2a}, B_2, and M_1, Competitive ELISAs showed the antiserum to be equally specific for aflatoxins B_{2s}, and B_1 and less reactive with aflatoxins B_2 and M_1. The relative sensitivities of RIA and ELISA for aflatoxin B_1 quantitation were 100 and 10 pg per assay, respectively.

1260. Goto, T. 1990. Comparison of some immunological aflatoxin analysis methods. Report National Food Research Institute Japan 54: 35-43.

A simple sample preparation method was developed for immunological aflatoxin analysis of maize. Maize and mixed feeds were extracted 2.5 times (v/w) with 80 % methanol. Recovery of aflatoxins using this solvent was high and adequate for further immunological analysis. Using samples extracted by this method, fice commercially available aflatoxin analysis kits : Aflatest, TD 100, Afla 10 Cup, Aflacheck UBE and Agriscreen were compared to determine their accuracy and practicality. The accuracy of these immunological methods was variable especially

with certain mixed feeds. However, these methods were considered as simple for aflatoxin analysis.

1261. Goto, T., and Manabe, M. 1988. Application of ELISA to aflatoxin analysis : Results of preliminary tests of aflatoxin kits on the market. Report National Food Research Institute Japan 52: 53-59.

Three types of enzyme immunoassay kits were tested for their ability to detect and/or quantitate aflatoxins. The E1A Kit (Japan), which uses 96 well micro-plates was suitable for the analysis of a large number of samples, had some difficulties regarding sample recovery and antibody specificity to aflatoxin B_1. Using the E-Z screen (USA), aflatoxins were detected rapidly, but could not be assayed quantitatively. The Aflatest 10 kit (U.K.), which uses a small Florisil chip for aflatoxin detection was suitable for semiquantitative analysis of aflatoxins but unfortunately the fluorescence of the chip occasionally masked the fluorescence of the aflatoxins, making quantitation difficult. It was concluded that there is a large possibility for the practical application of these kits for aflatoxin analysis.

1262. Goto, T., and Manabe, M. 1989. Methods for the analysis of aflatoxins in groundnut and other agricultural commodities. Pages 173-182 in Aflatoxin contamination of groundnut : Proceedings of the International Workshop, 6-9 October 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

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. Recently several enzyme-linked immunosorbent assay (ELISA) system kits for aflatoxin analysis have been developed and some of them found suitable for the kind of tesing 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.

1263. Groopman, J.D., and Donahue, K.F. 1988. Aflatoxin, a human carcinogen: Determination in foods and biological samples by monoclonal antibody affinity chromatography. Journal of the Association of Official Analytical Chemists 71(5): 861-867.

Monoclonal antibody technology has been used to produce antibodies that recognize aflatoxins in order to develop noninvasive methods in conduction with other chemical analytical techniques to monitor human exposure to the environmental carcinogens. These methods require the ability to quantitate aflatoxins and their metabolites, including DNA and protein adducts, in readily accessible compartments such as serum and urine. The techniques permit efficient analysis of many samples in a relatively short time. Also, these monoclonal antibody affinity columns have been extremely useful for rapid isolation of aflatoxins from food and grain samples, as well as aflatoxin M_1 from milk. Monoclonal antibody affinity methods are nondestructive to the aflatoxin molecule, so the sample aliquot can be used for confirmation. The use of monoclonal antibody preparative affinity columns represents a major, substantive breakthrough for analytical chemists and will be a generally applicable technology for isolation of many different substances.

1264. Kawamura, O., Nagayama, S., Sato, S., Ohtani, K., Ueno, I., and Ueno, Y. 1988. A monoclonal antibody-based enzyme-linked immunosorbent assay of aflatoxin B_1 in peanut products. Mycotoxin Research 4(2): 75-88.

An improved ELISA combined with monoclonal antibody (MAb) and one-step extraction method is described for the estimation of aflatoxin B_1 in groundnut products. AFB_1 was converted to AFB_1- oxime and then conjugated with bovine serum albumin (BSA). Spleen cells from mice immunized with AFB_1-BSA conjugates were fused with myeloma cells. After double selection with AFB_1-ovalbumin (OVA) and carbodiimide-modified OVA, 5 stable hybridoma cells secreting anti-AFB_1 MAbs (AFI. AF2. AF3, AF4 and AF5) were cloned. Using three anti-AFB, MAbs, the indirect competitive ELISA (cELISA) was developed with alkaline phosphatase (ALP)-labelled sheep anti-mouse IgG as marker and the direct cELISA with AFB_1-oxime horseradish peroxidase (POD) as marker. The minimum detectable limits of the indirect cELISA with AF1, AF2, AF3, AF4 and AF5 were 5, 5, 5, 5 and 50 pg of standard AFB, per assay, respectively, and those of the direct cELISA with AFI, AF3, AF4 and AF5 were 2.5, 5, 25 and 100 pg of standard AFB, assay^{-1}, respectively. The cross reactivity of each toxin with these MAbs in the indirect cELISA was as follows : (a) AF1 and AF2 were reactive with AFB2 as well as AFB1, weakly with AFG2>AFGl>aflatoxicol II (COL II) > aflatoxicol I (COL 1) and less weakly with other aflatoxins; (b) AF3 and AF4 were reactive with COL II as well as AFB,, weakly with COL I > AFQ, and less weakly with others; (c) AF5 was reactive with AFQ, as well as AFB,, weakly with COL II > AFG_2 > COL I and less weakly with others. The 60 % aqueous methanol extracts of oil-roasted blanched groundnuts ("peanut butter"), naturally contaminated with AFB, were assayed by the direct cELISA without further purification. The direct cELISA with the most sensitive MAb AFI was able to determine 1 ng of AFB_1 g^{-1} sample.

1265. Langone, J J., and Vunakis, H.Van. 1976. Aflatoxin B_1: Specific antibodies and their use in radioimmunoassay. Journal of the National Cancer Institute 56(3): 591-595.

The specificities of antisera produced by rabbits injected with aflatoxin B_1 or an analog were studied with respect to aflatoxins B_2, B_{2a}, G_1, G_2, Q_1 and P_1. Radioimmunoassays which can detect levels of 0.06 ng of aflatoxin B_1 were used to analyze serum, urine and crude extracts of maize and peanut butter supplemented with aflatoxin. In the foodstuffs 1 µg aflatoxin kg^{-1} could be measured. The immunoassay was at least as sensitive and specific as any other available analytical methods, but did not require purification of samples before analysis.

1266. Lawellin, D.W., Grant, D.W., and Joyce, B.K. 1977. Enzyme-linked immunosorbent analysis of aflatoxin B,. Applied and Environmental Microbiology 34(1): 94-96.

An enzyme-linked immunosorbent assay (ELISA) permitted the detection of less than 10 pg of aflatoxin B_1 mL^{-1}. The antitoxin was most specific for aflatoxins B_1 and B_{2a}, and least specific for aflatoxin G_1. The enzyme-linked immunosorbent assay for aflatoxin B_1 is of value for studying in vivo aflatoxin formation during experimental aspergillosis and as a diagnostic aid in cases of suspected aflatoxicosis.

1267. Morgan, M.R.A., Rang, A.S., and Chan, H.W.S. 1986. Aflatoxin determination in peanut butter by enzyme-linked immunosorbent assay. Journal of the Science of Food and Agriculture 37(9): 908-914.

High specificity, high titre antisera to aflatoxins B_1 and G_1 have been produced by immunisation of rabbits with a bovine serum albumin-aflatoxin B, conjugate. The antiserum has been used to set up an indirect, double antibody microtitration plate ELISA with a limit of detection of 0.1 pg toxin $well^{-1}$. The assay has been validated for application to peanut butter, and requires minimal sample preparation before assay, is technically simple and has high throughput.

1268. Mortimer, D.N., Shepherd, M.J., Gilbert, J., and Morgan, M.R.A. 1987. A survey of the occurrence of aflatoxin B_1 in peanut butters by enzyme-linked immunosorbent assay. Food Additives and Contaminants 5 (2): 127-132.

A survey was carried out in 1986 for the occurrence of aflatoxin B_1 in peanut butters (129 samples) obtained from specialist Health Food outlets. The results showed that 6.2 % of the samples contained > 10 µg kg^{-1} of aflatoxin, 8 % contained between 2.5 and 10 µg kg^{+-1} and in the remainder (86 %) aflatoxin could not be detected at a limit of 2.5 µg kg^{-1}. These results show a lower contamination by aflatoxin than found in these products in previous surveys (1982-1984). An aflatoxin B_1-specific enzyme- linked immunosorbent assay (ELISA) was employed for the first time in these analyses ; and to make an assessment of its performance positive aflatoxin results, together with a random selection of those below the ELISA limit of detection, were additionally analyzed by conventional extraction and clean-up followed by HPLC. The ELISA technique offered a significant improvement in speed of analysis over conventional approaches, enabling a six-fold increase in sample throughput compared to that required for conventioanl analysis, together with other advantages.

1269. Mortimer, D.N., Shepherd, M.J., Gilbert, J., and Clark, C. 1988. Enzyme-linked immunosorbent (ELISA) determination of aflatoxin B, in peanut butter.collaborative trial. Food Additives and Contaminants 5 (4): 601-608.

Fourteen laboratories in the UK participated in a collaborative trial of a commercially available ELISA test kit for the detection of aflatoxin B_1 in peanut butter. Each laboratory carried out four replicate analyses of each of six individual samples. Collaborators received a control, noncontaminated sample, together with samples prepared by blending naturally- contaminated and control material to give target levels of 8, 25 and 75 µg kg^{-1} aflatoxin B_1. Two of these samples (8 and 25 µg kg^{-1}) were supplied as undisclosed duplicates. The repeatabilities of the assay ranged from 6.2 to 16.7 µg kg^{-1}. The reproducibilities for aflatoxin B_1 concentration in naturally contaminated samples ranged from 3.6 to 18.7 µg kg^{-1} using noncontaminated peanut butter as a reference blank. Modifications to the format of the commercial kit were recommended as a result of the collaborative trial.

1270. Newsome, W.H. 1986. Potential and advantages of immunochemical methods for analysis of foods. Journal of the Association of Official Analytical Chemise 69(6): 919-923.

The technique of immunochemical analysis, including the principles involved, is described and various types of assay are discussed. Application of these methods to the analysis of foods for residues of pesticides, mycotoxins, contaminants, drug residues, and natural constituents is reviewed. Compared with the conventional methods, immunoassays offer similar detection limits and greatly simplified sample preparation procedures.

1271. Park, D.L., Miller, B.M., Hart, L.P., Yang, G., McVey, J., Page, S.W., Pestka, J.J., and Brown, L.H. 1989. Enzyme-linked immunosorbent assay for screening aflatoxin B, in cottonseed products and mixed feed: Collaborative study. Journal of the Association of Official Analytical Chemists 72(2): 326-332.

A joint AOAC/IUPAC interlaboratory study of an enzyme-linked immunosorbent screening assay (ELISA) for aflatoxins was conducted in laboratories in Canada,

France, Japan, South Africa, Switzerland, The Netherlands, Tunisia, and the United States. Twenty-eight samples of raw and roasted groundnuts, corn, whole cottonseed, cottonseed meal, ammoniated cottonseed meal, and poultry feed containing various quantities of natural aflatoxins, and supplemented when appropriate with aflatoxin B_1, were distributed to participating laboratories for testing. The assay is based on competition between an enzyme-conjugated aflatoxin B_1 and (free) aflatoxins in the test sample for aflatoxin-specific antibodies coated onto the microtiter wells. After a wash step to remove all unbound aflatoxins, a substrate added to each well is catalyzed from a colorless to a green solution by any bound enzyme-conjugated aflatoxin B_1 present. The intensity of the color decreases, as the amount of free aflatoxin B_1 in the test sample increases. Overall correlation was good between ELISA and thin-layer chromatography (TLC) results for cottonseed products and mixed feed. Variable results were reported for com and groundnut product samples. Although some positive samples (>15 ng g^{-1}) of cottonseed products and mixed feed were reported to contain <15 ng g^{-1} by visual determination, a review of data for absorbance measurements showed that the contamination level was close to the >15 ng g^{-1} standard and would not have been reported as negative under routine screening. Variation in ELISA results may have been due to several factors such as lack of homogeneity of the aflatoxin contamination in the samples (prestudy TLC analysis samples were collected randomly from a pool of subsamples) interferences that resulted from incomplete removal of hexane during the filtration step, and antibody strips at or past their expiration date. The ELISA method has been adopted as official first action as a screening method to determine the presence or absence of anatoxin B_1 at a concentration of >15 ng g^{-1} in cottonseed products and mixed feed.

1272. Park, D.L., Miller, B.M., Nesheim, S., Trucksess, M.W., Vekich, A., Bidigarc, B., McVey, J.L., and Brown, L.H. 1989. Visual and semiquantitative spectrophotometric ELISA screening method for anatoxin B_1 in corn and peanut products:Follow-up collaborative study. Journal of the Association of Official Analytical Chemists 72 (4): 638-643.

A joint AOAC/IUPAC interlaboratory study of an enzyme-linked immunosorbent screening assay (ELISA) for anatoxins was conducted in laboratories in Canada, France, Japan, The Netherlands, Switzerland, Tunisia, and The USA. Twelve raw and roasted groundnut and com portions containing various concentrations of natural anatoxins and supplemented when appropriate with anatoxin B_1 were distributed to participating laboratories for testing. The assay is based on competition between an enzyme-conjugated aflatoxin B_1 and (free) aflatoxins in the test sample for aflatoxin-specific antibodies coated onto microtiter wells. After a wash step to remove all unbound aflatoxins. a substrate added to each well is catalyzed from colorless to a blue solution by any bound enzyme-conjugated aflatoxin B, present. The intensity of the color decreases as the amount of free aflatoxin B_1 in the test sample increases. Final determination of aflatoxin concentrations can be made by either visual comparision with standard solutions or spectrophotometric comparisions (at 650 ntn) to knowns. Overall correlation was good between ELISA and thin-layer chromatographic results for corn and roasted groundnut products, with 93 and 98 % correct responses for visual and instrumental determinations, respectively. For instrumental determinations of aflatoxin in com and roasted groundnuts in the <20 ng g^{-1} range, the relative standard deviations for repeatability (RSDr) were 14.9 and 41.4 %, respectively, and the relative standard deviations for reproducibility (RSDR) were 45.7 and 43.5 %. respectively. For instrumental determination of >20 ng g^{-1}, the respective RSDr and RSDR values were 19.4 and 52.7 for com and 23.3 and 23.3 % for roasted groundnuts. For visual determinations in the <20 ng g^{-1} range, the respective RSDr and RSDR values for com were 38.5 and 60.7 % and for roasted groundnuts 73.7 and 73.7 %. The respective RSDr and RSDR values for determinations of >20 ng g^{-1} for com were 13.5 and 59.5 % and 24.3 and 57.3 % for roasted groundnuts. It is concluded that the ELISA method is approved interim official first action as a screening method to determine the presence or absence of aflatoxin B_1 at a concentration of >20 ng g^{-1} in com and roasted groundnuts.

1273. Putey, A.L., Sharman, M., Wood, R., and Gilbert, J. 1989. Determination of aflatoxin concentrations in peanut butter by enzyme-linked immunosorbent assay (ELISA): Study of three commercial ELISA kits. Journal of the Association of Official Analytical Chemists 72(6): 965-969.

Sixteen United Kingdom analytical laboratories participated in an evaluation of three commercially available enzyme-linked immunosorbent assay (ELISA) kits for analysis of aflatoxin in peanut butter. Each laboratory was sent three sets of 10 randomly numbered samples of peanut butter. Each set consisted of five pairs of undisclosed duplicates. Four of the sets of duplicates were naturally contaminated butters with "target" aflatoxin values (estimated by liquid chromatography) between 8 and 81 µg kg^{-1}. The fifth pair was a blank peanut butter containing approximately 3 µg kg^{-1} of total aflatoxins. A statistical treatment of the results of the studies is presented, together with discussion of the relative merits of the different kits.

1274. Patey, A.L., Sharman, M., and Gilbert, J. 1990. Determination of aflatoxin levels in peanut butter using HPLC and ELISA procedures : inter-laboratory comparison. Mycotoxin Research 6(1): 2-6.

Six laboratories analysed portions of the same aqueous acetonitrile extracts of 3 peanut butters for aflatoxin concentration by an HPLC procedure (using immunoaffinity column clean-up) and an ELISA procedure. The extracts were from a nominal "blank" peanut butter, a peanut butter naturally contaminated with anatoxins (mostly BI) and from a "blank" peanut butter to which equal amounts of aflatoxins B_1, B_2, G_1 and G_2 standards had been added. Similar results for the HPLC

and ELISA procedures were obtained for the blank (means 2.8 and 4.9 µg kg^{-1} respectively) and naturally contaminated (means 26.0 and 25.9 µg kg^{-1}, respectively) peanut butters. However, the results by ELISA (mean 16.7 µg kg^{-1}) for the spiked peanut butter was much lower than that obtained by HPLC (mean 28.3 µg kg^{-1}).

1275. Patey, A.L., Sharman, M., and Gilbert, J. 1990. Determination of aflatoxin B_1 levels in peanut butter using an immunoaffinity column clean-up procedure: inter-laboratory study. Pood Additives and Contaminants 7(4)): 515-520.

Ten United Kingdom laboratories participated in an evaluation of an immunoaffinity column sample preparation procedure used to prepare aflatoxin B_1 containing extracts obtained from peanut butters contaminated with aflatoxins. Each laboratory was sent 7 randomly numbered samples of roasted peanut butter which included 2 sets of undisclosed triplicates. These 2 peanut butters were naturally contaminated with aflatoxin B_1 at levels of about 12 and 35 µg kg^{-1}. The other sample was a nominal blank peanut butter containing approximately 2 µg kg^{-1} aflatoxin B_1 which was also employed by participants for recovery experiments. Participating laboratories were instructed to follow a protocol regarding the use of the immunoaffinity columns for extract preparation, but were allowed a free choice of instrumental technique for quantification of aflatoxin levels. Mean recovery for spikes was 72%. Coefficients of variation for the results from the 10 participants for the 2 contaminated roasted peanut butters were, respectively, 45% (on a mean of 13.6 µg kg^{-1}) and 36% (on a mean of 37.2 µg kg^{-1}).

1276. Pestka, J.J. 1988. Enhanced surveillance of foodborne mycotoxins by immunochemical assay. Journal of the Association of Official Analytical Chemists 71(6): 1075-1081.

Mycotoxins are a chemically diverse group of fungal secondary metabolities with a wide range of toxic effects. Conventional thin-layer and instrumental methods of mycotoxin analysis arc time-consuming and make routine safety and quality control screening of these compounds in agricultural commodities difficult. As an alternative, specific polyclonal and monoclonal antibodies have been produced against mycotoxin-protein conjugates and used in sensitive radio immunoassays (RIA) and enzyme-linked immunosorbent assays (ELISAs). One of the simplest ELISA approaches involves competition for a solid-phase antibody between a mycotoxin-enzyme conjugate and nonconjugated mycotoxin in the sample extract. ELISAs have been developed for aflatoxins B_1 and M_1, zearalenone, T-2 toxin, and deoxynivalenol, which are higly specific, rapid (10 min), easily adaptable for analyzing large numbers of samples, and directly applicable to assaying methonol-water extracts of a wide range of foods. Several commercial mycotoxin ELISAs using this approach (most typically for aflatoxin B_1) are currently being marketed. Since ELISAs will be used in a large part by personnel with limited technical expertise, individual kits must be critically evaluated by analytical chemists for suggested sampling procedures, efficiency of extraction, cross-reactivity, mycotoxin recovery, assay reproducibility, and product shelf-life prior to routine use in food safety and quality control screening.

1277. Pestka, J.J, and Chu, F.S. 1984. Aflatoxin B_1 dihydrodiol antibody: Production and specificity. Applied and Environmental Microbiology 47(3): 472-477.

A specific antibody for 2, 3-dihydro-2,3-dihydroxy aflatoxin B_1 (AFB_1-diol) was prepared, and its reactivity was characterized for the major aflatoxin B_1 (AFB_1) metabolites. Reductive alkylation was used to conjugate AFB_1-diol to ethylenediamine-modified bovine serum albumin (EDA-BSA) and horseradish peroxidase for use as an immunogen and an enzyme-linked immunosorbent assay (ELISA) marker, respectively. High reactant ratios, 1:5 and 1:10, for AFB_1-diol-EDA-BSA (wt/wt) resulted in precipitated conjugates which were poorly immunogenic. However, a soluble conjugate obtained by using a 1:25 ratio of AFB_1-diol to EDA-BSA could be used for obtaining high titer AFB_1-diol rabbit antibody within 10 weeks. Competitive ELISAs revealed that the AFB_1-diol antibody detected as little as 1 pmol of AFB_1-diol per assay. Cross reactivity of AFB_1-diol antibody in the compititive ELISA with AF analogs was as follows: AFB_1-diol, 100 %; AFB_1, 200 %; AFM_1. 130 %; AFB_{2a}, 100 %; AFG_1. 6 %; AFG_2, 4 %; aflatoxicol, 20 %; AFQ_1, 2 %; AFB_1-modified DNA, 32 %; and 2. 3-dihydro-2-(N7- guanyl)-3-hydroxy AFB_1, 0.6 %. These data indicated that the cyclopentanone and methoxy moieties of the AF molecule were the primary epitopes for the AFB_1-diol antibody. The AFB_1-diol competitive ELISA was subject to substantial interference by human, rat, and mouse serum albumins but not by BSA, Tris, human immunoglobulin G, or lysozyme. By using a noncompetitive, indirect ELISA with an AFB_1-modified DNA solid phase, a modification level of one AFB, residue for 200000 nucleotides could be determined.

1278. Pestka, J.J., Gaur, P.K., and Chu, F.S. 1980. Quantitation of aflatoxin B_1 and aflatoxin B_1 antibody by an enzyme-linked immunosorbent microassay. Applied and Environmental Microbiology 40; 1027-1031.

A specific microtest plate enzyme-immunoassay has been developed for the rapid quantitation of aflatoxin B, at levels as low as 25 pg $assay^{-1}$. Multiple-site injection of rabbits with an aflatoxin B,-carboxymethyloxime-bovine serum albumin conjugate was used for the production of hyperimmune sera. Dilutions of the purified antibody were air dried onto microplates previously treated with bovine serum albumin and glutaraldehyde and then incubated with an aflatoxin B_1-carboxymethyloxime-horseradish peroxidase conjugate. The amount of enzyme bound to antibody was determined by monitoring the change in absorbance at 414

nm after the addition of a substrate solution consisting of hydrogen peroxide and 2, 2-azino-di-3-ethyl-benzthiazoline--6-sulfonate. Antibody titers determined in this manner closely correlated with those determined by radioimmunoassay. Competition assays as performed by incubation of different aflatoxin analogs with the peroxidase conjugate showed that aflatoxins B_1 and B_2 and aflatoxicol caused the most inhibition of conjugate binding to antibody. Aflatoxins G_1 and G_2 inhibited the conjugate binding to a lesser degree, whereas anatoxins M_1 and B_2, had no efect on the assay.

1279. Ram, B.P., Hart, L.P., Cole, R.J., and Pestka, J.J. 1986. Application of ELISA to retail survey of aflatoxin B_1 in peanut butter. Journal of Food Protection 49(10): 792-795.

A simple procedure was devised for the routine screening of aflatoxin B_1 in peanut butter using enzyme-linked immunosorbent assay (ELISA). Peanut butter samples (5g) were spiked with aflatoxin B_1 and extracted by blending with 25 mL of 55 % methanol and 10 mL of hexane. The extract was filtered and aqueous filtrate analyzed by a indirect competitive ELISA. Recovery of aflatoxin B_1 added to peanut butter samples ranged from 85 to 112 %, with an average coefficient of variation of 18.4 %. Using this procedure, only three of 63 samples of peanut butter had detectable levels (>5.0 µg kg^{-1}) of aflatoxin B_1.

1280. Ram, B.P., Hart, L.P., Pestka, J.J., Cole, R.J., and Miller, B.M. 1986. Rapid analysis of peanuts and peanut products by enzyme immunoassay for aflatoxin. Proceedings of the American Peanut Research and Education Society, Inc. 18: 62.

A simple procedure was devised for the routine screening of aflatoxin B_1 in groundnuts and peanut butter using enzyme-linked immunosorbent assay (ELISA). Samples of peanut butter were spiked with aflatoxin B_1 and extracted by blending with 25 mL of 55 % methanol and 10 mL hexane. The extract was filtered and aqueous filtrate analyzed by a direct competitive ELISA. Recovery of aflatoxin B_1 added to peanut butter samples ranged from 85-112 %, with a mean recovery of 97 %. Coefficient of variation between test wells in the assay was 18.4 %. Using this procedure, only 3 of 63 commercial samples of peanut butter showed detectable aflatoxin B_1 (>5 ng g^{-1}).

1281. Rauch, P., Fukal, L., Brezina, P., and Kas, J. 1988. Interferences in radioimmunoassay of aflatoxins in food and fodder samples of plant origin. Journal of the Association of Official Analytical Chemists 71(3): 491-493.

Cross-reactions and resulting nonspecific binding of substances with structures resembling aflatoxins (derivatives of coumarin, and cinnamonic and benzoic acids. etc.) were investigated The concentrations of these substances causing erroneoussly high or false positive values in radioimmunoassay were determined. One |ig aflatoxin B_1 kg^{-1} sample may be simulated by the occurrence of 5 g coumarin, 10 g caffeic acid. 16 g chlorogenic acid, or 15 g vanillin kg^{-1} fodder or food sample.

1282. Singh, P., and Jang, L. 1987. A membrane-based enzyme immunoassay test for aflatoxin B_1. International Journal of Food Microbiology 5: 73-80.

A microtitre-based competitive enzyme immunoassay (EIA) earlier developed was adapted to a membrane-based EIA to show the feasibility of such a test in qualitative/semi-quantitative determination of aflatoxin B_1 (AFB_1) requiring no instrumentation. Anti-AFB_1 antibody was immobilized on 8 mm Immobilon TM membrane discs at different protein concentrations. Standard curves were prepared by direct competition between AFB, in standards and AFB_1-peroxidase conjugate for binding to specific antibody immobilized to membrane discs. Sensitivity of the membrane-based assay could be controlled to a desired level by adjusting the immobilized antibody on the solid support; decreasing the immobilized antibody concentration on the discs increased the sensitivity of the assay within a certain limit. A visual cut-off in color could be established at a level of 20 ng mL^{-1} AFB_1, with an appropriate amount of antibody on the membrane. Preliminary results for attaching the membrane to a solid support to develop a dipstick EIA test is discussed.

1283. Sun, P., and Chu, F.S. 1977. A simple solid-phase radioimmunoassay for aflatoxin B_1. Journal of Food Safety 1: 67- 75.

A solid-phase radioimmunoassay (RIA) for aflatoxin B_1 was developed. This method involved the incubation of aflatoxin B_1 both labelled and unlabelled, with immunoglobulin (IgG)-sepharose gel which was prepared by conjugation of the IgG highly specific to aflatoxin B_1 with CNBr-activated sepharose gel, followed by a filtration step. The binding capacity was determined by counting the radioactivity in the filtrate. Studies with different aflatoxin B_1 analogs revealed that the IgG-gel bound most effectively with B_1. Binding of aflatoxin B_2, G_1, G_2, and aflatoxicol to the IgG-gel was less effective in comparision with the IgG before couplig. Between 0.5-5.0 ng $assay^{-1}$, the displacement of radioactivity from gel was directly proportional to the amount of aflatoxin B_1 present. Using a simple extraction procedure without cleanup step, the recovery yields for aflatoxin B_1 in the contaminated corn or wheat at levels of 5 ng g^{-1} or above were 60 %.

1284. Trucksess, M.W., Stack, M.E., Nesheim, S., Park, D.L., and Pohland, A.E. 1989. Enzyme-linked immunosorbent assay of aflatoxins B_1, B_2. and G_1 in corn, cottonseed, peanuts, peanut butter, and poultry feed: Collaborative study. Journal of the Association of Official Analytical Chemists 72(6): 957-962.

A direct competitive enzyme-linked immunosorbent assay (ELISA) screening method for aflatoxins at 20 ng g^{-1} was studied by 12 collaborators. Test samples of peanut butter were extracted by blending with methanol-water-hexane (55:45:100) and heating the test extracts on a steam bath; test samples of other commodities were extracted by blending with methanol-water (80:20). All the test extracts were filtered and the filtrates were diluted with buffer to a final methanol concentration of <30 %. Each diluted filtrate was applied to a cup containing a filter with immobilized polyclonal antibodies specific to aflatoxins B_1, B_2, and G_1. Aflatoxin-B_1-peroxidase conjugate was added, the cup was washed with water, and a mixture of hydrogen peroxide and tetramethylbenzidine was added. The test sample was judged to contain >20 ng aflatoxins g^{-1} when, after exactly 1 min, no color was observed on the filter; when a blue or a grey color developed, the test sample was judged to contain <20 ng aflatoxin g^{-1}. All collaborators correctly identified naturally contaminated corn and raw peanut positive lest samples. No false positives were found for controls containing <2 ng aflatoxins g^{-1}. The correct response for positive test samples spiked at levels of 10, 20, and >30 ng aflatoxins g^{-1} (the ratio of B_1: B_2: G_1 was 10: 1: 3) were 52, 86, and 96 %, respectively. The method , which is rapid and simple has been adopted official first action for screening for aflatoxins at 20 ng g^{-1} in cottonseed and peanut butter and for aflatoxins at >30 ng g^{-1} in corn and raw groundnuts. Positive test samples may require reanalysis by an official, quantitative method.

1285. Trucksess, M.W., Young, K., Donahue, K.F., Morris, D.K., and Lewis, E. 1990. Comparison of two immunochemical methods with thin-layer chromatographic methods for determination of aflatoxins. Journal of the Association of Official Analytical Chemists 73(3): 425-428.

Three different methods were compared for the determination of total aflatoxins in corn and groundnuts naturally contaminated with aflatoxins and in corn, groundnuts, cottonseed, peanut butter, and poultry feed spiked with aflatoxins B_1, B_2, and G_1. The three methods were an enzyme-linked immunosorbent assay (ELISA) screening test, a monoclonal antibody-affinity column- solid phase separation method, and the AOAC official thin-layer chromatography (TLC) methods for all except poultry feed, for which Shannon's TLC method for mixed feed was used. The ELISA test is designed to provide only positive results for total aflatoxins at > 20 ng g^{-1} or negative results at < 20 ng g^{-1}. The affinity column separation is coupled with either bromination solution fluorometry to estimate total aflatoxins or liquid chromatography (LC) to quantitate individual aflatoxins. Fluorodensiiomctry was used to determine aflatoxins in commodities analyzed by the TLC methods. The LC and TLC results were in good agreement for all the analyses. The results for the affinity column using bromination solution fluorometry were similar except those for cottonseed, which were about 60 % higher. The ELISA screening method correctly identified naturally contaminated corn and groundnut positive samples. No flase positives were found for controls. The correct response for spiked corn, raw groundnuts, peanut butter, and cottonseed at > 20 ng aflatoxins g^{-1} was about 90 %. The correct response for spiked poultry feed at > 20 ng aflatoxins g^{-1} was about 50 %.

1286. Ueno, I. 1985. A simple and improved enzyme-linked immunosorbent assay method for microquantitation of aflatoxin B_1 in peanuts and blood plasma. Proceedings of the Japanese Association of Mycotoxicology No. 21: 24-27.

1287. Ward, CM., Wilkinson, A.P., Bramham, S., Lee, H.A., Chan, H.W.S., Butcher, G.W., Hutchings, A., and Morgan, M.R.A. 1990. Production and characterization of polyclonal and monoclonal antibodies against aflatoxin B_1 oxime-BSA in an enzyme-linked immunosorbent assay. Mycotoxin Research 6: 73-83.

From a single aflatoxin B_1 oxime-bovine serum albumin conjugate, polyclonal and monoclonal antibody preparations were produced. The four rabbit polyclonal antisera were specific for aflatoxin B_1 in a microtitration plate enzyme-linked immunosorbent assay. The monoclonal antibodies showed a wide range of differing specificities, recognizing, for example, aflatoxins B_1, B_2, G_1 and G_2; B_1 and B_2; B_1 and G_1; and G, alone. No antibody preparations reacted with aflatoxin M_1. The significance of these results to the strategy of anti-aflatoxin antibody production for use in quantitative enzyme immunoassays is discussed.

1288. Wilkinson, A.P., Rang, A.S., Chan, H.W.S., and Morgan, M.R.A. 1988. ELISA of aflatoxins. Pages 343-345 in Immunoassays for veterinary and food analysis -1 (Morris, B.A., Clifford, M.N., and Jackman, R., eds.). Barking, Essex 1G,1 8JU, UK : Elsevier Applied Science Publishers Ltd.

ELISAs for the determination of aflatoxins B_1 and G_1 in peanut butter and of sterigmatocystin in barley are described briefly.

1289. Wilson, D.M. 1989. Analytical methods for aflatoxins in com and peanuts. Archives of Environmental Contamination and Toxicology 18(3): 308-314.

This review includes discussion of safety, sampling, standards, presumptive and screening methods, quantitative methods, including thin-layer chromatography (TLC), high-pressure liquid chromatography (HPLC)and immunochemical methods, and selection of analytical approach in relation to the detection and estimation of aflatoxins in maize and groundnuts.

1290. Wilson, D.M., Beaver, R.W., Layton, R.C., Trantham, A.L., and McMillan, D.L. 1987. Comparison of immunochemical methods for aflatoxin

determination in raw peanuts. Proceedings of the American Peanut Research and Education Society 19: 36.

Three immunochemical and three chemical methods were compared for aflatoxin analysis, using fifty naturally contaminated lots of raw shelled groundnuts. The USDA/AMS analyzed all lots using the water slurry modification of the Best Foods (BF) method. The lots were chosen so that aflatoxin levels ranged from 0 to 100 µg kg^{-1}. The Federal-States Inspection Service modified Dickens mill was used to collect special 10 lb, subsamples from commercial lots. Liach 10 lb sample was divided, and duplicate analytical subsamples for each method were prepared to make each subsample as representative as possible. The Aflatesl-P mycotoxin testing system uses a monoclonal based affinity column and was provided by Cambridge Naremco, Springfiled. MO. The ELISA methods used were Agrichek aflatoxin test provided by the Agri Tech Systems, Inc., Portland, ME, and the Agri-Screen test supplied by Neogen Corporation, Lansing, MI. The chemical methods used were the water-slurry method , CB method and an HPLC method using normal phase separation and a silica gel packed cell with fluorescence detection following the CB extraction step. The overall mean from all analyses and methods was 19 µg kg^{-1} with a range of 0 to 100 µg kg^{-1} total aflatoxins ; the coefficient of variation (CV) was 36 %. Analysis of variance showed no significant differences between methods. The methods contributed little to variation, but samples contributed to 56 % and subsamples to 35 % of the variation. All methods showed significant relationships ($P< 0.01$) with regression using the water slurry method as the dependent variable. The R values ranged from 0.66 to 0.83. All methods performed well and were comparable. Any one of the immunochemical methods could be routinely used by trained personnel to perform aflatoxin analyses.

1291. Zajicek, G.J. 1989. New detection methods for aflatoxin. Bulletin, Association of Operative Millers Nov. 5587-5588.

Recent developments in the use of ELISA kits for the rapid detection of aflatoxin in corn, nuts, peanut butter and cottonseed are discussed and compared with other detection methods (TLC, UV and minicolumn screening). Advantages of ELISA are outlined with reference to ease of use, detection limits and cost.

7.2.8 Biological Methods

1292. Abedi, Z.H., and McKinley, W.P. 1968. Zebra fish eggs and larvae as aflatoxin bioassay test organisms. Journal of the Association of Official Analytical Chemists 51: 902-905.

Aflatoxin B_1 is acutely toxic to embryos and larvae of Zebra fish, Brachydanio rerio (Hamilton-Buchanan). These organisms are sensitive to sub-microgram quantities of the toxin and can he used as bioassay test organisms. The larval response is easier to measure man embryonic response, and a standard dosage-mortality curve for the larvae can be developed in 33 h. As an aflatoxin bioassay test organism, the larvae of zebra fish have several advantages over other test organisms, including ducklings, embryos, and tissue cultures. They require no special skill or equipment and can be obtained easily in the laboratory throughout the year.

1293. Abedi, Z.H., and Scott, P.M. 1969. Detection of toxicity of aflatoxins, sterigmatocystin, and other fungal toxins by lethal action on zebra fish larvae. Journal of the Association of Official Analytical Chemists 52(5): 963-969.

The effects of 13 fungal toxins (and one derivative) on the larvae of zebra fish, Brachydanio rerio, were investigated. The most toxic were sterigmatocystin, gliotoxin. and aflatoxin B,, which were lethal at concentrations of less than 1 µg mL^{-1}. Allatoxins B_2, G_1. and G_2, stemphone. diacetoxyscirpenol, ochratoxin A, aspertoxin, and patulin also showed toxicity to the larvae, while aflatoxin B_1 hemiacetal, penicillic acid, and griseofulvin were non-toxic at the levels tested. Specific morphological effects were noted with sterigmatocystin, aflatoxins, and stemphone. The usefulness of zebra fish larvae as bioassay test organisms for fungal toxins is discussed.

1294. Allcroft, R., and Carnaghan, R.B.A. 1963. Toxic products in groundnuts. Biological effects. Chemistry and Industry (London) 2 : 50-53.

Biological effects of toxic groundnut meal (meal containing aflatoxin) in various birds and animals are reviewed. Ducklings are the most susceptible to the toxin. They are suitable for bioassay of aflatoxin. Turkey poults are less susceptible, while chikens arc comparatively resistant. Among the larger farm animals, pigs arc most susceptible. Calves from one to six months of age are highly susceptible, becoming tolerant with age, and sheep are comparatively resistant.

1295. Allcroft, R., Carnaghan, R.B.A., Sergeant, K., and O'Kelly, J. 1961. A toxic factor in Brazilian groundnut meal. Veterinary Record 73: 428-429.

A toxic substance was found in the chloroform extract of Brazilian groundnut meal. This extract from 10 g meal was lethal to ducklings. Signs of poisoning were similar to those of Senecio poisoning of poultry, but the isolated poison was not a pyrrolizidine alkaloid or the N-oxide of such an alkaloid.

1296. Armbrecht, B.H., and Fitzhugh, O.G. 1964. Mycotoxins U. The biological assay of aflatoxin in Peking white ducklings. Toxicology and Applied Pharmacology 6: 421-426.

The Peking White duckling was found suitable for bioassay of aflatoxin. Aflatoxin caused liver damage in the birds 1-2 days after oral administration. The LD_{50} of the aflatoxin sample A16A was 1.40 mg kg^{-1}. The lower limit of liver damage detection after a single dose was 0.1 mg kg^{-1}; it could be reduced by a factor of 10 with multiple dosage. The multiple dosage protocol is suitable for qualitative screening, but an LD_{50} determination is recommended for quantitative measurements. It is recommended that mycological, chemical, and toxicological results be considered in a qualitative interpretation of aflatoxin poisoning.

1297. Asplin, F.D., and Carnaghan, R.B.A. 1961. The toxicity of certain groundnut meals for poultry with special reference to their effect on ducklings and chickens. Veterinary Record 73: 1215-1219.

Evidence is presented of the susceptibility of ducklings and chickens to the toxic principle in certain samples of groundnut meal. Ducklings were highly susceptible to the toxic principle in these meals, and it was suggested that they are eminently suitable for screening suscepted samples of groundnut meal and for other experimental work connected with this type of toxicity. A toxic agent was found in certain Brazilian and East African groundnut meals, and evidence is presented which suggests that the toxic principle in these meals is identical. The gross and microscopic lesions in ducklings and chickens fed on toxic groundnut meals arc described and the similarities and differences between the lesions in these birds and turkeys and in large animals are discussed.

1298. Banes, D. 1966. Food toxins of fungal origin : methodology and regulatory aspects. Food Technology 20(6): 51-52.

This paper very briefly reviews some analytical methods and bioassays for aflatoxins in groundnuts and groundnut products. Regulatory aspects are also discussed, and the actions of the Food and Drug Administration (FDA) of the US are highlighted.

1299. Bedi, J.S., and Chohan, J.S. 1986. Effect of culture filtrates of toxigenic and nontoxigenic isolates of Aspergillus flavus on different crop plants and weeds. Indian Journal of Mycology and Plant Palhology 16(1): 48-54.

Twenty-two isolates of Aspergillus flavus from seeds of groundnut, maize, cotton, rice and wheat were screened for production of aflatoxins on YES+salt medium by thin-layer chromatography (TLC) and the okra seedling chlorosis test. To develop a rapid bioassay, seeds of different crop plants and weed plants were soaked in culture filtrates of a toxigenic isolate from maize and in pure aflatoxin B_1 (50 ppm). Chlorosis and albinism of the primary leaves occurred in six species. The effects were most pronounced in sorghum, radish and okra and these could be used to screen aflatoxigenic strains of A. flavus.

1300. Blount, W.P., Fraser, D.McK., Knight, D., and Dowling, W.M. 1963. The use of ducklings for the detection of aflatoxin. Veterinary Record 75: 35.

Following the outbreaks of Turkey "X" disease in turkey poults in Britain in 1960, workers have reported the suitability of ducklings as biological assays for toxic principle in groundnut meals. This note reports that mere may be a difference in response whether one uses White Penine ducklings or Khaki Campbell ducklings. Both these ducklings were compared for sensitivity to the toxic principle. The authors conlcuded that Khaki Campbell ducklings were more sensitive to aflatoxin (toxic principle) than the White Penine ducklings, and as such should be used for biological assays until a more sensitive breed of species is known.

1301. Brown, R.F., Wildman, J.D., and Eppley, R.M. 1968. Temperature-dose relationships with aflatoxin on the brine shrimp, Anemia salina. Journal of the Association of Official Analytical Chemists 51(4): 905-907.

Temperature-dose relationships with aflatoxin B_1 were studied, using the brine shrimp, Artemia salina. Increase in temperature resulted in increased sensitivity by the brine shrimp to aflatoxin. Optimum sensitivity occurred at 37.5°C. Positive results were obtained at 0.5 µg aflatoxin B_1 mL^{-1} artificial sea water with a mortality of over 60 %. Greater than 90 % mortality occurred at dose levels of 1 µg mL^{-1} and above. The test can be conducted in 24 h.

1302. Buckle, A.E., and Sanders, M F. 1990. An appraisal of bioassay methods for the detection of mycotoxins - a review. Letters in Applied Microbiology 10: 155-160.

Bioassay methods for mycotoxins are reviewed. An outline of the range of bioassay methods is given and the role of cytotoxicity tests in particular is emphasized.

1303. Buckelew, A.R.Jr., Chakravati, A., Burge, W.R., Thomas, V.M.Jr., and Ikawa, M. 1972. Effect of mycotoxins and coumarins on the growth of Bacillus megaterium from spores. Journal of Agricultural Food Chemistry 20(2): 431-433.

The growth of Bacillus megaterium from spores is inhibited by a variety of toxic fungal metabolites and related compounds. Most of the mycotoxins possessing a carbonyl function conjugated with a double bond system, such as α, ß-unsaturated lactones, pyrones, and quinones, were inhibitory. The notable exception was rubratoxin B_1. Of a number of 3-substituted 4-hydroxycoumarins tested, only those possessing a 3 substituent terminated by a large group were highly inhibitory. A number of compounds which act as uncouplers of oxidative phosphorylation were found inhibitory. It is suggested that, among others, toxic fungal matabolites which possess ana. ß-unsaturated carbonyl system or which act as uncoupling agents of oxidative phosphorylation may be detected by the use of Bacillus spores.

1304. Butler, W.H. 1964. Acute liver injury in ducklings as a result o aflatoxin poisoning. Journal of Pathology and Bacteriology 88: 189-196.

This paper describes the histological changes in the liver produced by a single dose of aflatoxin and contrasts them with the lesions produced in day-old ducklings by other well-studied liver poisons. Day-old Khaki Campbell ducklings given 15 |ig aflatoxin by mouth developed an extensive biliary proliferation in the liver with fatty degeneration of the peripheral parenchyma cells. This lesion reached its maximum in 3 days, and men regressed with repair of the liver parenchyma. A comparable lesion was seen in ducklings given single doses of dimethylnitrosamine and cycasin, but not with other hepatotoxic agents such as carbon tetrachloride, ethionine and thioacetamide. The lesions varied greatly at the same dose level, and it was not possible to estimate the amount of aflatoxin by histological examination of the livers of poisoned birds. Evidence of possible direct action upon the biliary epithelium is given, and it is suggested that aflatoxin may be an alkylating agent.

1305. Butler, W.H. 1966. Acute toxicity of aflatoxin B_1 in guinea-pigs. Journal of Pathology and Bacteriology 91: 277-280.

The effects of single dose of aflatoxin B_1 on guinea-pigs were compared with the effects of feeding groundnut meal containing aflatoxin. The $LD_{5()}$ of aflatoxin B, in male and female guinea-pigs was estimated as 1.4 mg kg^{-1} bodyweight by interaperitoneal injection. The main hepatic lesion was a centrilobular zone of necrosis developing with biliary proliferation over 2 days. The lesion was healed over a few days and the biliary proliferation rapidly regressed. The pathological changes in other organs are described.

1306. Carnaghan, R.B.A., and Sargeant, K. 1961. The toxicity of certain groundnut meals to poultry. Veterinary Record 73: 726- 727.

Day-old ducklings in groups of six were given two turkey diets which had been associated with outbreaks of Turkey "X" disease. The diets had about 6 % Indian groundnut meal. Other groups were given similar amounts of Indian groundnut meal known to be non-toxic. Those given the toxic meal did not grow well and five in each group died within 5 weeks. Gross and microscopical lesions, similar to those produced by toxic Brazilian and East African groundnut meals, were found. Extracts of the Indian meals in amounts equivalent to 100, 200 and 750 g in 5, 5 and 11 days did not kill day-old ducklings, but liver lesions were found post mortem.

1307. Clements, N.L. 1968a. Note on a microbiological assay for aflatoxin B_1 : A rapid confirmatory test by effects on growth of Bacillus megaterium. Journal of the Association of Official Analytical Chemists 51(3): 611-612.

Growth inhibition of Bacillus megaterium (NRRL B-1368) in response to aflatoxin B_1 provides a simple and rapid bioassay of aflatoxin B_1. Inhibition was detectable with 1 µg of aflatoxin B_1 as early as 7 h after incubation: well-defined zones of inhibition were produced after 15-18 h. Crude aflatoxin, mixtures of aflatoxins B_1 and G_1. and pure aflatoxin B_1 all gave similar results.

1308. Clements, N.L. 1968b. Rapid confirmatory test for aflatoxin B_1 using Bacillus megaterium. Journal of the Association of Official Analytical Chemists 51(6): 1192-1194.

Growth inhibition of Bacillus megaterium (NRRL B-1368) in response to aflatoxin B_1 provides a simple and rapid technique to supplement thin-layer chromatographic (TLC) identification of aflatoxin. Less than an hour is required to set up the test, and results are obtained after overnight incubation (15-18 h). The test is sensitive to as little as 1 µg aflatoxin B_1.

1309. Fulsoundar, A.B., and Shukla, P.C. 1978. A note on chemical and biological assay of the aflatoxins extracted from cattle feeds available in Gujarat. Indian Journal of Animal Sciences 48(2): 145-146.

Aflatoxin was estimated with Bacillus megaterium. Of 133 samples of cattle feed tested, 56 were positive for aflatoxin. Some samples positive by thin-layer chromatography (TLC) were negative in inhibiting growth of B. megaterium.

1310. Legator, M.S. 1969. Biological assay for aflatoxins. Pages 107-146 in Aflatoxin : Scientific Background, Control, and Implications (Goldblatt, L.A., ed.). New York, USA : Academic Press. 472 pp.

This paper comprehensively reviews the available bioassays for aflatoxins. Both genetic and nongenetic effects of aflatoxin toxicity in microorganisms, animals, and birds are discussed. Guidelines for formulating aflatoxin for biological studies are given.

1311. Mehan, V.K., and Chohan, J.S. 1973. Aflatoxin Bl producing potential of isolates of Aspergillus flavus Link ex Pries from cotton, maize and wheat. Mycopathologia et Mycologia Applicata 49(4): 263-274.

Twenty-one isolates of Aspergillus flavus obtained from cotton, maize and wheat were screened for their ability to produce aflatoxins in two liquid media. Of these, 16 isolates were aflatoxigenic and produced only aflatoxin B_1 as assessed by bioassay on okra seedlings and thin-layer chromatographic (TLC) method. For screening isolates of A. flavus for aflatoxin formation, 0.7 % YES + Salt medium was found good as also for obtaining high yields of the toxin. Isolates of A. flavus

produced aflatoxin B_1 ranging from 0.85 to 17.2 mg 50 mL^{-1}. Maximum yield of aflatoxin was obtained when rice was used as the substrate in case of toxigenic isolates L-27 and C-9, and on maize in isolate M-11.

1312. Mehan, V.K., and Chohan, J.S. 1974. Effect of filtrates of aflatoxin producer and non-producer isolates of Aspergillus flavus on different crop plants. Indian Journal of Mycology & Plant Pathology 4(1): 74-76.

The effects of aflatoxin containing filtrates from aflatoxin producer and non-producer isolates of Aspergillus flavus on seed germination and seedlings of different crop plants arc reported, producer isolate M-11 and non-producer isolate M-7 of A. flavus were used ; the isolates were grown separately on 0.7 % YHS + salt liquid medium at 25 + 1°C for 14 days and the cultures were filtered and autoclaved. The effects of filtrates from the isolates were observed on seed germination of different crop plants. The soaking of seeds of different crop plants in the culture filtrates of M-11 for different durations inhibited seed germination of many crop plants tested. In some cases the toxic effects were observed on the germinating seedlings too. Seedlings of Raphanus sativus and Sorghum vulgare showed chlorosis and albinism in the cotyledonary and primary leaves, respectively. These plants can be used as bioassays for aflatoxin.

1313. Reiss, J. 1975. Mycotoxin bioassay, using Bacillus stearothermophilus. Journal of the Association of Official Analytical Chemists 58(3): 624.625.

Spores of Bacillus stearothermophilus in standardized spore strips are pretreated with solutions of the mycotoxins aflatoxin B,, polution, rubratoxin B, and diacetoxyscirpenol and subsequently incubated in a nutrient solution containing bromocresol purple as pH indicator. After 16.5 h of incubation the color of the indicator medium inoculated with nontreated spore strips of B. stearothermophilus changes from purple to yellow; no color change occurs in the indicator medium inoculated with spore strips treated 15 min with 0.01 µg of any of the mycotoxins mL^{-1} during a 60 h incubation.

1314. Sargeant, K., Allcroft, R., and Carnaghan, R.B.A. 1961. Groundnut toxicity. Veterinary Record 73: 865.

Samples of groundnut meals and decorticated groundnuts from Nigeria, French West Africa, and the Gambia were examined. Many were toxic in trials were ducklings. Some of the meals were already suspect, being associated with the deaths of calves and sheep, others were not. and among those several were not toxic. Some extracts from decorticated groundnuts were also toxic.

1315. Sargeant, K., O'Kelly, J., Carnaghan, R.B.A., and Allcroft, R. 1961. The assay of a toxic principle in certain groundnut meals. Veterinary Record 73: 1219-1223.

The toxic principle in certain samples of Brazilian groundnut meal was extracted and concentrated 250 times on a weight basis. This extract produced motality in ducklings and turkey poults and histological liver lesions identical to those seen in field outbreaks of so-called Turkey "X" disease. The dosing of concentrated extracts to young ducklings gives a rapid and sensitive method for testing the toxicity or otherwise of groundnut meals. The toxic principle is neither a pyrrolizidine alkaloid nor the N-oxide of such an alkaloid. It is suggested that the toxic principle is derived from a microorganism, possibly a fungus. A similar toxic principle was found in some groundnut meals from India, Uganda and Tanganyika, French West Africa. Nigeria, Gambia and Ghana.

1316. Sargeant, K., O'Kelly, J., Carnaghan, R.R.A., and Allcroft, R. 1961. The assay of a toxic principle in certain groundnut meals. Veterinary Record 73: 1215-1219.

The toxic substance present in some Brazilian and African groundnut meals which causes Turkey "X" disease was shown to be even more toxic to ducklings. The signs included loss of appetite, poor growth rate, a tendency to down or feather pulling, liver damage and sometime purple discoloration of the legs and feet in white-skinned birds over 3 weeks old, occasionally accompanied by lameness. Not all groundnut meals tested were toxic. Toxic groundnut meal as 10 % of the diet caused death within 6 to 20 days. This interval and the severity of the gross lesions depended on the toxicity of the sample, the amount eaten and the age of the ducklings; younger birds were more susceptible. With chicks, 10 % groundnut meal reduced growth rate but did not cause death ; with chickens 45 days old 15 % groundnut meal caused 3 deaths in the first 3 weeks but no more in the rest of the feeding period lasting 9 months.

1317. Schoental, R., and White, A.F. 1965. Aflatoxins and 'albinism' in plants. Nature 205:57-58.

Studies were carried out to investigate whether aflatoxins were the factors responsible for the inhibition of chlorophyll synthesis in plants. Crude extracts were prepared from two groundnut meal samples. One meal was known to be toxic to animals and to contain aflatoxins, and the other was a non-toxic meal. These extracts were tested on the rapidly germinating seeds of cress (Lepidium sativum). The leaves of the seedlings that had the toxic extract (5 mg mL^{-1} corresponding to approximately 0.5 g of meal) appeared yellowish, while those which had the same concentration of the non-toxic extract, or water, were bright green. The tests were repeated using various concentrations of aflatoxins, coumarin and several other

fungal metabolities. Aflatoxins, like coumarin, inhibited the germination of seeds in concentration of 25 µg mL^{-1} but its effect on the color of leaves was more marked. Complete absence of the green color occurred in the presence of 10 µg mL^{-1} of aflatoxin. The striking effect of aflatoxins in inhibiting the green color of cress seedlings suggests that this effect could serve as the bioassay for aflatoxin in foodstuffs.

1318. Tanaka, K., Goto, T., and Manabe, M. 1986. Biological test using Moina macrocopa Straus (Part 1). Rearing conditions of Moina macrocopa Straus and influence of mycotoxins on this animal plankton. Report National Food Research Institute 48: 36-42.

In order to develop a low cost and simple biological method for the detection of mycotoxins, the properties of Moina macrocopa, a kind of animal plankton in fresh water, were studied. City water was treated with charcoal in order to remove chlorine and this treated water was used for rearing the plankton. Vital yeast for bread-making was used as feed for this plankton. The temperature for rearing M. macrocopa was set between 22-24°C and the temperature for biological test was set at 20°C. The survival rate of M. macrocopa was found to be high for pH values ranging from 6.5 to 8.5 based on pH's tests using a mixture of water (2 parts) and Britton-Robinson buffer (1 part). The effects of salinity and organic matters of M. macrocopa which appear to be harmless were examined. When the concentration was below 0.23 % in case of sodium chloride, 0.45 % in case of sodium glutamate, and 2.0 % in case of glucose, these chemicals did not appear to affect the survival rate of the plankton directly. Based on the results of experiments on the survival rate of M. macrocopa using solvents such as acetone, methanol, ethanol and dimethyl sulfoxide (DMSO), DMSO was found to be the least toxic. Therefore DMSO could be used to dissolve mycotoxins into the rearing water of plankton. Of 8 mycotoxins tested, the toxicity levels of aflatoxin B_1, diacetoxyscirpenol, and sterigmatocystin were comparatively high. Comparison of these results with the toxicity levels of mycotoxins for the brine shrimp showed that the order of toxicity was similar. In most cases, however, M. macrocopa appeared to be more sensitive to mycotoxins than the brine shrimp. This method may be used for screening these mycotoxins.

1319. Tanaka, K., Manabe, M., and Matsuura, S. 1979. Biological test using brine shrimp (Part 2). The synergistic effects of mycotoxins to brine shrimp. Report National Food Research Institute 34: 84-88.

The synergistic effects of mycotoxins to brine shrimp were examined. Aflatoxin B,, diacetoxyscirpenol, fusarenon-X, ochratoxin A, sterigmatocystin and T-2 toxin were used as the mycotoxins. When aflatoxin B_1 and T-2 toxin were administered to the brine shrimp, the mortality was between 20 and 30 % at 1/2 median Tolerance Limit (TLm). The mortality increased almost linearly up to TLrn. When diacetoxyscirpenol, fusarenon-X and sterigmatocystin were administered, the mortality was less than 20 % at 1/2 TLm, then increased rapidly up to TLm. In the case of ochratoxin A, the mortality was more than 30 % at 1/2 TLm, then the mortality increased slowly up to TLm. When 1/2 TLm of two mycotoxins are to be administered at one time and if the mortality exceeds 60 %, it is regarded as synergistic efect. The synegisitic effect was observed at the administration of fusarenon-X and ochratoxin. In other cases the toxicities were considered, not synergistic, but additive.

1320. Tanaka, K., Manabe, M., and Matsuura, S. 1982. Biological tests using the brine shrimp (Artemia salina) (Part 3). The toxicities of metal compounds and the synergistic effects of metal compounds and mycotoxins on the brine shrimp {Artemia salina). Report National Food Research Institute 39: 58-63.

Biological tests carried out on the brine shrimp using metallic compounds and simultaneous administration of mycotoxins and metallic compounds. The survival rate of brine shrimp was high at pH 6.1 to 9.0, when examined at various pH's using a mixture of artificial sea water (2 parts) and Britton-Robinson buffer (1 part). Cl and SO_4^{2} were found less toxic to the brine shrimp compared to the metallic ions. Of the 17 metallic compounds tested, the toxicity levels of potassium chromate, potassium dichromate, cupric sulfate, and mercuric chloride were comparatively high. At toxic level, the pH of the solutions of each of the metallic compounds was measured ; pH was low and that itself could have influenced the survival rale of brine shrimp in the case of chromium potassium sulfate, ferrous sulfate, ferric sulfate and ferric chloride. The simultaneous administration of half the median Tolerance Limit (TLm) of each of the six mycotoxins and one of seven metallic compounds to the brine shrimp was carried out. A weak synergistic effect was observed with the administration of ochratoxin A and zinc sulfate while comparatively strong synergistic effects were obserrved with the administration of ochratoxin A and mercuric chloride, and aflatoxin B_1 and mercuric chloride. The toxicity level was weakened by the simultaneous administration of sterigmatocystin and cupric sulfate. The mean values of the toxicity levels of ochratoxin A when administered simultaneously with cadium chloride, or potassium chromate, or potassium dichromate, and sterigmatocystin when administered simultaneously with mercuric chloride were lower than the toxicity value of 1/2 TLm of each mycotoxin or metallic compound although the value varied widely. In the case of other mycotoxins and metallic compounds, the levels of toxicity were cumulative.

1321. Tanaka, K, Minamisawa, M., Manabe, M., and Matuura, S. 1975. Biological test using the brine shrimp (Part I). The influence of mycotoxins on the brine shrimp. Report National Food Research Institute Japan 30: 43-48.

Several conditions to rear the brine shrimp were examined, and sensitivity of the brine shrimp to mycotoxins was examined. Temperature-dose relationships with methanol on the brine shrimp were tested in "Kuroshio" artificial sea water. When 3 % methanol was used, the viability was high at 10 to 20"C. Of six artificial sea waters examined, Lyman and Fleming's artificial sea water proved good for hatching and rearing. The optimum temperature for rearing was 20°C while it was 30°C for hatching the brine shrimp. When the viability of the brine shrimp on solvents was tested, dimethylsulfoxide (DMSA) and propylene glycol were harmless to the brine shrimp. So DMSO was used to dissolve mycotoxins into artificial sea water. T-2 toxin, sterigmatocystin, diacetoxyscripenol, aflatoxin B_1, and aflatoxin G_1 were very toxic to the brine shrimp. This method may be used for screening these mycotoxins.

1322. Townsley, P.M., and Lee, E.G.H. 1967. Response of fertilized eggs of the mollusk Bankia setacea to aflatoxin. Journal of the Association of Official Analytical Chemists 50(2): 361-363.

Aflatoxin B, inhibits cell cleavage in fertilized mollusk eggs without preventing fertilization or nuclear division. The reaction is very dramatic and positive since fertilized eggs in the presence of aflatoxin are multinuclear, whereas the controls have multiplied to multicellular larvae. The bioassay requires a minimum of technique and training and is sensitive to concentrations of 0.05 µg mL^{-1}. Since the reaction is observed with the aid of a low-powered microscope (70 X). the concentration of aflatoxin required can be reduced considerably below 0.05 ug. The test required two to four hours for completion when observing egg division and 18 h for swimming larvae.

1323. Verrett, MJ., Marliac, J.P., and McLaughlin, J.Jr. 1964. Use of chicken embryo in the assay of aflatoxin toxicity. Journal of the Association of Official Agricultural Chemists 47: 1003-1006.

The possibility of using the chicken embryo as a test organism for the assay of aflatoxin toxicity was investigated. The injection of test solutions was made before incubation, in fertile white Leghorn eggs, by either of 2 routes: yolk or air cell. The development of the embryos was observed for the full 21-day incubation period. The injection of solutions of pure aflatoxins B_1 and G_1 and of extracts of aflatoxin-producing mold cultures indicated that the chicken embryo was sensitive to these compounds. A dose-response was exhibited in that the toxicity of the samples was related to the mortality at the time of hatching. Extracts of aflatoxin-free groundnut products were nontoxic to the chicken embryo. The addition of aflatoxin B_1 to such uncontaminated extracts produced the expected toxicity in the embryos. The injection of extracts from aflatoxin-contaminated groundnut products resulted in a toxic response that correlated well with that obtained by injection of pure aflatoxin B_1 solutions at the same dose levels, and in most cases the chemical analysis was confirmed. The presence of aflatoxins G_1, B_2, and G_2 had no apparent effect on the toxicity due to aflatoxin B_1, at the levels at which they occurred in the particular samples tested. The separation of aflatoxin B_1 from contaminated extracts by thin-layer chromatography, and its subsequent elution from the plates and injection into the eggs, confirmed that the toxicity of these extracts was due primarily to their aflatoxin B_1 content.

8. MANAGEMENT OF ASPERGILLUS FLAWS INFECTION AND AFLATOXIN CONTAMINATION OF GROUNDNUT

8.1 REVIEW

For management of A.flavus infection and aflatoxin contamination of groundnuts both preventative and curative procedures may be necessary. Aflatoxin management therefore starts in the farmer's field, continues through crop produce handling, marketing, storage, and processing, and ends with the consumer.

In general, cultural practices and use of crop protection chemicals are preventative in nature as are provision of effective storage procedures and care during transportation and processing. Curative measures concentrate on isolation and segregation of toxic groundnuts and various systems of detoxification. Varietal resistance is a desirable component of any integrated aflatoxin management system.

8.1.1 Cultural Control

To be effective, cultural control of aflatoxin contamination of groundnuts must take into consideration all the varied environmental and agronomic factors that influence pod and seed infection by the aflatoxin-producing fungi, and aflatoxin production. These factors can vary considerably from one location to another, and between seasons in the same location. Some environments may be particularly favorable to fungal infection and subsequent aflatoxin contamination of groundnuts, and this may even raise the question as to whether or not the crop should be grown in such places. However, for most situations it should be possible to devise cultural practices that can greatly reduce or even eliminate aflatoxin contamination from the saleable crop produce. Aflatoxin contamination may occur preharvest, postharvest or during both periods. The extent to which the aflatoxin problem occurs in one or other of the two periods is largely influenced by weather conditions. Where drought occurs late in the growing season aflatoxin contamination may occur prior to lifting of the crop. Where rainfall is well distributed and late season drought does not occur, wet and humid conditions postharvest may lead to fungal infection and aflatoxin contamination.

Growing groundnuts continuously on the same land may lead to a build-up of high populations of A. flavus and/or A, parasiticus in the soil, which in turn increases the probability of seed infection and aflatoxin contamination (Joffe and Lisker 1970, Pettit and Taber 1968, Subrahmanyam and Rao 1974). Limited research has been done on effects of crop rotations on fungal infection and aflatoxin contamination in groundnuts (Pettit and Taber 1968. Joffe and Lisker 1970, Griffin et al., 1981). In hot and arid environments, populations of A. flavus may be very high, and crop rotations may have little influence on the fungal activity. Cropping systems in some regions involve varied cultivation and fertilizer practices that individually or taken together may affect survival or build-up of populations of the toxigenic fungi. Evidence is accumulating that groundnuts grown in different soil types may have significantly different levels of seed infection by A. flavuslA. parasiticus (Graham 1982, Mehan et al. unpublished data). Light sandy soils and Alfisols favor rapid proliferation of the toxigenic fungi, particularly under dry conditions. Vertisols have high water-holding capacity and this may be partly responsible for the lower than average levels of aflatoxin contamination in groundnuts grown on such heavy black soils.

Some research has been done on possible effects of calcium on preharvest aflatoxin contamination of groundnuts. Davidson et al. (1983) reported that application of gypsum to a soil in Georgia, USA, reduced aflatoxin contamination, but Cole et al. (1985) and Wilson et al. (1985) did not observe any such effects. The groundnut pod obtains its calcium requirements from the surrounding soil and this may be difficult under drought conditions. This indicates possible interrelationships of drought, available calcium, pod development and A. flavus infection.

Choice of groundnut cultivar can be important. A cultivar should fit a particular growing season and mature at the end of the rains so that postharvest field drying can be done under favorable conditions (McDonald 1969, Mehan 1987). It is undesirable that a variety should suffer from drought stress during pod maturation and some compromise may have to be effected between harvesting under dry conditions and avoidance of drought stress by using short-duration cultivars that mature before the rains have ended. Also, planting dates may be adjusted so that the crop matures at the end of the rainy season and post-harvest conditions favor rapid and effective drying of the crop. Care should be taken to avoid drought stress during late stages of pod maturation. Optimum plant populations should be established bearing in mind that too high a population may lead to severe drought stress where rainfall is suboptimal in a growing season (Graham 1982). Excessive weed growth may also deplete available soil moisture, and effective weed control by use of herbicides, or cultivations is advisable. Care should be taken during cultivations to avoid damage to pegs and pods (McDonald 1969). Cultivations and crop protection practices that lower the incidence of soil insects, mites and nematodes should help in reducing aflatoxin contamination (Sellschop 1965).

Irrigation to ensure adequate soil moisture during the last 4-6 weeks of crop growth should prevent preharvest aflatoxin contamination of groundnuts (Sanders et al. 1986, Wilson and Stansell 1983). This may be achieved by growing a completely irrigated crop or by applying supplementary irrigation to a basically rainfed crop.

Individual plants that die from attack by pests and diseases should be lifted separately as their produce is likely to contain aflatoxin (McDonald 1969). It is very important to harvest the crop at optimum maturity, as excessive numbers of over-mature or very immature pods at harvest can be reflected in high levels of aflatoxin in the produce (McDonald 1969).

Where groundnuts are already invaded by aflatoxigenic strains of A. flavus/A. parasiticus at time of harvest, there may be a serious build-up of aflatoxin contamination if environmental conditions during crop drying favor development of the fungi (Mehan 1987). Where conditions before-harvest have not favored fungal infection there may still be significant invasion of shells of pods, and given unfavorable post-harvest drying conditions, the toxigenic fungi may invade the seed with subsequent aflatoxin contamination. Rapid field-drying of crop produce with protection from rain can largely prevent postharvest invasion of seeds by the aflatoxigenic fungi (Blatchford and Hall 1963a and b, McDonald 1969, Mehan 1987).

One of the most effective rapid curing procedures, developed to reduce postharvest aflaioxin contamination, is to invert the groundnut pods in the windrow. Pods are exposed to direct sunlight and air currents and they dry rapidly and effectively (Dickens and Khalsa 1967, Pettit et al. 1971). It should be borne in mind that too rapid drying may cause skin slippage and production of off-flavors in the seeds.

8.1.2 Chemical Control

Several attempts have been made to control or reduce A.flavus infection of pods and seeds by applying fungicides to soil, to groundnut foliage or to freshly-lifted groundnut pods (Jackson 1967a, b, Bell and Doupnik 1971, Pettit et al. 1971, Madaan and Chohan 1978).

Soil fumigation during land preparation has been tried to reduce populations of A. flavus developing around groundnut plants, but numbers of fungi on pod surfaces at maturity were not affected by use of vapam sodium-N-methyldithio-carbamate) or Vorlex or DD (1,2-dichloropropene) (Jackson 1967a). But Mixon et al. (1984) found that PCNB-fensulfothion or CGA 64250 reduced seed infection by A.flavus. Application of fungicides to freshly- lifted groundnut pods has been tried but with no definitive results in terms of their effects on subsequent fungal invasion and aflatoxin contamination of seeds (Jackson 1967a, b, Fonseca et al. 1976). However. Bell and Doupnik (1971. 1972) found that aflatoxin contamination of windrowed groundnuts could be substantially reduced by treatment with Manzatc, Benlate, or Botran. Madaan and Chohan (1978) reported prevention of A.flavus invasion of seeds by spraying freshly-lifted groundnut pods with propionic acid, sorbic acid, or chlorothalonil. No fungicide, combinations of fungicides, or other chemical treatments have been adopted for practical control of A. flavus infection and subsequent aflatoxin contamination of groundnuts in the field.

Biocides applied to the soil may have direct or indirect effects on the populations of the toxigenic fungi. For instance, they may affect other components of the soil microflora and fauna and stimulate biological control systems. Such interactions are likely to be complex and difficult to unravel. Similarly, addition of organic substances to soils, e.g., green-manure, crop residues, farmyard manure, is likely to have complex effects upon the soil microflora.

There are a number of reports (Ghewande and Nagaraj 1987, Bean et al. 1971. Bean and Rambo 1975, Premlata Singh and Sinha 1986) of the effects of various chemicals in preventing or inhibiting growth of A. flavus/A. parasiticus and aflatoxin production in vitro but their practical application is doubtful.

8.1.3 Biological Control

Aspergillus flavus is frequently found associated with several other fungi in groundnut pods and seeds (Hanlin 1970). Based on such observations it has been hypothesised that interactions between fungi as they compete for the substrate might, under favorable environmental conditions, restrict invasion of groundnuts by A. flavus and/or contamination with aflatoxin. Microbial competition or microbial breakdown may be responsible for lower levels of aflatoxin in parasite-damaged pods than in seed from mechanically broken pods. It has been reported that several fungi can break down aflatoxin in groundnuts and in aflatoxin-containing liquid culture media; A. niger and R. solani appeared to limit the development of A. flavus and aflatoxin production in the substrate (Diener 1973). In Israel, Joffe (1969) observed that a large number of viable propagules of A. niger in the geocarposphere and moderate invasion of seeds by this species was associated with very limited invasion of seeds by A.flavus, Fusarium solani, and Penicillium spp. Frequent invasion of seeds by A. niger has been suggested to be important in preventing the development ol' A. flavus (Joffe 1969). Aspergillus niger has been shown to inhibit growth of A. flavus and aflatoxin production in vitro (Burnett et al. 1972). Presence of normal

endogeocarpic mycoflora, including A. niger and M. phaseolina. appears to inhibit invasion of groundnuts by A. flavus. This notion is supported by the studies of Lindsey (1970) who found that A. flavus penetrated and colonized a high percentage of shells of living, attached, immature and mature pods under gnotobiotic conditions.

8.1.4 Genetic Resistance to Aspergillus flavus Invasion and Aflatoxin Production

The aflatoxin problem could be solved if a groundnut cultivar could be identified or bred that was immune to seed infection by the aflatoxin-producing fungi or, once infected, did not support aflatoxin production. Initially the aflatoxin problem in groundnut was linked more to the postharvest period than to the period of pod development in the soil, and interest was focussed mainly on groundnut products such as meal and cake. Thus early work tended to concentrate on resistance to aflatoxin production.

An absolute evaluation of genotypic resistance to aflatoxin contamination can only be obtained by comparing the aflatoxin contents of seeds of the different genotypes. However, it is easier to screen for resistance to A. flavus seed infection and this may be used to provide an indication of possible resistance to aflatoxin contamination. But it should be borne in mind that not all strains have similar aflatoxin-producing ability, and that A. flavus strains may interact with host genotypes to influence rate of aflatoxin production.

8.1.5 Resistance to Aflatoxin Production

Rao and Tulpule (1967) first reported varietal resistance in groundnut to aflatoxin production. In laboratory inoculation tests they found that the cultivar US 26 (PI 246388) did not support aflatoxin production when seeds were colonized by aflatoxin-producing strains of A. flavus. This finding was not confirmed by other workers, but did stimulate research on possible varietal resistance to aflatoxin production (Doupnik 1969. Doupnik and Bell 1969, Nagarajan and Bhat 1973, Tulpule et al. 1977, Aujla et al. 1978, Kiran Kalia et al. 1988). To test cultivars for their ability to support aflatoxin production most researchers autoclaved the groundnut seeds before inoculation with aflatoxigenic strains of A. flavus and/or A. parasiticus. However, any reaction measured on autocalved or otherwise killed seeds can have only limited relevance to genetic resistance. A laboratory method to screen live groundnuts for resistance to aflatoxin production was used at ICRISAT (Mehan and McDonald 1980) to test 502 genotypes. None was totally resistant to aflatoxin production but highly significant differences in aflatoxin production were found (Mehan et al. 1986). Recently, two wild Arachis species. A. cardenasii and A. duranensis, have been reported to support production of only trace levels of aflatoxins (Ghewande et al. 1989). These Arachis species were also found highly resistant to in vitro seed colonization by A. flavus. It is important that these findings should be confirmed and wild Arachis species accessions tested should be fully specified. A limited search has been made for groundnut genotypes that do not support, or support only very low levels of aflatoxin production following seed infection by aflatoxigenic strains of A. flavus/A. parasiticus. In most cases aflatoxin production tests have been carried out using only one or two aflatoxin- producing isolates of A. flavus and/or A. parasiticus. As interactions may occur between genotypes and aflatoxin-producing fungal isolates, it is obviously important to use several highly aflatoxigenic strains of A. flavus and A. parasiticus to establish low aflatoxin production supporting ability of the groundnut genotypes. Promising germplasm should be made available to all interested laboratories for validation of resistances to aflatoxin production.

8.1.6 Resistance to Aspergillus flavus Infection

The existence of seed resistance to infection by A. flavus/A. parasiticus was a logical assumption considering that seeds with damaged testae are more easily and rapidly invaded by fungi than are seeds with intact testae, and colored testae confer greater resistance to invasion by A. flavus than do white or variegated testae (Carter 1973).

Mixon and Rogers (1973a) first suggested that use of groundnut cultivars resistant to seed invasion and colonization by the aflatoxin-producing fungi could be an effective means of preventing aflatoxin contamination. They developed a laboratory inoculation method for screening groundnut genotypes for resistance to A. flavus/A. parasiticus invasion and colonization of rehydrated, mature, sound, stored seeds. Essentially, tests are carried out on sound, mature seeds from undamaged, hand-shelled pods that have been dried and stored for at least one month. Seeds are surface- sterilized, hydrated and then inoculated with a conidial suspension of an aflatoxigenic strain of A. flavus or A. parasiticus.

Mixon and Rogers (1973a) reported that two Valencia type genotypes. PI 337394F and PI 337409. had high levels of resistance to in vitro seed colonization by A. flavus and A. parasiticus. Six more breeding lines (GFA 1, GFA 2, AR 1, AR 2, AR 3, and AR 4) were later reported resistant (Mixon 1986). Other workers have used Mixon and Rogers' method, or modifications of it, in screening genotypes for resistance to seed colonization by aflatoxin-producing strains of A. flavus and A. parasiticus (LaPrade 1973, Bartz et al. 1978, Zambettakis et al. 1981, Mehan and McDonald 1980, Tsai and Yeh 1985, Pua and Medalla 1986). The methods have on the whole given comparable

results. When interpreting the results it is necessary to take into consideration the effects of environmental factors operating during pod development and postharvest curing and drying (Mixon and Rogers 1975a. Bartz et al. 1978, Mchan et al. 1983). A total of 37 genotypes have now been reported to have resistance of this type.

The resistance is likely to be of value if groundnuts dried in the field or in storage are wetted, or absorb moisture from the atmosphere. The resistance is of less value for decorticated seed that may have suffered damage to the testa in processing.

Resistance to A.flavus invasion and colonization of rehydrated, stored, dried seeds has relevance when aflatoxin contamination is largely postharvest. But significant invasion of undamaged groundnut pods by A. flavus and subsequent aflatoxin contamination can occur before harvest (Davidson et al. 1983, Mehan et al. 1986). In recent years there has been considerable research into possible genetic resistance in groundnuts to seed infection and aflatoxin contamination in the field (Davidson et al. 1983, Mehan and McDonald 1984a, b, Kisyombe et al. 1985. Mehan et al. 1986). In the first place, it was important to establish the relevance of in vitro "seed colonization" resistance to A. flavus invasion of developing pods in the field. A few studies (Mixon and Rogers 1973a, Mixon 1983a, b. Mixon 1986) indicated that the genotypes. PI 337394F, PI 337409, GAF 1 and GFA 2, resistant to in vitro seed colonization by A. flavus (IVSCAF-resistant), showed considerably lower levels of natural seed infection with A. flavus and of aflatoxin contamination than the susceptible (IVSCAF-susceptible) genotypes Florunner and PI 331326. In these studies, observations on natural seed infection were made primarily to determine the "initial" levels of A. flavusIA. parasiticus infection that could possibly interfere with the laboratory seed inoculation tests for resistance to seed colonization. The natural seed infection was estimated from occurrence of sporulating colonies of A. flavus on rehydrated, nontreated (no inoculation) seeds.

Davidson et al. (1983) found no significant differences at harvest in A. flavus infection or in aflatoxin contamination of seed between the cultivars Sunbelt Runner and Florunner, reported to be resistant and moderately susceptible respectively to in vitro seed colonization by A. flavus and A. parasiticus. Blankenship et al. (1985) reported that four genotypes (A 72118 (GFA 1), A 7404 (AR 3). UF 77316 and UF 791041) resistant to seed colonization, and the cultivar Florunner, grown under late season drought stress, were all highly susceptible to aflatoxin contamination. Other workers (Zambettakis et al. 1977, Zambettakis et al. 1981, Mehan et al. 1987) have reported several IVSCAF-resistant genotypes to be field resistant to A. Jlavus seed infection. Of 37 IVSCAF-resistant genotypes, only 10 (PI 337394F, PI 337409, UF 71513, Ah 7223, J 11, Var. 27, 55-437, 73-30, Monir 240-30. and RMP 12) have been tested for resistance to seed infection by A. Jlavus in the field (Zambettakis et al. 1981. Kisyombe et al. 1985, Mchan et al. 1987), and only three of them (PI 337409, PI 337394F, and J 11) have been evaluated in more than one location. Cultivar J 11 has been found resistant to seed infection in multilocational field trials in India and in the USA (Mehan et al. 1987, Kisyombe et al. 1985); PI 337409 showed resistance in Senegal (Zambettakis et al. 1981) but was susceptible in the USA (Kisyombe et al. 1985).

It should not be assumed that all IVSCAF-resistant genotypes will have resistance to seed infection in the field, or all IVSCAF-susceptible genotypes will show susceptibility to field infection by A.flavusIA. parasiticus (Mehan et al. 1987). The IVSCAF-resistant genotypes Var. 27, Monir 240-30. and RMP 12 have shown susceptibility to A. Jlavus seed infection in the field while some IVSCAF-susceptible genotypes such as Lampang and Exotic 6 have been found to have low levels of seed infection in the field (Mehan et al. 1987, Kisyombe et al. 1985).

Certain conditions should be met when screening groundnuts for resistance to seed infection by the aflatoxigenic fungi and/or aflatoxin production under field conditions. First, only undamaged pods should be analyzed as any kind of damage is likely to over-ride resistances. Second, environmental conditions such as temperature and moisture stress during pod maturation are important since A. Jlavus is a weak pathogen and its ability to invade undamaged pods and seeds is strongly influenced by environmental conditions. Third, the soil should contain aflatoxin-producing strains of A. flavus and A. parasiticus since some strains of these fungi may not be capable of producing aflatoxin. Therefore, to ensure uniform, high levels of seed infection and aflatoxin contamination, screening trials should be sited on a light sandy soil with high populations of A. flavusIA. parasiticus. A test site where late season drought stress is of common occurrence would be most effective. Otherwise, the screening might have to be carried out on early or late sown crops or on irrigated dry season groundnut crops where control of soil moisture during late stages of pod development can be assured.

8.1.7 Mechanisms of Resistance to Aspergillus flavus Colonization and Infection

In all reported cases of resistance to seed colonization by the aflatoxigenic fungi the protective role of the seed testa has been emphasized (Dieckert and Dieckert 1977. LaPrade et al. 1973. Mehan et al. 1983, Mixon and Rogers 1975, Zambettakis et al. 1977). Resistance depends upon the testa being complete and undamaged. Most research points to the testa resistance being physical, and it has

been correlated with thickness, density of 'pallisade cell' layers, absence of fissures and cavities, etc. (LaPrade et al. 1973, LaPrade and Bartz. 1972, Zambettakis 1975, Zambettakis and Bockelee-Morvan, 1976). Permeability could also be an important factor as presence of wax layers on the testa of A. flavus-resistant genotypes has been noted (Camara 1977; LaPrade 1973). Fungistatic phenolic compounds have been found in testae and may have a role in resistance. Some studies (Amaya et al. 1977 ; Amaya et al. 1980) have shown that testae of IVSCAF-resistant genotypes contained significantly lower levels of certain amino and carbohydrate compounds than testae of IVSCAF-susceptible genotypes. Resistance could result from combinations of physical, chemical and biological factors operating in the testa rather than being due to a specific mechanism.

The groundnut shell has logically been considered a barrier to penetration by A. flavus, as seeds from pods with damaged shells are more frequently contaminated with aflatoxin than those from undamaged pods (McDonald and Harkness 1967). The resistance of the groundnut fruit to A. flavus invasion appears to be associated with certain structural and biochemical characters of the pod and seed, and there is a possibility that genotypes may have differential effects upon the populations of A. flavus in the geocarposphere (Nahdi 1989).

8.1.8 Resistance Breeding

Breeding for resistance has relied on information obtained in the assessment of A. flavus infection in harvested, mature, dried seeds, as the laboratory screening test can readily be used to compare progenies for resistance (Mixon 1979, 1981, Rao et al. 1989). Several breeding lines with resistance to A. flavus colonization of seeds comparable to that of the resistance sources and with greater yield potential have been bred (Mixon 1986, Rao et al. 1989). However, when dealing specifically with resistance to natural seed infection in the field, resistance mechanisms may operate at the pod surface, within the shell, at the seed surface and within the testa/cotyledons. This makes it difficult for breeders to specify particular resistance traits to aim for. There appear to be different genes in conferring resistance to seed colonization, preharvest seed infection, and aflatoxin production by aflatoxin-producing fungi (Utomo et al. 1990).

Use of resistant varieties should be considered as part of an integrated aflatoxin management program incorporating cultural and crop handling procedures appropriate to different agroecological situations. Cultivars resistant to fungus invasion in the soil would be particularly desirable for semi-arid regions where preharvest aflatoxin contamination is a serious problem. The existence of significant resistance in the commercial cultivars J 11 and 55-437 could be immediately useful in minimizing aflatoxin contamination in some environments.

Resistance to A. flavus infection is also important for maintaining seed quality for planting as the fungus causes seed rots and aflaroot seedling disease.

8.1.9 Segregation and Decontamination

Groundnuts intended for human consumption may contain levels of aflatoxin in excess of the acceptable limit. But, in a toxic sample not all seeds are contaminated, and in many cases toxicity resides in a very small number of toxic seeds (Dickens 1977). Provided that seeds on which aflatoxigenic fungi have grown can be distinguished from apparently healthy seeds, it should be possible to recognize and remove the toxic seeds. This is the basis for procedures that have been developed and utilized in the groundnut industry in several countries (Dickens 1977, 1983. Tiemstra 1977, Read 1989). The methods arc based on such characteristics as color and kernel size and can be applied to raw kernels or to cotyledons alter blanching. Separation of toxic seeds may be done by hand-picking or by the use of electronic color-sorting equipment (Tiemstra 1977). Using these methods it is possible to significantly reduce aflatoxin levels in contaminated lots of groundnuts (Tiemstra 1977).

Sorting of raw kernels by visual examination may not be effective when healthy-appearing kernels have concealed damage (i.e.. mold growth between cotyledons but no mold damage evident on the seed surface) (Dickens and Whitaker 1975). This does not apply when scanning blanched kernels and emphasizes the need for such examinations at this later stage in processing. The most effective way to remove off-color, suspect kernels is by means of electronic color sorting. Kernels that differ substantially in color (i.e., are darker, or lighter, or molded) from the standard for the particular cultivar or cultivars being examined, should be rejected. Blanching followed by photoelectric color sorting and hand-picking effectively reduces aflatoxin contamination. Tiemstra (1977) has discussed in detail the use of electronic color sorters in removing contaminated kernels from blanched groundnuts. In processes where sorting is done alter roasting and blanching, the darker and nonblanched kernels may also be removed. One of the best methods of detecting suspect kernels is by partially drying them to about 2 to 3% moisture content, and then blanching (Tiemstra 1977). The cotyledons of contaminated kernels will darken perceptibly whereas sound cotyledons remain white. Sorting at this stage is more effective than after roasting since the roasting process can produce a range of color in sound kernels that obscures the off-colors of suspect kernels. Unfortunately, groundnuts treated in this way have very limited shelf life and should be used immediately.

In the USA, segregation of aflatoxin-contaminated groundnuts has been very successful (Tiemstra 1977, Dickens 1983). Contaminated lots are diverted for oil extraction and non-food uses (Dickens 1983). While such an approach can

be recommended for developed countries such as the USA and Australia, safeguards need to be established if segregation of contaminated groundnut lots is to be used in less developed countries where the reject groundnuts could find their way into local markets.

It is appreciated that while the primary need is to reduce aflatoxin levels by removal of toxic kernels, methods for inspection, separation and diversion should be efficient so that only contaminated kernels are removed and there is no wastage of non-contaminated kernels.

The potential exists to improve electronic sorting equipment. Polarized laser beams may be used to detect roughening and shrivelling of seed surfaces due to mold damage (Pettit and Chan 1980). It may be possible to detect hidden mold damage by measuring dielectric properties of groundnut seeds (Pettit and Geiger 1981).

8.1.10 Chemical Detoxification

Where removal and segregation of toxic kernels cannot be effectively carried out. or is only partially successful, there still remains the possibility of inactivating or destroying the aflatoxins in groundnuts or groundnut products by chemical and/or physical treatments. Chemical treatments, normally referred to as detoxification, should be technically and economically viable, and should meet the criteria listed by the FAO/WHO/UNEP Conference on Mycotoxins held in Nairobi, Kenya (1977). These criteria are that the process (a) destroys or inactivates the toxin, (b) does not produce or leave toxic or carcinogenic residues in the finished product, (c) destroys fungal spores and mycelia that could later proliferate and produce the toxin, (d) preserves the nutritive value and acceptability of the product, and (e) does not significantly alter important technological propenies of the product. Similar requirements have been established by the U.S. Food and Drug Administration (FDA); however, the FDA requires additional data on the impact of the process on the environment (Park et al. 1988). The International Union of Pure and Applied Chemistry Food Chemistry Commission has developed guidelines for safety evaluation of aflatoxin-contaminated feed (Jemmali 1982).

The polar solvents used in the solvent extraction methods for processing groundnuts remove most aflatoxins from the extracted oil. However, in the case of small-scale village level processing some aflatoxin may be left in the crude oil. Further processing of oil using alkali treatments (i.e., alkali refining) and filtration can largely remove this residual toxicity from the oil (Parker and Melnick 1966). Aflatoxin in oil may also be significantly reduced by exposing the contaminated oil to bright sunlight (Shantha and Sreenivasamurthy 1975, Shantha 1989). In some rural parts of India, unrefined, locally-expressed groundnut oil is preferred to refined oil because of its lower cost and preterred taste (Shantha and Sreenivasamurthy 1975). Extraction of such crude oil with 10% aqueous sodium chloride (1:4) at 80" C for 30 minutes removes almost 85% of contaminating aflatoxin (Shantha and Sreenivasamurthy 1975). It is generally agreed that most of the aflatoxin present in groundnuts remains in the cake after the oil is removed, contamination levels in the cake being almost double the levels in the raw kernels. There have been reports that the high temperatures that are reached during mechanical crushing of groundnuts might destroy some of the toxin, but considerable quantities are left in the cake.

Various chemicals have been tested for destruction of aflatoxins in groundnut cake and groundnut protein isolates : methoxymethane (Aibara and Yano 1977). formaldehyde (Codifer et al. 1976. Mann et al. 1969 , Ochomogo 1979), calcium hydroxide (Codifer et al. 1976, Giddey et al. 1977), ethylene oxide (Degesch 1978), sodium hydroxide (Dollear et al. 1968, Mann et al. 1969), methylamine (Dollear et al. 1968. Mann et al. 1969), chlorine (Sreenivasamurthy et al. 1967), sodium chloride (Farah et al. 1983), sodium hypochlorite (Natarajan et al. 1975, Ochomogo 1979, Rhee et al. 1977), isopropyl alcohol (Rayner and Dollear 1968, Ochomogo 1979), hydrogen peroxide (Srinivasamurthy et al. 1967. Rhee et al. 1977). and ammonia (Viroben et al. 1983. Coker et al. 1985a, Coker 1989, Conkerton et al. 1980). The most promising chemical detoxification procedure is that using ammonia; this was first demonstrated by Masri et al. (1969). and Gardener et al. (1971) reported on its development and application on a pilot plant scale. Several patented ammonification techniques are undergoing commercial tests (Coker 1989, Coker et al. 1985). Three pilot plant scale processes for ammoniation of groundnut cake and meal have been developed : the Lesieur process (Lesieur 1977), the Extechnik process (Extraktiostechnik) and the TDRI process (Coker et al. 1985). The Lesieur process was in operation in Senegal for some time but it has now been superceded by an ammonia/formaldehyde process (Coker et al. 1985). A pilot plant using the Extechnik process has been installed in the Sudan (Coker et al. 1985). The African Groundnut Council and its Member States actively support aflatoxin detoxification programs (FAO 1985). France, Ireland, The Netherlands, and Germany import ammonia-treated groundnut meal for animal feeds (Park et al. 1988). The TDRI process uses ammonia gas at high temperature but moderate pressure and a pilot plant has given satisfactory performance, reducing aflatoxin levels in groundnut cake by > 95%. Some loss of cystine and lysine occurs during the ammoniation process (Coker et al. 1982).

Treatment with ammonia produces complex reactions and only a small percentage of reaction products have been fully investigated (Cucullu et al. 1976).

Nutritional and toxicological evaluation of the ammoniated groundnut cake produced by these ammoniation processes has been carried out on a number of animal species (Viroben et al. 1978, DeLort-Laval et al. 1980, Frayssinet et al. 1976, Viroben et al. 1983). International acceptance of the ammoniation process will be delayed until safety of the treated products can be guaranteed (Coker et al. 1985a). Park et al. (1988) have presented a comprehensive review of the ammonia detoxification process and outlined current applications and regulatory status of the ammonia process for reducing aflatoxin levels in animal feeds.

8.1.11 Microbial Detoxification

Many microorganisms, including fungi, actinomycetes, bacteria and algae, have been tested for ability to destroy or transform aflatoxin (Ciegler et al. 1966, Lillehoj et al. 1967a, 1971, Mann and Rehm 1976, Cole and Kirksey 1971. Nout 1989). A bacterium, Flavobacterium aurantiacum, has been found to remove aflatoxin from solutions and preparations of peanut milk (Ciegler et al. 1966, Hao and Brackett 1988, Hao et al. 1989). But there could be problems in adopting this technology for commercial detoxification of groundnuts and groundnut products.

8.1.12 Physical Detoxification

Aflatoxins are stable up to their melting point of around 250° C (Feuell 1966). While dry heating has not been particularly effective, heating moist meal or autoclaving groundnuts has been found to reduce aflatoxin content (Coomes et al. 1966). Not all aflatoxins react equally to heating, for example aflatoxin B, is heat stable, but aflatoxin G1 can be destroyed by heat (Reegner 1967). Prolonged heating may adversely affect the quality of the protein or availability of lysine (Woodham and Dawson 1966). Roasting has been reported in some cases to reduce aflatoxin levels, but in no case has total destruction been achieved (Lee et al. 1968, Coker 1989).

Neither ultraviolet light nor gamma irradiation reduces aflatoxin levels in groundnut meal (Feuell 1966, Shantha 1989). However, several studies have shown that sunlight can be very effective in destruction of aflatoxin in oil (Shantha and Sreenivasamurthy 1975, 1977, Shantha 1989). Aflatoxin-contaminated unrefined groundnut oil in glass containers was subjected to direct sunlight (approximately 50,000 lux) and within one hour the aflatoxin was almost completely destroyed (Shantha and Sreenivasamurthy 1980, Shantha 1989). The safety and shelf life of the sunlight-exposed oil have been confirmed (Shantha 1989). Large-scale trials have not been done. The kind of container used could be critical as the sunlight has to penetrate to and within the oil. Sunlight has not proved to be effective for destruction of aflatoxin in contaminated groundnut seeds and cake (Shantha and Sreenivasamurthy 1981), as the toxin is bound to the protein molecules in these substrates and there are obvious problems of light penetration.

Aflatoxin in crude groundnut oil remains in finely suspended form and can easily be separated by filtration. Special filter pads have been developed by Basappa and Sreenivasamurthy (1979) which can easily be adopted in oil mills to remove aflatoxin from crude oil. This appears to be a simple approach to the problem of aflatoxin in unrefined groundnut oil.

Several types of clays can bind aflatoxin from oil (Miller et al. 1985, Pettit et al. 1990). As a follow up to this, hydrated sodium calcium aluminosilicate (HSCAS) was added to aflatoxin contaminated chicken feed and this reduced the toxicity of the feed to poultry (Pettit et al. 1990).

8.1.13 Control in Storage/Transit

Storage of groundnuts under clean, dry conditions with low kernel moisture content (about 8 %) and at low temperature, and with protection from insect infestation should avoid molding of groundnuts and consequent risk of aflatoxin contamination (Dickens 1977). However, trade groundnuts are often transported and stored in open containers and permeable bags and so are at risk. Various ways in which groundnuts may be wetted and absorb moisture have been highlighted. These include direct wetting by rainfall and by leakage through covers, and through seepage of ground water and rising damp, and by direct absorption of moisture from humid atmosphere, this being particularly important when the temperature of stored material falls below ambient temperature (Dickens 1977. Smith 1989). Moisture may also be generated in stored groundnuts from respiration of pests (insects and rodents). Any measure that reduces the risk of such rewetting of groundnuts will be effective in reducing risk of aflatoxin contamination.

As considerable quantities of groundnuts are shipped from tropical areas to temperate zones there does exist a problem of condensation in ship holds. Use of sealed containers could reduce this problem. The problem of mold damage and aflatoxin contamination can be further minimized by improving facilities for storage at port and transit points as well as on ships (Bhat 1988).

8.2 BIBLIOGRAPHY

8.2.1 MANAGEMENT OF ASPERGILLUS FLA VUS INFECTION AND AFLATOXIN CONTAMINATION OF GROUNDNUT

1324. Agboola, S.D., and Opadokun, J.S. 1982. A review of groundnut quality and storage in Nigeria. Pages 397-414 in Proceedings of the International Symposium in Africa on Production, World Oilseeds Market and Intra-African Trade in Groundnuts and Products, 7-11 June 1982, Banjul, The Gambia : African Groundnut Council, Lagos, Nigeria.

Research in Nigeria from 1948 to 1982 has identified the factors that determine quality in Nigerian groundnuts. These include, physical state of groundnuts, their moisture content, and storage conditions with emphasis on temperature, humidity, and infestations by pests, rodents and mold fungi. Procedures for ensuring purchase of only good quality produce and measures for prevention of damage to the groundnuts during storage are reviewed with particular emphasis on control of pests. The problem of aflatoxin contamination of groundnuts is discussed. Recommendations to growers, and those concerned with storage and transport of groundnuts on methods of preventing aflatoxin contamination are summarized.

1325. Baur, FJ., and Parker, W.A. 1984. The aflatoxin problem : industry-FDA-USDA cooperation. Journal of the Association of Official Analytical Chemists 67(1): 3-7.

The role of groundnut growers, shellers and manufacturers of consumer groundnut products, together with USDA and FDA, played in forming a joint task force to develop a program of research into incidence, causes and control of aflatoxin contamination of groundnuts and groundnut products is discussed.

1326. Basappa, S.C. 1983. Physical methods of detoxification of aflatoxin contaminated food materials. Pages 251-273 in Mycotoxins in Food and Feed (Bilgrami. K.S., Prasad, T., and Sinha, K.K., eds.). Bhagalpur, India: Allied Press.

This paper discusses several physical methods for detoxification of aflatoxin-contaminated groundnuts, groundnut oil, maize, cottonseed, and rice. Removing discolored seeds by handpicking or by electronic color sorting devices is efficient for reducing aflatoxin content in groundnuts, maize and cottonseed. The possibility of using air classification to separate aflatoxin-contaminated groundnuts and cottonseed has been explored and found to be a useful tool. Milling of contaminated brown rice has been found to reduce aflatoxin in the polished rice. Roasting, frying, boiling, baking and cooking of contaminated groundnuts have been found to inactivate aflatoxin to a certain extent. Detoxification of groundnut oil by exposure to bright sunlight has proved useful. Decontamination of groundnut oil is also possible by the use of filterpads in the processing line of oil mills. Washing the oil with sodium chloride solution is another promising method for removal of aflatoxin. These detoxification/decontamination procedures are discussed with special reference to their economic and technical feasibility.

1327. Bedi, P.S., and Singh, P.P. 1989. Aflatoxin production in food crops and its management. Pages 79-91 in Perspectives in Phytopathology (Agnihotri, V.P., Singh, N., Chaube, H.S., Singh, U.S., and Dwivedi, T.S., eds.). New Delhi, India : Today and Tomorrow's Printers and Publishers.

In this review the occurrence of aflatoxin in food crops and the magnitude of the problem, aflatoxin production (by Aspergillus flavus and A. parasiticus) under different conditions with special reference to pre-harvest contamination and the management of aflatoxin contamination are discussed.

1328. Bhat, R.V. 1987. Review of activities in mycotoxin prevention and control : Strategies for improvement based on experience in Asia and East Africa, in Proceedings of the Joint FAO/WHO/UNEP Second International Conference on Mycotoxins, 28 September - 3 October 1987, Bangkok. Thailand.

This paper gives a brief background to the mycotoxin problems in major food crops including maize and groundnut. Problems in implementing prevention and control of mycotoxin contamination with special emphasis on aflatoxins are outlined. Several measures for prevention are discussed, including use of appropriate cultural practices, e.g., irrigation, crop drying, storage, chemical control, and use of resistant cultivars. Need for education and extension activities is emphasized. Need for regulatory programs to ensure quality of produce for export and for local consumption is stressed. Surveillance studies are needed for all regions.

1329. Blanc, M. 1981. [Measures for the control of aflatoxins (the case of groundnut and groundnut products).]. Moyens de prevention et de destruction des aflatoxines (cas des graines d'arachide et de leurs derives). Actualites Scientifique et Techniques en Industries Agro-Alimentaires (France) 27, 120 pp.

This report on aflatoxin control in groundnut production and processing reviews available information on : (1) preventive measures; (2) contamination of groundnut oil and press-cake; and (3) detoxification methods. Basically, there are two possible solutions of the aflatoxin problem, viz. prevention of invasion of groundnuts by the toxigenic fungus Aspergillus flavus, and elimination of aflatoxins from the contaminated groundnuts.

1330. Bockelee-Morvan, A., and Gillier, P. 1976. [Reducing aflatoxin in groundnuts at the level of agricultural production.]. Reduction de l'aflatoxine de l'arachide au niveau de la production agricole. Cahiers de Nutrition et de Dietctique (2): 101-104.

Possibilities for reducing the aflatoxin contamination of groundnut include the selection of varieties resistant to Aspergillus flavus.

1331. Carl Bro International A/S. 1976. Aflatoxin-free groundnut production in developing countries. : 153pp.

Specific recommendations are made as to which aflatoxin control measures developing countries could introduce for producing groundnuts with an acceptable low aflatoxin content. Tanzania was used as the reference country for a study of the revelant agricultural conditions. Topics discussed included the production and handling of groundnuts, drying and storage, control, quality regulations and processing, preservation of groundnuts and use of insecticides and fumigation, detoxification, and economic aspects.

1332. Chohan, J.S. 1979. Aflatoxins and pollution hazards in groundnut and their control. Pages 313-317 in Environmental pollution and toxicology. New Delhi, India : Today & Tomorrow's Printers and Publishers.

This is a review of work done at the Punjab Agricultural University on Aspergillus flavus infection of groundnut seeds and seedlings. Issues discussed include the physiology and pathology of aflatoxin production by A. flavus, a bioassay technique for detection of aflatoxin-producing strains of the fungus, varietal response for aflatoxin production, field control of A.flavus infection and postharvest technology for aflatoxin control.

1333. Coker, R.D. 1979. Aflatoxin : past, present and future. Tropical Science 21(3): 143-162.

This paper briefly discusses various aspects of the groundnut aflatoxin problem. The aspects discussed include, susceptibility of major food crops to aflatoxin contamination, the acute and chronic effects of aflatoxins, the natural occurrence of aflatoxicosis, and the metabolic fate of the anatoxins. Various approaches to the reduction of aflatoxin contamination are outlined, including appropriate cultural practices, chemical control, sampling and analysis together with detoxification procedures. Areas of research which need to be investigated in the future are briefly discussed.

1334. Committee on Fungus Infection of Groundnuts. Northern Nigeria. 1970. Recommendations on the control of aflatoxin contamination in groundnut products. 4 pp.

In Nigeria groundnut is sold as unsorted kernels, groundnut cake, oil, hand picked selected (HPS) kernels, and groundnut flour. All these products may contain aflatoxin. Interdisciplinary committee meeting on behalf of the Ministries of Natural Resources of the six northern states of Nigeria recommended to growers, and to those concerned with purchase, storage, transport and processing of groundnuts various procedures for reducing aflatoxin contamination.

1335. Coulibaly, B. 1990. The African Groundnut Council : purpose and achievements. Pages 75-77 in Summary Proceedings of the first ICRISAT Regional Groundnut Meeting for West Africa, 1CR1SAT Sahelian Center, Niamey, Niger, 13-16 Sep 1988. Patancheru, Andhra Pradesh 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

The African Groundnut Council (AGC) aimed at promoting production, consumption and international trade of groundnuts in its six member states (The Gambia, Mali, Niger, Nigeria. Senegal and Sudan) operates through its 3 bodies (a council of ministers, a council of representatives and a permanent executive secretariat), and had several installations in member states either wholly or partially financed by this organization. Several research projects including one on aflatoxins and another a regional Variety Trial are executed in collaboration with each member state and with the technical/financial assistance of national and international institutions. The AGC organizes various forms of training for scientists, conducts conferences, publishes on-going research and statistical data, and standardizes the price and sales policies in member states.

1336. Delassus, M. 1967. [Plant health studies on groundnut and cereals in Senegal.|. Etudes phytosanitaires sur l'arachide et les cereales alimentaires au Senegal. L'Agronomic Tropicale 22(12): 1226-1234.

The general situation with respect to the occurrence of aflatoxin in groundnuts is reviewed, and problems related to methods of aflatoxin analysis are discussed. Effects of inoculating groundnut pods, in the field or after harvesting, with Aspergillus flavus under varying environmental conditions in Senegal are described. The effects of rate of drying of pods under natural conditions on aflatoxin contamination are described. Recommendations to reduce aflatoxin contents in exported groundnuts, included removal of diseased plants and diseased or discolored pods and kernels, and provision of proper drying and storage facilities. Some diseases occurring in cereals, particularly millets and rice, are briefly mentioned.

1337. Delassus, M, Goarin, P., and Goarin, S. 1966. [Review of mycological and agronomical studies on aflatoxin.]. Revue d'etudes mycologiques et agronomiques faites sur l'aflatoxine. L'Agronomic Tropicale 21(12): 1398-1406.

A review is presented on aflatoxin contamination of groundnuts and groundnut cake due to infection by the fungus Aspergillus flavus as influenced by environmental conditions and postharvest treatments. Control measures recommended in Senegal include : elimination of plants prematurely dried in the field before harvesting; rapid drying of the lilted plants, first in small heaps or windrows and then in stacks; breaking-up of stacks if these become wetted by late rains; reduction of toxicity of the product by elimination of all perforated pods and all discolored kernels.

1338. Dickens, J.W. 1975a. Some approaches to a solution of the aflatoxin problem through research and education. Proceedings of the American Peanut Research and Education Association 7: 54-61.

Approaches to solution of the aflatoxin problem in groundnut are discussed. None of the approaches is perfect. A progressive aflatoxin control program by all segments of the groundnut industry is necessary to achieve a final solution to the aflatoxin problem.

1339. Dickens, J.W. 1975b. [Some approaches to a solution of the aflatoxin problem through research and education.|. Approches d'une solution au probleme de l'aflatoxine a travers la recherche et linformation. Oleagineux 30(12): 517-522.

The control of Aspergillus flavus infection of groundnuts and contamination with aflatoxin is discussed in relation to seed infection, detection of infection/contamination and divergence of contaminated groundnuts to non-food uses. Sampling and aflatoxin analysis protocols for use at various stages in groundnut production and processing are described.

1340. Dickens, J.W. 1977a. Aflatoxin occurrence and control during growth, harvest and storage of peanuts. Pages 99-105 in Mycotoxins in human and aninmal health. (Rodricks, J.V., Hesseltine, C.W., and Mehlman, M.A., eds.). Illinois. USA : Pathotox Publishers, Inc. 807 pp.

This paper discusses causes of aflatoxin contamination and methods of prevention and segregation to control the aflatoxin problem in farmers' stock groundnuts from the time the groundnuts arc growing in the field until they are marketed, stored, and shelled.

1341. Dickens, J.W. 1977b. Aflatoxin control programme for peanuts. Journal of the American Oil Chemists' Society 54: 225A-228A.

Under provisions of a USDA Marketing Agreement, an aflatoxin control programme for groundnuts produced in the United States is administered by the Peanut Administrative Committee composed of peanut (groundnut) growers and sellers. Regulations of this committee contain provisions about the quality of groundnuts acquired from farmers, storage of unshelled groundnuts, aflatoxin testing, quality and disposition of processed lots, and indemnification of handlers for losses caused by lots which contain over 25 µg Kg^{-1} aflatoxin. Effects of the control programme on aflatoxin concentration in peanut products are discussed.

1342. Dorner, J.W. 1989. Prevention of preharvest aflatoxin contamination. Proceedings of the American Peanut Research and Education Society 21 : 63.

Many years of study have shown that preharvest aflatoxin contamination of groundnuts is preventable when groundnuts arc provided with adequate moisture (through rainfall or irrigation) during the pod maturation period. However, since all groundnuts cannot be grown under such conditions, other strategies to prevent contamination arc being investigated. Two of those discussed include : (1) prevention of aflatoxigenic fungal invasion by development of groundnut varieties resistant to invasion and use of biocompetitive agents to exclude aflatoxin-producing fungi from the soil ; and (2) drought-tolerant varieties, enhancement of phytoalcxin-based as well as other natural defense mechansims, and incorporation of resistance characteristics through genetic manipulation.

1343. Howell, F.E., Dorner, J.W., Davidson, J.I.Jr., Cole, R.J., and Ferguson, W.G. 1988. Detection of aflatoxin in various components of farmers stock peanuts. Proceedings of the American Peanut Research and Education Society 20: 25.

Ten 50 lb pneumatic samples from 17 loads of farmers stock groundnuts were collected during the 1987 harvest season in Georgia. Grade samples were run over a 24/64 belt screen. Loose- shelled kernels (LSK) and small pods that fell through the belt screen were separated, pods were shelled, and the kernels were screened over a 16/64 and 14/64 slotted screen. Pods that rode the 24/64 belt screen were similarly shelled and screened, and in addition, the damaged kernels were removed. The resulting components were analyzed separately for aflatoxin by high performance liquid chromatography (HPLC). From the grade samples, the LSK, other kernels (OK), and damaged kernels were combined and the sound mature kernels (SMK) and sound splits (SS) were combined. These two grade components were analyzed separately for aflatoxin by HPLC. Results showed the distribution of aflatoxin within each component and provided variance data to determine sample sizes needed to detect specified levels of aflatoxin in each respective component. Results also provided additional information needed for finalizing the design of a pilot study to determine the performance of the belt screen separator in removing

foreign material and poor quality groundnuts prior to marketing of farmers stock groundnuts.

1344. Food and Agriculture Organization. 1979. Prevention of mycotoxins. FAO Food and Nutrition Paper No. 10. pp. 71. FAO, Rome.Italy.

This publication is in three parts. Part 1 gives a schedule of practices recommended respectively for standing crops, for harvesting and drying, for storage of crops, for transportation and for processing (postharvest, including decontamination), for prevention and control/detoxification of aflatoxins and other mycotoxins. Part II is a discussion of factors relating to the recommended practices. Part III focuses on needs and aids for implementation of the recommendations. The commodities mentioned in the discussion are those that form the bulk of the food and feedingstuffs in some countries. References are also given to existing practices, current trends in research, and their likely outcome in relation to the problem posed by mycotoxins.

1345. Goarin, P., and Goarin, S. 1970. [Contribution to the knowledge of groundnut aflatoxin in Senegal.]. Contribution a la connaissance de l'aflatoxine de l'arachide au Senegal. L'Agronomie Tropicale 25(3): 277-293.

Research conducted mainly in Senegal on the significance of aflatoxin contamination of groundnut, methods of analysis, and control measures are reviewed. Contrary to the general assumption that high aflatoxin contents are associated with wetting of crop produce, in northern Senegal it was found that aflatoxin contamination was attributed to failure of crops to attain physiological maturity during dry years. Less arid production area, where infection is less, improved crop drying and possibly grading of produce should allow the production of acceptable edible or confectionery groundnuts and even groundnut meals. In general, the use of varieties better adapted to the environmental conditions, and careful harvesting, will keep the aflatoxin contents of groundnut and oil-cakes at an acceptable level.

1346. Goldblatt, L.A. 1966. Some approaches to the elimination of aflatoxins from protein concentrates. Advances in Chemistry Series 57: 216-226.

Prevention, removal and inactivation of aflatoxin in groundnut protein concentrates is considered. Prevention is best and may be achieved by careful harvesting, handling, drying and storage of the crop produce. Removal of toxic kernels is also possible and this can be done by handpicking of discolored and moldy kernels. Aflatoxin may also be removed by polar solvents, by exposure to moist heat, and by treatment with ammonia.

1347. Goldblatt, L.A. 1968. Aflatoxin and its control. Economic Botany 22 : 51-62.

This paper reviews the problem of aflatoxin contamination in groundnut and other agricultural commodities and discusses preventive and control measures. Prevention is the first and best approach and preventive measures should be taken at all stages of cultivation, harvest, transportation, storage and processing. Physical separation of contaminated seed has proved feasible in some cases. Effective removal of aflatoxins by extraction with polar solvents has been accomplished. Several chemicals such as ammonia, methylamine, sodium hydroxide, hydrogen peroxide and ozone have been used with success to substantially reduce, inactivate or destroy aflatoxins in contaminated oilseed meals, but some reduction in protein quality occurs during most of the treatments.

1348. Goldblatt, L.A. 1970. Chemistry and control of aflatoxin. Pure and Applied Chemistry 21: 331-353.

This paper briefly discusses various aspects of the groundnut aflatoxin problem. The aspects discussed include, analytical methods and chemistry of aflatoxins, the acute and chronic effects of aflatoxins, and the natural occurrence of aflatoxicosis. Various approaches to the prevention/reduction of aflatoxin contamination of agricultural commodities are outlined, including cultural and crop handling practices, inactivation and detoxification procedures.

1349. Goldblatt, L.A. 1971. Control and removal of aflatoxin. Journal of the American Oil Chemists' Society 48: 605-610.

Approaches to solution of the aflatoxin problem in groundnut are discussed. Prevention of aflatoxin contamination is the best approach and preventive measures should be taken at all stages of cultivation, harvest, transportation, storage and processing. If preventive measures have failed or are not used, aflatoxin-contaminated groundnuts can still be salvaged by mechanical removal of contaminated seed, by extraction with polar solvents, or by destruction of aflatoxins with appropriate chemicals. Heat is relatively ineffective for destruction of aflatoxin although normal roasting, as of groundnuts for the preparation of peanut butter, results in considerable reduction in aflatoxin content. Treatment with Flavobacterium aurantiacum removes aflatoxin and may be useful for beverages. Oxidizing agents readily destroy aflatoxin, and treatment with hydrogen peroxide may be useful. Aflatoxins that may be present in crude oil are effectively removed in conventional refining operations. Treatment of defatted oilseed meals with ammonia can reduce aflatoxin content to very low or nondeteciable levels with only slight reduction in protein quality.

1350. Goldblatt, L.A., and Dollear, F.G. 1976. Review of prevention, elimination.

and detoxification of aflatoxins. Page 12 in Abstracts of the Third International I.U.P.A.C. Sponsored Symposium on Mycotoxins in Foodstuffs, 16-18 September, Paris, Prance.

To the extent feasible, the best approach to contain aflatoxin contamination is prevention. Good farm management practice is essential. This includes use of sound, fungus-free, viable seed, proper fertilization, adequate control of insects and diseases, and harvesting at maturity with equipment properly adjusted and operated to avoid damaging the crop and picking up excessive leaves, trash, and dirt. Special attention should be given to diverting aflatoxin-containing lots from food and feed channels as early as possible in the marketing chain to avoid contamination of uncontaminated lots. Provision of, clean, dry, adequately cooled and ventilated storage is important, and good sanitation is essential to minimize mold contamination during storage and processing. Genetic approaches which result in resistance to elaboration of aflatoxins and use of preservatives or mold inhibitors may be helpful in specific situations. When prevention fails, removal or destruction by physical, chemical, or other means must be considered. Removal of contaminated seeds or kernels may be achieved by hand or electronic sorting as is done with groundnuts or pneumatic sorting of Brazil nuts. Aflatoxin may be removed during processing, as in alkali refining of crude oils obtained from contaminated oilseeds, or by extraction of contaminated oilseed meals with polar organic solvents. Cells of Flavobacterium aurantiacum have been reported to completely remove aflatoxin in liquid media, including milk. A host of chemicals, especially oxidizing and reducing agents and acids and bases, have been screenec as reagents for the destruction of aflatoxin in contaminated feeds. Such processes should result in minimal damage to nutritive value and may not produce other noxious materials. Several processes have been patented.

1351. Goldblatt, L.A., and Dollcar, F.G. 1977. Review of prevention, elimination, and detoxification of aflatoxins. Pure and Applied Chemistry 49: 1759-1764.

The best approach to contain aflatoxin contamination is prevention. Good farm management practices are essential. This includes use of sound, fungus-free, viable seed, proper fertilization, control of insects and diseases, prevention of lodging, and harvesting practices that avoid damaging the crop and picking up excessive leaves, trash, and dirt. Special attention should be given to diverting aflatoxin-containing lots from food and feed channels as early as possible in the marketing chain. Clean, dry, adequately cooled and ventilated storage and good sanitation are essential to minimize mold contamination. Genetic approaches and use of mold inhibitors may be helpful. When prevention fails, hand or electronic sorting can remove contaminated seeds. Aflatoxin may be removed during processing by alkali refining of crude oils or by extraction of contaminated oilseed meals with polar organic solvents. Some allatoxin is destroyed or degraded during normal preparation of some foods. Many chemicals, especially oxidizing and reducing agents and acids and bases, have been screened as reagents for destroying allatoxin in contaminated feeds. Several processes have been patented. In the USA aflatoxin in copra has been destroyed on a commercial scale by hot aqueous calcium hydroxide and in cottonseed meal by treatment with ammonia under pressure.

1352. Golumbic, C. 1968. United States Marketing programs for the control of mycotoxins. Document 17/23, Rev. 1. P.A.G. (FAO/WHO/UNICEF), September 1968 Meeting - Rome. 5 pp.

In the United States, the effort to prevent the entry of mycotoxins into foodstuffs is a cooperative one between industry and government. This effort is concentrated mainly on preventing aflatoxin-contaminated lots of groundnuts from entering food marketing channels. The US groundnut industry has significantly contributed to this objective by the adoption of a code of good practices for purchasing, handling, storage, and processing of groundnuts. Strict limits are placed on the grades and qualities of farmers' stocks of unshelled groundnuts that can go into food channels, and even stricter limits on shelled groundnuts that can be sold for edible use. These classifications or "segregations" are principally based on the percentage of damaged kernels (Aspergillus flavus-infested kernels) in the loads of groundnuts as delivered by farmers and in the outturn from the shelling plants as determined by the official inspection service.

1353. Graham, J. 1982. Aflatoxins in peanuts : occurrence and control. Queensland Agricultural Journal 108(3): 119-122.

The problem of aflatoxin contamination, which was serious in the Queensland groundnut industry in drought years, is reviewed with special reference to factors influencing aflatoxin formation including moisture stress, insect damage, and cultivar resistance. Control measures included crop hygiene, resistant varieties, avoiding moisture stress, harvesting the crop at optimum maturity, avoiding pest and mechanical injury, drying rapidly and segregating contaminated batches of groundnuts.

1354. Hanafi, MO., and Hassan, S.H. 1982. Prospects for improved control of aflatoxin in Sudanese groundnuts through traditional marketing systems. Pages 417-420 in Proceedings of the International Symposium in Africa on Production, World Oilseeds Market and Intra-African Trade in Groundnuts and Products, 7-11 June 1982, Banjul, The Gambia : African Groundnut Council, Lagos, Nigeria.

Groundnut is an important crop in the Sudan, both in rainfed areas where end-of-season droughts are common, and under irrigation in the Gezira. Quality factors are important in determining acceptability and market price. Aflatoxin

contamination is a problem in the rainfed crop. Aflatoxin levels are important in determining quality grade and price. The groundnut marketing system is described. A detoxification plant is under construction and will be used to detoxify groundnut cake and meal.

1355. Hanssen, E., and Birn, K. 1979. Measures taken by groundnut growers and processors to achieve standard quality. Deutsche Lebensmittel-Rundschau. 75: 43-49.

Cultivation, harvesting, threshing, sorting, storage and transportation of groundnuts are briefly discussed. Problems due to contamination of groundnuts with aflatoxins are considered, with reference to allatoxin concentrations in groundnuts from various countries. Methods for manual sorting of groundnuts to eliminate broken and discolored kernels (which have a relatively high incidence of contamination with aflatoxins) are briefly discussed, together with sampling plans. An electronic device for sorting of groundnuts on the basis of their color relative to standard color sheets is described. Use of this device permits a 90 % reduction in aflatoxin content of the batch. 8-18 % of kernels are rejected; approximately 85 % of these are in fact free from aflatoxins.

1356. Hanssen, E., and Jung, M. 1972. Control of aflatoxins in the food industry. Pages 239-250 in Control of Mycotoxins - Special lectures presented at the Symposium on the Control of Mycotoxins, 21-22 August 1972, Goteborg, Sweden. (Krogh. P.. ed.). Butterworth & Co. (Publishers) Ltd. London.

The results of aflatoxin assays of foodstuffs including groundnuts are presented. Conditions for controlling aflatoxins in stored products and for preventing mold growth and allatoxin production are discussed. Storage conditions for certain raw materials are oudined.

1357. Harkness, C, McDonald, D., Stonebridge, W.C., A'Brook, J., and Darling, H.S. 1966. The harvesting, handling and storage of crops in relation to development of toxic fungi, with special relation to groundnut production in Tropical Africa. Food Technology 20: 72-78.

Problems adherent to the cultivation and storage of food crops in tropical Africa are reviewed in relation to the development of mycotoxins in the stored produce due to fungal activity. Attention is paid in particular to the production and storage of groundnuts, and to the studies conducted in northern Nigeria over the period 1961-64 on contamination of groundnut pods and kernels by Aspergillus flavus causing the occurrence of aflatoxin. Recommendations given to reduce the incidence of aflatoxin in groundnuts in that region mainly comprise early harvesting, avoidance of damaging the pods during harvest, removal of damaged or molded pods and kernels, rapid and effective drying of produce, and storage under adequately dry conditions.

1358. Indian Standards Institution 1979a. Code of practice for control of aflatoxin in groundnut. 1. Harvesting, transport and storage of groundnuts. Indian Standard IS : 9071 (Part 1). 9 pp.

Methods are recommended for the harvesting, curing, drying, transport and storage in warehouses or markets of groundnuts to minimize the development of aflatoxin by growth of the mold Aspergillus flavus.

1359. Indian Standards Institution 1979b. Code of practice for control of aflatoxin in groundnut. II. Plant storage and processing flour and oil. Indian Standard IS : 9071 (Part II). 9 pp.

Methods are recommended for the control of aflatoxin during the buying of groundnuts, their storage, and their processing to oilcake or edible flours, and during the treatment of raw groundnut oil prior to edible use. Guidelines are given for selection of groundnut kernels/pods as regards chemical characteristics for food and feed categories.

1360. Jewers, K. 1988. [Mycotoxins and their effects on poultry production.]. Mikotoksini i njihovi efekti na zivinarsku proizvodnju. Peredarstvo 23(11-12): 69-73.

To prevent the losses resulting from the presence of mycotoxins in poultry feed the EEC has introduced regulations limiting the level of allatoxin in poultry feeds, and has set maximum levels for aflatoxin in six major raw materials used in animal feed production : babassu, copra, cottonseed, groundnut, maize, and palm kernels. However, the sampling, sample preparation and analytical methodologies required for the monitoring of allatoxin in these commodities are not available at present, and further work is required to ensure that the heterogenous distribution of aflatoxin in a commodity is taken into account when new quality control procedures are developed. At present no limits have been set for the other mycotoxins known to produce adverse effects in poultry. Care must be taken by animal feeds processors and poultry producers to ensure mycotoxins do not enter the food chain, and this may necessitate them introducing quality control procedures even though a legal framework for such testing is not in place. Utilization of highly contaminated raw materials presents a major problem. Blending of highly contaminated and noncontaminated raw materials is not advisable, as most mixing techniques involving unground material are likely to lead to pockets of highly contaminated material which could have a disastrous effect on poultry production. An alternative strategy would be to decontaminate the highly contaminated raw material or the feed

prior to the addition of vitamins and other additives. This approach is being investigated in a project in Pakistan.

1361. Jones, B.D. 1975. Aflatoxin in feedingstuffs - its incidence, significance and control. Pages 273-290 in Proceedings of the Conference on animal feeds of tropical and subtropical origin. Tropical Products Institute. London (UK).

The nature of the aflatoxin problem and the significance of the aflatoxin contamination of feeds is discussed from both the animal production and the public health aspects. Procedures that can be adopted to minimize aflatoxin contamination of products and detoxification of aflatoxin-contaminated material are reviewed. Legislative and quality control measures which regulate the use of contaminated material and the world situation regarding the incidence of aflatoxin in feedstuffs and feed-materials are discussed.

1362. Kensler, C.J., and Natoli, D.J. 1969. Processing to ensure wholesome products. Pages 334-353 in Aflatoxin - Scientific Background, Control, and Implications. (Goldblatt,, L.A., ed.). New York, USA : Academic Press. 472 pp.

This paper describes testing, removal, and segregation of aflatoxin-contaminated groundnuts in relation to the USA groundnut industry. Sorting and removal of discolored, mold-damaged groundnuts can greatly reduce aflatoxin levels in raw groundnuts. These selective procedures have been very effective in the USA so that very few lots exceed the acceptance level of 25 µg kg^{-1} aflatoxin.

1363. Kisyombe, C.T. 1989. Aflatoxin contamination of groundnuts : Control strategies in Malawi. Pages 71-76 in Aflatoxin contamination of groundnut : Proceedings of the International Workshop, 6-9 October 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

In Malawi the rains start in October and end in April so that long-duration groundnut cultivars are harvested under dry conditions after the rains. The dry conditions favor rapid postharvest drying of groundnuts thus limiting seed infection by Aspergillus flavus and A. parasiticus and aflatoxin contamination. However, certain pratices used by small holder farmers to process groundnuts in readiness for sale provide conditions that favor the rapid development of the toxigenic fungi and 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 groundnuts from small holder farmers, and electronically sorts and tests the groundnuts for aflatoxin contamination at the Liwonde Groundnut Factory. The process of handlshelling and handgrading of groundnuts by smallholder farmers, followed by re- grading, and aflatoxin testing of the groundnuts has earned Malawi a reputation as a source of high-quality groundnuts for the confectionery trade.

1364. Manzo, S.K., and Misari, S.M. 1989. Status and management of aflatoxin in groundnuts in Nigeria. Pages 77-90 in Aflatoxin contamination of groundnut : Proceedings of the International Workshop, 6-9 October 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

A committee to coordinate action on the problem of aflatoxin contamination in Nigeria was formed in 1961 with representatives from four ministries, i.e., the Institute for Agricultural Research (IAR), Zaria, the Nigerian Stored Products Research Institute (NSPRI), and the Northern Nigerian Marketing Board. This Committee was given 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 investigated the aflatoxin contamination of the groundnut crop up to the stage where the produce was sold by farmers, while NSPRI studied the problem from the time of storage until produce was exported or consumed. IAR investigated the time of invasion of groundnut kernels by Aspergillus flavus, and when, and under what conditions it produced aflatoxin. An interplay of temperature, relative humidity, drought, erratic rainfall patterns, and maturity of the crop at lifting was found to affect 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, while in the major groundnut growing areas that lie in the drier Northern Guinea and Sudan Savanna the problem is largely 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 groundnuts to either 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 government 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 with an aflatoxin content that does not exceed the maximum permissible limit of 20 µg kg^{-1} set by the European Economic Community. 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.

1365. McDonald, D. 1969. Aspergillus flavus on groundnut (Arachis hypogaea L.) and its control in Nigeria. Journal of Stored Products Research 5: 275-280.

Groundnuts are produced in two distinct zones in Nigeria; the dry northern zone where 95 % of the crop is grown, and the wetter riverain zone. In the northern zone the crop is normally harvested after the rains have ended, but in the riverain zone harvesting takes place during the rains. Investigations on groundnuts from the 1961 crop showed that A. flavus infection of kernels and aflatoxin production occurred in both zones. The condition of the shell had a marked influence on the fungal infection and toxicity of the kernels. Kernels from undamaged pods were rarely infected by A, flavus, whereas kernels from perforated pods had a high degree of infection with this and other fungi. Kernels from termite-scarified pods were intermediate in this respect. Kernels from all pod grades showed higher fungal infection in material from the riverain zone than in that from the northern zone, indicating that poor drying conditions could be important. Trials at Mokwa in the riverain zone and at Kano in the northern zone in which various methods of drying were tested demonstrated that rapid drying gave kernels with low fungal infection and little or no toxicity. Slow drying with kernel moisture contents above 20 % for extended periods resulted in heavily infected, toxic kernels. Kernels from undamaged, mature pods were free from fungal infection at lifting, infection by A. flavus not normally occurring until 4-6 days after harvest. Over-mature pods and pods from plants that had wilted and died before harvest had kernels infected by fungi at lifting in the northern zone. On the basis of these findings recommendations were provided for management of A. flavus in groundnut.

1366. McDonald, D. 1976. Aflatoxins : Poisonous substances that can be present in Nigerian groundnuts. Samaru Miscellaneous Paper 53, Institute for Agricultural Research, Samaru, Ahmadu Bello University, Zaria, Nigeria, 14 pp.

This paper outlines events leading up to the discovery of aflatoxin, describes briefly research done on the groundnut aflatoxin problem in the northern states of Nigeria, considers the implications of aflatoxin in relation to animal and human health, and discusses measures for elimination of aflatoxin from Nigerian groundnuts, or to at least greatly reduce incidence of aflatoxin.

1367. Mehan, V.K. 1985. The Aflatoxin problem in groundnut - Approaches to prevention and control. Pages 1-2 in Proceedings of the XXVI Annual AICORPO Workshop, 15-19 April 1985. Nagpur, India.

This paper reviews the problem of aflatoxin contamination in groundnuts and underlines some of the areas where additional research is most needed. Possible preventive and control measures are also discussed with special reference to conditions in the semi-arid tropics.

1368. Mehan, V.K. 1987. The aflatoxin contamination problem in groundnut - Control with emphasis on host plant resistance. Pages 63-92 in Proceedings of the first Regional Groundnut Plant Protection Group Meeting and Tour, 15-21 February 1987, Harare, Zimbabwe.

The status of the global aflatoxin problem is reviewed with special reference to African groundnut producing countries, and research needs are highlighted. Possible practical control measures are discussed with emphasis on use of host plant resistance to the aflatoxin-producing fungus Aspergillus flavus.

1369. Mehan, V.K., and McDonald, D. 1983. Mycotoxin contamination in groundnut - prevention and control. Pages 237-250 in Mycotoxins in Food and Feed. (Bilgrami, K.S., Prasad, T., and Sinha, K.K.. eds.). Bhagalpur. India : The Allied Press.

This paper reviews the problem of aflatoxin contamination in groundnuts and discusses possible preventive and control measures. The possible use of genetic resistance to the aflatoxin-producing fungus Aspergillus flavus is considered. Reports of natural occurrence of mycotoxins in groundnuts are reviewed.

1370. Mehan, V.K., and McDonald, D. 1984. [Mycotoxin-producing fungi in groundnuts: Potential for mycotoxin contamination.]. Champignons producteurs de mycotoxines chez l'arachide: Potentiel de contamination par les mycotoxines. Oleagineux 39(1): 25-29.

The possible use of genetic resistance to seed invasion by Aspergillus flavus and to aflatoxin production is considered and some research data presented. Many species of fungi have been found associated with groundnut seeds and several are known to be capable of producing mycotoxins on suitable substrates. Reports of natural occurrence of mycotoxins in groundnuts are reviewed, and the natural occurrence of citrinin and zearalenone reported. Preliminary data on mycotoxin production by fungi isolated from groundnuts are presented.

1371. Mixon, A.C. 1980. Potential for aflatoxin contamination in peanuts (Arachis hypogaea L.) before and soon after harvest - a review. Journal of Environmental Quality 9(3): 344-349.

Cultural and preharvest conditions and early postharvest conditions which influence the vulnerability of groundnuts to seed invasion by Aspergillus flavus and to aflatoxin contamination are discussed. Management practices, including chemical control and genetic resistance are considered.

1372. Nakayama, T.O.M. 1989. Aflatoxin research in the peanut-CRSP : an overview. Pages 203-207 in Aflatoxin contamination of groundnut : Proceedings of the International Workshop. 6-9 October 1987, ICRISAT Center, India.

Patancheru. A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

The global problem of aflatoxin is being pursued by the Peanut Collaborative Research Support Program (Peanut-CRSP) through : (1) development of cultivars resistant to seed 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.

1373. National Peanut Council. 1968. Voluntary Code of good practices for Purchasing. Handling, Storage, Processing and Testing of Peanuts. (Sixth Edition). National Peanut Council, Washington D.C., USA. 3 pp.

The code of practices for purchasing, handling, sorting, processing, and testing of groundnuts is described. Adherence to these voluntary practices should ensure groundnuts and groundnut products being of good quality and meeting required limits for aflatoxin content.

1374. National Peanut Council. 1976. Voluntary Code of Good Practices for Peanut Product Manufacturers. (Eleventh Edition). National Peanut Council, Washington D.C.. USA.

The code of practices for groundnut produci manufacturers to follow in relation to aflatoxin contamination in groundnuts and groundnut products is presented in detail. While these practices are voluntary their application should reduce the chances of products being rejected.

1375. Parpia, H.A.B., and Sreenivasamurthy, V. 1969. Report on recent studies on aflatoxin. Document 2.17/25. P.A.G. (FAO/WHO/UNICEF), September 1969 Meeting - Geneva.

Detoxification of groundnut flour with hydrogen-peroxide is feasible and effective in destroying aflatoxin. This treatment is now used commercially, particularly in the preparation of milk substitutes containing groundnut protein. A mixture of phosphine and ammonia can be used as a fungicide for treatment of groundnuts pods immediately after harvest for controlling growth of Aspergillus flavus. Aqueous ethanol can be used effectively to extract about 90 % of aflatoxin from split groundnuts without removing any significant amounts of fat. A study of excretory metabolites of aflatoxin in rats, guinea-pigs and monkeys showed considerable differences in the excretion of aflatoxin M and B between these species. In an exploratory study of the possible role of aflatoxin in infantile liver cirrhosis about 8 % of the urine samples examined contained 10-50 µg of aflatoxin B_1 in a 24 h sample. The pattern of muscular damage in cirrhotic children was similar to the muscular damage caused by aflatoxin fed to albino rats.

1376. Peers, F.G. 1967. Aflatoxin - A summary of recent work. Tropical Science IX(4): 186-203.

The important published information on aflatoxin during the period January 1964 to March 1967 is summarized. The topics covered include (a) assay of aflatoxins, (b) toxicology of aflatoxins, (c) biosynthesis of aflatoxins. (d) biochemical and intercellular effects of aflatoxins. (e) metabolism of aflatoxins in animals, (f) incidence, control and detoxification, and (g) implication in man.

1377. Sanders, T.H. 1983. The aflatoxin crisis. Pages 151-164 in Peanuts : Production, Processing, Products. Third Edition. (Woodroff, J.G., ed.). Westport, Conn., USA : AVI Publishing Co., Inc.

This paper very briefly reviews all aspects of the aflatoxin problem in groundnut. Control measures are listed and cover all stages from crop production through processing and storage. Occurrence of aflatoxicosis in man is also reviewed. Methods of detoxification of groundnut products are discussed.

1378. Sanders, T.H., Blankenship, P.D., and Cole, R.J. 1988. Aflatoxin content of peanuts cleaned on a peanut belt screen before drying. Proceedings of the American Peanut Research and Education Society 20: 25.

Removal of aflatoxin-contaminated groundnuts from the edible market is of high priority in the groundnut industry. A recently developed belt screen was utilized to evaluate quality improvements in moisture, foreign material, and aflatoxin content when farmers' stock groundnuts were cleaned before drying. Four drying wagon loads from each of seven farm fields were evaluated. Two loads were screened over a belt screen which had a 10.3 mm gap between belts and two loads were left unscreened. Fall- through weight of the screened loads ranged from 2-26% (weight) of the loads. One screened and one unscreened load were artificially dried at 8.3°C rise above ambient and the other loads were dried at a 16.7°C rise above ambient. Approximately 100 pounds were shelled and the seed were sized. Groundnuts from three of the seven locations contained detectable levels of aflatoxin. All size categories of groundnuts from the three locations contained aflatoxin regardless of whether or not they had been screened and regardless of whether pods rode on fell through the screen. Jumbo, medium, and No. 1 size groundnuts from pods riding the screen were hand-picked to remove damaged seed and, with one exception, aflatoxin levels were less than 2.5 µg kg^{-1} in subsequent analyses.

1379. Schmidt, F.R., and Esser, K. 1985. Anatoxins: medical, economic impact, and prospects for control. Process Biochemistry 20(6): 167-174.

Current knowledge about mode of action of aflatoxins, their medical and economic importance and prospects for their control are reviewed, considering the following aspects : structure, nomenclature and biosynthesis; mode of action; health risks and legislative regulation; postharvest control (segregation, decontamination, storage); and preharvest control (pesticides, and biological control by breeding or microbial interactions, particularly repression of aflatoxin synthesis by Aspergillus flavus following infection with a virus from Penicillium chrysogenum).

1380. Stoloff, L. 1980. Aflatoxin control : past and present. Journal of the Association of Official Analytical Chemists 63(5): 1067- 1073.

History of control of aflatoxin levels in foods, especially groundnut products, in the USA is surveyed, and data presented show changes in aflatoxin contamination from 1967 to 1978 for raw shelled groundnuts, consumer groundnut products, almonds, pecans and walnuts, and imported Brazil and pistachio nuts. The effect of establishment of an FDA action level of 0.5 ng aflatoxin M1 mL^{-1} milk on aflatoxin concentration in market milk is discussed. Aflatoxin contamination of food is effectively controlled in the USA, with the possible exception of some commeal and corn grits products locally made and consumed in rural South Eastern USA.

1381. Tiemstra, P.J. 1977. Aflatoxin control during food processing of peanuts. Pages 121-137 in Mycotoxins in human and animal health. (Rodricks. J.V., Hesseltine, C.W., and Mehlman, M.A., eds.).Illinois, USA : Pathotox Publishers, Inc.

Testing, removal, and segregation of aflatoxin-contaminaled groundnuts is described in relation to the USA groundnut industry. Checking at all stages from growers to consumers is necessary. Sorting and removal of discolored groundnuts can reduce aflatoxin levels in raw groundnuts by 16 %. Reduction may be improved if sorting is done after roasting and blanching. About one third of the aflatoxin is destroyed during roasting. Raw groundnuts containing 25 µg kg^{-1} of aflatoxin will, as a finished product, contain approximately 8 µg kg^{-1} of aflatoxin. Selective procedures in the USA have been very effective so that few lots exceed the acceptance level of 25 µg kg^{-1}. The average aflatoxin content of groundnut products sold in the USA is less than 2 µg kg^{-1}.

1382. U.S. Department of Agriculture. 1968. Preventing mycotoxins in farm commodities. ARS 20-16, March 1968, 7 pp.

The problem of mycotoxins in farm commodities is reviewed. Concerning groundnuts, it is stressed that controlling pod insects and diseases, careful harvesting and postharvest drying to prevent damage to pods and ensure rapid drying of seeds should help in reducing risk of aflatoxin contamination. Drying the crop produce to below 8 % seed moisture content will inhibit colonization of seeds by aflatoxin-producing fungi.

1383. Vaishnav. K.A., Savalia, R.L., Patel, VJ., and Patel, R.S. 1989. Aflatoxin in groundnut, problems and remedies. Seeds and Farms 15(2): 12-15.

A general account is given of the aflatoxin contamination problems in stored groundnuts. Brief accounts are given of the natural occurrence of aflatoxins. Aspergillus flavus causes aflaroot disease of groundnuts ; the aflatoxigenic fungus being seedborne results in infected seeds becoming rancid. Of 18 cultivars listed as having resistance to seed infection by A. flavus, only J 11 is widely grown in India. Regulations concerning trade in affected commodities are discussed. Preventive and curative measures for controlling aflatoxin contamination are listed.

1384. Wogan, G.N. 1968. Aflatoxin risks and control measures. Federation Proceedings 27(3): 932-938.

This paper reviews research on toxicity and carcinogenicity of aflatoxins to animals, and summarizes control measures applied or proposed for protecting human food supplies from aflatoxin contamination. Control measures include use of effective crop handling, processing and storage procedures, segregation of contaminated produce by various sorting and diversion systems, and detoxification.

8.2.2 Cultural Control

1385. Bharat Singh. 1982. Pre- and post-harvest handling of peanuts. Pages 127-132 in World Peanut Production, Utilization and Research (Cummins, D.G., and Jackson. C.R., eds). University of Georgia, College of Agriculture Experiment Stations Special Publication No. 16, April 1982.

Quality of groundnut depends on the effectiveness of the curing, drying, storage, and decortication processes. It is important to harvest at optimum maturity as late harvest may result in seed infection with Aspergillus flavus and aflatoxin contamination. In the USA, almost all commercially grown groundnuts are mechanically harvested, artificially dried and mechanically handled and shelled. They are therefore unlikely to become contaminated with aflatoxin during these processes. In less developed countries groundnuts are harvested and processed manually and are often stored under conditions favorable to mold growth.

1386. Blatchford, S.M., and Hall, D.W. 1963a. Methods of drying groundnuts : 1. Natural methods (Literature survey). Tropical Science 5(1): 6-33.

Natural methods used by fanners in several countries for drying groundnuts are described. The necessity of adequate drying to prevent the development of the aflatoxin-producing mold Aspergillus flavus in seeds is emphasized.

1387. Blatchford, S.M., and Hall, D.W. 1963b. Methods of drying groundnuts : II Artificial methods. Tropical Science 5(2): 82-98.

Information on the artificial drying of groundnuts, which is of special importance in preventing the development of the mold Aspergillus flavus is summarized. It is recommended that, before drying, the nuts should be cured on the plant in windrows and should then be threshed rather than left on the haulms. Reference is made to the extensive experimental work carried out in the USA ; various types of dryers used there and in Australia, Israel. Nigeria and Tanzania are described with special reference to batch dryers. In the tropics, where ambient temperatures can be above 29°C, the use of forced air without additional heat may be sufficient for drying groundnuts.

1388. Burrell, N.J., Grundey, J.K., and Harkness, C. 1964. Growth of Aspergillus flavus and production of aflatoxin in groundnuts. V . Tropical Science 6(2): 74-90.

Investigations of sun-drying methods in relation to moisture content of groundnuts were carried out in two areas in Northern Nigeria. Groundnuts are harvested during the wet season in one area and during the dry season in the other. In the wet area, of the several sun-drying methods tried, the only one whicn was satisfactory was to pick the nuts from the haulms as soon as practicable after lifting and to expose them to the sun on materials, such as matting, which could be carried under cover at night and during rain. In the dry area, a variety of sun-drying methods were used and in all cases the groundnuts dried rapidly.

1389. Cole, R.J., Csinos, A.S., Blankenship, P.D., Sanders, T.H., Gaines, T.P., and Davidson, J.I.Jr. 1985. Evaluation of soil calcium as methods of prevention of preharvest aflatoxin contamination of peanuts. Proceedings of the American Peanut Research and Education Society 17: 71.

In 1984, studies were conducted to evaluate the role of calcium in controlling preharvest aflatoxin contamination of groundnuts using the environmental control plots at Dawson, Georgia, USA. Florunner groundnuts were grown under soil environmental stress conditions optimum for preharvest aflatoxin contamination. Calcium treatments were 0, 64.7, 256.7 kg ha^{1} calcium as $CaSO_4$ added to pretreatment levels. The levels of calcium in the kernels from the 0 and 256.7 kg treatment levels were significantly different, reflecting a response to added calcium in spite of relatively high pretreatment levels of soil calcium. There were no significant differences in calcium levels of kernels between the 0 and 64.7 kg calcium treatments. No significant relationship between aflatoxin contamination and kernel calcium levels was found. The authors concluded that application and uptake of calcium by the groundnut seed was not a viable method to prevent preharvest aflatoxin contamination.

1390. Davidson, J.I.Jr., Blankenship, P.D., Sanders, T.H., Cole, R.J., Hill, R.A., Henning, R.J., and Guerke, W.R. 1983. Effect of row spacing, row orientation, and gypsum application on the production and quality of nonirrigated Florunner peanuts. Proceedings of the American Peanut Research and Education Society 15(1): 46-57.

In field trials in Georgia, USA, in 1981-82 Florunner groundnuts were grown without irrigation in rows 90 cm apart or in paired rows 15 or 25 cm apart with 90 cm between pairs. Gypsum application (1120 kg ha^{-1}) at flowering was compared with no gypsum, and East-West orientation of rows was compared wim North-South orientation. Soil temperature was lower, and seed yields and germination percentage higher, in groundnuts grown with North-South orientation. Close spacing of rows gave lower soil temperatures during crop growth, and seeds produced had higher germination percentages, but there was no effect on yield. Soil moisture content was highest in close rows with North-South orientation. Gypsum application increased seed germination from 71.2 to 83.8 % in 1981 and from 83.2 to 86.8 % in 1982 and reduced aflatoxin concentration by 40 %.

1391. Dickens, J.W., and Khalsa, J.S. 1967. Windrow orientation and harvesting damage to peanuts. Oleagineux 22(12): 741-746.

Studies on the effects of groundnut plant orientation in windrows were conducted in North Carolina, USA. Groundnuts on inverted plants dried much more rapidly than did those on plants randomly oriented in windrows. Both plant orientation and moisture content at time of combining affected pod damage during combining, seed germination, and the degree of kernel damage caused by subsequent shelling operations. Apical kernels were more subject to mechanical damage during combining man basal kernels. In groundnuts inoculated with Aspergillus flavus, kernels in broken pods and shelled kernels were more often contaminated with aflatoxin during bulk curing man were groundnuts in sound pods.

1392. Graham, J. 1982. Aflatoxins in peanuts : occurrence and control. Queensland Agricultural Journal 108(3): 119-122.

The problem of aflatoxin contamination, which was serious in the Queensland groundnut industry in drought years, is reviewed with special reference to factors influencing aflatoxin formation including moisture stress, insect damage, and cultivar resistance. Control measures included crop hygiene, resistant varieties, avoiding moisture stress, harvesting the crop at optimum maturity, avoiding pest and mechanical injury, drying rapidly and segregating contaminated batches of groundnuts.

1393. Griffin, G.J., Garren, K.H., and Taylor, J.D. 1981. Influence of crop rotation and minimum tillage on the population of Aspergillus flavus group in peanut field soil. Plant Disease 65(11): 898-900.

The effects of various crop sequences of maize, soybean, groundnut, green manuring with sorghum sudangrass, fallow, and minimum-tillage soybean cropping on Aspergillus flavus group populations in soil were examined in field plots on a farm in Southampton County, Virginia, from 1975 to 1979. Plantings of maize in 1975 and groundnut in 1976 were associated with significant increases in the population of A. flavus group in soil in following years, compared with the fallow treatment. Populations increased primarily in the lower half of the plough layer following maize planting. Other crop sequences did not significantly affect A. flavus group populations.

1394. Joffe, A.Z., and Lisker, H. 1970. Effects of crop sequence and soil types on the mycoflora of groundnut kernels. Plant and Soil 32: 531-533.

In Israel, the effects of crop sequence and soil types on the mycoflora of groundnuts were examined over three years in 81 fields. Some fields were previously fallowed, others continuously cropped with or without groundnuts. The mycoflora of the groundnut kernels from fields previously sown with groundnuts was more abundant than the mycoflora of kernels from fallow fields and from fields cropped without groundnuts. In the cropped fields, whether groundnuts were included in the previous crops or not, Aspergillus niger made up 50-60 % of the total mycoflora. Penicillium funkulosum and P. rubrum were considerably more prevalent in groundnuts produced in previously fallowed fields. Incidence of A. flavus was always low and it was not influenced by any crop sequence. Total kernel mycofloras were consistently higher on medium and heavy soils than on light soils.

1395. McDonald, D. 1969. Aspergillus flavus on groundnut (Arachis hypogaea L.) and its control in Nigeria. Journal of Stored Products Research 5: 275-280.

Groundnuts are produced in two distinct zones in Nigeria; the dry northern zone where 95 % of the crop is grown, and the wetter riverain zone. In the northern zone the crop is normally harvested after the rains have ended, but in the riverain zone harvesting takes place during the rains. Investigations on groundnuts from the 1961 crop showed that A. flavus infection of kernels and aflatoxin production occurred in both zones. The condition of the shell had a marked influence on the fungal infection and toxicity of the kernels. Kernels from undamaged pods were rarely infected by A. flavus, whereas kernels from perforated pods had a high degree of infection with this and other fungi. Kernels from termite-scarified pods were intermediate in this respect. Kernels from all pod grades showed higher fungal infection in material from the riverain zone than in that from the northern zone, indicating that poor drying conditions could be important. Trials at Mokwa in the riverain zone and at Kano in the northern zone in which various methods of drying were tested demonstrated that rapid drying gave kernels with low fungal infection and little or no toxicity. Slow drying with kernel moisture contents above 20 % for extended periods resulted in heavily infected, toxic kernels. Kernels from undamaged, mature pods were found to be free from fungal infection at lifting, infection by A. flavus not normally occurring until 4-6 days after harvest. Over-mature pods and pods from plants that had wilted and died before harvest had kernels infected by fungi at lifting in the northern zone. On the basis of these findings recommendations were provided for management of A. flavus in groundnut.

1396. Mehan, V.K. 1987. The aflatoxin contamination problem in groundnut - Control with emphasis on host plant resistance. Pages 63-92 in Proceedings of the first Regional Groundnut Plant Protection Group Meeting and Tour, 15-21 February 1987, Harare, Zimbabwe.

The status of the global aflatoxin problem is reviewed with special reference to African groundnut producing countries, and research needs arc highlighted. Possible practical control measures are discussed with emphasis on use of host plant resistance to the aflatoxin-producing fungus Aspergillus flavus.

1397. Pettit, R.E., and Taber, R.A. 1968. Factors influencing aflatoxin accumulation in peanut kernels and the associated mycoflora. Applied Microbiology 16(8): 1230-1234.

Levels of aflatoxin in Spanish groundnut kernel samples from different geographical areas in Texas during 1966 were low. Of the 334 samples tested, 239 (71.6 %) contained no aflatoxin and only 2.7 % contained > 30 µg kg^{-1} and 25.8 % contained trace-29 µg kg^{-1}. Analysis of samples obtained from growers using artificial drying equipment (forced air and supplemental heat), when windrow conditions were unfavorable for rapid drying, suggests that this practice reduces the possibility of aflatoxin accumulation. In general, groundnuts harvested from land planted to groundnuts the previous year were more highly infected by fungi and contained more aflatoxin than groundnuts grown on land planted to rye, oats, melons, or potatoes the previous year. Aflatoxin incidence tended to decrease from south to

north Texas. Detection of aflatoxin in sound mature kernels (kernels screened for minimal size) indicates that the practice of screening for removal of small immature kernels and removal of obviously damaged kernels does not completely eliminate aflatoxin contamination.

1398. Pettit, R.E., Taber, R.A., Schroeder, H.W., and Harrison, A.L. 1971. Influence of fungicides and irrigation practice on aflatoxin in peanuts before digging. Applied Microbiology 22(4). 629-634.

Groundnuts (cultivar Starr) grown rainfed under drought stress conditions had markedly higher levels of Aspergillus flavus infection and aflatoxin contamination of kernels before digging than had groundnuts grown under irrigation, in field trials at Yoakum and Stephenville, USA. in the 1967, 1968 and 1969 seasons. Levels of seed infection and aflatoxin contamination were considerably higher in 1967 and 1969 than in 1968, A. flavus infection and aflatoxin contamination levels were higher in drought-stressed groundnuts produced at Yoakum than in less stressed groundnuts produced at Stephenville. In 1969 some samples of freshly-dug groundnuts from irrigated plots had high levels of A. flavus infection (16-59 %), but no aflatoxin was detected in these samples. Absence of aflatoxin in these samples is attributed to high seed moisture levels and to low soil temperatures (10 to 21°C). Several fungicides applied to the soil and foliage did not affect fungal infection and aflatoxin contamination of groundnuts.

1399. Sanders, T.H., Blankenship, P.D., Cole, R.J., and Smith, J.S. 1986. Role of agrometeorological factors in postharvest quality of groundnut. Pages 185-192 in Agrometeorology of groundnut: proceedings of an International Symposium, ICRISAT Sahelian Centre. Niamey, Niger, Patancheru. India : International Crops Research Institute for the Semi-Arid Tropics.

Postharvest quality of groundnut is influenced by the particular set of environmental and cultural practices that influence physiology and maturation. Groundnut composition, although related to environment, changes dramatically as groundnuts mature. There is biochemical basis for inferior quality in immature groundnut. Drought stress and soil temperature influence maturation rate and thus have an indirect effect on postharvest quality. Aspergillus flavus invasion and aflatoxin contamination in groundnuts arc related to drought stress, soil temperature, and maturity. Small, immature seed are more likely to be contaminated with A. flavus than larger, mature seed. The biochemical composition, fungal contamination, and the tendency toward higher moisture content complicate storage of immature seed. Each of these factors predisposes immature seed to rapid quality deterioration in storage. Agrometeorological studies must include an awareness of the interrelationships of environment, maturity, and postharvest quality.

1400. Sellschop, J.P.F. 1965. Field observations on conditions conducive to the contamination of groundnuts with the mould Aspergillus flavus Link ex Fr. Pages 47-52 in Symposium on Mycotoxins in Foodstuffs. Agricultural Aspects (Abrams, L., Sellschop, J.P.F.. and Rabie, C.J., eds.). Dept. of Agriculture, Technical Services, Pretoria, South Africa.

Environmental and biological factors responsible for aflatoxin contamination of the South African groundnuts are discussed. During the 1963/64 growing season, maturing groundnuts in the North-Western Transvaal areas were damaged by certain species of termites; this predisposed groundnuts to seed invasion by Aspergillus flavus. Protracted droughts followed by the late rains in these areas were also conducive to infection of groundnuts by the aflatoxigenic fungus. Most of the infection of groundnuts by the fungus was believed to occur in the groundnut before harvest.

1401. Subrahmanyam, P., and Rao, A.S. 1974. Effect of crop sequence on Aspergillus flavus infestation and aflatoxin accumulation in groundnut (Arachis hypogaea L.). Current Science 43(21): 671-673.

The effect of the previous season's crop (vegetables, rice or groundnut) on populations of Aspergillus flavus and other fungi in the soil, rhizosphere and geocarposphere of groundnuts and of shells and seeds at various stages of development, and on aflatoxin contamination at harvest was investigated. A. flavus populations in the rhizosphere and geocarposphere were high where groundnut was the previous crop and infection of shells and seeds were also high. Aflatoxin levels were higher in seeds from damaged pods than in seeds from undamaged pods.

1402. Wilson, D.M., and Stansell, J.R. 1981. Effects of irrigation on aflatoxin contamination of peanuts. Proceedings of the American Peanut Research and Education Society 13(1): 60.

Florunner and Florigiant groundnuts were grown in 1974, 1975, 1976 and 1977 and inoculated with Aspergillus parasiticus 30 days after sowing. Four replicates were grown in plots for 140 to 145 days under rainfall controlled shelters with six irrigation treatments: (1) wet from 0-140, (2) dry from day 36-70, (3) dry from day 71-105, (4) dry from day 106-140, (5) dry from day 36- 105, (6) dry from day 71-140. Aflatoxin concentrations from Florunner groundnuts showed significant differences among treatments (P=0.01) in 1974 and 1976 but not in 1975 or 1977. In 1974 and 1976, Florunner sound mature kernels had significantly more aflatoxin in treatments 4 and 6 than in other treatments. Aflatoxin concentrations from Florigiant treatments 4 and 6 were significantly greater (P=0.01) than other treatments in 1974 and 1975, but not in 1977. No data were taken in 1976 for Florigiant groundnuts. Water stress during the last 35 or 70 days of the season

affected aflatoxin contamination of sound mature kernels in three of the four years on one or both cultivars. Because of year to year variation, drought stress alone will not account for high levels of field aflatoxin contamination. However, in all treatments with irrigation during the last 35 days of the season no significantly high levels of aflatoxin contamination occurred in any year or cultivar.

1403. Wilson, D.M., and Stansell, J.R. 1983. Effect of irrigation regimes on aflatoxin contamination of peanut pods. Peanut Science 10: 54-56.

Effects of irrigation and drought stress treatments on preharvest aflatoxin contamination of groundnuts were investigated in the 1974, 1975, 1976 and 1977 seasons. Two groundnut cultivars, Florunner and Florigiant, were grown under rainfall controlled shelters with six irrigation treatments : (1) full irrigation throughout the growing season, (2) no irrigation from 36 to 70 days after sowing (DAS). (3) no irrigation from 71 to 105 DAS, (4) no irrigation from 106 to 145 DAS, (5) no irrigation from 36 to 105 DAS. and (6) no irrigation from 71 to 145 DAS. Groundnut plants in each replicated plot were inoculated by sprinkling them with a spore suspension of an aflatoxigenic isolate (NRRL 2999) of Aspergillus parasiticus. Significant differences in anatoxin contents of Florunner groundnuts were attributable to the irrigation treatments in 1974 and 1976 but not in 1975 and 1977. In 1974 and 1976, sound mature kernels of Florunner from the treatments 4 and 6 had significantly higher levels of aflatoxins than had the kernels from other treatments. Aflatoxin contamination levels in sound mature kernels of cultivar Florigiant from treatments 4 and 6 in 1975 and from treatment 6 in 1974 were significantly greater than in sound mature kernels from other treatments. Such differences in aflatoxin contamination were not evident in 1977. Drought stress during the last 40 to 75 days of the season favored aflatoxin contamination in three of the four seasons in one or both cultivars. Because of season to season variation, drought stress was not alone responsible for preharvest aflatoxin contamination. In some seasons, other environmental factors interacted with drought stress to either favor or inhibit aflatoxin contamination. No aflatoxin was detected in seeds of the test cultivars from treatments where irrigation was applied during the last 40 days of the season.

1404. Wilson, D.M., and Walker, M.E. 1988. Effects of gypsum and irrigation on Aspergillus flavus group colonization of peanuts. Proceedings of the American Peanut Research and Education Society 20: 24.

Field experiments were conducted in the 1984, 1985. 1986 and 1987 seasons on a calcium deficient Lakeland sand at Tifton, Georgia, USA. Florunner groundnuts were planted in 1984 and 1987 while NC 7 was planted in 1985 and 1986. Irrigation and non-irrigation treatments were the whole plots, split-plots were rates of gypsum corresponding to 0, 112, 224 and 336 kg ha^{-1} of added calcium. Split-plots were inoculated or not by spinkling a spore suspension of Aspergillus parasiticus (NRRL 2999) over the plants at early bloom. Soil populations of the A. flavus group fungi were monitored four times each year. Harvested hulls and kernels were plated to assess A. flavus incidence. Aflatoxin contents of kernels were determined using high-pressure liquid chromatography (HPLC). Gypsum applications increased yield, value, per cent sound mature kernels (SMK) and reduced damage in all years. A. parasiticus inoculation decreased yield of Florunner but not NC 7. Inoculation increased soil populations of the A. flavus group fungi. Both irrigation and gypsum decreased hull and kernel infection by A. flavus. Aflatoxin contamination was infrequently observed and occurred in a random manner.

1405. Wilson, D.M., Walker, M.E., Gaines, T.P., Csinos, A.S., Win, T., and Mullinix, B.G.Jr. 1985. Effects of Aspergillus parasiticus inoculation, calcium rates and irrigation on peanuts. Proceedings of the American Peanut Research and Education Society 17: 72.

The effects of gypsum, irrigation and inoculation with Aspergillus parasiticus (NRRL 2999) on mycoflora and aflatoxin contamination of groundnuts were examined. Two rows of groundnut plants in each plot were inoculated by sprinkling them with a spore suspension of the A. parasiticus isolate. Two irrigation regimes and four gypsum rates were tested. Soil samples were collected three times during the season and at harvest to monitor populations of A. flavus group fungi (A. parasiticus and A. flavus). Groundnut pods were collected at harvest for P, K, Ca, Mg, aflatoxin and mycoflora analyses. No aflatoxins were found in groundnuts from any treatment. Populations of A. flavus group fungi were significantly higher in soil from inoculated plots for the first two sampling dates only; A. parasiticus apparently did not persist in the soil throughout the growing season. More kernels were infected with the A. flavus group fungi and other fungi in plots with no gypsum treatments than in plots with gypsum treatments. The relationship between calcium nutrition and A. flavus infection in groundnuts may be important in preharvest aflatoxin contamination.

8.2.3 Chemical Control

1406. Ahmad, S., and Branen, A.L. 1981. Inhibition of mold growth by butylated hydroxyanisole. Journal of Food Science 46(4): 1059- 1063.

Direct addition of 200 ppm of butylated hydroxyanisole (BHA) inhibited growth of some species of Penicillium. Aspergillus, or Geotrichum in a glucose salts broth (GSB). and of Aspergillus flavus and P. expansum in potato dextrose agar (PDA) or applesauce. In processed cheese spread, direct addition of 400 ppm of BHA was

required to inhibit growth of A. flavus or P. expansion, while the concentration required for inhibition of A. flavus, by a BHA emulsion sprayed on the surface was 150-200 ppm. BHA was effective in GSB over a wide range of p11 values and incubation temperature against A. flavus at a concentration of 200 ppm. A combination of 150 ppm BHA + 0.2 % potassium sorbate gave total inhibition of growth of A. flavus in GSB.

1407. Badii, F., and Moss, M.O. 1988. The effect of the fungicides tridemorph, fenpropimorph and fenarimol on growth and aflatoxin production by Aspergillus parasiticus Speare. Letters in Applied Microbiology 7(2): 37-39.

The effects of the systemic fungicides tridemorph, fenpropimorph and fenarimol (at 240, 250 and 0.75 µg mL^{-1} respectively) on growth and aflatoxins B_1 and G_1 production by Aspergillus parasiticus (NRRL 3145) was studied in a chemically defined medium. All three fungicides inhibited growth and simultaneously decreased aflatoxin production. The ratio of aflatoxin B, to G, produced remained relatively unchanged in the presence of tridemorph (the NRRL 3145 strain characteristically produces more G_1 than B_1). In the presence of fenpropimorph the ratio of B_1 to G_1 was more pronounced than in the presence of fenarimol (considered to be an inhibitor of cytochrome P_{450}).

1408. Bank, J., and Marth, E.H. 1983a. Growth and synthesis of aflatoxin by Aspergillus parasiticus in the presence of ginseng products. Journal of Food Protection 46(3): 210-215.

Red ginseng saponin (0.36 %) inhibited mycelial growth, sporulation and aflatoxin production by Aspergillus parasiticus in a culture medium during 9 days at 28°C. The mold caused no change in pH of the medium when it contained red ginseng saponin or ginseng tea (9 %). Most ginseng products supported mycelial growth and production of aflatoxin B_1, but inhibited production of aflatoxin G_1. However, when compared to the control, aflatoxin production by -4. parasiticus was reduced by most of the ginseng products tested. Ginseng tea (9 %) resulted in a higher index of maximum accumulation of aflatoxins per interval of mold growth than occurred in the control. Red ginseng was more effective than white ginseng for inhibiting mold growth and aflatoxin production.

1409. Bank, J., and Marth, E.H. 1983b. Aflatoxin production is inhibited by selected herbal drugs. Mycopathologia 83: 129-134.

Effects of several herbal substrates on growth and aflatoxin production by Aspergillus parasiticus were studied. The mold was grown in the presence of the selected herbal extracts: burdock, cromwell, honeysuckle, licorice, and ginger. Two % of each herb was used in an enriched medium which was inoculated with spores of the aflatoxigenic strain of A. parasiticus and incubated at 28°C for 9 days. Mycelial growth was inhibited by honeysuckle and no sporulation occurred in me presence of burdock and honeysuckle. Burdock, cromwell, ginger and licorice enhanced mycelial growth over that in the control. All the herbs inhibited accumulation of aflatoxins B_1 and G_1, especially extracts of honeysuckle flower and root-stem, which inhibited both mycelial growth and aflatoxin production. In the presence of licorice, loss of aflatoxin from the medium during later stages of incubation was greatest.

1410. Batt, C, Solberg, M., and Ceponis, M. 1980. Inhibition of aflatoxin production by carrot root extract. Journal of Food Science 45(5): 1210-1213.

This study was conducted to determine the potential of carrot root to support Aspergillus parasiticus growth and aflatoxin production. Raw carrot tissue did not support the germination of A. parasiticus spores. Autociaved carrot tissue supported germination, growth, sporulation. and aflatoxin production by A. parasiticus. There was no measurable difference in water activity before or after autoclaving the carrot tissue. There was an increase in water extractable carbohydrate and protein as a result of autoclaving the carrot tissue. Chloroform extracts of carrot tissue contained a compound that inhibited differentiation and aflatoxin production by A. parasiticus in both synthetic and semisynthetic media. The inhibitor was optimally active within a pH range 3.5 - 4.0. The minimum inhibitory concentration of the extract at pH 4.5 and at 28°C in minimal salts medium containing 6 % glucose and 1.7 x 10^5 A. parasiticus spores was 3.84 g equivalent weights of tissue mL^{-1} assay medium.

1411. Batt, C, Solberg, M., and Ceponis, M. 1983. Effect of volatile components of carrot seed oil on growth and aflatoxin production by Aspergillus parasiticus Journal of Food Science 48(3): 762-764.

Effects of the volatile fraction of carrot seed oil (VCSO) and its components on growth and aflatoxin production by Aspergillus parasiticus were studied. Geraniol. citral and terpineol prevented growth and aflatoxin production. VCSO inhibited growth and prevented aflatoxin production. Limonene and terpinene did not affect growth but inhibited aflatoxin production. VCSO, limonene and terpinene reduced growth rate, measured by incorporation of [^{3}H] amino acids into trichloracetic acid insoluble protein. Addition of VCSO at any time up to 5 days reduced aflatoxin accumulation in the culture medium at 7 days.

1412. Bauer, J., Gareis, M., Montgelas, A.von, and Gedek, B. 1983. Effects of food preservatives on mycotoxin production. Microbiologic-Aliments-Nutrition 1(2): 203-209.

Investigations were undertaken into the effects of subinhibitory concentrations of

food preservatives on mycotoxin production. Methyl-p-hydroxybenzoaie. propyl-p-hydroxybenzoate, benzoic acid and sorbic acid stimulated aflatoxin production by Aspergillus flavus. No stimulation was observed with propionic acid. Fusarium acuminatum grown on maize meal supplemented with 0.025-0.05 % sorbic acid produced more T-2 toxin than was produced without this chemical. It is assumed that attack of the krebs cycle by preservatives leads to high amounts of acetyl coenzyme A which is essential for aflatoxin B_1 and T-2 toxin synthesis.

1413. Bean, G.A., Klarman, W.L., Rambo, G.W., and Sanford, J.B. 1971. Dimethyl sulfoxide inhibition of aflatoxin synthesis by Aspergillus flavus. Phytopathology 61: 380-382.

Aspergillus flavus was grown at 22, 28, and 35°C in a culture medium containing different concentrations of dimethyl sulfoxide (DMSO). Mycelial growth and aflatoxin production were monitored. Optimum temperature for aflatoxin production in the medium was 35°C after 1 week, 28°C after 2 weeks and 22°C after 4 and 6 weeks. Addition of DMSO (5,000 ppm) to the medium influenced aflatoxin concentration; either increasing or decreasing concentration depending upon temperature and periods of incubation.

1414. Bean, G.A., and Rambo, G.W. 1975. Use of dimethyl sulfoxide to control aflatoxin production. Annals of the New York Academy of Sciences 243: 238-245.

Aflatoxin production by Aspergillus flavus was reduced by dimethyl sulfoxide (DMSO) being added to culture media. Mycelial growth of A. flavus on inoculated shelled or unshelled groundnuts was reduced when they had been treated with 5, 10 or 20 % DMSO. Chick embryo bioassays were also carried out to confirm the inhibitory effect on aflatoxin production of DMSO.

1415. Bean, G.A., and Southall, A. 1983. Effect of pyridazinone herbicides on growth and aflatoxin release by Aspergillus flavus and Aspergillus parasiticus. Applied and Environmental Microbiology 46(2): 503-505.

The influence of pyridazinone herbicides on aflatoxin production by Aspergillus flavus and A. parasiticus was studied in liquid media. Mycelia production was not affected by 20, 40, or 60 µg of herbicide mL^{-1} ; however, aflatoxin production by A. parasiticus was higher in media with herbicide, whereas A. flavus produced lower aflatoxin levels in the presence of herbicides.

1416. Bell, D.K., and Doupnik, B. 1971. Chemical treatment of peanuts in the windrow to control Aspergillus flavus and aflatoxins. Proceedings of the American Peanut Research and Education Association 3: 31-32.

The effects of various chemicals on seed infection by Aspergillus flavus and aflatoxin contamination of windrowed groundnuts (cultivar Starr) were studied. Aqueous solutions or suspensions of 24 chemicals were applied to pods immediately after harvest, and the plants were covered with polyethylene film (PEF). After 24 h the PEF was removed, pods were inoculated with an aqueous spore suspension of an aflatoxigenic strain (NRRL 2999) of A. flavus, and the PEF replaced. After 6-day incubation, pods were sampled and seeds assayed for infection with A. flavus and for aflatoxins. Chemicals that proved effective in reducing A. flavus infection and aflatoxin contamination were p-aminobenzoic acid (PABA) + dimethylsulfoxide (DMSO), Bordeaux 8-8-100D, and Captafol - DMSO. Seed infection of freshly-dug groundnuts averaged 15 %, and aflatoxin contamination 21 µg kg^{-1}. Aflatoxin contamination was not correlated with A. flavus seed infection levels.

1417. Bell, D.K., and Doupnik, B. 1972. Chemicals in the windrow for controlling aflatoxins in peanuts. Proceedings of the American Peanut Research and Education Association 4(1): 18-20.

The efficacy of chemicals for controlling Aspergillus flavus infection and aflatoxin contamination of groundnuts (cultivar Starr) on windrowed plants in the field was examined. Plants were dug and inverted in the windrows 135 days after planting. Approximately 6.4 mm of water was then applied to the plot area by overhead irrigation. After irrigation, aqueous solutions or suspensions of 27 chemicals (fungicides and some industrial chemicals) were applied to pods in each of four replicated plots. Then, the plants were covered with Tri-Pli (T) white, opaque moisture barrier, which was sealed around the edges with moist soil. After 24 h, the Tri-Pli was removed, pods were inoculated with spore suspension of an aflatoxin-producing isolate of A. flavus (NRRL 2999), and the Tri-Pli was replaced and sealed with moist soil. Both inoculated and noninoculated windrows treated with water only were maintained as controls. After six days of incubation, pod samples were collected and kernels from each replicate were assayed for infection by A. flavus and aflatoxins. Results indicated that aflatoxin contamination of windrowed groundnuts could be substantially reduced by chemicals. Manzate, Benlate, and Botran treated pods had no detectable aflatoxins. A. flavus, however, was recovered from kernels from these treatments.

1418. Beuchat, L.R. 1981. Influence of potassium sorbate and sodium benzoate on heat inactivation of Aspergillus flavus, Penicillium puberulum and Geotrichum candidum. Journal of Food Protection 44(6): 450-454.

Experiments were conducted to determine if two food perservatives, potassium sorbate and sodium benzoate, had a synergistic effect with heat on inactivation of conidia of Aspergillus flavus and Penicillium puberulum and vegetative cells of Geotrichum candidum. Investigations were also made to determine if heated conidia

had increased sensitivity to preservatives in a recovery medium. As the pH of heating menstrua was decreased from 7.0 to 2.5, the rates of inactivation of molds were increased. Conidia were not as adversely affected by acid pH as were vegetative cells. At 50 ppm, potassium sorbate caused a significant increase in the rale of thermal inactivation of A. flavus and G. candidum, while 100 ppm had a significant effect on P. puberulum. Sodium benzoate caused significant decreases in decimal reduction times of A. flavus and P. puberulum when present at a concentration of 50 ppm in heating media. Viable heated conidia of A. flavus and P. puberulum had increased sensitivity to potassium sorbate and sodium benzoate, indicating heat injury. However, the relative effects of the preservatives on colony formation in recovery agar were reversed from those noted in heating media, i.e., at comparable concentration potassium sorbate was more effective than sodium benzoate for inhibiting colony formation.

1419. Beuchat, L.R., Smith, D.H., and Young, C.T. 1974. Effect of foliar fungicides on aflatoxin, oil and protein content and maturing rate of peanut kernels. Journal of the Science of Food and Agriculture. 25(5): 477-482.

Groundnuts (cvs. Tifspan and Florunner) were treated with selected foliar fungicides for control of leafspots. Groundnuts were harvested on three different dates and analysed for aflatoxin. oil and protein content, and degree of maturity. No significant differences in aflatoxin levels were associated with either fungicide treatments or harvest dates. However, significant differences were found in the protein and oil contents of the groundnut kernels, depending upon the fungicide treatment. Delayed maturity was caused by specific fungicide treatments.

1420. Bhatnagar, D., and McCormick, S.P. 1987. The inhibitory effect of neem (Azadirachta indica) leal' formulations on aflatoxin synthesis in Aspergillus parasiticus. Journal of American Oil Chemists' Society 64(5): 654. (Abstract)

The effect of neem leaf extract on Aspergillus parasiticus growth and aflatoxin synthesis was studied. The extracts were prepared either by blending 50 g (wet weight) of fresh leaves in 1L of 10 mM potassium phosphate (pH 7.0) or by boiling the leaves in the buffer. The extracts were added to a culture medium at 1, 5, 10 and 20 % concentrations prior to inoculation. The neem leaf extracts did not affect fungal growth but completely inhibited (> 98 %) aflatoxin synthesis. The inhibitory effect was somewhat diminished (60-70 % inhibition) in the heated leaf extracts. The volatile components of the extracts were stripped with air onto Tenax tubes and analyzed using capillary gas chromatography/mass spectrometry. The major volatile present, 3-methyl 2 buten-1-ol, was nearly 400-fold greater in the blended extract than in the heated extract. The observed inhibition is probably at the level of the regulation of the synthesis of the secondary metabolic enzymes, because once the secondary metabolism was initiated, the inhibitory effect of the neem leaf constituents was lost.

1421. Bilgrami, K.S., Sinha, K.K., and Singh, P. 1981. Inhibition of aflatoxin production by ferulic acid on some cereals and oilseeds. Current Science 50(22): 997-998.

Soaking seeds of rice, wheat, maize, groundnut and mustard in 500 ppm aqueous ferulic acid solution for 3 h inhibited aflatoxin production in them by Aspergillus parasiticus. Seed germination was not affected by the treatment.

1422. Bilgrami, K.S., Sinha, K.K., and Singh, P. 1982. Prevention of aflatoxin production on some cereal and oilseeds by O-vanillin. Current Science 51(3): 138.

Seeds of rice (var. Sita), wheat (var. S308), maize (var. Ganga 2), groundnut (var. Ak 12-24), and mustard (var. BR 13) were soaked for 2 h in 500 ppm O-vanillin solution. Seeds were then inoculated with an aflatoxin-producing strain of Aspergillus parasiticus. The seed treatment successfully checked aflatoxin production without having any adverse effect on seed germination.

1423. Bowen, K.L., and Backman, P.A. 1990. Fungicide effectiveness for control of fungal invasion and aflatoxin contamination in peanut kernels. Proceedings of the American Peanut Research and Education Society 22: 32.

Fungicides used for control of southern stem rot (Sclerutium rolfsii) and limb and pod rot (Rhizoctonia solani) have also been found to reduce fungal damage affecting seed quality. Starting in 1988, the fungicides, terbutrazole, flutolanil, and diniconazole were applied to groundnuts in addition to standard foliar sprays for leafspot control with chlorothalonil. Kernels harvested from these plots were evaluated for infection by Aspergillus spp., other soil-borne fungi, and aflatoxins. In 1988, groundnuts from irrigated plots, that were treated with these fungicides, showed no significant differences in incidence of fungal invasion. However, groundnuts from plots treated with each of these fungicides had lower aflatoxin contamination than plots treated with only chlorothalonil. In 1989, similar studies were conducted in non-irrigated plots. Groundnuts from plots treated with each of these fungicides had lower fungal infection and flutolanil-treated groundnuts had lower aflatoxin contamination than groundnuts from non-treated (control) plots. In both years, the use of these fungicides resulted in higher yields and improved crop value.

1424. Buchanan, R.L., and Fletcher, A.M. 1978. Methylxanthine inhibition of aflatoxin production. Journal of Food Science 43: 654-655.

The effects of caffeine and theophylline on growth and afiatoxin B, production by Aspergillus parasiticus (NRRL 2999) were studied in AMY medium at pH 4.5. Caffeine levels of 0.5, 1.0 and 2.0 mg mL^{-1} decreased afiatoxin production by 86, 96 and 100 %, respectively. Theophylline levels of 2.0, 4.0 and 8.0 mg mL^{-1} were tested, but only the highest concentration was inhibitory, decreasing afiatoxin production by 54 %. Inhibition of growth was noted, but did not completely account for the reduction in afiatoxin production. The data help explain why aflatoxins are not usually reported from caffeine-containing commodities.

1425. Buchanan, R.L., and Shepherd, A.J. 1981. Inhibition of Aspergillus parasiticus by thymol. Journal of Food Science 46: 976-977.

Thymol concentrations equal to or greater than 500 µg mL^{-1} completely inhibited the growth of Aspergillus parasiticus, while lower concentrations of the flavor compound caused either partial or transitory growth inhibition. Afiatoxin production was also inhibited to a degree equal to or lesser than that of growth.

1426. Bullerman, L.B. 1983. Effects of potassium sorbate on growth and afiatoxin production by Aspergillus parasiticus and Aspergillus flavus. Journal of Food Protection 46(11): 940-942.

Growth and afiatoxin production by selected strains of Aspergillus flavus and A. parasiticus in the presence of potassium sorbate in yeast-extract sucrose broth were studied. Potassium sorbate at 0.05, 0.10 and 0.15 % delayed or prevented spore germination and initiation of growth, and slowed growth of these fungi at 12°C. Increasing the concentration of sorbate resulted in a decrease in total mycelial weight. Potassium sorbate also greatly reduced or prevented production of afiatoxin B, by A. parasiticus and A. flavus for up to 70 days at 12°C. At 0.10 and 0.15 % concentrations of sorbate, afiatoxin production was essentially prevented. At 0.05 % sorbate, afiatoxin production was greatly decreased in A. flavus over the control, but only slightly decreased in A. parasiticus.

1427. Bullerman, L.B., Lieu, F.Y., and Seier, S.A. 1977. Inhibition of growth and afiatoxin production by cinnamon and clove oils, cinnamic aldehyde and eugenol. Journal of Food Science 42(4): 1114-1116.

The effects of cinnamon oil, clove oil, cinnamic aldehyde and eugenol on growth and afiatoxin production by Aspergillus parasiticus were studied using yeast-extract sucrose broth as the substrate. All four substances inhibited mold growth and afiatoxin production. Cinnamon and clove oils were inhibitory at 200-250 ppm, cinnamic aldehyde at 150 ppm and eugenol at 125 ppm. Since cinnamic aldehyde and eugenol are the respective major components of cinnamon and clove oils, it was concluded that these are the major active antifungal ingredients of these two essential oils. The inhibitory effect of these substances was judged to be inhibition of growth rather than of afiatoxin production. Given sufficient time, cultures which were inhibited initially, but which subsequently grew, produced afiatoxin levels equivalent to control cultures. Levels of the oils above 250 ppm and of cinnamic aldehyde and eugenol above 250 ppm completely inhibited mold growth, or supported only a small amount of growth that never reached secondary metabolism and never produced afiatoxin during the time of this study.

1428. Chipley, J.R., Story, L.D., Todd, P.T., and Kabara, J.J. 1981. Inhibition of Aspergillus growth and extracellular afiatoxin accumulation by sorbic acid and derivatives of fatty acids. Journal of Food Safety 3(2): 109-120.

A study was conducted to determine the effects of sorbic acid and several derivatives of fatty acids (amides, aminimides, and monoglycerides) on aflatoxigenic cultures of Aspergillus flavus and A. parasiticus. A synthetic medium was inoculated with spores of the aflatoxigenic fungi, incubated for 48 h at 27°C, and then supplemented with sorbic acid and fatty acid derivatives. Cultures were then incubated for an additional five days. Mycelial mats were dried, weighed, and analysed for lipid and mineral content. Aflatoxins were also quantitated. Cerulenin (8 µg mL^{-1}) was the most effective fatty acid derivative examined, reducing mycelial growth by 37 % and completely inhibiting extracellular accumulation of aflatoxins. Other derivatives, in decreasing order of effectiveness included M-20 (an aminimide), lauribic and lauricidin. Inhibition by sorbic acid was nonspecific, affecting both mycelial growth and extracellular afiatoxin accumulation to the same extent. Utilization of fatty acid derivatives for determining mechanisms of afiatoxin accumulation and lipid biosynthesis appears promising.

1429. Chipley, J.R., and Uraih, N. 1980. Inhibition of Aspergillus growth and afiatoxin release by derivatives of benzoic acid. Applied and Environmental Microbiology 40(2): 352-357.

A study was conducted to determine the effect of o-nitrobenzoate, p-aminobenzoate, benzocaine, ethyl benzoate, methyl benzoate, salicylic acid, trans-cinnamic acid, trans-cinnamaldehyde, ferulic acid, aspirin, and anthranilic acid on growth and afiatoxin release in Aspergillus flavus (NRRL 3145) and A. parasiticus (NRRL 3240). Inhibition of mycelial growth and afiatoxin production by various concentrations of these aromatic compounds indicates the possibility of their use as fungicides. However, further investigation is needed to determine the possible toxic effects of any residues. At concentrations of 2.5 and 5.0 mg 25 mL^{-1} medium, methyl benzoate and ethyl benzoate were the most effective in reducing both mycelial growth and afiatoxin production.

1430. Davis, N.D., and Diener, U.L. 1967. Inhibition of aflatoxin synthesis by p-aminobenzoic acid, potassium sulfite, and potassium fluoride. Applied Microbiology 15(6): 1517-1518.

The effects of some chemicals (p-aminobenzoic acid (PABA), potassium sulfite, and potassium fluoride) on growth and aflatoxin production by Aspergillus parasiticus (ATCC 15517) were studied in a culture medium. PABA inhibited aflatoxin production at all concentrations used, and, at higher concentrations, inhibited growth as well. Also, aflatoxin production was reduced up to 50 % in groundnuts that had been treated with PABA solutions. Potassium sulfite inhibited aflatoxin production without significant effects on the growth of the fungus. Potassium fluoride inhibited aflatoxin production only at very high concentration used.

1431. DeLucca, A.J., Palmgren, M.S., and Daigle, D.J. 1987. Depression of aflatoxin production by flavonoid-type compounds from peanut shells. Phytopathology 77(11): 1560-1563.

Groundnut shells contain luteolin, eriodictyol, and 5,7- dihyroxychromone. These flavonoid-related compounds, and a mixture of them, were tested to determine whether they would affect aflatoxin production. Broth medium (50 mL) was amended with 0.01, 0.02 and 0.06 mg mL^{-1} of the individual and mixed compounds, inoculated with 0.1 mL of a spore suspension (1 x 10^6 conidia mL^{-1}) of an aflatoxigcnic isolate of Aspergillus parasiticus and incubated at 27°C. At 4, 7, 11 and 14 days after inoculation, the mycelium was removed, dried and weighed. The medium was extracted and tested for aflatoxin. No differences in mycelial weight were observed among the controls and amended cultures. However, each individual compound, at all concentrations, depressed aflatoxin production as compared with the controls. The mixture of compounds was the most effective on a percentage basis in reducing aflatoxin concentration.

1432. Doupnik, B.Jr, and Bell, D.K. 1971. Inhibition of aflatoxin production in liquid culture by biological dyes. Proceedings of the American Peanut Research and Education Association. 3: 33-34.

Of 24 dyes tested for their inhibitory effects on growth, sporulation. and aflatoxin production by an aflatoxigcnic isolate (NRRL 2999) of Aspergillus flavus in a liquid medium, four (brilliant green, malachite green, gentian violet, and crystal violet) significantly inhibited growth, sporulation, and aflatoxin production at 100 ppm. These four dyes completely inhibited growth at 500 ppm.

1433. Doyle, M.P., Applebaum, R.S., Brackett, R.E., and Marth, E.H. 1982. Physical, chemical and biological degradation of mycotoxins in foods and agricultural commodities. Journal of Food Protection 45(10): 964-971.

Aflatoxin is partially or completely degraded by irradiation, heat, or treatment with strong acids or bases, oxidizing agents or bisulfite. Hydrogen peroxide plus riboflavin denature aflatoxin in milk. Mycelia of Aspergillus parasiticus can degrade aflatoxin, possibly via fungal peroxidase. Such degradation is affected by strain of A. parasiticus, amount of mycelium, temperature, pH and concentration of aflatoxin. Adsorbants, including bentonite and activated charcoal, can physically remove aflatoxin and patulin from liquid foods. Patulin can be degraded by fermenting yeasts and rubratoxin can be degraded by the mycelium of Penicillium rubrum.

1434. Doyle, M.P., and Marth, E.H. 1978. Bisulfite degrades aflatoxin : Effect of temperature and concentration of bisulfite. Journal of Food Protection 41(10) 774-780.

Bisulfite reacted with aflatoxins B_1 and G_1 resulting in their loss of fluorescence This was a first order reaction whose rate depended on bisulfite concentration Aflatoxin G_1 reacted more rapidly with bisulfite than did aflatoxin B_1. In the presence of 0.035 M potassium acid phthalate - Na OH buffer (pH 5.5) + 1.3 % (v/v) methanol at 25°C, the reaction rate constant for degradation of aflatoxin G_1 was 2.23 x 10 h^{-1} and that for aflatoxin B_1 was 1.87 x 10 h^{-1} when 50 mL of reaction mixture contained 1.60 g of K_2SO_3. Besides bisulfite concentration, temperature influenced reaction rates. The Q_{10} for the bisulfite - aflatoxin reaction was approximately 2 while activation energies for degrading aflatoxin B_1 and aflatoxin G_1 were 13.1 and 12.6 Kcal mol^{-1}, respectively. Data suggest that treating foods with 50-500 ppm of SO_2 probably would not effectively degrade appreciable amounts of aflatoxin. Treating foods with 2000 ppm of SO_2, or more, and increasing the temperature, might reduce aflatoxin to an acceptable level.

1435. Doyle, M.P., and Marth, E.H. 1978. Bisulfite degrades aflatoxin : Effect of citric acid and methanol and possible mechanism of degradation. Journal of Food Protection 41(11): 891-896.

Citric acid retarded degradation of aflatoxins B_1 and G_1 by bisulfite. Methanol also retarded degradation of aflatoxins by bisulfite. Presence of citric acid and various concentrations of methanol also reduced rates at which free bisulfite concentration changed. From these observations, known effects of methanol and probable effects of citric acid on bisulfite oxidation, it is suggested that degradation of aflatoxin by bisulfite is dependent on bisulfite oxidation. Most of the degradation product(s) were in the water soluble phase, indicating that a structural modification of aflatoxin occurred.

1436. Draughon, F.A., and Ayres, J.C. 1981. Inhibition of aflatoxin production by selected insecticides. Applied and Environmental Microbiology 41(4): 972-976.

This investigation determined growth and aflatoxin production by Aspergillus parasiticus when selected insecticides, i.e., naled (NL). dichlorvos (DC), Landrin (LD), pyrethrum (PY), Sevin (SV), malathion (ML), Diazinon (DZ), methoxychlor and piperonyl butoxide, were incorporated into liquid medium, and identified some factors contributing to insecticide inhibition of aflatoxin production. NL at 100 ppm completely inhibited production of aflatoxins B_1, B_2, G_1 and G_2 and growth of A. parasiticus. DC, LD, PY, SV, ML and DZ at concentration of 100 ppm significantly inhibited production of aflatoxins, but at 10 ppm inhibition was found only with NL, DC, SV, LD and PY. Growth of the fungus was inhibited by 28.9, 18.9, 15.7 and 100 % by 100 ppm of DC. LD. SV and NL, respectively. Stimulation of growth occurred when DZ was added to cultures.

1437. Dutton, M.F., and Anderson, M.S. 1980. Inhibition of aflatoxin biosynthesis by organophosphorous compounds. Journal of Food Protection 43(5): 381-384.

Effects of a range of organophosphorus and various other compounds on production of aflatoxin by Aspergillus flavus were investigated. Five organophosphorus compounds. Chlormephos, Ciodrin, Naled, Phosdrin and Trichlorphon, at concentrations of 20 and 100 µg mL^{-1} of liquid medium were found to have activity similar to dichlorvos, in that they inhibited aflatoxin production and caused formation of several anthraquinone pigments. Two of these pigments have not previously been described; one was named Versicol whilst the other was its acetate derivative. A rationale is presented for the required elements of structure that are necessary for an organophosphorus compound to have dichlorvos-type activity. Two unrelated compounds, ammonium nitrate and tridecanone were also found to elicit dichlorvos-type activity. It is likely that tridecanone or its breakdown products competitively inhibit enzymes involved in aflatoxin biosynthesis. It is possible that this inhibition effect explains the inhibition of aflatoxin production in lipid- rich commodities infected by A. flavus

1438. El-Gazzar, F.E., Rural, G., and Marth, E.H. 1986. Growth and aflatoxin production by Aspergillus parasiticus in the presence of sodium chloride. Journal of Food Protection 49(6): 461-466.

The effect of sodium chloride on growth and aflatoxin production by an aflatoxin-producing strain of Aspergillus parasiticus NRRL 2999 was studied in glucose-yeast-salt broth. The culture was incubated at 13 or 28°C. Increasing the concentration of sodium chloride (2 to 10 %) reduced accumulation of aflatoxin and also induced a lag in growth of the culture. At 13°C, the mold produced small amounts of aflatoxin after an extended lag phase. Sodium chloride was markedly more inhibitory at 13 than at 28°C.

1439. Farag, R.S., Daw, Z.Y., and Abo-Raya, S.H. 1989. Influence of some spice essential oils on Aspergillus parasiticus growth and production of aflatoxins in a synthetic medium. Journal of Food Science 54(1): 74-76.

Effects of some essential oils from herbs and spices on growth and aflatoxin production by Aspergillus parasiticus were studied. Gas-liquid chromatography was used to determine me essential oil compositions of thyme, cumin, clove, caraway, rosemary, and sage. The basic components of these oils were thymol, cumin aldehyde, eugenol, carvone. bomeol and thujone, respectively. The essential oils caused complete inhibition of both mycelial growth and aflatoxin production. The effectiveness followed the sequence : thyme > cumin > clove > caraway > rosemary > sage. The major components of the essential oils produced an inhibitory effect at minimum inhibitory concentrations equal to those obtained with the oils.

1440. Farag, R.S., El-Leithy, M.A., Basyony, A.E., and Daw, Z.Y. 1987. Growth and aflatoxin production by Aspergillus parasiticus in a medium containing plant hormones, herbicides or insecticides. Journal of Food Protection 50(12): 1044-1047.

Aflatoxin production by Aspergillus parasiticus in a synthetic medium was significantly increased in the presence of 10, 20. or 30 ppm of growth regulator indole-3-acetic acid (IAA) or 10 ppm (but not 20 or 30 ppm) of gibberellic acid (GA) ; mycelial growth was increased in the presence of 10 ppm of GA and decreased in the presence of 20 or 30 ppm of IAA or GA. The herbicide Treflan [trifluralin] at 5, 10 and 20 ppm had a stimulatory effect upon growth and aflatoxin production, whereas the herbicide Stomp |pendimethalin| had the reverse effect at 10 and 20 ppm, and Gramoxone [paraquat | at 5 ppm increased and at 20 ppm decreased aflatoxin production. Malathion at 5 ppm stimulated aflatoxin production, but malathion at 10 and 20 ppm and actellic [pirimiphos-methylj and guthion [azinphos-methyl], at 5, 10 and 20 ppm, decreased both growth and aflatoxin production, effectiveness being in the order guthion > actellic > malathion. At the recommended application rates, all products, except IAA and trifluralin, were inhibitory to growth and aflatoxin production.

1441. Fonseca, H., FIlho, A.S., Soave, J., and Filho, V.C. 1976. |Study of the chemical control of Aspergillus flavus and aflatoxin production in groundnut (Arachis hypogaea L.) in the windrow.]. Estudo do controle quimico do Aspergillus flavus e producao de aflatoxina no amendoim (Arachis hypogaea L.) no campo. Anais da Escola Superior de Agricultura "Luiz de Queiroz". 33: 337-347.

The possibility of chemical control of Aspergillus flavus to prevent the production of aflatoxin in groundnuts by spraying fungicides to the freshly-dug pods in me windrows was studied. Four fungicides, viz., ferbam, thiram, sodium orthophenylphenate and captafol were used in experiments run for 4 years in

different regions. When the harvest was carried out in rainy periods, the fungicides seemed to be inefficient, and when rains were absent the weather condition by itself seemed to inhibit the growth of A. flavus and so prevented the occurrence of aflatoxin. Nevertheless, more studies on the subject are suggested.

1442. Ghewande, M.P., and Nagaraj, G. 1987. Prevention of aflatoxin contamination through some commercial chemical products and plant extracts in groundnut. Mycotoxin Research 3: 19-24.

The effect of rock salts, sodium chloride, propionic acid, NCP.75, plant products-asafoetida, turmeric powder and aqueous leaf extracts of Azadirachta indica, Lawsonia alba, Pongamia glabra and Tridax procumbens on seed colonization and aflatoxin production by Aspergillus flavus was studied in two Spanish bunch groundnut varieties (J 11 and JL 24). All these treatments inhibited in vitro seed colonization and aflatoxin production to varying degrees. Inhibition of seed colonization with chemicals, plant products and aqueous leaf extracts ranged from 17 to 96 %, 27 to 100 % and 8 to 75 %, while inhibition of aflatoxin production ranged from 14 to 74 %, 42 to 71 %, and 6 to 64 %. respectively. In general, salts (20 g L^{-1}), propionic acid (10 mL L^{-1}), asafoetida (pure 1 g L^{-1} and impure 20 g L^{-1}), and Azadirachta indica aqueous leaf extract (20 g L^{-1}) proved better in inhibiting aflatoxin production in both the varieties than other chemicals, plant products and aqueous leaf extracts tested.

1443. Ghewande, MP., Nagaraj, G., and Reddy, P.S. 1989. Aflatoxin research at the National Research Centre for Groundnut. Pages 237-243 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 October 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

The potential of Aspergillus flavus isolates to produce anatoxins, and the toxicity of their culture filtrates to germinating seeds and seedlings of groundnut were studied. Isolates NRRL 3000 and V3734/K) produced high levels of aflatoxins in culture. Culture filtrates from the isolates and from NRCGAFA 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, S230, derivatives of S230 x PI 337394F, Latur 33 x PI 337394F. and the wild species. Arachis 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 by NRRL 3000, but only trace levels were produced in A. cardenasii and A. duranensis. Aflatoxins were found (range of 27-146 µg kg^{-1}) in commercial groundnut cake and in de-oiled 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 colonization by A. flavus and of aflatoxin production in in vitro inoculation tests.

1444. Hitokoto, H., Morozumi, S., Wauke, T., Sakai, S., and Ueno, I. 1978. Inhibitory effects of condiments and herbal drugs on the growth and toxin production of toxigenic fungi. Mycopathologia 66(3): 161-167.

The effects of 13 kinds of powdered herbal drugs and seven kinds of commercial dry condiments on the growth and toxin production of Aspergillus parasiticus, A. flavus, A. ochraceus, and A. versicolor were studied by introducing these substances/drugs into culture media. Of the twenty substances tested, cinnamon bark completely inhibited the fungal growth, while the others only inhibited the toxin production. The inhibitors were easily extracted from the substances/drugs with solvents such as hot water, chloroform, or ethanol. The extracts from coptis. philodendron bark, mustard, green tea leaves, and zanthoxylum completely inhibited aflatoxin production by A. parasiticus, however, they had little or no inhibitory effect against A. flavus.

1445. Hitokoto, H., Morozumi, S., Wauke. T., Sakai, S., and Kurata, H. 1980. Inhibitory effects of spices on growth and toxin production of toxigenic fungi. Applied and Environmental Microbiology 39(4): 818-822.

The inhibitoy effects of 29 commercial powdered spices on growth and toxin production of three species of toxigenic Aspergillus were investigated by introducing these materials into culture media. Three spices, namely, cloves, star anise seeds, and allspice, completely inhibited growth of toxigenic strains of A. flavus, A. versicolor and A. ochraceous, whereas most of the other spices inhibited only the toxin production (aflatoxin, ochratoxin and sterigmatocystin). Eugenol extracted from cloves and thymol from thyme caused complete inhibition of the growth of A. flavus and A. versicolor at 0.4 mg mL^{-1} or less. At a concentration of 2 mg mL^{-1}, anethol extracted from star anise seeds inhibited the growth of all the toxigenic strains of Aspergillus spp.

1446. Holomquist, G.V., Walker, H.W., and Stahr, H.M. 1983. Influence of temperature, pH, water activity and antifungal agents on growth of Aspergillus flavus and A. parasiticus. Journal of Food Science 48: 778-782.

Effects of temperature, pH, water activity, and nine antifungal agents on growth of Aspergillus flavus and A. parasiticus were studied using Sabouraud-dextrose agar and corn as the substrates. Maximal growth of the two molds occurred at 33°C, the highest temperature used, pH of 5.0 and water activity of 0.99. At 15°C, growth was observed at water activity of 0.95 but not 0.90. Slight growth was observed at water

activity of 0.85 at 27°C and 33°C. All antifungal agents (Botran, Orthocide, Folyram 80, Topsin-M, Thiram. Imazalil, sodium propionate, sodium sulfite and DDVP) showed antifungal activity, but Imazalil and DDVP were the most effective at the lowest concentrations. Activity of the antifungal agents increased as the water activity was decreased.

1447. Hsieh, D.P.H. 1973. Inhibition of aflatoxin biosynthesis of dichlorvos. Journal of Agricultural and Food Chemistry 21: 468- 470.

Dichlorvos (dimethyl 2,2-dichlorovinyl phosphate) was found to possess a strong inhibitory effect on aflatoxin biosynthesis by Aspergillus parasiticus ATCC 15517. At 10 ppm it inhibited 90 % of aflatoxin production in three types of liquid media without affecting the fungal growth. When the actively synthesizing cultures were supplemented with 10 ppm of dichlorvos and [1-^{14}C] acetate, a reduced amount of aflatoxin B_1 was produced which contained almost no label from the acetate, suggesting that dichlorvos inhibits an early step in the secondary metabolic pathway for aflatoxin biosynthesis. Experiments with other organophosphorus insecticides showed that dichlorvos was particularly inhibitive to the biosynthesis of aflatoxin.

1448. Jackson, C.R. 1967a. Studies on control of peanut pod fungi. Part 1. Effects of pre-planting soil fumigants on peanut pod surface fungi at harvest. University of Georgia, College of Agriculture Experiment Stations, Athens, Georgia, Research Report 11 : 1-8.

In 1966 and 1967 field experiments in Tifton, Georgia, Vapam was used at rates of 115 and 260 mL per 100 feet of row and Vorlex at 115 mL per 100 feet of row as pre-planting treatments for groundnuts. DD was included as a nematicide check treatment. Use of soil fumigants did not increase yield, reduce pod mycoflora, or indirectly suppress the formation of aflatoxins in Argentine groundnuts.

1449. Jackson, C.R. 1967b. Studies on control of peanut pod fungi. Part II. Value of fungicidal treatment of windrowed peanuts in post-harvest reduction of pod-borne fungi and aflatoxins. University of Georgia, College of Agriculture Experiment Stations, Athens, Georgia, Research Report No. 11 : 9-18.

The possibility of chemical control of fungi invading pods and seeds of groundnuts by spraying fungicides onto the freshly-dug pods in the windrows was studied. Fungicides were applied 12 h after lifting groundnuts (cultivar Early Runner). Fungicides applied were : (i) Difolatan - 0.67 kg ; (ii) triphenyltin hydroxide (DuTer) - 0.67 kg ; (iii) Tri-basic copper sulfate (TBCS) - 0.67 kg ; (iv) tetrachloroisophthalonitrile (DAC 2787) - 0.67 kg ; (v) Captan - 0.89 kg ; (vi) sodium propionate - 1.78 kg and 3.57 kg ; (vii) sulfur - 3.57 kg and (viii) tap water (control). Difolatan and TBCS reduced development of pod surface fungi. Kernels from these treatments also had fewer fungi than the nontreated controls. Aflatoxin contents of kernels from slowly dried pods, which had been treated previously with various fungicides in the windrow, were not related closely to observed efficacy of fungicides in controlling pod surface fungi or fungi from kernels.

1450. Kannaiyan, J., Sandhu, R.S., and Phiri, A.L. 1989. Aflatoxin and Aspergillus flavus contamination problems of groundnuts in Zambia. Pages 65-70 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 Oct 1987, ICRISAT Center. India. Patancheru. A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

In Zambia, groundnut kernels meant for export are routinely monitored for aflatoxin contamination. Since 1979, 6.3 % of the 28410 samples analyzed had contamination levels of more than 5 µg aflatoxin kg^{-1}. A 2-year study with promising varieties revealed the variability of Aspergillus flavus seed infection. Seed treatment with Benlate$^{(R)}$/Labilite$^{(R)}$ at 3 g kg^{-1} seed was found to control A. flavus in groundnut seed and can improve crop stand.

1451. Lindsey, D.L., and Turner, R.B. 1975. Inhibition of growth of Aspergillus flavus and Trichoderma viride by peanut embryos. Mycopathologia 55(3): 149-152.

Growth of Aspergillus flavus and Trichoderma viride on agar media was inhibited around embryos of green groundnut seeds but not around those of cured, mature seeds, intact seeds or testae ; both colonized intact seeds and testae. Substances inhibitory to the fungi were extracted with acetone from cotyledons of freshly harvested seeds. Four compounds inhibitory to A. flavus were detected in crude acetone extracts ; three of these had phenolic properties. The inhibitory compounds in groundnut cotyledons may be factors in protecting the embryo from fungal infection.

1452. Madaan, S.L., and Chohan, J.S. 1978. Efficacy of antimicrobial chemicals to control postharvest occurrence of Aspergillus flavus in groundnut kernels. Indian Phytopathology 31(1): 57-59.

When mature, freshly-harvested groundnut pods were sprayed with propionic add (5 %), sorbic acid (0.1 %) or chlorothalonil (0.15 %), invasion by Aspergillus flavus and the consequent formation of aflatoxins in the kernels was prevented.

1453. Madhyastha, M.S., and Bhat, R.Y. 1984. Aspergillus parasiticus growth and aflatoxin production on black and white pepper and the inhibitory action of their chemical constituents. Applied and Environmental Microbiology 48(2): 376-379.

Growth and aflatoxin production by Aspergillus parasiticus (NRRL 2999) in black

and white pepper over various incubation periods were studied. The effects of piperine and pepper oil on growth and aflatoxin production were also studied. Black and white pepper supported aflatoxin production (62.5 and 44 ng g^{-1}, respectively) over 30 days of incubation. Fungal growth measured in terms of chitin was considerably less in white than black pepper. A histological study of black pepper corns showed the fungus penetrating up to the inner mesocarp and establishing itself in the middle mesocarp. Piperine and pepper oil inhibited fungal growth and aflatoxin production in a dose-dependent manner.

1454. Marshall, D.L., and Builerman, L.B. 1986a. Effect of sucrose esters in combination with selected mold inhibitors on growth and aflatoxin production by Aspergillus parasiticus. Journal of Food Protection 49 (5): 378-382.

The effects of sucrose esterified with a mixture of palmitic and stearic acids (commonly referred to as sucrose ester) in combination with cinnamon, potassium sorbate, or calcium propionate on growth and aflatoxin production by Aspergillus parasiticus were studied in broths at two pH values. Cinnamon in combination with sucrose ester did not result in additive or synergistic inhibitory effects on growth or aflatoxin production. At pH 4.0, subinhibitory levels of cinnamon were stimulatory toward growth and antagonistic to inhibition of growth by sucrose ester. Complete inhibition of growth and aflatoxin production was observed with a cinnamon level of 1.0 %, alone and in combination with sucrose ester. Low levels (0.1 %) of calcium propionate or potassium sorbate combined with sucrose ester did not enhance inhibition of growth or aflatoxin production. A synergistic effect on inhibition of growth was observed with high levels of propionate or sorbate in combination with sucrose ester, while aflatoxin production remained relatively unaffected. However, subinhibitory levels of propionate resulted in a 10-fold increase of aflatoxin production and a shift in the ratio of aflatoxin B_1 to G_1 from 1:1 to 1:8. Subinhibitory levels of sorbate also caused a stimulation of aflatoxin production during the latter stages of incubation, though to a lesser degree than propionate.

1455. Marshall, D.L., and Builerman, L.B. 1986b. Antimicrobial activity of sucrose fatty acid ester emulsifiers. Journal of Food Science 51(2): 468-470.

Six sucrose esters substituted to different degrees with a mixture of fatty acids (palmitic and stearic) were examined for antimicrobial properties. Growth and acid production of several lactic acid bacteria and growth of Saccharomyces cerevisiae were not inhibited by 0.2 % of the sucrose esters in the test medium. Antimycotic activity was detected against several mold species of Aspergillus, Penicillium, Cladosporium, and Alternaria. The least substituted sucrose ester was the most active in reducing mold growth. Reduction of mold growth ranged from 37 to 91 % with this ester at a 1 % concentration. Inhibitory activity did not appear to be influenced by changes in pH. Aflatoxin production by A. parasiticus was not affected by 0.1 % sucrose ester.

1456. Mixon, A.C., Bell, D.K., and Wilson, D.M. 1984. Effect of chemical and biological agents on the incidence of Aspergillus flavus and aflatoxin contamination of peanut seed. Phytopathology 74(12): 1440-1444.

Chemical and biological soil amendments were investigated for effects on seed infection by fungi of the Aspergillus flavus group (A. flavus and A. parasiticus) and, in certain instances, on aflatoxin contamination of one or more genotypes of groundnut. During 1976, 1977, 1981, and 1982, granular and/or liquid pesticide formulations and Trichoderma harzianum were applied either as soil amendments or as postharvest liquid applications to groundnut pods. In 1981 and 1982, soil treatments also were applied to plots treated with surface applications of 0, 673, and 1345 kg of gypsum $hectare^{-1}$. Genotypes grown with chemicals applied to either the soil (both alone and in certain combinations) or to the pods of these genotypes varied in infection of the seed by the A. flavus group fungi. Gypsum applications reduced the percentage of seed infected by these fungi. Applications of gypsum also enchanced the control of seed infection in plots treated with T. harzianum, PCNB-fensulfothion, or CGA 64250, but treatment and genotype interactions were noted. No aflatoxin was detected in groundnuts harvested from gypsum- treated plots, but it was occasionally found in groundnuts from the non-gypsum treatments resulting in a highly significant treatment x genotype interaction.

1457. Niyomca, P., and Suttajit, M. 1988. Inhibitory effects of benzoic, propionic and sorbic acids on growth and aflatoxin production of Aspergillus flavus in peanuts and corn. Proceedings of the Japanese Association of Mycotoxicology Supplement 1: 83-84.

Growth of Aspergillus flavus (ATCC 15546) and its aflatoxin production were completely inhibited in groundnuts and maize treated with acid mixtures of benzoic : propionic, benzoic : sorbic and propionic : sorbic at 0.2:0.1, 0.2:0.3 and 0.1:0.5 %, respectively. When the chemicals were mixed with feed given to male Wistar rats, there was no decrease in growth of the rats. Aqueous and alcoholic extracts from the treated seeds showed neither toxicity upon development of chick embryo nor mutagenicity using Ames bacterial test.

1458. Paulsen, M.R., Brusewitz, G.H., Clary, B.L., Odell, G.V., and Pominski, J. 1976. Aflatoxin content and skin removal of Spanish peanuts as affected by treatments with chemicals, water spray, heated air, and liquid nitrogen. Journal of Food Science 41(3): 667-671.

Unshelled Spanish groundnuts contaminated with aflatoxin were shelled and dried with heated air, liquid nitrogen, H_2O_2, HCl, sodium oleate and water spray. After passing through a whole nut blancher the ailatoxin content and percentages of blanched and whole kernels were determined. Blanching percentages were significantly higher for sodium oleate, water spray, liquid nitrogen and M_2O_2 than for HCl or heat treatments. The heat treatments produced the highest percentage of whole kernels. In most tests, the non-blanched kernels had higher levels of aflatoxin than had those that blanched fully. The treatments which were most effective in producing low levels of aflatoxin in the blanched kernels were H_2O_2, water spray and HCl.

1459. Pettit, R.E., Taber, R.A., Schroeder, H.W., and Harrison, A.L. 1971. Influence of fungicides and irrigation practice on aflatoxin in peanuts before digging. Applied Microbiology 22(4): 629-634.

Groundnuts (cultivar Starr) grown rainfed under drought stress conditions had markedly higher levels of Aspergillus flavus infection and aflatoxin contamination of kernels before digging than had groundnuts grown under irrigation, in field trials at Yoakum and Stephenville, USA, in the 1967, 1968 and 1969 seasons. Levels of seed infection and aflatoxin contamination were considerably higher in 1967 and 1969 than in 1968, A. Jlavus infection and aflatoxin contamination levels were higher in drought-stressed groundnuts produced at Yoakum than in groundnuts produced at Stephenville. In 1969 some samples of freshly-dug groundnuts from irrigated plots had high levels of A. flavus infection (16-59 %), but no aflatoxin was detected in these samples. Absence of aflatoxin in these samples is attributed to high seed moisture levels and to low soil temperatures (10 to 21°C). Several fungicides applied to the soil and foliage did not affect fungal infection and aflatoxin contamination of groundnuts.

1460. Premlata Singh, and Sinha, K.K. 1986. Inhibition of aflatoxin production on some agricultural commodities through aqueous plant extracts. Journal of the Indian Botanical Society 65(1): 30-32.

Of 22 plant extracts screened, aqueous extracts of Adiantum sp., Euphorbia hirta, Gynandrupsis pentaphylla, Justicia gendarussa and Thuja orientalis significantly inhibited aflatoxin production by Aspergillus parasiticus on agricultural commodities, including rice, wheat, maize and groundnut.

1461. Rambo, G.W., and Bean, G.A. 1971. Reduction of aflatoxin production in peanuts in the presence of dimethyl sulfoxide. Phytopathology 61: 907.

The effect of dimethyl sulfoxide (DMSO) on the production of aflatoxin by Aspergillus flavus (ATCC 2221) in groundnuts was determined. Fifty g of dry groundnuts (cultivar Early Runner) were soaked for 30 min in distilled water containing 0.0, 0.6, 1.2, 2.5, 5.0, 10.0 or 20 % DMSO. After soaking, they were autoclaved and inoculated with a spore suspension of A. Jlavus. The cultures were incubated for 7 days at 24°C, and aflatoxins were extracted. Toxin production in cultures containing 0.6 and 1.2 % DMSO was comparable to the controls. However, at concentrations of 2.5 % and above, aflatoxin production decreased rapidly. At 20 % DMSO, little or no fungal growth was detected. These results were verified by a bioassay using chick embryos.

1462. Rambo, G.W., and Bean, G.A. 1973. Treatment of peanuts with dimethyl sulfoxide and its effect on aflatoxin production by Aspergillus Jlavus. Phytopathology 63(7): 936-937.

Treatment of groundnut seeds (cultivar Early Runner) with 2.5 % or higher concentration of dimethyl sulfoxide (DMSO) prior to inoculation with the aflatox in-producing fungus Aspergillus Jlavus caused an inhibition of normal condial pigmentation and 62-64 % inhibition of aflatoxin production.

1463. Rao, H.R.G., and Harein, P.K. 1972. Dichlorvos as an inhibitor of aflatoxin production on wheat, corn, rice, and peanuts. Journal of Economic Entomology 65(4): 988-989.

In laboratory tests, wheat, maize, rice and groundnuts were treated with various concentrations of dichlorvos either before or after infection with Aspergillus Jlavus. Treatment with dichlorvos at concentration of 20 ppm before infection prevented aflatoxin production on wheat. However, production of aflatoxin occurred when the dichlorvos concentration before infection was less than 20 ppm, when the treatment followed infection, and when the substrate was maize, rice or groundnut.

1464. Ray, L.L., and Bullerman, L.B. 1982. Preventing growth of potentially toxic molds using antifungal agents. Journal of Food Protection 45(10): 953-963.

Inhibition of mold growth and mycotoxin production by use of simple chemicals, antibiotics and natural plant products was reviewed, especially considering treatment of foods. Topics discussed included effects of organic acids, antibiotics, herbs and spices, essential oils, insecticides and fumigants, phenolic antioxidants, methylxanthines, and chlorine.

1465. Rusul, G., and Marth, E.H. 1988. Food additives and plant components control growth and aflatoxin production by toxigenic aspergilli : A review. Mycopathologia 101: 13-23.

This review paper summarizes information on effects of commonly used food

additives or preservatives and chemicals on growth and aflatoxin production by Aspergillus parasiticus and A. flavus. Growth and aflatoxin production by toxigenic aspergilli are partially or completely inhibited by the undissociated form of acetic, benzoic, citric, lactic, propionic and sorbic acids. Salts such as sodium chloride, potassium chloride and sodium nitrate, at low concentrations, can enhance aflatoxin production. At higher concentrations they become inhibitory, but marked inhibition requires amounts of the salts greater than are commonly used in foods. Phenolic antioxidants, sometimes added to foods to prevent oxidative deterioration, also are inhibitory to toxigenic aspergilli. Other inhibitory agents include certain insecticides, methylxanthines (caffeine and theophylline and components of some herbs, spices and other plants.

1466. Schroeder, H.W., Cole, RJ., Grigsby, R.D., and Hein, H. 1974. Inhibition of aflatoxin production and tentative identification of an aflatoxin intermediate 'versiconal acetate' from treatment with dichlorvos. Applied Microbiology 27: 394-399.

Aflatoxin production by Aspergillus flavus and A. parasiticus was greatly reduced in vitro in the presence of the insecticide dichlorvos. Reductions in the production of aflatoxins were accompanied by the appearance of an orange pigment. Spectral analyses of the pigment and of its methylated and acetylated derivatives indicated the compound to be versiconal acetate (IV). The data suggest that IV is an intermediate in the metabolic cycle that may terminate in the production of aflatoxin or of the versicolorins, or both. Dichlorvos apparently inhibits biosynthesis of the difurano ring structure common to the aflatoxins and the versicolorins.

1467. Sharma, A., and Padwal-Desai, S.R. 1989. A note on aflatoxin biogenesis in the presence of benomyl and carbendazim. Journal of Food Science and Technology 26(6): 366-367.

Aflatoxin biogenesis by Aspergillus parasiticus in the presence of benomyl and carbendazim was studied in a culture medium. Sub- inhibitory concentrations of the fungicides stimulated biosynthesis of all the four aflatoxins B_1, B_2, G_1 and G_2 in culture. At a higher concentration (2.4 µg kg^{-1}) of the fungicides, the fungus lost the ability to form a mycelial mat. Instead, abnonnal growth in the form of giant cells and a poorly differentiated mycelium was observed in such cultures, which failed to produce aflatoxin.

1468. Sharma, A., Padwal-Desai, S.R., and Nadkarni, G.B. 1985. Possible implications of reciprocity between ethylene and aflatoxin biogenesis in Aspergillus flavus and Aspergillus parasiticus. Applied and Environmental Microbiology 49(1): 79-82.

Aspergillus flavus and A. parasiticus produced ethylene during early growth. However, the onset of aflatoxin biosynthesis was marked by the absence of ethylene evolution. 2-chloroethyl phosphoric acid, an ethylenc-gcnerating compound, inhibited aflatoxin biosynthesis in vivo. The reciprocal relationship between the production of aflatoxin and ethylene by the organism may indicate the involvement of the latter in the regulation of aflatoxin biogenesis. Prevention of aflatoxin biogenesis by ethylene and ethylene-generating compounds could help in devising newer methods of storage of agricultural products.

1469. Sharma, A., Padwal-Desai, S.R., and Nadkarni, G.B. 1987. A new method for aflatoxin-free storage of agricultural commodities. Journal of Food Science 52(2): 497-499.

Aflatoxin production was observed in the 2-kg lots of groundnuts and maize that were stored for 90 days at ambient temperatures 28-30°C and R1I 100 % after infection with an aflatoxin-producing strain of Aspergillus parasiticus (NRRL 3145). Treatment of the samples with an aqueous solution of 2-chloro ethylphosphonic acid (ethephon) prevented aflatoxin formation in both commodities, whereas the nontreated lots supported aflatoxin production.

1470. Sharma, A., Shrikhande, A.J., Padwal-Desai, S.R., and Nadkarni, G.B. 1978. Inhibition of aflatoxin-producing fungi by ethyl acetate extracts from gamma-irradiated potatoes. Potato Research 21(1): 31-34.

Ethyl acetate extracts prepared from potatoes exposed to a sprout-inhibiting dose (10 Krad) of gamma irradiation were tested for inhibitory activity towards Aspergillus flavus and A. parasiticus. The treatment did not adversely affect the naturally occurring compounds which inhibit growth of these aflatoxin-producing fungi and which were still evident 4 weeks after irradiation following storage at 15°C.

1471. Sharma, A., Tewari, G.M., Shrikhande, A.J., Padwal-Desai, S.R., and Bandyopadhyay, C. 1979. Inhibition of aflatoxin-producing fungi by onion extracts. Journal of Food Science 44: 1545-1547.

Various extracts of onion were tested for their inhibitory activity against the growth of the aflatoxigenic fungi, Aspergillus flavus and A. parasiticus. Ether extract and lachrymatory factor (LF), which has been earlier identified as thiopropanal-s-oxide, were found to have potent antifungal activity. Steam-distilled onion oil, which is devoid of LF, was not as potent as ether extract and LF. Its major component, dipropyldisulfide was ineffective as a fungal inhibitor. Ethyl acetate extract containing phenolics was also ineffective. Exposure of onions to gamma-irradiation at a sprout-inhibiting dose (6 krad) did not alter the inhibitory potency of the onion extracts which, however, appeared to be heat-labile.

1472. Stewart, R.G., Wyatt, R.D.. and Ashmore, M.D. 1977. The effect of various antifungal agents on aflatoxin production and growth characteristics of Aspergillus parasiticus and Aspergillus flavus in liquid medium. Poultry Science 56: 1630-1635.

Effects of selected antifungal compounds on growth and aflatoxin production by the aflatoxin-producing fungi, Aspergillus flavus and A. parasiticus, in a liquid medium were studied. Of the antifungal agents evaluated, propionic acid and crystal violet were the most effective in retarding mold growth. Propionic acid was fungicidal at concentrations > 3.0 µg mL^{-1} whereas crystal violet exhibited a mold retarding activity at levels ≥ 2.0 µg mL^{-1}. Crystal violet retarded the growth rate of the mold during the initial stages of growth ; however, this retardation was overcome after 10 days of incubation. Crystal violet also inhibited aflatoxin production and sporulation of A. parasiticus. A survey involving 12 toxigenic isolates of A. parasiticus and A. flavus indicated that these species vary markedly in susceptibility to crystal violet.

1473. Swaminathan, B.J., and Kuehler, P.E. 1976. Isolation of an inhibitor of Aspergillus parasiticus from white potatoes {Solarium tuberosum). Journal of Food Science 41: 313-319.

A study was conducted to evaluate growth of Aspergillus parasiticus on white potato. White potatoes did not support the growth of A. parasiticus (NRRL 2999) unless they were previously heated to above 60°C. The mold grew well on autoclaved potatoes and produced, on an average, 8 µg g^{-1} total aflatoxins (mainly B_1 and G_1) at 27°C and 95-97 % relative humidity in 20 days. When soluble potato starch was substituted for sucrose in yeast-extract sucrose (YES) medium, the maximum growth of A. parasiticus was 80 % of that observed in YES medium, but total aflatoxin production was only 0.5-1 % of the production in YES medium. Chlorogenic acid and solamine. at levels normally found in the white potato, did not show appreciable fungitoxic activity towards A. parasiticus in vitro. Caffeic acid partially inhibited the growth of A. parasiticus and the production of aflatoxins at 0.01 M concentration in YES medium. A compound with high inhibitory activity towards A. parasiticus was isolated from the potatoes. The compound is cxtractable with ethyl acetate. From its behavior on TLC systems, its ultraviolet spectrum and its reactions with chromagenic spray reagents, it is inferred that the compound is phenolic in nature. It appears to be a hydroxy-cinnamic acid derivative structurally similar to caffeic acid but lacking the ortho-dihydroxy structure of caffeic acid.

1474. Tango, J.S., and Tela, R. 1971. [Control of Aspergillus flavus in peanuts during drying period.]. Controle de Aspergillus flavus en amendoim durante o pcriodo de secagem. Coletanea do Instituto de Tecnologia de Alimentos (Brazil) 4: 83-90.

Application of oil no. 3, Maneb. 1 % sulphur powder, 1 % calcium hydroxide solution, and 70 % TMTD powder to groundnut pods during the rainy season reduced aflatoxin contamination.

1475. Tripathi, S.C., Singh, S.P., and Dube, S. 1986. Studies on antifungal properties of essential oil of Trachyspermum ammi (L.) Sprague. Journal of Phytopathology 116(2): 113-120.

The essential oil from Trachyspermum ammi fruits completely inhibited mycelial growth on agar medium of Aspergillus flavus and A. niger at 800 ppm. Its toxicity was not affected by treatment at 100°C. autoclaving or storage for up to at least 1 year. It killed the test fungi within 50 seconds, withstood heavy inoculum density and was inhibitory at 800 ppm to 24 other species of fungi tested. No fungi were recovered from groundnut seeds treated with the oil at 5000 ppm and stored for 1 year, indicating its grain protectant ability. The oil was characterized by various physico-chemical properties and thymol and p-cymene were isolated as its antifungal principles. They completely inhibited mycelial growth of A. flavus and A. niger at 1000 ppm.

1476. Tsuboi, S., and Iwamura, N. 1984. The inhibitory action of mustard on the growth of fungus. ICMR Annals 4: 205-207.

The growth of Aspergillus flavus and other fungi on groundnuts was completely inhibited by mustard. Compenents of mustard were obtained and the inhibitory activity was shown to be due to allyl isothiocyanate.

1477. Uraih, N., Cassity, T.R., and Chipley, J.R. 1977. Partial characterization of the mode of action of benzoic acid on aflatoxin biosynthesis. Canadian Journal of Microbiology 23: 1580-1584.

Aflatoxin production by a toxigenic strain of Aspergillus flavus was greatly reduced by benzoic acid and sodium benzoate in synthetic media. The reduction was accompanied by the appearance of a yellow pigment. Spectral analyses partially characterized this pigment as closely related to an acetyl derivative of a versiconal-type compound. A cell-free extract prepared from A. flavus grown in synthetic media was active in converting this yellow compound into aflatoxin B_1 in the presence of reduced nicotinamide adenine dinucleotide phosphate at 25° C (pH 7.4). In the presence of benzoic acid and its salt or autoclaved cell-free extract, conversion of yellow coumpound to aflatoxin B_1 was prevented. These results suggest that the yellow compound is an intermediate in the secondary metabolic cycle involved in aflatoxin B_1 production. Benzoic acid, sodium benzoate, or autoclaving the cell-free extract appear to block or denature an enzymatic step late in the biosynthetic pathway of aflatoxin B_1.

1478. Uraih, N., and Offonry, S. 1981. Inhibition of aflatoxin production in groundnut with benzoic acid derivatives and possible toxic effect of their aromatic residues. Microbios 31(124): 93-102.

Benzoic acid (10 mg g^{-1}), sodium benzoate (24 mg g^{-1}), ethyl-p- aminobenzoate (10 mg g^{-1}), and salicylic acid (20 mg g^{-1}) inhibited mycelial growth and aflatoxin production by Aspergillus flavus in groundnut. At their effective concentrations these aromatic compounds were non-toxic to 1-day-old chicks. Chicks fed aflatoxin-contaminated diets experienced depressed body and liver weights and their livers manifested various pathological changes. Groundnut samples supplemented with various concentrations of these aromatic compounds did not give a foul odour.

1479. Valcarcel, R., Bennett, J.W., and Vitanza, J. 1986. Effect of selected inhibitors on growth, pigmentation, and aflatoxin production by Aspergillus parasiticus. Mycopathologia 94: 7-10.

Effects of several chemicals on growth and aflatoxin production by Aspergillus parasiticus were investigated. In a defined medium, benzoic acid (2 and 3 mg mL^{-1}), cinnamon (1 mg mL^{-1}), and sodium acetate (5 mg mL^{-1}) were fungitoxic. Benzoic acid (0.5 and 1 mg mL^{-1}), chlorox (5 µg mL^{-1}), and dimethyl sulfoxide (5 µL mL^{-1}) did not affect dry weight or mycelial pigmentation. Soidum benzoate (1, 2, 4 and 8 mg mL^{-1}) added after two days' growth inhibited aflatoxin production in two defined culture media. The authors were unable to confirm previously published reports that an uncharacterized yellow pigment accumulated with benzoate-inhibition of aflatoxin production.

1480. Wales, P., and Somers, E. 1968. Susceptibility of aflatoxin- producing strains of Aspergillus flavus to a range of fungicides. Canadian Journal of Plant Science 48: 377-379.

Twenty-three fungicides were tested for fungistatic activity against three strains of Aspergillus flavus (two aflatoxigenic strains and one nontoxigenic strain). With only three exceptions, no differnce among the three strains in susceptibility to a given fungicide was found. The two most effective fungistats were dichlofluanid and Difolatan; only the latter showed fungicidal activity.

1481. Wei, C.I., Tan, H., Fernando, S.Y., and Ko, N.J. 1986. Inhibitory effect of beta-ionone on growth and aflatoxin production by Aspergillus parasiticus on peanuts. Journal of Hood Protection 49(7): 515-518.

The volatile ketone ß-ionone showed a dose-related inhibition of growth and aflatoxin production by aflatoxigenic Aspergillus parasiticus on groundnuts after they were soaked in distilled water for 25 or 50 min, inoculated with conidia of the fungus, and incubated at 28°C for up to 2 weeks. Aflatoxin B_1 production after 1 week incubation was reduced to < 11.0 and 6.7 % of the control when 2.5 and 5 mL ß-ionone 100 g^{-1}. respectively, were added to water-soaked (25 min) groundnuts. Aflatoxin G_1 production was reduced to 4.7 % (2.5 mL) or 3.3 % (5.0 mL) under the same treatment conditions. Groundnuts treated with > 0.25 mL ß-ionone had only sparse mycelial growth and supported only limited sporulation.

1482. Wheeler, M.H., Bhatnagar, D., and Bennett, J.W. 1988. Inhibition of aflatoxin biosynthesis with chlobenthiazone. Phytopathology 78(12): 1617. (Abstract)

Studies were made to determine if the melanin pathway reductase inhibitor, chlobenthiazone, inhibits aflatoxin synthesis in Aspergillus spp. A. flavus and A. parasiticus were grown in shake cultures containing up to 8 µg mL^{-1} chlobenthiazone. This compound had a strong inhibitory effect on the accumulation of allatoxins B_1 and B_2 in cultures of both fungi. At 8 µg mL^{-1}, it caused a 24 % decrease in the mycelial dry weight of both fungi. Levels of aflatoxin B, in cultures of A. flavus were decreased by 90 and 99 % at 1 and 4 |ig mL^{-1} chlobenthiazone, respectively. levels of aflatoxin B_1 in cultures of A. parasiticus were decreased by 64, 81. and 86 % at 1, 4, and 8 µg mL^{-1} chlobenthiazone, respectively.

1483. Wilson, D.M., Gueldner, R.C., Mckinney, J.K., Lievsay, R.H., Evans, B.D., and Hill, R.A. 1981. Effect of B-ionone on Aspergillus flavus and Aspergillus parasiticus growth, sporulation, morphology and aflatoxin production. Journal of the Association of Oil Chemists' Society 58(12): 959A-961A.

ß-ionone inhibited mycelial growth and sporulation of Aspergillus flavus and A. parasiticus on potato dextrose agar medium. The fungal colonies were restricted, remained buff-colored and had little or no sporulation. No sporulation occurred at levels of 5 µl or above of ß-ionone even after 4 week's incubation. Concentrations of 100 µl and above of ß-ionone L^{-1} in liquid cultures of A. parasiticus (NRRL 2999) inhibited aflatoxin production.

1484. Yang, C.Y. 1972. Comparative studies on the detoxification of aflatoxins by sodium hypochlorite and commercial bleaches. Applied Microbiology 24(6): 885-890.

Cultures of Aspergillus flavus and aflatoxins were destroyed in 5 days by a commercial bleach (Clorox; active ingredient, NaOCl) or analytical reagent grade NaOCl at 7.0 x 10^{-3} M NaOCl. Addition of Clorox or NaOCl at 2.8 x 10^{-3} M to the fungal growth medium prior to inoculation completely inhibited fungal growth. Aflatoxin production was inversely proportional to the logarithm of NaOCl concentration and time of treatment. Clorox and NaOCl were equally effective on

aflatoxins. but fungal cells were lysed more readily by Clorox than by NaOCl. Myceiia older than 8 days lysed more readily than younger ones. Most conidia survived concentrations below 1.4 x 10^{-3} M. The lowest effective concentration for a 2-hr treatment was 8.8 x 10^{-3} M which is well below the Clorox concentration recommended for routine laboratory decontamination of aflatoxins. Mice and rats injected with aflatoxins and aflatoxins incompletely destroyed by Clorox died within 72 hr and had typical liver and kidney damage caused by aflatoxins. However, animals injected with NaOCl or Clorox or Clorox-destroyed aflatoxin extracts survived and showed no obvious liver or kidney damage.

1485. Yao, R.C., and Hseih, D.P.H. 1974. Step of dichlorvos inhibition in the pathway of aflatoxin biosynthesis. Applied Microbiology 28(1): 52-57.

Dichlorvos (dimethyl 2,2-dichlorovinyl phosphate) inhibits the biosynthesis of aflatoxin by Aspergillus parasiticus. Cultures treated with dichlorvos excrete an orange pigment which can be converted into aflatoxin B_1 by the nontreated mycelia. The orange pigment was partially identified as an acetyl derivative of a versiconal-type compound. In the presence of dichlorvos, sterigmatocystin is converted into aflatoxin B_1 without being interfered, but averufin is converted into the orange pigment instead of aflatoxin B_1. Therefore, dichlorvos appears to block an enzymatic step in the aflatoxin biosynthetic pathway, which lies beyond averufin but before sterigmatocystin, at the formation of the orange pigment.

8.2.4 Biological Control

1486. Burnett, C, Rambo, G.W., and Bean, G.A. 1972. Inhibition of Aspergillus flavus and aflatoxin production by Aspergillus niger. Phytopathology. 62(6): 668.

A metabolite produced by Aspergillus niger grown on groundnut kernels inhibited the production of aflatoxins by Aspergillus flavus. A. niger was incubated on autoclaved groundnuts at room temperature for 3,6,9 and 14 days, then the cultures were re- autoclaved and inoculated with A. flavus, incubated at 24°C for 14 days and then the aflatoxins were extracted. The concentration of aflatoxin produced decreased with increasing length of incubation with A niger, those incubated for 14 days with A. niger had only 12 % as much aflatoxins as the controls (without incubation with A. niger). When A. flavus was grown on a 9-day- old culture filtrate of A. niger no aflatoxins were detected and mycelial growth was inhibited Germination of conidia of A. flavus was also retarded in the filtrates although the final percentage germination was the same as in the controls.

1487. Diener, U.L. 1973. Deterioration of peanut quality caused by fungi. Pages 523-557 jn Peanuts : Culture and Uses. American Peanut Research & Education Association, Stillwater, OK, USA.

This paper reviews seed infection by Aspergillus flavus and other fungi, and aflatoxin contamination of groundnuts from the time the groundnuts are growing in the field until they are marketed, stored and shelled. Causes of aflatoxin contamination and methods to control the aflatoxin problem are discussed.

1488. Dorner, J.W., Cole, R.J., and Blankenship, P.D. 1990. The use of a biocompetitive agent to control preharvest aflatoxin in drought stress peanuts. Proceedings of the American Peanut Research and Education Society 22: 34.

A three year study was conducted to evaluate the use of a biocompetitive agent as an effective management strategy for preharvest aflatoxin contamination, The strategy involved the incorporation of a non-aflatoxin producing strain of Aspergillus parasiticus into the soil of an environmental control plot facility. The agent was tested by subjecting groundnuts to ideal conditions for preharvest aflatoxin contamination and comparing the effects with non-treated controls. The biocompetitive agent maintained a dominance over the wild, toxigenic strains of A. flavuslparasiticus for the three year period with no further addition of fungal propagules after the first year. This treatment also resulted in a significant reduction in aflatoxin in edible grade groundnuts compared to non-treated controls. Results from the first year showed that control, non-treated groundnuts averaged 522 µg kg^{-1} aflatoxin, while biocontrol treated groundnuts averaged 11 µg kg^{-1}. The second year, controls contained 96 µg kg^{-1} compared to 1.1 µg kg^{-1} in treated groundnuts. The third year controls had 241 µg kg^{-1} and treated groundnuts 40 µg kg^{-1}. Also of significance, soil populations of the biocompetitive agent were not higher than populations of wild strains of A. flavusl parasiticus that were present in untreated groundnut soils subjected to late-season drought stress. This is an important ecological consideration related to ultimate implementation of this strategy.

1489. Doyle, M.P., Applebaum, R.S., Brackett, R.E., and Marth, E.H. 1982. Physical, chemical and biological degradation of mycotoxins in foods and agricultural commodities. Journal of Food Protection 45(10): 964-971.

Aflatoxin is partially or completely degraded by irradiation, heat, or treatment with strong acids or bases, oxidizing agents or bisulfite. Hydrogen peroxide plus riboflavin denature aflatoxin in milk. Myceiia of Aspergillus parasiticus can degrade aflatoxin, possibly via fungal peroxidase. Such degradation is affected by strain of A. parasiticus, amount of mycelium, temperature. pH and concentration of aflatoxin. Adsorbants, including bentonite and activated charcoal, can physically remove aflatoxin and patulin from liquid foods. Patulin can be degraded by fermenting yeasts and rubratoxin can be degraded by the mycelium of Penicillium rubrum.

1490. Idziak, E.S., and Coallier-Ascah, J. 1984. Streptococcus lactis inhibition of aflatoxin production by Aspergillus flavus. Pages 281-283 in Microbial associations and interactions in food.

Streptococcus lactis is known to produce an extracellular compound which inhibits the production of aflatoxin by Aspergillus flavus. Attempts made to identify the compound revealed that it appears to be a low molecular weight (<500) phosphoglyco-lipid containing an aromatic ring structure. No mutagenic or carcinogenic activity was detected by the Ames test extracts of A. flavus grown in mixed cultures with S. lactis.

1491. Joffe, A.Z. 1969. Relationship between Aspergillus flavus, A. niger, and some other fungi in the mycoflora of groundnut kernels. Plant and Soil 31: 57-64.

Relationships between Aspergillus flavus. A. niger, Penicillium funiculosum, P. rubrum, and Fusarium solani were studied in plate culture with 234 samples of freshly lifted and stored groundnuts obtained from the 1965 and 1966 seasons' crops in different parts of Israel. Pronounced antagonism was noted between A. flavus and A. niger, and rather lower level antagonism between each of these species and P. funiculosum, P. rubrum and F. solani.

1492. Lindsey, D.L. 1970. Effect of Aspergillus flavus on peanuts grown under gnotobiotic conditions. Phytopathology 60: 208-211.

Two varieties of groundnut (Tennessee Red and Virginia Bunch 46- 2) were grown under gnotobiotic conditions, and the pods were inoculated with a conidial suspension of the aflatoxin-producing fungus Aspergillus flavus. No evidence of pathogenicity to groundnut plants or pod rot symptoms was observed. A. flavus penetrated the shell tissue consistently, but was limited in its seed invasion to the testa. Invasion and colonization of the embryos by A. flavus appeared to be limited.

1493. Mixon, A.C., Bell, D.K., and Wilson, D.M. 1984. Effect of chemical and biological agents on the incidence of Aspergillus flavus and aflatoxin contamination of peanut seed. Phytopathology 74(12): 1440-1444.

Chemical and biological soil amendments were investigated for effects on seed infection by fungi of the Aspergillus flavus group (A. flavus and A. parasiticus) and, in certain instances, on aflatoxin contamination of one or more genotypes of groundnut. During 1976, 1977, 1981, and 1982, granular and/or liquid pesticide formulations and Trichoderma harzianum were applied either as soil amendments or as postharvest liquid applications to groundnut pods. In 1981 and 1982, soil treatments also were applied to plots treated with surface applications of 0, 673, and 1345 kg of gypsum ha^{-1}. Genotypes grown with chemicals applied to either the soil (both alone and in certain combinations) or to the pods of these genotypes varied in infection of the seed by the A. flavus group fungi. Gypsum applications reduced the percentage of seed infected by these fungi. Applications of gypsum also enchanced the control of seed infection in plots treated with 7'. harzianum, PCNB-fensulfothion, or CGA 64250, but treatment and genotype interactions were noted. No aflatoxin was detected in groundnuts harvested from gypsum-treated plots, but it was occasionally found in groundnuts from the non-gypsum treatments resulting in a highly significant treatment x genotype interaction.

GENETIC RESISTANCE TO ASPERGILLUS FLAVUS INVASION AND AFLATOXIN PRODUCTION

8.2.5 Resistance to Aspergillus flavus Infection and Colonization

1494. Azaizeh, A.K., and Pettit, R.E. 1987. Influence of tannin-related compounds from peanut seed coats and cotyledons on Aspergillus parasiticus growth and aflatoxin production. Phytopathology 77(12): 1703.

Of 23 genotypes evaluated in a humidity chamber, Florunner. PI 337409, 55-437 and Texas 7 were the most resistant, with low infection by Aspergillus parasiticus and low aflatoxin contamination. Levels of tannin compounds in seed coats and cotyledons differed among genotypes, with levels being higher in seed coats. Some compounds significantly decreased the growdi of A. parasiticus and inhibited the production of aflatoxin.

1495. Azaizeh, H.A., Pettit, R.E., Sarr, B.A., and Phillips, T.D. 1990. Effect of peanut tannin extracts on growdi of Aspergillus parasiticus and aflatoxin production. Mycopathologia 110(3): 125-132.

Twenty-three groundnut genotypes were evaluated for resistance to seed colonization by Aspergillus parasiticus and aflatoxin production when incubated under high relative humidity. Tannin- containing extracts from seed testae and cotyledons of these genotypes were prepared and tested for their effect on growdi of A. parasiticus and aflatoxin production. Seed colonization was low (<30 per cent) in the genotypes Toalson X UF 73-4022 (selections TX-798731 and TX-798736), A72118, 55-437, PI 337409 and Florunner. Genotypes with low levels of seed colonization also had low aflatoxin contamination. Higher levels of tannins were detected in testae

(23.9-97.2 mg g^{-1}) compared with cotyledons (0.17-0.82 mg g^{-1}). Some of the methanol-extracted and water-soluble tannin extracts from testae and cotyledons, when incorporated' into yeast extract sucrose liquid medium (100 mg L^{-1}), significantly inhibited growth of A. parasiticus and aflatoxin production. There was no overall correlation between geotypes and the influence of tannin extracts on A. parasiticus growth and aflatoxin production.

1496. Azaizeh, A.K., Pettit, R.E., Smith, O.D., and Taber, R.A. 1989. Reaction of peanut genotypes under drought stress to Aspergillus flavus and A. parasiticus. Peanut Science 16(2): 109-113.

Seven groundnut genotypes were tested in greenhouse and microplot experiments during 2 consecutive years to determine peg colonization by Aspergillus flavus and to determine the effect of 2 drought stress treatments on the susceptibility of shells and kernels to Aspergillus colonization and aflatoxin contamination. Moisture tensions within the soil and temperature of the soil and air were monitored during these experiments. Colonization of pegs was inconsistent among genotypes and between years. In general, low soil moisture tension enhanced colonization of shells and kernels. Shells of most gentoypes were highly colonized after harvest from each moisture regime. Kernels of all genotypes were more susceptible to A. flavus and A. parasiticus colonization under both long and short drought stress conditions compared with non-stressed conditions. However, no significant differences were obtained in the degree of Aspergillus infection of kernels from PI 337409, Starr and J 11 in the greenhouse experiments. Under microplot conditions, a comparison of several gentoypes revealed that kernels of genotypes TX811956 and TX798736 (short stress treatments) contained significantly lower Aspergillus infection and kernels of PI 337409 and TX811956 TX798736 contained significanUy less aflatoxin.

1497. Bartz, J.A., Norden, A.J., LaPrade, J.C., and DeMuynk, T.J. 1978. Seed tolerance in peanuts (Arachis hypogaea L.) to members of the *Aspergillus* flavus group of fungi. Peanut Science 5(1): 53-56.

Hand-shelled seeds of various groundnut genotypes cured and dried in different ways were assayed for seed colonization by Aspergillus parasiticus in Florida in the years 1971-1974. The assay involved exposing groundnut seed at 20-30 % moisture content to conidia of A. parasiticus in petri plates and incubating at 25°C. After 1 week, the percentage of the seeds with sporulating colonies of the test fungus was determined. Typically, individual lines or cultivars were evaluated on the basis of the average of three plates. However, second or third assays of the same seed lots were done on 45 occasions during the 4-year period. Repeat assays yielded data similar to those from the original assay. But assays of specific lines from different seed lots could give different results unless the date of digging, methods of curing and production location were the same. Some apparent shifts in susceptibility of seed to fungal colonization were quite extreme. One lot of stackpole-cured cultivar 'Altika' had 12 % of seed colonized whereas a windrow-cured seed lot, dug on the same day from the same plot had 77 % of seed colonized. No particular change in the harvesting procedure was consistently associated with increases or decreases in apparent susceptibility. Based on tests of all seed lots of 15 commonly grown cultivars during the years 1971-1974, 'Florunner' was the most tolerant and 'Tifspan' the most susceptible to seed colonization. The three resistant lines PI 337394F, PI 337409 and UF 71513 had markedly lower percentages of seed colonized man had the other cultivars.

1498. Blankenship, P.D., Cole, R.J., and Sanders, T.H. 1985. Comparative susceptibility of four experimental peanut lines and the cultivar Florunner to preharvest aflatoxin contamination. Peanut Science 12: 70-72.

Four peanut genotypes, selected as resistant to invasion by Aspergillus flavus in laboratory screening with rehydrated, stored seed and the cultivar Florunner were subjected to preharvest drought and temperature conditions conducive to A. flavus invasion and aflatoxin contamination. Preharvest ailatoxin contamination of peanuts has been previously correlated with geocarposphere temperature and moisture conditions during drought. All genotypes were highly contaminated with aflatoxin. This study indicates that a critical assessment should be made of the value of using the current laboratory method to select germplasm for resistance to A. flavus invasion and assuming resistance to aflatoxin contamination under field conditions.

1499. Bockelee-Morvan, A., and Giller, P. 1976. [Reducing aflatoxin in groundnuts at the level of agricultural production.]. Reduction de l'aflatoxine de l'arachide au niveau de la production agricole. Cahiers de Nutrition et de Dietetique (2): 101-104.

Possibilities for reducing the aflatoxin contamination of groundnut include the selection of varieties resistant to Aspergillus flavus.

1500. Carter, J.B.H. 1973. The influence of the testa, damage and seed dressing on the emergence of groundnut (Arachis hypogaea). Annals of Applied Biology 74: 315-323.

The effects of damaging the testa and the application of seed dressings were examined in field trials on several short-and long-season cultivars of groundnut with differendy-pigmented testa at Samaru, Nigeria, in 1967. There was a high correlation between the number of seedlings which emerged and the resistance or susceptiblility of the seed to invasion by the fungus Aspergillus flavus, as assessed by laboratory tests. When the testa was not damaged the emergence of white

(susceptible) seed was only 50 % while that of colored (resistant) seed was between 95 and 98 %. Damage to the testa greatly reduced emergence. The application of seed dressing increased the emergence of susceptible seed and also restored the emergence of damaged seeds to the level of undamaged seed. The effect of the complete removal of the testa was not counteracted by seed dressing; naked seeds, with and without dressing, gave 10 % emergence. The importance of pigment in the testa, the condition of the seed and the effects of seed dressing arc discussed.

1501. Davidson, J.I.Jr., Hill, R.A., Cole, R.J., Mixon, A.C., and Henning, R..I. 1982. Field performance of two peanut cultivars relative to resistance to invasion by *Aspergillus flavus* and subsequent aflatoxin contamination. Proceedings of the American Peanut Research and Education Society 14(1): 74.

Two runner type groundnut cultivars, Sunbelt Runner and Florunner, identified by a laboratory method as having large differences in seed resistance to colonization by *Aspergillus flavus,* were evaluated for resistance to natural seed infection by *A. flavus* and subsequent aflatoxin contamination. Groundnuts were grown on three nonirrigated farms during 1980 using two planting dates and three harvest dates for each cultivar. Groundnuts grown on two farms experienced moderate to severe drought stress and both cultivars contained high levels of aflatoxin. Groundnuts on the third farm had adequate rainfall and contained only very low levels of aflatoxin. Sunbelt Runner (reported to be resistant to *A. flavus* colonization of seeds) had no advantage over Florunner (reported to have only moderate resistance to seed colonization) in respect of levels of *A. flavus* and subsequent aflatoxin contamination under field conditions. Levels of *A. flavus* infection and aflatoxin contamination were related primarily to environmental conditions, especially drought stress, during pod maturation. Genetic resistance to invasion by *A. flavus* and subsequent aflatoxin production must be verified in field environments or under conditions simulating those environments.

1502. Davidson, J.I.Jr., Hill, R.I., Cole, R.J., Mixon, A.C., and Henning, R.J. 1983. Field performance of two peanut cultivars relative to aflatoxin contamination. Peanut Science 10(1): 43-47.

Two runner type groundnut cultivars, "Sunbelt Runner" and "Florunner", were compared under differing field conditions for natural seed infection by *Aspergillus flavus* and aflatoxin contamination. Laboratory tests had shown marked differences in seed resistance to colonization by *A. flavus.* Groundnuts were grown on three nonirrigated farms during 1980 using two planting dates and three harvesting dates for each cultivar. Groundnuts grown on two farms experienced moderate to severe drought stress and both cultivars contained high levels of allatoxin. Groundnuts on the third farm received adequate rainfall and contained only very low levels of aflatoxin. Sunbelt Runner (reported to be resistant to *A. flavus* colonization of seeds) had no advantage over Florunner (reported to have moderate resistance to seed colonization) in respect of levels of *A. flavus* infection and subsequent aflatoxin contamination under field conditions. Levels of *A. flavus* infection and aflatoxin contamination were related primarily to environmental conditions, especially drought stress, during pod maturation. These results show that the current laboratory assay method for selecting resistant lines should be carefully reassessed.

1503. Ghewande, M.P., Nagaraj, G., and Raj Jhala. 1986. Aflatoxin production and detoxification in groundnut. Pages 15-16 in Proceedings of the National Seminar on Plant Protection in Field Crops, 29-31 January 1986, Central Plant Protection Training Institute, Hyderabad 500 030, India.

Twenty-eight groundnut genotypes were tested for resistance to seed colonization by *Aspergillus flavus* and for aflatoxin content. Of these, J 11, TMV 12. and Ah 7223, in general, were found to have less seed colonization. Ten cross derivatives, involving J 11 as pollen parent in some cases, were also tested for seed colonization. Genotypes OGO-2 and 1-4 had seed colonization of 8-9 %. Seed treatment with solid sodium chloride at 2 % level inhibited seed colonization considerably. Aflatoxin content was found to vary among genotypes. Those which had more than 3 % phenols were found to contain less aflatoxins. Fumigation with ammonia was found to be highly effective in detoxification of aflatoxins (68 *%).* NaOH spray (2 %) also reduced aflatoxins by about 70 %. Fumigation with burning cowdung fumes and sun drying for one day were found effective in reducing aflatoxin (16-25 %).

1504. Ghewande, M.P., Nagaraj, G., and Reddy, P.S. 1989. Aflatoxin research at the National Research Centre for Groundnut. Pages 237-243 in Aflatoxin contamination of groundnut : Proceedings of the International Workshop, 6-9 October 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

The potential of *Aspergillus flavus* isolates to produce aflatoxins, and the toxicity of their culture filtrates to germinating seeds and seedlings of groundnut were studied. Isolates NRRL 3000 and V3734/10 produced high levels of aflatoxins in culture. Culture filtrates from the isolates and from NRCGAFA 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, S230, derivatives of S230 x PI 337394F. Latur 33 x PI 337394F, and the wild species, *Arachis 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 by NRRL 3000, but only trace levels were produced in *A. cardenasii* and *A, duranensis,* Aflatoxins were found (range of 27-146 µg kg^{-1}) in

Commercial groundnut cake and in de-oiled 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 colonization by *A. flavus* and of aflatoxin production in *in vitro* inoculation tests.

1505. Kisyombe, C.T., Beute, M.K., and Payne, G.A. 1985. Field evaluation of peanut genotypes for resistance to infection by *Aspergillus parasiticus.* Peanut Science 12(1): 12-17.

Fourteen groundnut genotypes were evaluated for resistance to *Aspergillus parasiticus* infection in 1983 and 1984 in rain-shaded field microplots where water stress conditions were simulated, and in unshaded microplots under normal rainfall conditions. A brown color mutant of A. *parasiticus* (ATCC 24690) was grown on cracked corn and the colonized corn was spread on the soil in each microplot. Infection ol groundnut pegs, pods, kernels and tap and fibrous roots was enhanced by low soil moisture. Infection of kernels differed among genotypes; all other tissues were found to be infected at moderate to high frequency and no genotypic differences were observed. Genotypes J11 and Lampang were characterized as resistant to *A. parasiticus* under both dry and moist field conditions. Although percentage infection of kernels varied with genotype, ranking of genotypes reported to have drought resistance was consistent under both dry and moist field conditions. Thirty-four genotypes, including those tested in field microplots, were also evaluated for resistance to seed colonization by *A. parasiticus* in the laboratory. Genotypes J 11 and PI 337409 were highly resistant. Except for J 11, there was no correlation between genotype rankings for resistance to seed colonization and resistance to seed infection under field conditions.

1506. Kushalappa, A.C., Bartz, J.A., and Norden, A.J. 1976. Influence of the shell on the colonization of intact peanut *(Arachis hypogaea* L.) pods and seeds of different breeding lines by *Aspergillus flavus.* Page 255 in Proceedings of the American Phytopathological Society 3.

The percentage of intact groundnut pods with sporulating colonics of *Aspergillus flavus* varied widely following inoculation of pods of different groundnut breeding lines and incubation at 25°C for 14 days. The pods had been windrow-cured for 2-3 days and then artificially dried in a forced air drier at 35°C. Pod moisture content was adjusted to 25 % at the time of inoculation. Although 0-100 % of the pods of the different lines were colonized, only 0-40 % of the seeds within those pods were invaded. The presence of *A. flavus* colonies on the surface of intact pods was not correlated with the presence of infected seeds within these pods. In addition, some apparently noncolonized pods contained infected seeds. With similar shell colonization, groundnut lines with tolerance to seed colonization had fewer seeds colonized than had the more susceptible lines. With similar seed susceptibility, those lines with lower shell colonization also had fewer seeds invaded. Differences in shell susceptibility appeared to be due to the presence of antagonistic microflora.

1507. Kushalappa, A.C., Bartz, J.A., and Norden, A.J. 1979. Susceptibility of pods of different peanut genotypes to *Aspergillus flavus* group fungi. Phytopathology 69(2): 159-162.

Pods and seeds from 17 groundnut genotypes were separately tested for resistance to colonization by *Aspergillus parasiticus* after each of two successive growing seasons. In each year's tests, pods of three genotypes remained completely free from colonies of the test fungus, whereas all the pods of two genotypes in the first year and of three genotypes in the second year had at least one colony. The percentages of inoculated pods and seed colonized by *A. parasiticus* were inversely correlated (r= -0.5 and -0.6 in tests 1 and 2, respectively). The shell of the intact pod seemed to provide an effective barrier to *A. parasiticus.* The quantity of aflatoxin B_1 in seeds of four genotypes was correlated (r = 0.89) with the percentage of pods with surface colonies at 21 days after inoculation. The latter values also were correlated (r = 0.89 and 0.94) with the percentage of seeds that had been penetrated and those with surface colonies, respectively. Resistance of pods to *A. parasiticus,* however, may not be a true plant resistance, since genotypes that were relatively resistant one year were susceptible the next, and vice versa. Colonies arising from natural infections occurring before the pods were artificially inoculated accounted for only three of the seven significant changes in the percentage of pods with colonics between the first and second tests.

1508. LaPrade, J.C. 1973. Physical and chemical properties of resistance exhibited by certain genotypes of *Arachis hypogaea* to invasion by aflatoxin producing *Aspergillus* spp. University of Florida, USA, Ph.D. thesis. 76 pp.

Hand-shelled, intact seeds of 165 groundnut breeding lines were assayed for seed colonization by three isolates of *Aspergillus flavus* (NRRL 3794, NRRL 2999, and one Florida isolate). Significant differences in tolerance to colonization by the fungus were noted between the lines. Seed coats of the tolerant lines were not as permeable as those of the susceptible lines. An intact testa was required for tolerance and appeared to act as a mechanical barrier to penetration by the fungus. Seeds of tolerant lines appeared to possess more wax-like accumulations on the testa than did susceptible lines. Several preconditioning factors that fluctuate under growing and storage conditions were found to affect the tolerance expressed by two breeding lines and the moderately tolerant Florunner variety. Tolerance was lost after 1 year of storage of shelled groundnuts compared to non- shelled groundnuts of the same tolerant genotypes.

1509. LaPrade, J.C., and Bartz, J.A. 1972. Mechanical resistance of selected genotypes of dried peanuts to colonization by strains of aflatoxin-producing *Aspergillus* spp. Phytopathology 62(7): 771.

Hand-shelled, intact seeds of 165 groundnut breeding lines were assayed for seed colonization by three isolates of *Aspergillus flavus* (NRRL 3794, NRRL 2999, and one Florida isolate). Significant differences in tolerance to seed colonization were noted between the lines. No differences in tolerance were found when seed coats were punctured with a needle or abraded with carborundum before inoculation. When intact seeds were soaked in an aqueous solution of 1.0 % 2,3,5-triphenyl-2H-tetrazolium chloride (TZC), a red stain occurred in the cotyledons of the susceptible lines, but not in those of the tolerant lines, indicating that the seed coats of the tolerant lines were not as permeable as those of the susceptible lines. Aqueous extracts of intact seeds of both tolerant and susceptible lines stimulated germination of *Aspergillus flavus* conidia when compared to conidia incubated in distilled water. Diethyl ether extracts of intact seeds of tolerant groundnut lines did not inhibit germination of conidia. Thus, an intact testa was required for tolerance and appeared to function as a mechanical barrier to penetration by the fungus.

1510. McDonald, D., and Mehan, V.K. 1982. Post harvest programme in ICRISAT. Pages 163-171 in Proceedings of the Workshop on Post Harvest Losses and Small Farmer Storage for South Asia and ECS Africa, 19-24 April, 1982, New Delhi, India. Vol II Food Production and Rural Development Division, Commonwealth Secretariat, Marlborough House, Pall Mall, London SW1, UK.

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has comprehensive responsibility for research on its mandate crops of sorghum, pearl millet, pigeonpea, chickpea, and groundnut. However, priority has been given to field problems and only limited research has been done on postharvest problems. The highest priority project with important postharvest and storage aspects is the aflatoxin problem in groundnut. The research emphasis at ICRISAT has been placed on utilization of genetic resistance to develop groundnut cultivars with pods or seeds which the fungus cannot invade, or which if invaded do not support aflatoxin production. A number of breeding lines and cultivars have been identified which have seeds with good resistance to invasion by *A, flavus.* All genotypes so far tested have supported production of aflatoxins but some have given much slower rates of toxin accumulation than have others.

1511. Mehan, V.K. 1987. The aflatoxin contamination problem in groundnut - Control with emphasis on host plant resistance. Pages 63-92 in Proceedings of the first Regional Groundnut Plant Protection Group Meeting and Tour, 15-21 February 1987, Harare, Zimbabwe.

The stams of the global aflatoxin problem is reviewed with special reference to African groundnut producing countries, and research needs are highlighted. Possible practical control measures are discussed with emphasis on use of host plant resistance to the aflatoxin-producing fungus *Aspergillus flavus.*

1512. Mehan, V.K. 1989. Screening groundnuts for resistance to invasion by *Aspergillus flavus* and to aflatoxin production. Pages 323-334 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 October 1987, ICRISAT Center, India. Patancheru, A.P. 502 324. India : International Crops Research Institute for the Semi-Arid Tropics.

Research in several countries into evaluation of responses of groundnuts to seed infection and colonization 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 provided 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.* The usefulness of these resistances in management of aflatoxin contamination is discussed.

1513. Mehan, V.K., Ba, A., and Renard, J.L. 1989. Evaluation of groundnut genotypes for field resistance to seed infection by *Aspergillus flavus* and to aflatoxin contamination : report of work done during May 1988-April 1989. Paris, France : Institut de Recherches pour les Huiles et Oleagineux. 76 pp.

Twenty-one groundnut genotypes reported resistant and susceptible to in vitro seed colonization by *Aspergillus flavus* were tested for field resistance to seed infection, particularly preharvest infection and for aflatoxin contamination in Senegal. Seven genotypes (Ah 7223, J 11. U4-47-7. UF 71513. PI 337394F. 55-437, and 73-30) with resistance to in vitro seed colonization had significantly greater field resistance to A. flavus and had lower aflatoxin contamination. Some genotypes (U4-7-5, VRR 245, and Exotic 6) susceptible to in vitro seed colonization by *A. flavus* also showed field resistance, while four of the 5 resistant breeding lines (ICGV 86016, -86169, -86171 and -86174) were highly susceptible in field trials. These results emphasize that there is not an absolute relationship between resistance to preharvest seed infection and resistance to in vitro seed colonization by *A. flavus* in groundnut

genotypes. Low levels of infection (1-3 %) found in the cultivar 55-437 in all the northern groundnut growing regions of Senegal indicated its stable resistance to field infection of seed by *A. flavus.*

1514. Mehan, V.K., and McDonald, D. 1980. Screening for resistance to *Aspergillus flavus* invasion and aflatoxin production in groundnuts. ICRISAT Groundnut Improvement Program Occasional Paper-2. 15 pp.

This paper describes laboratory procedures to screen groundnuts for resistance to seed infection and colonization by *Aspergillus flavus,* and to aflatoxin production. Some methods for aflatoxin analysis are also outlined.

1515. Mehan, V.K., and McDonald, D. 1984a. [Mycotoxin-producing fungi in groundnuts: Potential for myeotoxin contamination.]. Champignons producteurs de mycotoxines chez l'arachide: Potentiel de contamination parles mycotoxines. Oleagineux 39(1): 25-29.

The possible use of genetic resistance to seed invasion by *Aspergillus flavus* and to aflatoxin production is considered and some research data presented. Many species of fungi have been found associated with groundnut seeds and several are known to be capable of producing mycotoxins on suitable substrates. Reports of natural occurrence of mycotoxins in groundnuts are reviewed, and the natural occurrence of citrinin and zearalenone reported. Preliminary data on mycotoxins production by fungi isolated from groundnuts are presented.

1516. Mehan, V.K., and McDonald, D. 1984b. Research on the aflatoxin problem in groundnut at ICRISAT. Plant and Soil 79(2): 255-260.

Aflatoxin contamination of groundnut is a serious problem in most groundnut producing countries and as such is given high priority by the Groundnut Improvement Program of ICRISAT. Since 1979 the emphasis has been on selecting cultivars resistant to seed invasion and colonization by toxigenic suains of *Aspergillus flavus,* and/or to aflatoxin production following invasion by the fungus. Of 850 germplasm lines screened for reaction to seed invasion and colonization by *A. flavus* in laboratory tests, eight proved resistant. Three of these lines (PI 337394F, PI 337409 and UP 71513) had been reported resistant in the USA but the other five were new sources of resistance. Several groundnut cultivars have been screened for seed resistance in the field, both under natural conditions and with the inoculum of the fungus added to the soil in the pod zone. Some cultivars with resistance to seed colonization also showed resistance to seed invasion by *A. flavus.* None of the cultivars tested was completely resistant to aflatoxin production but significant cultivar differences occurred in the amounts of aflatoxin produced in seeds inoculated with a toxigenic strain of *A. flavus.*

1517. Mehan, V.K., McDonald. D., and Gibbons, R.W. 1982. [Seed colonization and aflatoxin production in groundnut genotypes inoculated with different strains of *Aspergillus flavus.].* Colonisation de la graine et production d'aflatoxine dans des genotypes d'arachide inocules avec differentes souches d' *Aspergillus flavus.* Oleagineux 37(4): 185-191.

Nine groundnut genotypes were tested for resistance to seed colonization by five different strains of *Aspergillus flavus.* These genotypes, and the cultivar J 11 which has been shown to be resistant to seed colonization by *A. flavus,* were also checked for production of aflatoxin following infection of scarified, surface-sterilized seeds by three aflatoxigenic strains of *A. flavus.* The genotypes PI 337394F and PI 337409 showed significantly less seed colonization and internal invasion than the other genotypes. The *A. flavus* strains differed significantly from one another in their ability to colonize seeds and produce internal infection. Strain NRRL 3000 was the least effective. Of the three strains used in the aflatoxin production tests. AF 8-3-2A produced the highest levels of aflatoxin B_1 on all genotypes while AFS-3 produced the least; NRRL 3000 being intermediate in this respect. Aflatoxin G_1 was produced on all genotypes by NRRL 3000, and on J 11 by AF 8-3-2A. There was no obvious correlation between seed resistance to *A. flavus* colonization and aflatoxin production when seeds were infected. Significantly higher amounts of aflatoxin B_1 were produced in the two genotypes resistant to *A. flavus* colonization than in the highly susceptible genotype FESR-11-P11-B2-B1.

1518. Mehan, V.K., McDonald, D., and Lalitha, B. 1983. [Effect of season, location and field-drying treatment on in vitro seed colonization of groundnut genotypes by *Aspergillus flavus.].* Effets de la saison de culture, de la localisation el du mode de sechage au champ sur la colonisation in vitro des graines de differents genotypes d'arachide par *Aspergillus flavus.* Oleagineux 38(10): 553-559.

Aspergillus flavus colonization levels on 10 groundnut genotypes were significantly higher on seed from the 1979/80 and 1980/81 postrainy season crops than on seed from the 1979 and 1980 rainy season crops. Sixty-four groundnut genotypes were tested for resistance to seed colonization by *A. flavus* in relation to crop season, location (fields) and period of windrow drying. Seed colonization levels on the genotypes were higher on seed from the 1980/81 postrainy season crops than on seed from the 1981 rainy season crops. Windrow drying treatment for 48 h in the postrainy season resulted in significantly higher percentages of seed colonized compared to the 24 h treatment. Significant interactions occurred between genotypes and locations (fields) in both the rainy and postrainy seasons. Levels of seed colonization by *A. flavus* can be influenced by growing season, crop location, and postharvest drying treatment.

1519. Mehan, V.K., McDonald, D., Nigam, S.N., and Lalitha, B. 1981. [

Groundnut cultivars with seed resistant to invasion by *Aspergillus flavus.].* Cultivars d'arachide avec graines resistantes a l'invasion par *Aspergillus flavus.* Oleagineux 36(10): 501-507.

Eleven groundnut genotypes were tested for resistance to in vitro seed colonization by *Aspergillus flavus.* One Indian commercial groundnut cultivar J 11 and two germplasm lines PI 337409 and PI 337394F were found resistant to invasion and colonization by *A. flavus* of intact, dried seeds when, these were rehydrated and inoculated with Indian strains of the fungus. Storage of seeds for periods of 55, 70 and 90 days before testing did not significantly affect the results. Inoculation of seeds of seven genotypes with three different toxigenic strains of *A. flavus* showed marked differences in invasive potential between them. The strain NRRL 3000 was less virulent than the other two on all genotypes. The cultivar J 11, which also shows resistance to pod rots, could be useful in areas where aflatoxin contamination is a serious problem.

1520. Mehan, V.K., McDonald, D., and Rajagopalan, K. 1987. Resistance of peanut genotypes to seed infection by *Aspergillus flavus* in field trials in India. Peanut Science 14(2): 17-21.

Eleven groundnut genotypes, six resistant and five susceptible to in vitro seed colonization by *Aspergillus flavus* (IVSCAF), were evaluated for field resistance to seed infection by *A. flavus* and other soil fungi, and for aflatoxin contamination, in seven environments in southern India. Five of the IVSCAF-resistant genotypes had significantly greater resistance to infection of seed by *A. flavus* in the field and had lower aflatoxin contamination than the IVSCAF-susceptible genotypes. Resistance to field infection of seed by *A. flavus* was stable across the seven environments. Significant interactions were found between environments and IVSCAF-susceptible genotypes for infection by *A. flavus, Aspergillus niger* and *Maerophomina phaseolina.* Genotypes with field resistance to *A. flavus* also had significantly less seed infection by *A. niger, M. phaseolina,* and *Fusarium* spp. than had the *A.* flavus-susceptible genotypes. Significant positive correlations were found between IVSCAF-resistance and field resistance to *A. flavus* seed infection, and between the seed infection and aflatoxin B_1 contamination. The field resistant genotypes J 11, Ah 7223, UF 71513, and U4-47-7 have yield levels and pod and seed characters acceptable in India.

1521. Mehan, V.K., McDonald, D., and Ramakrishna, N. 1988. Effects of adding inoculum of *Aspergillus flavus* to pod-zone soil on seed infection and aflatoxin contamination of peanut genotypes. Oleagineux 43(1): 21-28.

Several groundnut genotypes resistant or susceptible to in vitro seed colonization by *Aspergillus flavus* were grown in replicated field trials at ICRISAT Center, Patancheru, India, during 1981 to 1984. Addition of inoculum of an aflatoxigenic strain of *A. flavus* to the pod-zone soil increased preharvest *A. flavus* seed infection and aflatoxin contamination. Levels of aflatoxin B1 contamination were positively correlated with percentage of seed infected by *A. flavus,* irrespective of whether or not inoculum was added to the soil. Genotypic differences for seed infection by *A. flavus* and for aflatoxin contamination were about the same in both control and inoculum treated plants. Levels of seed infection by other soil fungi were not significantly influenced by addition of *A. flavus* inoculum to the pod-zone soil. Most of the genotypes included as resistant to in vitro seed colonization by *A. flavus* of rehydrated, mature, undamaged, stored seed, also showed resistance to invasion by the fungus in the field.

1522. Mehan, V.K., McDonald, D., Ramakrishna, N., and Williams, J.H. 1986. Effects of genotype and date of harvest on infection of peanut seed by *Aspergillus flavus* and subsequent contamination with aflatoxin. Peanut Science 13(2): 46-50.

Several groundnut genotypes reported as resistant, susceptible or highly susceptible to in vitro colonization of rehydrated, mature, stored, undamaged seed by *Aspergillus flavus* (IVSCAF) were tested for natural seed infection by *A. flavus* and other fungi in two or more replicated field trials at ICRISAT Center, Patancheru, India, in 1979-1984. Undamaged pods were sampled before maturity, at optimum maturity (normal harvest) and when over-mature (late harvest) and seed examined for infection by *A. flavus* and other fungi. In the 1983 and 1984 rainy and 1983/84 postrainy seasons, only four genotypes (one resistant and three susceptible) were tested, and seed were also tested for aflatoxin content. In all seasons the genotypes reported as IVSCAF-resistant had significantly lower levels of seed infection with *A. flavus* and other fungi than did genotypes reported as IVSCAF-susceptible. Genotypic differences in levels of seed infection by *A. flavus* were consistent over seasons. The resistant cultivar J 11 had a significantly lower aflatoxin content than the other three IVSCAF-susceptible genotypes tested in the 1983 and 1984 rainy and 1983/84 postrainy seasons. Drought stress in the 1984 season apparently increased susceptibility to seed infection by *A. flavus* and other fungi, and to aflatoxin contamination, in all genotypes. Seed infection by *A. flavus* and other fungi, and aflatoxin contamination increased with increasing maturity of pods, indicating the importance of lifting the groundnut crop at optimum maturity.

1523. Mehan, V.K., Rao, R.C.N., McDonald, D., and Williams, J.H. 1988. Management of drought stress to improve field screening of peanuts for resistance to *Aspergillus flavus.* Phytopathology 78(6): 659-663.

Drought stress during late stages of pod maturation in an irrigated groundnut crop during the postrainy season significantly increased the level of seed infection by *A. flavus.* A line-source sprinkler irrigation system imposing a drought- stress gradient

was used for field screening of groundnut genotypes for resistance to seed infection by *A. flavus. A* significant, positive, linear relationship was found between water deficit (drought intensity) and seed infection in groundnut genotypes. Gcnotypic differences for seed infection by *A. flavus* were evident at all levels of drought stress, but, under the more severe drought stress conditions, the genotypes resistant to *A. flavus* had low but positive levels of seed infection giving improved statistical precision.

1524. Mixon, A.C. 1976. Peanut breeding strategy to minimize aflatoxin contamination. Proceedings of the American Peanut Research and Education Association 8(1): 54-58.

Screening, selection and breeding procedures for increasing the resistance of groundnut varieties to aflatoxin-producing strains of *Aspergillus flavus* are reviewed and discussed. This review includes consideration of sources and nature of resistance, reaction of genotypes to seed colonization, variation among fungal isolates of *A. flavus,* and factors associated with the interaction of aflatoxin-producing strains of the fungus and seed of groundnut genotypes. The pros and cons of breeding an improved groundnut variety with greater resistance to aflatoxin contamination are presented.

1525. Mixon, A.C. 1977. Influence of plant genetics on colonization by *Aspergillus flavus* and toxin production (peanuts). Pages 163-172 in Mycotoxins in human and animal health. (Rodricks, J.V., Hesscltine, C.W., and Mehlman, M.A., eds.). Illinois, USA : Pathotox Publishers, Inc. 807 pp.

The potential for using genotypes identified to be resistant to seed colonization by aflatoxin-producing strains of *Aspergillus flavus* and *A. parasiticus* for breeding agronomically suitable groundnut cultivars with resistance to the toxigenic fungi is discussed. Factors affecting seed colonization in groundnuts are discussed and factors associated with resistance to colonization of the groundnut seed testa are reviewed.

1526. Mixon, A.C. 1979. Developing groundnut lines with resistance to seed colonization by toxin-producing strains *of Aspergillus* species. Pest Articles News and Summaries 25(4): 394-400.

Evidence for the possible development of groundnut cultivars with favourable agronomic characteristics and resistance to aflatoxin-producing strains *of Aspergillus flavus* is presented. In studies of F_2 and F_3 progenies from crosses of resistant and susceptible genotypes data on the frequency distribution and least square estimates of genetic effects showed the possibility of selecting for resistance to seed colonization by the fungus. Yield, value and seed quality data for seven advanced lines developed by using pedigree selection from crosses showed that potentially favorable groundnut cultivars can be developed.

1527. Mixon, A.C. 1980a. [Comparison of pod and seed screening methods on *Aspergillus* spp. infection of peanut genotypes.]. Comparaison de tests d'evaluation de l'infection par *Aspergillus* spp. de gousses et de graines de differents genotypes d'arachide. Oleagineux 35(1): 33-35.

In laboratory studies, six groundnut genotypes grown at three locations were tested for the effect of pod and seed inoculation methods on seed colonization by *Aspergillus parasiticus* and/or incidental prior infection in the field or storage by *A. flavus* or *A. parasiticus.* The genotypes had been identified as having varying levels of resistance to seed colonization by *A. parasiticus* in laboratory tests. Noninoculated seed (incubated like the inoculated samples) was considerably less colonized. Pod inoculation resulted in a lower subsequent seed colonization of the more susceptible genotypes when compared to seed inoculation (genotype x method interaction). For all three methods, seed colonization was consistently less for the resistant genotypes than for the 'Florunner' cultivar or the highly susceptible check PI 343419. There was a location x genotype interaction but the resistant genotypes were colonized less frequently than susceptible genotypes. Pod inoculation resulted in a noticeable reduction in seed colonization of the more susceptible genotypes when compared to seed inoculation (genotype x method interaction). Genotypes resistant to seed colonization by *A. parasiticus* were best identified by seed inoculation.

1528. Mixon, A.C. 1980b. Comparison of pod and seed screening methods on *Aspergillus* spp. infection of peanut genotypes. Peanut Science 7(1): 1-3.

In laboratory studies, six groundnut genotypes grown at three locations were tested for the effect of pod and seed inoculation methods on seed colonization by *Aspergillus parasiticus* and/or incidental prior infection in the field or storage by *A. flavus* or *A. parasiticus.* The genotypes had been identified in laboratory tests as having varying levels of resistance to seed colonization by *A. parasiticus.* Pod inoculation resulted in noticeably less seed colonization of the more susceptible genotypes compared to inoculated seed (genotype x method interaction). Noninoculated seed incubated similarly to the inoculated samples exhibited considerably less colonization. For all three methods, seed colonization was consistently less for the resistant genotypes than for the 'Florunner' cultivar or the highly susceptible check PI 343419. A location x genotype interaction resulted from the difference in the magnitude of % colonization but the resistant genotypes were colonized less frequendy than were susceptible genotypes. Genotypes resistant to seed colonization by *A. parasiticus* could be best identified by seed inoculation.

1529. Mixon, A.C. 1981. Reducing aflatoxin contamination in peanut genotypes by selection and breeding. Journal of the American Oil Chemists' Society 58: 961A-966A.

The potential for developing agronomically suitable cultivars using groundnut genotypes that exhibit resistance to seed colonization by aflatoxin-producing strains of *Aspergillus flavus* is discussed. The practical implications of developing resistant cultivars are presented in data for yield, value, and seed quality for six advanced groundnut lines that were developed by selection from crosses.

1530. Mixon, A.C. 1983a. Two peanut germplasm lines, GFA-1 and GFA-2. Crop Science 23(5): 1020.

Two groundnut lines GFA-1 and GFA-2 are resistant to seed colonization by aflatoxin-producing strains *of Aspergillus flavus* and *A. parasiticus.* GFA-1 was derived from a 1972 cross between lines A137 and A5-5, while GFA-2 was derived from a 1972 cross between line A137 and the cultivar Florunner. Both these lines were developed from one to four single-plant selections made within each F_2 through F_{10} progeny-row generation. Seed and pod yields over a three-year period were equal to or greater than those of Florunner. Average seed colonization of both lines by aflatoxin-producing strains of A. *flavus* and/or *A. parasiticus* was equal to or less than that shown by the resistant control (PI 337409) and much less than that of the Florunner control. These lines have growth habit, testa and flower colors, and plant height similar to that of Florunner. Both are runner market types.

1531. Mixon, A.C. 1983b. Peanut germplasm lines, AR-1, -2, -3, and -4. Crop Science 23(5): 1021.

Four groundnut lines AR-1, AR-2. AR-3, and AR-4 are resistant to seed colonization by aflatoxin-producing strains of *Aspergillus flavus* and *A. parasiticus.* These lines are semi-upright in growth habit. AR-1 was derived from a cross between Florunner and a selection from PI 337409, an A. flavus-resistant line. AR-2 was derived from a cross between an experimental line A5-5 and a selection from PI 337409. AR-3 was derived from a cross between a selection from PI 337432 and Tifspan, while AR-4 was derived from a cross between PI 337394F *(A. flavus*-resistant line) and Florunner. All four lines have for several years shown greater resistance to aflatoxin-producing strains of *A. flavus* and *A. parasiticus* than have standard cultivars and hundreds of germplasm accessions and breeding lines. The yield potential of these Ones is too low for their practical use as commercial cultivars.

1532. Mixon, A.C. 1983c. Aflatoxin resistant germplasm lines developed at Coastal Plain Station. Peanut Journal or Nut World 62(7): 15- 16.

In laboratory inoculation tests, four groundnut lines were identified as resistant to seed colonization by aflatoxin-producing strains of *Aspergillus parasiticus.* Although their yields were poor, it is suggested that their resistance could be used in breeding.

1533. Mixon, A.C. 1986. Reducing *Aspergillus* species infection of peanut seed using resistant genotypes. Journal of Environmental Quality 15(2): 101-103.

Studies were conducted to determine the potential for reducing aflatoxin contamination using recently released groundnut genotypes found to be resistant to seed invasion by aflatoxin-producing strains of *Aspergillus flavus* and *A. parasiticus.* When rehydrated groundnut seeds inoculated with *A. parasiticus* were incubated under conditions favoring infection, the resistant genotypes GFA-1, GFA-2, AR-1, AR-2. AR-3, and AR-4 were more resistant than the commercial cultivars, 'Florunner' and 'Sunbelt Runner", in successive years of evaluating. Aflatoxin contamination of seed in the field was markedly less in the resistant genotypes than in the commercial cultivars. Pod yields of GFA-1 and GFA-2 were similar to those of the commercial cultivars. Use of such resistant genotypes could reduce aflatoxin contamination of seed immediately following harvest or in short term storage when environmental conditions are conducive to infection by *A. flavus* and A. *parasiticus.*

1534. Mixon, A.C, Hammons, R.O., and Branch, W.D. 1983. Germplasm for use in genetic enhancement of peanut genotypes. Proceedings of the American Peanut Research and Education Society 15(1): 15-38.

Sources of resistance or tolerance to various diseases and pests of groundnut art-listed. Groundnut genotypes have been reported to be resistant or tolerant to bacterial wilt, early leaf spot, late leaf spot, rust, Cylindrocladium black rot, pod breakdown, stem rot, Diplodia collar rot, Verticillium wilt, and Sclerotinia blight. Seventeen genotypes are listed as resistant to in vitro seed colonization by aflatoxin-producing strains of *Aspergillus flavus or Aspergillus parasiticus.* Several sources of resistance to bud necrosis, clump and rosette virus diseases are also given. Some genotypes resistant to the major insect pests or nematode diseases of groundnut are presented.

1535. Mixon, A.C, and Rogers, K.M. 1973a. Peanut accessions resistant to seed infection by *Aspergillus flavus.* Agronomy Journal 65: 560-562.

A laboratory procedure for screening groundnut germplasm for resistance to *A. flavus* has been developed: seeds are rehydrated then inoculated with a spore suspension of the fungus and incubated for 7 days, after which seed colonization can be assessed. Of 1406 accessions, selections and varieties tested by this method, two germplasm accessions, PI 3373941- and PI 337409, were resistant to two aflatoxin-producing strains of the fungus. Seed with flesh seedcoat color from PI

337394 had an average of 5 % seed colonization for hand-picked and hand-shelled samples evaluated at optimum seed maturity in five separate evaluations during 4 years. Also, seed colonization for samples of PI 337409 averaged 9 % for similar evaluation. For two years, comparable checks of two susceptible accessions, PI 331326 and PI 343419, had an average of 92 % and 91 % seed colonization, respectively, and 'Argentine'. 'Florunner', and 'Wilco I ' had an average of 34 %, 39 %, and 30 % respectively.

1536. Mixon, A.C., and Rogers, K.M. 1973b. Breeding peanuts for resistance to *Aspergillus flavus.* Proceedings of the American Peanut Research and Education Association 5(1): 206.

Two groundnut accessions averaged less than 5 % seed colonization by aflatoxin-producing strains of *Aspergillus flavus* following laboratory inoculation of samples at optimum seed maturity and incubating under conditions highly conducive to fungal development. Comparable checks of two susceptible accessions averaged 89 % and 91 % seed colonization, and Florunner, Goldin I and Argentine varieties averaged 22 %, 23 %, and 38 %, respectively. Three maturity separations of the resistant accessions indicated that sound mature seed were less susceptible to *A. flavus* than immature and overmature seed. Seed from the two resistant accessions and a susceptible check harvested at four 2- week intervals beginning near optimum maturity revealed that delayed harvest increased the incidence of infection, but the resistant selections were considerably more tolerant to delayed harvest than the susceptible check.

1537. Mixon, A.C., and Rogers, K.M. 1973c. Peanuts resistant to seed invasion by *Aspergillus flavus.* Oleagineux 28(2): 85-86.

Laboratory evaluation of groundnut accessions and varieties for their reaction to two strains of *Aspergillus flavus* revealed two accessions (PI 337394F and PI 337409) with a high degree of resistance to the fungus.

1538. Mixon, A.C., and Rogers, K.M. 1975a. Factors affecting *Aspergillus flavus* Lk. ex Fr. colonization of resistant and susceptible genotypes of *Arachis hypogaea* L. Peanut Science 2(1): 18-22.

The effects of initially adjusted seed moisture, incubation and storage time, seed maturity, harvest time and seed handling on seed colonization by *Aspergillus flavus* of the two groundnut genotypes PI 337394F and PI 337409 (resistant to seed colonization by aflatoxin-producing strains of *Aspergillus flavus),* and the susceptible genotype PI 331326 were studied. Seed colonization in PI 337394F was greater at 20 % adjusted seed moisture than at 25 % seed moisture. Colonization was least at 15 and 30 % adjusted moisture. *Aspergillus flavus* colonized a low percentage (17 %) of the cotyledons of PI 337409 after 48 hours of incubation, whereas 100 % of the cotyledons of PI 331326 were colonized. Colonization of PI 337394F seed with intact seed coats increased with each increase in storage time from 0 to 6 and 12 weeks, and for each increase in temperature from 5 to 20 and 35°C. Seed colonization in the immature and overmature seed of both resistant genotypes was greater than in the mature seed. Mature seed of the resistant genotypes were colonized at a low level, with no difference for seed harvested at 4 successive 2-week intervals, whereas PI 331326 had greater colonization for each successive harvest date. Seed coat abrasion, soaking for 5 min. in a H_2SO_4 solution, machine picking or machine-shelling increased colonization of seed over check treatments. Seed colonization of PI 331326 was greater than that of the resistant genotypes under all conditions.

1539. Mixon, A.C., and Rogers, K.M. 1975b. Registration of *Aspergillus* flavus-resistant peanut germplasms. Crop Science 15: 106.

Two groundnut genotypes PI 337394F (Reg. No. GP3) and PI 337409 (Reg. No. GP4) are resistant to seed colonization by aflatoxin- producing strains of *Aspergillus flavus.* Both lines arc of the Valencia botanical type and were found in a collection brought from South America in 1968. The original population of PI 337394 was a mixture of genotypes with seed testa color ranging from light pink to purple. A true-breeding genotype with light pink or flesh testa was designated PI 337394F.

1540. Murari Singh, Mehan, V.K., and McDonald, D. 1989. Screening groundnuts for seed resistance to *Aspergillus flavus* : Statistical approaches to data evaluation. Pages 335-344 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 October, 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

Environmental factors influence 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 categories and to ensure a stable basis for comparisons of control and test entries across environemts. This approach was also adopted to compare the degree and distribution of resistance in Spanish and Valencia groundnuts. The establishment of such procedures would facilitate interpretation of screening data from different environments.

1541. Nahdi, S. 1989. The geocarposphere mycoflora and resistance of groundnut to *Aspergillus flavus.* Pages 365-378 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 October, 1987, 1CRISAT Center,

India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

Four groundnut genotypes, two resistant (J 11 and PI 337394F) and two susceptible (TMV 2 and EC 76446 (292)) to in vitro seed colonizttion by *Aspergillus flavus*, 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 markedly higher in the geocarpospheres of the susceptible genotypes than in those of the resistant genotypes. Genotypes were also evaluated for levels of seed infection by *A. flavus* at harvest. The A. flavus-susceptible genotypes had significantly higher levels of seed infection than the *A. flavus-resistant* genotypes. In a greenhouse experiment pod exudates were collected from potted plants. Exudates from the two resistant genotypes inhibited in vitro germination of *A. flavus* conidia.

1542. Pettit, R.E., Azaizeh, H.A., Tuber, R.A., Szerszen, J.B., and Smith, O.D. 1989. Screening groundnut cultivars for resistance to *Aspergillus flavus, Aspergillus parasiticus,* and aflatoxin contamination. Pages 291-304 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 October, 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

Factors determining resistance to aflatoxin contamination are reviewed. Possible factors influencing resistance in groundnut pods and seeds to invasion by *Aspergillus flavus* and to aflatoxin production are considered. A range of groundnut genotypes were examined for presence of inhibitory compounds in testae and cotyledons and considerable variation was found. Tannin-like compounds found in seed testae of some genotypes inhibited *A. flavus* growth and aflatoxin production. Polypeptide contents of seeds varied between genotypes susceptible and resistant to seed colonization by *A. flavus.*

1543. Pins, O.de. 1983. [Cultivar improvement of groundnut and production of selected seed.]. Amelioration varietale de l'arachide et production de semences selectionnees. Oleagineux 38(2): 61-71.

A groundnut breeding programme is outlined, of which the major aims are high yields, resistance to drought, *Aspergillus flavus, Puccinia arachidis, Cercospora arachidicola, Cercospora personata* and rosette virus, and high technological qualities. The breeding methods briefly discussed include mass selection, pedigree selection, the bulk method, single seed descent and multifamilies (a composite of isogenic families).

1544. Pua, A.R., and Medulla, E.C. 1986. Screening for resistance to *Aspergillus flavus* invasion in peanui. in Seventeenth Anniversary and Annual Convention of the Pest Control Council of the Philippines, 8-10 May 1986. Hoila City. Philippines. (Abstract).

Results of screening of various groundnut genotypes for resistance to seed colonization by *Aspergillus flavus* are presented. Four genotypes (ACC 63, CES 48-30, Celebes, and UPL PN 4) are reported resistant to seed colonization by *A. flavus.*

1545. Rao, M.J.V., Nigam, S.N., Mehan, V.K., and McDonald, D. 1989. *Aspergillus flavus* resistance breeding in groundnui : Progress made at ICRISAT Center. Pages 345-356 in Aflatoxin contamination of groundnut : proceedings of the International Workshop. 6-9 October, 1987. ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

Progress worldwide in breeding groundnuts resistant to seed colonization by *Aspergillus flavus* and aflatoxin contamination is summarized, and research at ICRISAT is 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. The need for breeders to incorporate other resistance traits is stressed, and future prospects for breeding for resistance to aflatoxin production are discussed.

1546. Sanders, T.H., Blankenship, P.D., Cole, R.J., Ashley, K.H., and Hill, R.A. 1985. Conclusive test for aflatoxin resistance in peanuts. Journal of the American Oil Chemists' Society 62(4): 654-655.

Data compiled over 4 years showed that 5 cm of soil at a mean of 29.4° C and severe drought 40-50 days before harvest resulted in *Aspergillus flavus* infection and aflatoxin contamination in preharvest groundnuts. Four genotypes, which were rated as resistant to seed colonization by *A. flavus* in a laboratory screening assay, and 2 other genotypes were grown in plots and subjected to the above stress conditions 98 days alter sowing. Tests of dried groundnuts harvested 143 days after sowing showed that nuts of all the genotypes contained aflatoxin and were extensively infected by *A. flavus.*

1547. Tavasolian, B. 1977. Native Iranian peanut resistance to seed infection by *Aspergillus.* Journal of Agricultural and Food Chemistry (6): 1422-1423.

Seeds of three groundnut cultivars (Flori Spanish 334A, Georgia 119-20 and Local Gilan Iran) obtained from the 1974 and 1975 crops of the Seed and Plant Improvement Institute, Karaj, Iran, were stored under conditions conducive to mold attack. When these cultivars were analysed for aflatoxin contamination (using a minicolumn and thin-layer chromatography methods), seeds of Mori Spanish 334 A and Georgia 119-20 were contaminated, but the cultivar Local Gilan Iran showed no contamination. This interesting observation should be followed up.

1548. Tsai, A.H., and Yen, C.C. 1985. Studies on aflatoxin contamination and screening for disease resistance in groundnuts. Journal of Agricultural Research of China 34(1): 79-86.

In a study of four Virginia and four spanish groundnut varieties at 2 sites, preharvest pod damage, by insects or microorganisms, led to increased aflatoxin contamination during storage. Virginia varieties were more easily damaged than Spanish varieties at both sites. Of 350 varieties and lines screened for reaction to seed colonization by *Aspergillus flavus* in laboratory tests, 16 proved resistant. The commercial cultivars Tainan 9, Tainan 10, Tainung 4 (all Spanish types) and Penghu 2 (Virginia type) were highly susceptible.

1549. Waliyar, F. 1978. [Pod and seed contamination by *Aspergillus flavus* Link, in peanut.]. La contamination des gousses et des graines d'arachide par 1'*Aspergillus flavus* Link. Bulletin de la Societe Mycologique de France 94(3): 305-327.

Differences in resistance to seed colonization by *Aspergillus flavus* were observed in 36 groundnut varieties, which could be divided into four classes on this basis. In conditions of drought, some varieties usually susceptible were classified as resistant, although *A.* flavus-resistant varieties remained the most resistant.

1550. Waliyar, F. 1990. *Aspergillus flavus* and *A. niger* contamination of groundnut in Niger. Proceedings of the American Peanut Research and Education Society 22: 32.

During the 1989 rainy season, 25 lines, including germplasm of advanced *Aspergillus flavus-resistant* breeding lines and some cultivars from West Africa, were tested in three locations (Sadore, Bengou and Maradi) in Niger. More than 50 % of seed were infected by *A. flavus* at Sadore. Average seed infection depended on the location (25 % at Sadore, 13 % at Bengou. and 13 % at Maradi). Significant differences between genotypes were found. Genotypes 55-437 and J 11 were the least infected lines. Among the 1CR1SAT advanced *A.flavus*-resistant breeding lines ICGV 87107, ICGV 87094 and ICGV 87110 were the least infected. At the same locations trials were conducted to estimate the yield and plant losses from seedling diseases of groundnut, using 2 fungicides to control seedling diseases. Seeds were treated with thiran or corvet CM at the rate of 3 g kg^{-1} seed. The percentage of unprotected plants that died after emergence in the non- fungicide treatment ranged from 19-43 + 3.2 %. Seed treated with fungicide produced higher yields than the untreated seed. ICGS 11 showed a high percentage of plant losses in all three locations in the untreated plots. There were no significant differences between the two fungicides for these traits.

1551. Waliyar, F., and Bockelee-Morvan, A. 1989. Research into the resistance of groundnut varieties to *Aspergillus flavus.* Pages 305-310 in. Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 October. 1987, ICR1SAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

Some 40 groundnut genotypes were screened for field resistance to seed infection by *Aspergillus flavus* in trials at Bambey and Darou in Senegal during the period 1977-1980. Significant varietal differences were observed at harvest for levels of natural seed infection by *A. flavus.* Field resistance to *A. flavus* in genotypes were positively correlated with previously measured resistance to seed colonization by *A. flavus* in laboratory inoculation tests. The commercially grown cultivar 55- 437 had high levels of resistance to *A. flavus* in both field and laboratory screening, while two other cultivars (73-30 and 73-33) also grown in Senegal had moderate levels of resistance. Genotypes with seed resistance to *A. flavus* had a lower proportion *of A. flavus* in their rhizosphere mycoflorac than had genotypes susceptible to seed infection by the fungus.

1552. Zambettakis, Ch. 1975. [Study of the contamination of several varieties of groundnut by *Aspergillus flavus.].* Etude de la contamination de quelques varietes d'arachide par *Aspergillus flavus.* Oleagineux 30(4): 161-167.

Experiments were carried out on 24 varieties of groundnut for their reaction to pod and seed infection by *Aspergillus flavus* in the field in Darou, Senegal, and for seed colonization in artificial inoculation tests in the laboratory in Paris. The varietal differences observed in seed colonization in inoculation tests and in infection of pods and seeds in the soil before harvest appeared to be linked to the shell and seed coat structure. Drought at the end of the growth cycle was a contributing factor to increased infection.

1553. Zambettakis, Ch. 1983. [Results of studies on groundnut hybrids selected to restrict infestation by *Aspergillus flavus.*]. Resultats de rechcrches sur les hybrides

selectionnes de l'arachide pour limiter l'infestation *par Aspergillus flavus.* Comptes Rendus des Seances de l'Academie d'Agriculture de France 69(1): 44-50.

A brief survey of breeding groundnut for resistance to *Aspergillus flavus* is presented. It is noted that marked varietal differences exist and that the extent of seed infection in natural field conditions was correlated with that obtained using in vitro inoculation. Varieties bred using resistant material from India, Senegal and the USA gave good results in Senegal; they included 55-437, 73-30. 57-422 and 73-33.

1554. Zambettakis, Ch., Bockelec-Morvan, A., Waliyar, F., and Rossion, J. 1977. [Varietal differences in susceptibility of groundnut to infection by *A. flavus* in the field and under artificial conditions.]. Differences varie'tales de sensibilite de l'arachide a la contamination par *A. flavus* aux champs et en conditions artificiclles. Oleagineux 32(8-9): 377-383.

In field trials at Darou, Senegal in 1975. 33 groundnut varieties were tested under drought conditions favourable for infection by *Aspergillus flavus.* Varieties were sown on two dates (normal sowing date, late sowing date) and were harvested at maturity and 14 days before maturity. Significant differences were found between varieties for pod infection (0.8-8 %) and for seed infection (0.4-4 %) by *A. flavus.* Among the 10 varieties least infected were PI 337394F and PI 337409 (previously reported resistant to seed colonization by *A. flavus* in artificial inoculation tests in the laboratory), Florunner. and seven varieties selected in Senegal for resistance to drought. In laboratory inoculation tests, varieties showing field resistance in general showed resistance to seed colonization by the fungus. However, the results varied considerably for certain varieties depending on the sowing and harvesting dates.

1555. Zambettakis, Ch., Waliyar, F., Bockelee-Morvan, A., and Pins, O.de. 1981. [Results of four years of research on resistance of groundnut varieties to *Aspergillus flavus*.]. Resultats de quatre annees de recherches sur la resistance de varietes d'arachide a l'*Aspergillus flavus.* 0leagineux 36(7): 377-385.

Seven trials, each on some 40 varieties of groundnut at 2 locations (Bambey and Darou) in Senegal, were carried out in 1976-1980. Natural seed infection by *Aspergillus flavus* in the field varied with varieties, and was strongly correlated to seed colonization in in vitro inoculation tests, indicating the value of the latter method in selecting resistant varieties. A Senegalese variety of proven agronomic value, 55-437, showed resistance similar to that of the resistant control genotype PI 337409. Two other varieties, 73-30 and 73-33, were considered tolerant to *A. flavus.*

8.2.6 Mechanisms of Resistance to *Aspergillus flavus* Colonization and Infection

1556. Amaya, F.J., Young, C.T., Mixon, A.C., and Norden, A.J. 1977. Soluble amino and carbohydrate compounds in the testae of six experimental peanut lines with various degrees *of Aspergillus flavus* resistance. Journal of Agricultural and Food Chemistry 25(3): 661-663.

The testae of three *Aspergillus flavus-resisumi* (PI 337394F, UF 734022, PI 337409) and three susceptible (UF 73515, PI 331326, PI 343419) lines of groundnut were analyzed for soluble amino compounds and carbohydrates. Water-soluble nitrogenous compounds were found within the testae of resistant lines in significantly, lower concentrations (17.09-28.09 µmol g^{-1}) than those in testae of susceptible lines (33.94-65.28 umol g^{-1}). Arginine, glycine, lysine, ammonia, methionine, and aspartic acid contents were most strongly correlated with susceptibility. These results suggest that the nonavailability of readily soluble, small molecular weight amino compounds on or within the testa matrix may play a role in the mechanism of *A. flavus* resistance exhibited by some groundnut lines.

1557. Amaya, F.J., Young, C.T., Norden, A.J., and Mixon, A.C. 1980. [Chemical screening for *Aspergillus flavus* resistance in peanuts.]. Methode chimique d'evaluation de la resistance des arachides a *Aspergillus flavus.* Oleagineux 35(5): 255-259.

In 18 groundnut genotypes of known resistance or susceptibility to *Aspergillus flavus* grown in the USA or in Puerto Rico, the total content of soluble amino compounds in the testa was directly correlated with susceptibility except in two susceptible lines, UF 71513 and Tifspan, which had a low content. In a further study of 12 genotypes, including UF 71513 and Tifspan. susceptibility was associated with a low content of acid-hydrolysable arabinose in the testa.

1558. Azaizeh, A.K., and Pettit, R.E. 1987. Influence of tannin- related compounds from peanut seed coats and cotyledons on *Aspergillus parasiticus* growth and aflatoxin production. Phytopathology 77(12): 1703.

Of 23 genotypes evaluated in a humidity chamber, Florunner, PI 337409, 55-437 and Texas 7 were the most resistant, with low infection by *Aspergillus parasiticus* and low aflatoxin contamination. Levels of tannin compounds in seed coats and cotyledons differed among genotypes, with levels being higher in seed coats. Some compounds significantly decreased the growth of *A. parasiticus* and inhibited the production of aflatoxin.

1559. Azaizeh, A.K., Pettit, R.E., Sarr, B.A., and Phillips, T.D. 1990. Effect of peanut tannin extracts on growth of *Aspergillus parasiticus* and aflatoxin production. Mycopathologia 110(3): 125-132.

Twenty-three groundnut genotypes were evaluated for resistance to seed colonization by *Aspergillus parasiticus* and aflatoxin production when incubated under high relative humidity. Tannin- containing extracts from seed testae and cotyledons of these genotypes were prepared and tested for their effect on growth of A. *parasiticus* and aflatoxin production. Seed colonization was low (< 30 %) in the genotypes Toalson X UF 73-4022 (selections TX-798731 and TX-798736), A72118, 55-437, PI 337409 and Florunner. Genotypes with low levels of seed colonization also had low anatoxin contamination. Higher levels of tannins were detected in testae (23.9-97.2 mg g^{-1}) compared with cotyledons (0.17-0.82 mg g^{-1}). Some of the methanol-extracted and water-soluble tannin extracts from testae and cotyledons, when incorporated into yeast extract sucrose liquid medium (100 mg L^{-1}), significantly inhibited growth of A. *parasiticus* and aflatoxin production. There was no overall correlation between geotypes and the influence of tannin extracts on *A. parasiticus* growth and aflatoxin production.

1560. Azaizeh, A.K., Pettit, R.E., and Taber, R.A. 1987. Influence of tannin-related compounds on the growth of *Aspergillus parasiticus* and aflatoxin production. Proceedings of the American Peanut Research and Education Society 19: 35.

Several tannin-like compounds were tested in a liquid nutrient medium at concentrations of 100, 500, and 1000 mg L^{-1} to determine their influence on growth of *Aspergillus parasiticus* and aflatoxin production. Fungal growth inhibition was greatest using tannic acid, catechol, and methyl catechol. Aflatoxin production was significantly decreased by methyl catechol, naringenine, umbelliferone, and hydrobenzoic acid. Ferulic acid caused significant growth inhibition at concentration of 1000 mg L^{-1}. Tannin-like compounds extracted from groundnut seed coats also inhibited fungal growth and aflatoxin production.

1561. Azaizeh, A.K., Waniska, R.D., and Pettit, R.E. 1988. Isolation and characterization of phenolic acids in mature peanut seed coats from twenty-three peanut genotypes. Proceedings of the American Peanut Reserach and Education Society 20: 27.

Phenolic acids were extracted from seed coats of twenty-three groundnut genotypes with an acetone-water solution. The acetone was evaporated and the extract partitioned in ethyl acetate. This phase was roto-evaporated to dryness at 35-40° C, redissolved in methanol, filtered, and injected into a C_{18} column with a 10 um particle size. Phenolic acids were detected at a wavelength of 254 nm. Twelve different compounds were separated. Comparisons with known compounds revealed a preliminary identification of protoatechnic acid, genetisic acid, catechin, methyl catechin. epicatechin and p-coumaric acid. Correlation analysis of the ability of the initial testae extracts to inhibit *Aspergillus flavus* and *A. parasiticus* growth and aflatoxin production in liquid culture media revealed R^2 values which ranged from 0.36 to 0.89. Testae extracts from seed of genotypes which contained relatively high levels of phenolic acids caused the least inhibition of fungal growth and aflatoxin production. Dark testae color was negatively correlated with kernel resistance and aflatoxin production. The concentration of specific phenolic acids was variable among genotypes and was related to testae color, market type, and selections within a genotype. Some positive correlations were obtained with specific phenolic acids and inhibition of fungal growth and aflatoxin formation in extracts from groundnut genotypes classified in the Virginia market type.

1562. Camara, P.A. 1977. [Development of a test of the permeability of the testa of seeds of groundnut *(Arachis hypogaea* L.).]. Mise au point d'un test de permeabilite de la pellicule des graines d'arachide (*Arachis hypogaea* L.). 016agineux 32(6): 273-276.

After soaking the seeds of four varieties of groundnut in deionized water for periods ranging from 5 to 60 min, the water from soaking cultivar 28-206 had the highest electrical conductivity and that from Florunner the least, showing that these varieties had respectively the most and least permeable testae. It is suggested that these measurements could constitute a rapid test of seed resistance to penetration by *Aspergillus flavus.*

1563. Cole, R.J., Sanders, T.H., Dorner, J.W., and Blankenship, P.D. 1989. Environmental conditions required to induce preharvest aflatoxin contamination of groundnuts : summary of six years' research. Pages 279-287 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 Oct 1987, ICRISAT Center, India. Patacheru 502 324, A.P., India : International Crops Research Institute for the Semi-Arid Tropics.

Environmental conditions necessary for preharvest aflatoxin contamination of visibly sound groundnuts are reviewed on the basis of studies conducted at Dawson, Georgia, USA during six 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 infection 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, immature kernels. Phytoalexin-based resistance can readily explain the resistance in immature kernels, but it does not explain the broader-based resistance observed in 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.

1564. Daigle, D.J., Mixon, A.C., DeLucca, A.J., and Coffelt, T.A. 1984. Flavonoids and *A. flavus* resistant peanuts. Proceedings of the American Peanut Research and Education Society 16(1): 47.

Acetone extracts of a variety of groundnut were shown by Lindsey and Turner (1975) to inhibit the growth of *Aspergillus flavus*. They identified one of the inhibitory substances as 5,7- dimethoxyisoflavone. This present work with the use of standards and high performance liquid chromatography shows that a huge number of groundnut genotypes contain not the dimethoxy compound but 5,7-dihydroxyisoflavone. Twenty genotypes were screened for resistance to *A. flavus* in the laboratory. The resistance of these genotypes and their correlation to 5,7-dihydroxyisoflavone content are discussed. The fungal inhibition characteristics of the dihydroxyisoflavone are described.

1565. Dieckert, M.C., and Dieckert, J.W. 1977. Genetically determined structural parameters of the seed coat affecting the colonization of peanut seeds by aflatoxin-producing *Aspergilli.* Annales de Technologie Agricole 26: 353-366.

The groundnut genotypes PI 337394F and PI 337409, resistant to seed colonization by *Aspergillus parasiticus,* had thinner testae in which the collapsed cell walls were more tightly packed than in the susceptible genotypes PI 343360 and PI 343326. It is suggested that the structure of the testa is genetically determined.

1566. Dieckert, J.W., Dieckert, M.C., Pettit, R.E., Benedict, C.R., and Ketring, D.L. 1973. Comparison of *Aspergillus-flavus* tolerant and susceptible lines II. Electronmicroscopy. Proceedings of the American Peanut Research and Education Association 5(1): 207.

The ultrastructures of seed coats of mature seeds of an *Aspergillus flavus* -resistant genotype, PI 337394F and of a susceptible genotype, PI 343326 were studied. Observations were made on thin sections of epoxy-embedded samples by transmission electron microscopy and the ultrastructures of the seed coats of the resistant and susceptible genotypes are described. The groundnut seed coat may serve as a structural barrier to the hyphae of the aflatoxigenic fungus *A. flavus.*

1567. Dorner, J.W., Cole, R.J., Sanders, T.H., and Blankenship, P.D. 1989. Interrelationship of kernel water activity, soil temperature, maturity, and phytoalexin production in preharvest aflatoxin contamination of drought-stressed peanuts. Mycopathologia 105(2): 117-128.

Samples of Florunner groundnuts were collected throughout a period of late-season drought stress with mean geocarposphere temperature of 29 and 25"C, and determinations of maturity, kernel water activity (aw), percentage moisture, capacity for phytoalexin production and aflatoxin contamination were made. Results showed an association between the loss of the capacity of kernels to produce phytoalexins and the appearance of aflatoxin contamination. Kernel water activity (aw) appeared to be the most important factor controlling the capacity of kernels to produce phytoalexins. Mature groundnuts possessed additional resistance to contamination that could not be attributed solely to phytoalexin production. Kernel moisture loss was accelerated in the 29° treatment compared with the 25°C treatment, and data indicated that the higher soil temperature also favored growth and aflatoxin production by *Aspergillus flavus* in groundnuts susceptible to contamination.

1568. Ghewande, M.P., Pandey, R.N., Shukla, A.K., and Misra, D.P. 1985. Spore germinability of *Aspergillus* species in seed coat leachates of groundnuts. Indian Journal of Mycology and Plant Pathology 14(2): 134-137.

Seed coat leachates from some of the 36 groundnut cultivars were inhibitory to spore germination of *Aspergillus niger* and *A. flavus* and others were stimulatory. Maximum inhibition of A. *niger* (89.74 %) and *A. flavus* (74.47 %) was observed in leachates of Jyoti and TMV-12, respectively. The inhibition and/or stimulation is attributed to the presence of chemicals in seed coats which may effect invasion of the seed by *Aspergillus* spp.

1569. Jambunathan, R., Mehan, V.K., and Santosh Gurtu. 1989. Polyphenols in groundnut genotypes resistant and susceptible to seed colonization by *Aspergillus flavus*. Pages 357-364 in Aflatoxin contamination of groundnut: proceedings of the International Workshop, 6-9 October. 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

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 cured, mature seeds of these genotypes were analyzed for polyphenols using different methods. No significant correlation was observed between seed colonization and polyphenol content.

1570. Ketring, D.L., Benedict, C.R., and Yeager, M. 1976. Growing season and location effects on water uptake and drying rates of peanut seeds from genotypes

resistant and susceptible to invasion by *Aspergillus flavus* Lk. ex. Fr. Agronomy Journal 68(4): 661- 665.

Seeds from two *Aspergillus* flavus-resistant and three A. flavus- susceptible genotypes grown in 1972 and 1973 in Puerto Rico and in Texas in 1973, were tested for water uptake and drying characteristics. Growing season and location significantly affected rate and total water uptake of the cured seeds. The rate and total water uptake were greater for seeds from Puerto Rico than those from Texas. Seeds of the two *A. flavus-resistant* genotypes and Florunner had slower rates of water uptake and dried out at a faster rale than two of the *A. flavus*-susceptible genotypes. Lipid and protein contents of seeds were not correlated with water uptake or drying characteristics, but varied with growing season and site.

1571. Kushalappa, A.C., Bartz, J.A., and Norden, A.J. 1976. Influence of the shell on the colonization of intact peanut *(Arachis hypogaea* L.) pods and seeds of different breeding lines by *Aspergillus flavus*. Page 255 in Proceedings of the American Phytopathological Society 3.

The percentage of intact groundnut pods with sporulating colonies of *Aspergillus flavus* varied widely following inoculation of pods of different groundnut breeding lines and incubation at 25°C for 14 days. The pods had been windrow-cured for 2-3 days and then artificially dried in a forced air drier at 35°C. Pod moisture content was adjusted to 25 % at the time of inoculation. Although 0-100 % of the pods of the different lines were colonized, only 0-40 % of the seeds within those pods were invaded. The presence of A. *flavus* colonies on the surface of intact pods was not correlated with the presence of infected seeds within these pods. In addition, some apparently noncolonized pods contained infected seeds. With similar shell colonization, groundnut lines with tolerance to seed colonization had fewer seeds colonized than had the more susceptible lines. With similar seed susceptibility, those lines with lower shell colonization also had fewer seeds invaded. Differences in shell susceptibility appeared to be due to the presence of antagonistic microflora.

1572. Lansden, J.A. 1981. Fungistatic properties of peanut polyphenols. Proceedings of the American Peanut Research and Education Society 13(1): 61.

Various fractions of polyphenols were isolated from groundnut seedcoats and hulls and were assayed for their fungistatic properties on *Aspergillus parasiticus* NRRL 2999. The fractions exerted different degrees of inhibition. The fractions were also assayed for their ability to inhibit aflatoxin production. Isolation and partial characterization of the polyphenol fractions were performed.

1573. LaPrade, J.C. 1973. Physical and chemical properties of resistance exhibited by certain genotypes of *Arachis hypogaea* to invasion by aflatoxin producing *Aspergillus* spp. University of Florida, USA. Ph.D. thesis. 76 pp.

Hand-shelled, intact seeds of 165 groundnut breeding lines were assayed for seed colonization by three isolates of *Aspergillus flavus* (NRRL 3794, NRRL 2999, and one Florida isolate). Significant differences in tolerance to colonization by the fungus were noted among the lines. Seed coats of the tolerant lines were not as permeable as those of the susceptible lines. An intact testa was required for tolerance and appeared to act as a mechanical barrier to penetration by the fungus. Seeds of tolerant lines appeared to possess more wax-like accumulations on the testa than did susceptible lines. Several preconditioning factors that fluctuate under growing and storage conditions were found to affect the tolerance expressed by two breeding lines and the moderately tolerant Florunner variety. Tolerance was lost after 1 year of storage of shelled groundnuts compared to non- shelled groundnuts of the same tolerant genotypes.

1574. LaPrade, J.C., and Bartz, J.A. 1972. Mechanical resistance of selected genotypes of dried peanuts to colonization by strains of aflatoxin-producing *Aspergillus* spp. Phytopathology 62(7): 771.

Hand-shelled, intact seeds of 165 groundnut breeding lines were assayed for seed colonization by three isolates of *Aspergillus flavus* (NRRL 3794, NRRL 2999, and one Florida isolate). Significant differences in tolerance to seed colonization were noted between the lines. No differences in tolerance were found when seed coats were punctured with a needle or abraded with carborundum before inoculation. When intact seeds were soaked in an aqueous solution of 1.0 % 2,3,5-triphenyl-2H-tetrazolium chloride (TZC), a red stain occurred in the cotyledons of the susceptible lines, but not in those of the tolerant lines, indicating that the seed coats of the tolerant lines were not as permeable as those of the susceptible lines. Aqueous extracts of intact seeds of both tolerant and susceptible lines stimulated germination of *Aspergillus flavus* conidia when compared to conidia incubated in distilled water. Diethyl ether extracts of intact seeds of tolerant groundnut lines did not inhibit germination of conidia. Thus, an intact testa was required for tolerance and appeared to function as a mechanical barrier to penetration by the fungus.

1575. LaPrade, J.C., Bartz, J.A., Norden, A.J., and DeMuynk, T.J. 1973. Correlation of peanut seed-coat surface wax accumulations with tolerance to colonization by *Aspergillus flavus*. Proceedings of the American Peanut Research and Education Association 5(1): 89-94.

Wax-like accumulations were noted in scanning electron micrographs on the testae of dried groundnut seeds. Seeds of a breeding line resistant to colonization by *Aspergillus flavus* (NRRL 2999) appeared to possess more of the wax-like accumulations than did several lines that were highly susceptible to colonization by

the fungus. Extraction of waxes and lipids from intact seeds with a chloroform-methanol (2:1, v/v) mixture, for up to five minutes increased the susceptibility of the seeds without reducing their germination. Conidial germination was slightly stimulated when a conidial suspension of *A. flavus* was placed on the dried solvent residue after extraction of intact resistant groundnut seed. It apppears that the wax-like accumulations help prevent *A. flavus* from penetrating the intact seed coat.

1576. McDonald, D., and Harkness, C. 1967. Aflatoxin in the groundnut crop at harvest in Northern Nigeria. Tropical Science IX(3): 148-161.

Serial harvesting trials were carried out at Mokwa and Kano Agricultural Research Stations, Northern Nigeria, in 1963 and 1964 to investigate the occurrence of aflatoxin in the groundnut crop at lifting, when harvested before, at, and after the normal time. This work formed part of the programme of work on the toxicity of the groundnut crop in Northern Nigeria. Crops harvested either at or earlier than the normal time were free from aflatoxin, but late harvesting usually resulted in some toxicity. The weather, as well as the age of the crop, had some effect on toxicity; wet weather delayed the appearance of aflatoxin. Pods with shells damaged while the crop was in the ground were more likely to contain toxic kernels than were pods with undamaged shells.

1577. Mehan, V.K., McDonald, D., and Lalitha, B. 1983. [Effect of season, location and field-drying treatment on in vitro seed colonization of groundnut genotypes by *Aspergillus flavus*.]. Effets de la saison de culture, de la localisation et du mode de sechage au champ sur la colonisation in vitro des graines de differents genotypes d'arachide *par Aspergillus flavus.* Oleagineux 38(10): 553-559.

Aspergillus flavus colonization levels on 10 groundnut genotypes were significantly higher on seed from the 1979/80 and 1980/81 postrainy season crops than on seed from the 1979 and 1980 rainy season crops. Sixty-four groundnut genotypes were tested for resistance to seed colonization by *A. flavus* in relation to crop season, location (fields) and period of windrow drying. Seed colonization levels on the genotypes were higher on seed from the 1980/81 postrainy season crops than on seed from the 1981 rainy season crops. Windrow drying treatment for 48 h in the postrainy season resulted in significantly higher percentages of seed colonized compared to the 24 h treatment. Significant interactions occurred between genotypes and locations (fields) in both the rainy and postrainy seasons. Levels of seed colonization by *A. flavus* can be influenced by growing season, crop location, and postharvest drying treatment.

1578. Mixon, A.C., and Rogers, K.M. 1975a. Factors affecting *Aspergillus flavus* Lk. ex Fr. colonization of resistant and susceptible genotypes of *Arachis hypogaea* L. Peanut Science 2(1): 18-22.

The effects of initially adjusted seed moisture, incubation and storage time, seed maturity, harvest time and seed handling on seed colonization by *Aspergillus flavus* of the two groundnut genotypes PI 337394F and PI 337409 (resistant to seed colonization by aflatoxin-producing strains of *Aspergillus flavus),* and the susceptible genotype PI 331326 were studied. Seed colonization in PI 337394F was greater at 20 % adjusted seed moisture than at 25 % seed moisture. Colonization was least at 15 and 30 % adjusted moisture. *Aspergillus flavus* colonized a low percentage (17 %) of the cotyledons of PI 337409 after 48 hours of incubation, whereas 100 % of the cotyledons of PI 331326 were colonized. Colonization of PI 337394F seed with intact seed coats increased with each increase in storage time from 0 to 6 and 12 weeks, and for each increase in temperature from 5 to 20 and 35°C. Seed colonization in the immature and overmature seed of both resistant genotypes was greater than in the mature seed. Mature seed of the resistant genotypes were colonized at a low level, with no difference for seed harvested at 4 successive 2-week intervals, whereas PI 331326 had greater colonization for each successive harvest date. Seed coat abrasion, soaking for 5 min. in a H_2SO_4 solution, machine picking or machine-shelling increased colonization of seed over check treatments. Seed colonization of PI 331326 was greater than that of the resistant genotypes under all conditions.

1579. Mixon, A.C., and Sanders, T.H. 1979. Seed coat and tannin influence on the mycelial growth of *Aspergillus parasiticus* for peanuts varying in fungal infection. Agronomy Abstracts : 70.

Seed coat tannins caused radial growth inhibition of *Aspergillus parasiticus,* on agar plates, after 52 h, and only minor differences were noted between the six groundnut genotypes resistant and susceptible to *A. flavus* seed colonization. Growth inhibition by seed coat extracts or seed coat tannins showed considerable seed coat x genotype interaction in relation to seed colonization by the fungus.

1580. Nahdi, S. 1989. The geocarposphere mycoflora and resistance of groundnut to *Aspergillus flavus.* Pages 365-378 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 October, 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

Four groundnut genotypes, two resistant (J 11 and PI 337394F) and two susceptible (TMV 2 and EC 76446 (292)) to in vitro seed coloniztion by *Aspergillus flavus,* 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 markedly higher in the geocarposphcres of the susceptible genotypes than in those of the resistant genotypes. Genotypes were also evaluated for levels of seed infection by A. *flavus* at harvest. The A. flavus-susceptible genotypes had significantly higher levels of seed infection than the A. *flavus*-resistant genotypes. In a greenhouse experiment pod exudates were collected from potted plants. Exudates from the two resistant genotypes inhibited in vitro germination of A. *flavus* conidia.

1581. Pettit, R.E., Azaizeh, H.A., Taber, R.A., Szerszen, J.B., and Smith, O.D. 1989. Screening groundnut cultivars for resistance to *Aspergillus flavus, Aspergillus parasiticus,* and aflatoxin contamination. Pages 291-304 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 October, 1987. ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

Factors determining resistance to aflatoxin contamination are reviewed. Possible factors influencing resistance in groundnut pods and seeds to invasion by *Aspergillus flavus* and to aflatoxin production are considered. A range of groundnut genotypes were examined for presence of inhibitory compounds in testae and cotyledons and considerable variation was found. Tannin-like compounds found in seed testae of some genotypes inhibited *A.flavus* growth and aflatoxin production. Polypeptide contents of seeds varied between genotypes susceptible and resistant to seed colonization by *A.flavus*.

1582. Pettit, R.E., Taber, R.A., Ives, P.J., Thurston, E.L., Smith, O.D., and Boswell, T.E. 1976. Peanut pods: Structural differences among cultivars as revealed by scanning electron microscopy. Pages 506-512 in Proceedings of Scanning Electron Microscopy Workshop. Johari, Om. ed. Vol II.

Pod structures in three groundnut genotypes were examined by scanning electron microscopy. An attempt was made to correlate structural differences with variations in resistance to fungal invasion with particular reference to *Sclerotium rolfsii, Rhizoctonia solani,* and *Aspergillus flavus.* Fungal hyphae were restricted to the imrafasicular parenchyma and parenchyma in the exocarp tissue of intact pods. Physical injuries which disrupted the sclerenchyma mantle provided avenues for penetration of hyphal strands into the parenchyma and gave access to the kernels. Sclerenchyma layers were more developed in mature pods of Tamnut 74 and TP 1025 genotypes than in the more susceptible Florunner cultivar.

1583. Pettit, R.E., Taber, R.A., Smith, O.D., and Jones, B.L. 1977. Reduction of mycotoxin contamination in peanuts through resistant variety development. Annales de Technologic Agricole 27(3): 343- 351.

Pods and seed coats of groundnut genotypes resistant and susceptible to seed colonization by *Aspergillus flavus* were examined histochemically with the light microscope and structurally with the transmission and scanning electron microscopes. Significant biochemical and structural differences were associated with an ability to restrict fungal penetration. Seed coats of the *A. flavus-resistant* genotypes PI 337394F and PI 337409 had a more even deposition of wax on their surfaces and more compact arrangement of palisade-like cells than had the *A.* flavus-susceptible genotypes tested. The resistant genotypes had smaller hila than the susceptible cultivars. Pods of genotype PI 337409 stained heavily for tannin and callose, and pods of Florunner (moderately resistant to *A. flavus)* stained heavily for tannin.

1584. Sanders, T.H. 1979. Effect of variety, location and year on tannin content of peanut seed coats. Peanut Science 6(1): 62-64.

Tannin concentrations in the seed coats of six groundnut varieties grown in seven geographical locations for 2 years were determined. Tannin content within variety varied significantly between locations. Levels among varieties at a location were also variable. Tannin concentrations for a specific variety at a specific location in 1975 and 1976 were compared and 60 % of the comparisons differed significantly. These data indicate that any resistance to *Aspergillus parasiticus* colonization associated with groundnut seed coat tannin concentration would probably vary somewhat with production location and year.

1585. Sanders, T.H., and Mixon, A.C. 1978. Effect of peanut tannins on % seed colonization and in vitro growth by *Aspergillus parasiticus.* Mycopathologia 66(3): 169-173.

The relationship between tannin content of mature, intact, cured groundnut seed and % seed colonization by *Aspergillus parasiticus* was examined. Tannin content in 9 groundnut cultivars, 7 of which were grown in both Tifton, Georgia, and Puerto Rico, was significantly correlated with % seed colonization. For data expressed as mg tannin g^{-1} intact seed and mg tannin g^{-1} seed coat, correlation coefficients with % seed colonization were 0.74 and 0.76, respectively. Seedcoat tannin, methanol-extracted, water-soluble material from groundnut seed coats, was tested in vitro for effects on growth of A. *parasiticus.* As concentrations of tannins were increased to 7.5 %, inhibition of fungal growth increased linearly to 88 %, a concentration of 20 % produced over 96 % inhibition.

1586. Souza, V.L.F.De., Amaya, F.J., Pompeu, A.S., and Young, C.T. 1978. A correlation between the amount of soluble amino compounds in the testae of peanuts and colony development of inoculated *Aspergillus flavus.* Proceedings of the American Peanut Research and Education Association 10(1): 66.

The possible link between the level of water-soluble amino compounds in the testae of groundnut genotypes and the ability of inoculated spores of *Aspergillus flavus* to develop on the surface of seeds was examined. Some 500 genotypes from the germplasm bank of the Agronomic Institute of Campinas, Brazil, were sampled for the determination of soluble amino compounds. Levels of soluble amino compounds ranged between 24 and 419 µ equivalent glutamic acid g^{-1} of testa. Seeds of those genotypes containing either <50 or >250 µ equivalent glutamic acid g^{-1} in the testae were inoculated with spores of *A. flavus* (NRRL 6108) and incubated at 28°C for 7 days. Four genotypes among those with low levels of amino compounds had less than 1 % seed colonization. Seed of genotypes with high levels of amino compounds were colonized to a greater extent.

1587. Taber, R.A., Pettit, R.E., Benedict, C.R., Dieckert, J.W., and Ketring, D.L. 1973. Comparison of *Aspergillus flavus* tolerant and susceptible peanut lines I. Light microscope investigation. Proceedings of the American Peanut Research and Education Association 5: 206-207.

Seeds of several groundnut genotypes with varying degrees of resistance to colonization by *Aspergillus flavus* were compared to determine why some genotypes exhibited more resistance than others. Seed coats were sectioned and examined under a light microscope for morphological differences between varieties that might account for such differences. The seed coat of groundnut differs from that of other legumes including a difference in the definition of the light line, presence of osteosclereids and Malpighian cells. Groundnut plant introductions differed from each other in several respects including the size and shape of the hila, amount of articular wax secretion, thickness of the palisade-like layers and size and arrangement of cells within these layers. The hila of the most resistant line was small and closed. The seeds of susceptible lines had longer, more open hila. *A. flavus* has a definite affinity for the open hilar area as opposed to other parts of the seed coat. Breaks in the seed coats of both resistant and susceptible lines allow the fungus to establish colonies at such points. Cotyledonary material of both resistant and susceptible lines served as an excellent nutrient source for *A. flavus*. It appears that a number of factors may influence varietal resistance in the groundnut.

1588. Waliyar, F., and Abadie, M. 1978. [The penetration of mycelium of *Aspergillus flavus* Link, into the groundnut seed coat after artificial contamination : Ultrastructural analysis. J. La penetration du mycelium d'*Aspergillus flavus* Link, dans le tegument seminal de l'Arachide, apres contamination artificielle.- Analyse ultrastructurale. Oleagineux 33(8-9): 447-453.

The ultrastructural analysis of the sequence of penetration of *Aspergillus flavus* mycelium into the groundnut seed coat after artificial inoculation revealed that the fungus acted on the protective layer of the testa as from the 4th day after surface inoculation. On the 5th and 6th days it reached the central cavity of the epidermal cell. Most of the epidermal and parenchymatous cells were invaded on the 7th day. Some hyphae re- emerged on the outside of the testa on the 8th day to form characteristic fructifications of *A. flavus;* others formed similar fructifications in the intercotyledonary space.

1589. Zambettakis, Ch. 1975. [Study of the contamination of several varieties of groundnut by *Aspergillus flavus*.]. Etude de la contamination de quelques varietes d'arachide *par Aspergillus flavus*. Oleagineux 30(4): 161-167.

Experiments were carried out on 24 varieties of groundnut for their reaction to pod and seed infection by *Aspergillus flavus* in the field in Darou, Senegal, and for seed colonization in artificial inoculation tests in the laboratory in Paris. The varietal differences observed in seed colonization in inoculation tests and in infection of pods and seeds in the soil before harvest appeared to be linked to the shell and seed coat structure. Drought at the end of the growth cycle was a contributing factor to increased infection.

1590. Zambettakis, Ch. 1976. [Study of the factors affecting penetration by *Aspergillus flavus* in seeds of *Arachis hypogaea* before and alter harvest.).] Etude des facteurs qui interviennent dans la penetration de l'*Aspergillus flavus* dans les graines d'*Arachis hypogaea* avant et apres la recolte. Oleagineux 31(8-9): 390.

In a study of 36 groundnut genotypes, seed infection or seed colonization by *Aspergillus flavus* was dependent on the genotype. Factors implicated in resistance to fungal penetration included a high proportion of sclerenchyma in the pods and particularly the structure of the seed testa.

1591. Zambettakis, Ch. 1978. [Factors of resistance to *Aspergillus flavus* Link ex Fr. in stored groundnuts.]. Facteurs de resistance, a l'*Aspergillus flavus* Link ex Fr., des arachides en conservation. Fruits 33(1): 30-33.

Thirty-six groundnut genotypes were tested for resistance to *Aspergillus flavus* seed infection in the field in Senegal, and for resistance to seed colonization in in vitro inoculation tests in the laboratory. Seed testae were examined under the light microscope and by scanning electron microscopy. Great differences in structure were found in the seed tegument (seed testa) and these appeared to be related to fungal penetration. Certain structural features might obstruct seed invasion by the fungus.

1592. Zambettakis, Ch., and Bockelee-Morvan, A. 1976. [Research on the structure of the groundnut seed coat and its influence on the penetration of *Aspergillus flavus*.]. Recherches sur la structure du tegument seminal de la graine d'arachide et son influence sur la penetration de l'*Aspergillus flavus*. Oleagineux 31(5): 219- 228.

The groundnut seed coat plays an important part in resistance to seed invasion and colonization by *Aspergillus flavus.* An examination of the surface of the seed coat by scanning electron microscope and of transverse sections by light microscope was carried out on 36 genotypes of groundnut. Great differences in structure were found In the seed coat and these appeared to be related to fungal penetration. These genotypes could be classified according to certain characteristics which may affect resistance to fungal penetration: thickness of the wax layer, junction between the epidermal cells, thickness of cell walls, presence of cracks or detachment of the epidermal foundation.

1593. Zambettakis, Ch., Bockelee-Morvan, A., Waliyar, F., and Rossion, J. 1977. [Varietal differences in susceptibility of groundnut to infection by *A. flavus* in the field and under artificial conditions.]. Differences varietales de sensibilite de l'arachide a la contamination par *A.flavus* aux champs et en conditions artificielles. Oleagineux 32(8-9): 377-383.

In field trials at Darou, Senegal in 1975, 33 groundnut varieties were tested under drought conditions favourable for infection by *Aspergillus flavus.* Varieties were sown on two dates (normal sowing date, late sowing date) and were harvested at maturity and 14 days before maturity. Significant differences were found between varieties for pod infection (0.8-8 %) and for seed infection (0.4-4 %) by *A. flavus.* Among the 10 varieties least infected were PI 337394F and PI 337409 (previously reported resistant to seed colonization by *A. flavus* in artificial inoculation tests in the laboratory), Florunner, and seven varieties selected in Senegal for resistance to drought. In laboratory inoculation tests, varieties showing field resistance in general showed resistance to seed colonization by the fungus. However, the results varied considerably for certain varieties depending on the sowing and harvesting dates.

8.2.7 Resistance to Aflatoxin Production

1594. Aujla, S.S., Chohan, J.S., and Mehan, V.K. 1978. The screening of peanut varieties for the accumulation of aflatoxin and their relative reaction to the toxigenic isolate *of Aspergillus flavus* Link ex Fries. Punjab Agricultural University Journal of Research 15:400-403.

Thirty-seven cultivars of groundnut were tested for their ability to support aflatoxin production following inoculation of seeds with an aflatoxigenic isolate of *Aspergillus flavus.* One hundred genotypes were tested for resistance to aflaroot disease, when their seeds were artificially inoculated with the aflatoxigenic fungus. All cultivars tested supported substantial levels of aflatoxin production. None of the genotypes showed resistance to the aflatoxigenic isolate of the fungus. Only two genotypes, U4-7-2 and U2-1-14, were moderately resistant to aflaroot disease

1595. Bhat, R.V., Nagarajan, V., and Tulpule, P.G. 1974. Aflatoxin production in agricultural commodities : Variations due to fungal isolates and crop genotypes and their scope in prevention of aflatoxin contamination. Pages 199-209 in Proceedings of the Symposium on Biological Approach to Problems in Medicine, Industry and Agriculture, Bhabha Atomic Research Center, Bombay, 12-14 March 1974, Bombay, India.

Screening of 36 groundnut varieties, seven maize varieties, and five varieties each of soyabean and sunflower, using several aflatoxin-producing isolates of *Aspergillus flavus* and *A. parasiticus,* showed that there were varietal differences in resistance to aflatoxin production. The groundnut variety J 11 supported the least toxin production even by the most aflatoxigenic isolates of *A. flavus* and *A. parasiticus.* The low toxin-producing attribute of this variety was confirmed in further tests. The scope of the genetic resistance approach for minimising aflatoxin contamination in agricultural commodities is discussed.

1596. Dange, S.R.S., and Prasad, S.R. 1989. Aflatoxin production on seeds of selected groundnut cultivars. Bulletin of Grain Technology 27(1): 60-62.

Seeds of 15 selected groundnut genotypes were evaluated for production of aflatoxins following inoculation with an aflatoxigenic strain *of Aspergillus flavus.* The data after 8 days of incubation at 28 + 1°C showed aflatoxin production in all genotypes. The cultivar J 11 supported the lowest amount of aflatoxin production (2658 µg kg^{-1}) followed by Robut 33-1 (6558 µg kg^{-1}), var. 27 (7030 µg kg^{-1}) and Faizpur (8262 µg kg^{-1}) whereas high amounts of aflatoxins were supported by OG 43-4-1 (23956 µg kg^{-1}) followed by TMV2 (22746 µg kg^{-1}) and GAUG-10 (21102 fig kg^{-1}). The rest of the cultivars supported production of aflatoxins in the range 10011-18906 µg kg^{-1}. It is emphasized that J 11 is a released cultivar and can be adopted in areas where aflatoxin contamination of groundnut is a serious problem.

1597. Dorner, J.W., Cole, R.J., Sanders, T.H., and Blankenship, P.D. 1987. The relationship of water activity and phytoalexin production to preharvest aflatoxin contamination of peanuts subjected to late-season drought stress. Proceedings of the American Peanut Research and Education Society 19: 34.

Florunner groundnuts were grown in environmental control plots, and 103 days after sowing (DAS) the following treatments were imposed : irrigated, drought with mean 2 in. soil temperature of 29°C (optimum for aflatoxin contamination) and drought with mean 2 in. soil temperature of 25°C (less conducive for aflatoxin contamination). Beginning at 114 DAS (11 treatment days) samples of groundnuts were taken at weekly intervals from the three treatments. Groundnuts were hand-picked and classified into maturity stages by the Hull-Scrape method. Water activity (Aw), moisture, capacity for phytoalexin production, and aflatoxin

contamination were measured in groundnuts from five maturity stages (yellow 1, yellow 2, orange, brown, black). The objectives of the study were to determine (1) what role, if any, stilbene phytoalexins have in natural resistance of groundnuts to aflatoxin contamination, and (2) the role of elevated soil temperature in aflatoxin contamination of groundnuts subjected to late-season drought stress. Results showed that kernels from the irrigated treatment maintained high Aw, high capacity for phytoalexin production, and essentially no aflatoxin contamination throughout the study. As the drought period progressed in the other two treatments, kernel Aw decreased, phytoalexin production ceased, and aflatoxin contamination appeared. This rate of change was faster in the 29°C treatment than in the 25°C treatment, indicating that the primary role of elevated soil temperature is in the rate at which groundnuts become susceptible to *Aspergillus flavus* infection and aflatoxin contamination. Evidence for phytoalexin involvement in natural resistance of groundnuts to aflatoxin contamination was strong. Regardless of maturity, there was no significant aflatoxin contamination of groundnuts until the ability to produce phytoalexins was lost as a result of decreased water activity.

1598. Doupnik, B.,Jr. 1969. Aflatoxins produced on peanut varieties previously reported to inhibit production. Phytopathology 59: 1554.

Four groundnut genotypes (PI 268893, PI 295170, PI 246388 and Starr) were tested for their ability to support aflatoxin production following inoculation of seeds with two aflatoxin- producing isolates of *Aspergillus flavus.* The inoculated seed samples were incubated for 7 days at 25°C, and then analyzed for aflatoxins. All four genotypes supported substantial levels of aflatoxins. The genotype PI 246388 (US 26) was earlier reported to be resistant to aflatoxin production.

1599. Doupnik, B., and Bell, D.K. 1969. Screening peanut breeding lines for resistance to aflatoxin accumulation. Proceedings of the American Peanut Research and Education Association 1: 80-82.

Twenty groundnut breeding lines were tested for their ability to support aflatoxin production following inoculation of seeds with an aflatoxin-producing isolate of *Aspergillus flavus.* The inoculated seed samples were incubated for 7 days at room temperature (25-27°C), and then analyzed for aflatoxins. All lines supported substantial aflatoxin production.

1600. Ghewande, MP., Nagaraj, G., and Reddy, P.S. 1989. Aflatoxin research at the National Research Centre for Groundnut. Pages 237-243 in Aflatoxin contamination of groundnut : Proceedings of the International Workshop, 6-9 October 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

The potential of *Aspergillus flavus* isolates to produce aflatoxins, and the toxicity of their culture filtrates to germinating seeds and seedlings of groundnut were studied. Isolates NRRL 3000 and V3734/10 produced high levels of aflatoxins in culture. Culture filtrates from the isolates and from NRCGAFA 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, S230, derivatives of S230 x PI 337394F, Latur 33 x PI 337394F. and the wild species. *Arachis 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 by NRRL 3000, but only trace levels were produced in *A. cardenasii* and *A. duranensis.* Aflatoxins were found (range of 27-146 μg kg^{-1}) in commercial groundnut cake and in de-oiled cake. Moisture intake capacity, levels of seed coal 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 colonization by *A. flavus* and of aflatoxin production in *in vitro* inoculation tests.

1601. Gopalan, C. 1966. Studies on aflatoxin. Nutrition Document: Aflatoxin/19. P.A.G. (WHO/FAO/UNICEF) August 1966 Meeting - Geneva. 7 pp.

Research on aflatoxins carried out at the National Research Laboratories, Hyderabad (India), is presented. Aflatoxicosis was found in buffaloes and ducks at government farms near Hyderabad. Aflatoxin contamination was found in the livestock feeds. Biological effects of aflatoxins in ducklings and rats are described. Of 60 varieties of groundnut screened for resistance to aflatoxin production by a toxigenic strain of Aspergillus flavus, one variety "U.S. 26" did not support aflatoxin production.

1602. Kiran Kalia, Desai, H.M., and Chakraborty, M.K. 1988. Resistance of groundnut (*Arachis hypogaea)* to aflatoxin. Indian Journal of Agricultural Sciences 58(2): 121-123.

Fifty-three varieties of groundnut were tested for their ability to support aflatoxin production following inoculation of autoclaved seeds with an aflatoxigenic isolate of *Aspergillus flavus.* All varieties tested supported production of aflatoxins B_1 and B_2. High-yielding lines supported high levels of aflatoxin production. The cultivar J 11, earlier reported to have resistance to aflatoxin production, supported moderate levels of aflatoxin production. The line OG 35-1 showed the least aflatoxin production, but had a low yield potential.

1603. McDonald, D., and Mehan, V.K. 1982. Post harvest programme in ICRISAT. Pages 163-171 in Proceedings of the Workshop on Post Harvest Losses and Small Farmer Storage for South Asia and ECS Africa, 19-24 April, 1982, New

Delhi, India. Vol II Food Production and Rural Development Division, Commonwealth Secretariat, Marlborough House, Pall Mall, London SW1, UK.

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has comprehensive responsibility for research on its mandate crops of sorghum, pearl millet, pigeonpea, chickpea, and groundnut. However, priority has been given to field problems and only limited research has been done on postharvest problems. The highest priority project with important postharvest and storage aspects is the aflatoxin problem in groundnut. The research emphasis at ICRISAT has been placed on utilization of genetic resistance to develop groundnut cultivars with pods or seeds which the fungus cannot invade, or which if invaded do not support aflatoxin production. A number of breeding lines and cultivars have been identified which have seeds with good resistance to invasion by *A. flavus*. All genotypes so far tested have supported production of aflatoxins but some have given much slower rates of toxin accumulation than have others.

1604. Mehan, V.K. 1989. Screening groundnuts for resistance to invasion by *Aspergillus flavus* and to aflatoxin production. Pages 323-334 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 October 1987. ICRISAT Center, India. Patancheru. A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

Research in several countries into evaluation of responses of groundnuts to seed infection and colonization 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 provided 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*. The usefulness of these resistances in management of aflatoxin contamination is discussed.

1605. Mehan, V.K., and McDonald, D. 1980. Screening for resistance to *Aspergillus flavus* invasion and aflatoxin production in groundnuts. ICRISAT Groundnut Improvement Program Occasional Paper-2. 15 pp.

This paper describes laboratory procedures to screen groundnuts for resistance to seed infection and colonization by *Aspergillus flavus,* and to aflatoxin production. Some methods for aflatoxin analysis are also outlined.

1606. Mehan, V.K., and McDonald, D. 1983. Aflatoxin production in groundnut cultivars resistant and susceptible to seed invasion by *Aspergillus flavus.* Pages 221-226 in Proceedings of the International Symposium on Mycotoxins, National Research Centre, Cairo, 6-8 September 1981. Cairo, Eygpt.

The use of groundnut cultivars resistant to seed invasion and colonization by *Aspergillus flavus* is a possible means of preventing or reducing aflatoxin contamination. Such resistance was identified in several cultivars, one of which was the released commercial Indian cultivar J 11. No direct relationship was found between resistance to seed colonization by *A. flavus* and the quantity of aflatoxins produced when seeds were colonized by toxigenic strains of the fungus. Some of the cultivars with seed resistance to *A. flavus* colonization have been found resistant to fungal pod rot.

1607. Mehan, V.K., and McDonald, D. 1984. Research on the aflatoxin problem in groundnut at ICRISAT. Plant and Soil 79(2): 255-260.

Aflatoxin contamination of groundnut is a serious problem in most groundnut producing countries and as such is given high priority by the Groundnut Improvement Program of ICRISAT. Since 1979 the emphasis has been on selecting cultivars resistant to seed invasion and colonization by toxigenic strains of *Aspergillus flavus,* and/or to aflatoxin production following invasion by the fungus. Of 850 germplasm lines screened for reaction to seed invasion and colonization by *A. flavus* in laboratory tests, eight proved resistant. Three of these lines (PI 337394F, PI 337409 and UF 71513) had been reported resistant in the USA but the other five were new sources of resistance. Several groundnut cultivars have been screened for resistance to seed invasion by *A. flavus* in the field, both under natural conditions and with the inoculum of the fungus added to the soil in the pod zone. Some cultivars with resistance to seed colonization also showed resistance to seed invasion by *A. flavus*. None of the cultivars tested was completely resistant to aflatoxin production but significant cultivar differences occurred in the amounts of aflatoxin produced in seeds inoculated with a toxigenic strain of *A. flavus*.

1608. Mehan, V.K., McDonald, D., and Gibbons, R.W. 1982. [Seed colonization and aflatoxin production in groundnut genotypes inoculated with different strains of *Aspergillus flavus.].* Colonisation de la graine et production d'aflatoxine dans des genotypes d'arachide inocules avec differentes souches d'*Aspergillus flavus.* Oleagineux 37(4): 185-191.

Nine groundnut genotypes were tested for resistance to seed colonization by five

different strains *of Aspergillus flavus.* These genotypes, and the cultivar J 11 which has been shown to be resistant to seed colonization by *A.flavus,* were also checked for production of aflatoxin following infection of scarified, surface-sterilized seeds by three aflatoxigenic strains of *A.flavus.* The genotypes PI 337394F and PI 337409 showed significantly less seed colonization and internal invasion than the oilier genotypes. The *A. flavus* strains differed significantly from one another in their ability to colonize seeds and produce internal infection. Strain NRRL 3000 was the least effective. Of the three strains used in the aflatoxin production tests, AF 8-3-2A produced the highest levels of aflatoxin B, on all genotypes while AFS-3 produced the least; NRRL 3000 being intermediate in this respect. Aflatoxin G, was produced on all genotypes by NRRL 3000, and on J 11 by AF 8-3-2A. There was no obvious correlation between seed resistance to *A. flavus* colonization and aflatoxin production when seeds were infected. Significantly higher amounts of aflatoxin B_1 were produced in the two genotypes resistant to *A. flavus* colonization than in the highly susceptible genotype FESR-11-P11-B2-B1.

1609. Mohan, V.K., McDonald, D., and Ramakrishna, N. 1986. Varietal resistance in peanut to aflatoxin production. Peanut Science 13(1): 7-10.

Rehydrated, mature, undamaged seed of 502 groundnut genotypes were scarified, inoculated with an aflatoxigenic strain *of Aspergillus flavus,* and tested for aflatoxin B_1 production after incubation at 25°C for 10 days. All genotypes supported production of aflatoxin B_1 but significant genotypic differences in levels of aflatoxin B_1 production were found. Genotypes U 4-7-5 and VRR 245 supported the lowest levels of aflatoxin B_1 (<10 µg g^{-1} seed), whereas the commonly grown Indian cultivar TMV 2 supported production of aflatoxin B_1 at levels of over 150 µg g^{-1} seed. Eight genotypes with low, moderate or high capacity to support aflatoxin B_1 production were further tested using seed from one rainy season crop, and two irrigated postrainy season crops. Genotypic differences in levels of aflatoxin B_1 production were consistent over seasons. Production levels were slightly lower in seed from the rainy season crop than in seed from the two postrainy season crops.

1610. Nagarajan, V., and Bhat, R.V. 1973. Aflatoxin production in peanut varieties by *Aspergillus parasiticus* Speare. Applied Microbiology 25(2): 319-321.

Tests with the groundnut varieties TMV 2 and US 26, three isolates of *Aspergillus flavus* (producing aflatoxins B, and B_2) and two isolates *of A. parasiticus* (producing aflatoxins B_1, B_2, G_1 and G_2) showed that aflatoxin production in groundnut depended both on the host variety and on the species or strain of the fungus.

1611. Naguib, Kh., Naguib, M.M., Diab, M.M., Sahab, A.F., and Amra, H. 1989. Occurrence of aflatoxins and aflatoxin-producing strains of *Aspergillus flavus* in groundnut cultivars in Egypt. Pages 311-315 in Aflatoxin contamination of groundnut: proceedings of the International Workshop, 6-9 October 1987, ICR1SAT Center, India. Patancheru, A.P. 502 324, India : International Crops Reserach Institute for the Semi-Arid Tropics.

Twenty-one groundnut genotypes obtained from the International Crops Research Institute for the Semi-Arid Tropics (1CRISAT), Patancheru, Andhra Pradesh. India, and the United States Department of Agriculture (USDA), and one cultivar (Giza 4) from Egypt included as a local susceptible control, were tested for their ability to support aflatoxin production. All the genotypes supported production of aflatoxins B_1 and B_2, although the amount produced differed among genotypes. The lowest level of total aflatoxin production being 19180 µg kg^{-1} seed in genotype Ah 7223, and the highest 44290 µg kg^{-1} seed in cultivar Giza 4.

1612. Pettit, R.E., Azaizeh, H.A., Taber, R.A., Szerszen, J.B., and Smith, O.D. 1989. Screening groundnut cultivars for resistance to *Aspergillus flavus, Aspergillus parasiticus,* and aflatoxin contamination. Pages 291-304 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 October. 1987. ICRISAT Center, India. Patancheru. A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

Factors determining resistance to aflatoxin contamination are reviewed. Possible factors influencing resistance in groundnut pods and seeds to invasion by *Aspergillus flavus* and to aflatoxin production are considered. A range of groundnut genotypes were examined for presence of inhibitory compounds in testae and cotyledons and considerable variation was found. Tannin-like compounds found in seed testae of some genotypes inhibited *A. flavus* growth and aflatoxin production. Polypeptide contents of seeds varied between genotypes susceptible and those resistant to seed colonization by *A. flavus.*

1613. Priyadarshini, E, and Tulpule, P.G. 1978. Relationship between fungal growth and aflatoxin production in varieties of maize and groundnut. Journal of Agricultural and Food Chemistry 26(1): 249-252.

Seeds of 13 maize and six groundnut varieties were inoculated with a spore suspension of *Aspergillus parasiticus.* Wide variations were observed between varieties of both crops for fungal growth, assessed by glucosamine content, and aflatoxin production. Aflatoxin production per unit growth of the fungus differed between varieties, and maize varieties with soft endosperm, such as Shakti, supported greater fungal growth than hard-endosperm varieties like composite H3. although aflatoxin production did not differ. Both fungal growth and aflatoxin production increased linearly with incubation time up to 7 days, but there was no correlation between growth rate and aflatoxin production in the varieties used,

indicating that some varieties are less suitable for afiatoxin production than others, irrespective of how well they support fungal growth.

1614. Rao, K.S., and Tulpule, P.G. 1967. Varietal differences of groundnut in the production of aflatoxin. Nature (London) 214: 738-739.

Sixty varieties of groundnut were tested for their ability to support afiatoxin production following inoculation of seeds with an aflatoxin-producing strain of *Aspergillus flavus*. All varieties except US 26 supported production of afiatoxin. Resistance to afiatoxin production in US 26 was confirmed in further tests.

1615. Tulpule, P.G., Bhat, R.V., Nagarajan, V., and Priyadarshini, E. 1977. Variations in afiatoxin production due to fungal isolates and crop genotypes and their scope in prevention of afiatoxin production. Archives de l'Institut Pasteur de Tunis 54(3-4): 487-493.

Varietal differences in afiatoxin production by *Aspergillus flavus* and *A. parasiticus* were studied in 78 varieties of groundnut, 38 varieties of maize, five varieties of soybean, and five varieties of sunflower. Varieties supporting minimal afiatoxin production were identified.

1616. Wilson, D.M., Branch, W.D., Beaver, R.W., and Maw, B.W. 1990. Screening peanut genotypes for resistance to afiatoxin accumulation. Proceedings of the American Peanut Research and Education Society 22: 33.

During 1988 and 1989, preliminary screening trials were conducted under two rainout shelters to determine possible differential afiatoxin production in four groundnut genotypes (Florunner, Sunbelt Runner, Tifrun and Tifton-8). In 1988, the rainout shelters failed due to old sensors malfunctioning during critical rainy periods. Thus, this extra moisture eliminated afiatoxin and any test results. However, in 1989 after repairs the shelters performed as expected, and drought stress was severe and uniform. Significant genotypic differences were obtained in one shelter but not another. Overall. Tifton-8 had a significantly lower afiatoxin content than Tifrun and Florunncr; Sunbelt Runner was intermediate. These data strongly suggest that differences do exist among certain groundnut genotypes for afiatoxin production.

8.2.8 Breeding for Resistance to *Aspergillus flavus* Infection and Colonization

1617. Mixon, A.C. 1976. Peanut breeding strategy to minimize afiatoxin contamination. Proceedings of the American Peanut Research and Education Association 8(1): 54-58.

Screening, selection and breeding procedures for increasing the resistance of groundnut varieties to aflatoxin-producing strains *of Aspergillus flavus* are reviewed and discussed. This review includes consideration of sources and nature of resistance, reaction of genotypes to seed colonization, variation among fungal isolates of A. flavus, and factors associated with the interaction of aflatoxin-producing strains of the fungus and seed of groundnut genotypes. The pros and cons of breeding an improved groundnut variety with greater resistance to afiatoxin contamination are presented.

1618. Mixon, A.C. 1977. Influence of plant generics on colonization by *Aspergillus flavus* and toxin production (peanuts). Pages 163-172 in Mycoioxins in human and animal health. (Rodricks, J.V.. Hesseltine, C.W., and Mehlman, M.A., eds.). Illinois, USA : Pathotox Publishers, Inc. 807 pp.

The potential for using genotypes identified to be resistant to seed colonization by aflatoxin-producing strains of *Aspergillus flavus* and *A. parasiticus* for breeding agronomically suitable groundnut cultivars with resistance to the toxigenic fungi is discussed. Factors affecting seed colonization in groundnuts are discussed and factors associated with resistance to colonization of the groundnut seed testa are reviewed.

1619. Mixon, A.C. 1979. Developing groundnut lines with resistance to seed colonization by toxin-producing strains of Aspergillus species. Pest Articles News and Summaries 25(4): 394-400.

Evidence for the possible development of groundnut cultivars with favourable agronomic characteristics and resistance to afiatoxin- producing strains of *Aspergillus flavus* is presented, in studies of F_2 and F_3 progenies from crosses of resistant and susceptible genotypes data on the frequency distribution and least square estimates of genetic effects showed the possibility of selecting for resistance to seed colonization by the fungus. Yield, value and seed quality data for seven advanced lines developed by using pedigree selection from crosses showed that potentially favorable groundnut cultivars may be developed.

1620. Mixon, A.C. 1981. Reducing afiatoxin contamination in peanut genotypes

by selection and breeding. Journal of the American Oil Chemists' Society 58: 961A-966A.

The potential for developing agronomically suitable cultivars using groundnut genotypes that exhibit resistance to seed colonization by aflatoxin-producing strains of *Aspergillus flavus* is discussed. The practical implications of developing resistant cultivars are presented in data for yield, value and seed quality for six advanced groundnut lines that were developed by breeding and selection from crosses.

1621. Pins, O.de. 1983. [Cuitivar improvement of groundnut and production of selected seed.]. Amelioration varietale dc l'arachide et production de sentences se'lectionnees. Oleagineux 38(2): 61-71.

A groundnut breeding programme is outlined, of which the major aims are high yields, resistance to drought, *Aspergillus flavus,* Puccinia arachidis, Cercospora arachidicola, Cercospora personata and rosette virus, and high technological qualities. The breeding methods briefly discussed include mass selection, pedigree selection, the bulk method, single seed descent and multifamilies (a composite of isogenic families).

1622. Rao, M.J.V., Nigam, S.N., Mehan, V.K., and McDonald, D. 1989. *Aspergillus flavus* resistance breeding in groundnut : Progress made at ICRISAT Center. Pages 345-356 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 October, 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

Progress worldwide in breeding groundnuts resistant to seed colonization by *Aspergillus flavus* and aflatoxin contamination is summarized, and research at ICRISAT is 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. The need for breeders to incorporate other resistance traits is stressed, and future prospects for breeding for resistance to aflatoxin production are discussed.

1623. Utomo, S.D., Anderson, W.F., Wynne, J.C., Beute, M.K., Hagler, Jr., and Payne, G.A. 1990. Estimates of heritability and correlation among three mechanisms of resistance to *Aspergillus parasiticus* in peanut. Proceedings of the American Peanut Research and Education Society 22: 26.

The heritability of three mechanisms of resistance to aflatoxin including seed colonization resistance (SCR), aflatoxin production resistance (APR), and preharvest infection resistance (PIR), as well as phenotypic correlations among the mechanisms, was estimated. Forty-five F_2-derived F_6 families of two crosses, AR-4/NC 7 and GFA-2/NC 7, were evaluated using a randomized complete block design with three replications. To evaluate SCR, sterilized rehydrated seeds were inoculated by a spore suspension of *Aspergillus parasiticus* strain NRRL 2999 (5×10^5 conidia mL^{-1}). Percent seed colonization was recorded after 8 days of incubation at 25°C. To evaluate APR, seed coat was removed from five seeds which were then sterilized, rehydrated, inoculated, and incubated for 4 days at 29°C. Samples were analyzed using an HPLC to determine aflatoxin production. Plants of each family were evaluated for PIR in the greenhouse and inoculated at 40 days after sowing (DAS). Drought stress was imposed at 60 DAS to induce *A. parasiticus* infection. Seeds from each plant were harvested, dried, and plated onto malt salt agar, and percent infection was recorded after 8 days. In cross AR-4/NC 7. heritability estimates of SCR, APR. and PIR were 0.55 ± 0.23, 0.20 + 0.25, and 0.27 + 0.25, with family means ranging from 7-95 %, 7.95-43.62 mg kg^{-1}, and 0-100 %, respectively. In cross GFA- 2/NC 7, heritability estimates of SCR, APR, and PIR were 0.63 ± 0.21, 0.47 ± 0.22, and 0.33 ± 0.26, with family means ranging from 25-98 %, 14.73-69.62 mg kg^{-1}, and 0-100 %, respectively. The means of the three traits in cross AR-4/NC 7 were generally lower than those of GFA-2/NC 7. In both crosses, there was no significant correlation among three mechanisms of resistance, indicating that the three mechanisms are controlled by different genes. Selections from cross GFA-2/NC 7 should produce a number of progenies with resistance than selections using AR-4/NC 7.

8.2.9 Segregation and Decontamination

1624. BaikalofT, A., and Read, M.J. 1985. Aflatoxin - Incidence, segregation and destination in Australia. Proceedings of the American Peanut Research and Education Society 17: 55.

The occurrence of aflatoxin in the Australian groundnut crop is mainly due to late season drought stress. The Australian Peanut Marketing Board uses a minicolumn test to segregate aflatoxin- contaminated (aflatoxin > 16 µg kg^{-1}) farmers' stock groundnuts at the point of delivery. To check the effectiveness of this segregation, an investigation was made to quantify the incidence of aflatoxin in groundnuts at several major points in the deshelling, grading and blanching operations. The aflatoxin "positive" segregation lots contained aflatoxin levels eight times higher than the aflatoxin "negative" lots. Seventy-one % of the aflatoxin through the

shellers was concentrated into the oil milling kernels, which comprised 16 % of the total kernels. Oil kernels from "negative" and "positive" stock averaged 48 µg kg^{-1} and 253 µg kg^{-1} aflatoxin respectively. It appears that in excess of 50 % of the aflatoxin in the product which was roasted and blanched was lost and/or degraded in the operation.

1625. Blankcnship, P.D., Davidson, J.I.Jr., Sanders, T.H., and Bennett, C.T. 1984. Separation and removal of aflatoxin contaminated peanuts at peanut cleaning and shelling plants. Proceedings of the American Peanut Research and Education Society. 16(1): 43.

Approximately 7 tonnes of Segregation 3 official grade check samples from farmers' stock groundnuts marketed in 1980 were cleaned and shelled in the USDA pilot shelling plant. Large samples were removed at 28 different points in the shelling process within the plant. The portion of material that was removed from each point was blended and divided into four samples, ground, blended and subsampled. The subsamplcs were analyzed for aflatoxin using minicolumn chromatography. Pod damage and pod strength were direcdy correlated with aflatoxin levels while pod size, seed density, and pod terminal velocity were inversely correlated with aflatoxin levels. Use of these findings in designing farmers' stock cleaning and shelling systems are discussed.

1626. Bockelee-Morvan, A., and Gillier, P. 1974. [Trial of the elimination of aflatoxin in groundnuts by physical methods.]. Essai d'elimiation de l'aflatoxine de l'arachide par des methodes physiques. Oleagineux 29(11): 513-516.

In a trial in 1972, 8 samples of unshelled groundnuts from Mali, Niger and Senegal were sorted manually. Intact pods averaged 73 % of the total of all samples and contained 24 % of the total aflatoxin content. Pods damaged by millipedes were only 4.3 % of the total, but contained 40 % of the total aflatoxin content. In a similar trial in Senegal, 4 samples of shelled seeds were sorted manually, using the criteria employed for confectionery groundnuts, into intact seeds, broken seeds, seeds which had lost their testa, (a) abnormally-colored seeds and (b) seeds showing growth *of Aspergillus flavus.* Almost all the total aflatoxin content was in (a) and (b). The results are discussed in relation to development of mechanical/electronic methods of sorting pods and seeds to reduce aflatoxin content.

1627. Bockelee-Morvan, A., and Gillier, P. 1975. Trial of the elimination of aflatoxin in groundnuts by physical methods. Pages 291-293 in in Proceedings of the Conference on animal feeds of tropical and subtropical origin, 1974, Tropical Products Institute, London, UK.

Aflatoxin contamination of groundnut kernels from francophone West Africa is largely confined to those from pods which become physically damaged by pests before harvesting, or during harvesting. Trials are described in which it is shown that a significant reduction in overall aflatoxin contamination of unshelled groundnuts can be achieved by the removal of defective pods by hand or pneumatic sorting. The removal of abnormal kernels from shelled groundnuts by mechanical or hand sorting also greatly reduces overall aflatoxin contamination.

1628. Coker, R.D., Jewers, K., and Jones, B.D. 1986. The treatment of anatoxin contaminated commodities. International Biodeterioration Supplement 22:103-108.

This paper very briefly reviews several methods for decontamination and detoxification of aflatoxin-contaminated foods and feeds. The utilization of ammonia gas has been extensively investigated and commercial facilities exist for the detoxification of groundnut cake by ammoniation.

1629. Cole, R.J., Dorner, J.W., and Dowell, F.E. 1989. Effect of belt screening on aflatoxin in farmers stock peanuts. Proceedings of the American Peanut Research and Education Society 21: 63.

Data from two different belt screening studies have shown that belt screening reduced aflatoxin in all three groundnut types. A further reduction was achieved when oil stock and damaged kernels were removed. The amount of reduction for each step depended on the distribution of aflatoxin contamination in a farmers' stock load. When the loose shelled kernels contribute a significant amount of the aflatoxin in a farmers' stock load, the belt screen will be very effective for aflatoxin reduction. When the oil stock and damage contribute more of the aflatoxin in a farmers' stock load, the belt screen will be less effective. Ideally, both risk components need to be removed for efficient aflatoxin reduction.

1630. Davidson, J.I.Jr., Holaday, C.E., and Bennett, C.T. 1981. Separation and removal of aflatoxin contaminated kernels in peanut shelling plants : Part 1 A case study. Proceedings of the American Peanut Research and Education Society 13(1): 29-45.

A 20-tonne lot of Segregation 3 groundnuts grown in 1979 was shelled in the USDA pilot shelling plant and 50 to 250 kg of groundnut samples were removed at each of 42 different locations throughout me plant. The groundnut sample mat was removed from each location was blended, divided into four samples, ground, blended, subsampled, and the subsamples analyzed for aflatoxin. Aflatoxin contamination was direcdy related to the stage of shelling, resistance to shelling, and inversely related to seed thickness and specific gravity. Use of these findings in designing shelling and processing plants is discussed.

1631. Dickens, J.W. 1974. Selective harvesting may help reduce segregation-3 peanuts. Southeastern Peanut Farmer 12: 3.

1632. Dickens, J.W. 1977. Aflatoxin occurrence and control during growth, harvest and storage of peanuts. Pages 99-105 in Mycotoxins in human and aninmal health. (Rodricks, J.V., Hesseltine, C.W.. and Mehlman, M.A., eds.). Illinois, USA : Pathotox Publishers, Inc.

This paper discusses causes of aflatoxin contamination and methods of prevention and segregation to control the aflatoxin problem in farmers' stock groundnuts from the time the groundnuts are growing in the field until they are marketed, stored, and shelled.

1633. Dickens, J.W. 1983. Mycotoxins - Prevention and control. Pages 131-141 in Proceedings of the International Symposium on Mycotoxins, 6-9 September 1981, Cairo, Egypt.

The availability of mycotoxin-free food products depends upon a successful program of mycotoxin prevention and control. Such a program must be designed according to the growing, harvesting, drying, storing, transporting and processing methods employed for each food commodities. The aflatoxin prevention and control program for groundnuts is broader in scope and better organized than any other mycotoxin control program employed in the United States. Many features of this program may be used for mycotoxins in other commodities. Rapid aflatoxin detection methods are employed when groundnuts arc marketed from the farm to avoid mixing lots of groundnuts containing high concentrations of aflatoxin with those lots which contain either low concentrations or no aflatoxin. Lots with high concentrations of aflatoxin are diverted to non-food uses. Sorting techniques and other processes are utilized to remove aflatoxin-contaminated kernels from those lots of groundnuts intended for food use. An extensive aflatoxin sampling and testing program is used to prevent marketing for food processing those lots of shelled groundnuts which test more than 25 μg kg^{-1} aflatoxin. The finished product is than tested for aflatoxin by the Food processor. Although less well developed than the program for groundnuts, aflatoxin prevention and/or control methods are also employed for corn, cottonseed, almonds, pistachio nuts, Brazil nuts, animal feeds, milk and other commodities.

1634. Dickens, J.W., and Sattcrwhite, J.B. 1971. Diversion program for fanners' stock peanuts with high concentrations of aflatoxin. Oleagineux 26(5) : 321-328.

In the USA. determination of visible *Aspergillus flavus* growth on groundnut kernels has been reported to be an effective method for detecting aflatoxin contamination and for classification of farmers' stock groundnuts. Lots containing *A. flavus* on kernels are designated "Segregation-3" groundnuts and are diverted to non-food use, except for the oil which is aflatoxin-free. Lots not found to contain kernels with visible *A. flavus* and with low levels of damage are designated "Segregation-I" groundnuts. This article discusses the method and the results of the tests to determine the efficacy of the method for determining lots of farmers' stock groundnuts with high concentrations of aflatoxin.

1635. Dickens, J.W., and Whitaker, T.B. 1975. Efficacy of electronic color sorting and hand picking to remove aflatoxin contaminated kernels from commercial lots of shelled peanuts. Peanut Science 2(2): 45-50.

Samples (89.3 kg) from 40 commercial lots of shelled groundnuts each containing an average concentration of 48 μg kg^{-1} aflatoxin were sorted, using an electronic color sorter, 3 to 5 times and subsequently discolored kernels were removed by hand. Prediction equations indicted that cumulative removal of 2, 4, 6, 8 and 10 % of the kernels from each sample by electronic sorting would remove an average of 16, 28, 37, 45, and 51 % of the aflatoxin content respectively. Electronic sorting became less selective for aflatoxin-contaminated kernels during each additional sorting operation. Careful hand picking of discolored kernels was far more selective for aflatoxin-contaminated kernels than electronic color sorting. An average 72 % of the aflatoxin was in kernels that were removed by electronic sorting and subsequent hand picking. The efficacy of aflatoxin removal with electronic sorting varied considerably among the lots, indicating that each lot should be pretested to determine if aflatoxin can be effectively removed before the expense of electronic color sorting is incurred.

1636. Dowell, F.E., Dorner, J.W., Cole, R.J., and Davidson, J.I.Jr. 1990. Aflatoxin reduction by screening farmers stock peanuts. Peanut Science 17(1): 6-8.

Samples from 17 loads of farmers' stock groundnuts suspected of containing aflatoxin were screened over a belt cleaner, shelled and sorted into grade components. Tests showed that removal of loose shelled kernels (LSK) and small pods by belt screening reduced aflatoxin levels by an average of 35 %. Belt screening removed 97 % by weight of the LSK, but only 4 % of the sound mature kernels and sound splits (SMK+SS). Further removal of other edibles (OE), oil stock (OS), LSK and damaged kernels (DM) from the groundnuts riding over (OVERS) the belt screen reduced aflatoxin levels from an average of 110.7 μg kg^{-1} in the unscreened load to 3.8 *μg* kg^{-1} in SMK+SS. The OE, OS, LSK and DK were removed from the OVERS through the use of slotted screens and by sorting.

1637. Gnanasekharan, V., Chinnan, M.S., and Dorner, J.W. 1990. Density distribution of aflatoxin contaminated peanuts. Proceedings of the American Peanut Research and Education Society 22: 24,

Samples of groundnuts (cv Florunner) were randomly drawn from a commercial color sorter reject stream, to increase the chances of acquiring aflatoxin contaminated samples. Two hundred samples from each size range (jumbo, medium and No. 1) were utilized to map the density distributions of allatoxin contaminated groundnuts. A water displacement technique developed in the Food Science and Technology laboratory. Georgia Agricultural Experiment Station, that practically eliminates water absorption by the nuts, was used to determine individual kernel densities. A two parameter logistic function was found to be most appropriate for modelling the density data. To facilitate widespread practical applicability, predictive models were developed for the cumulative density distribution of each size range. The same kernels were split and studied for internal discoloration and/or fluorescence under long range UV light, these being indicators of possible contamination. The suspect kernels were individually quantitated for aflatoxins by reverse phase high performance liquid chromatography. This data would allow correlation of aflatoxin levels with density on an individual kernel basis, minimizing the extreme variability associated with sampling for aflatoxin contamination. The predictive models developed in this study could be used in the design and assessment of density based separation techniques for aflatoxin contaminated groundnuts from non-contaminated groundnuts.

1638. Hanssen, E., and Birn, K. 1978. Efficacy of electronic sorting devices for separation of discolored groundnut kernels. Lebensmittelchemie und Gerichtliche Chemie 32: 81-82.

Legal requirements concerning freedom of groundnuts from allatoxin in the Federal Republic of Germany are briefly considered. A study on batches of groundnuts from various countries, studied in 1976/77. showed that the percentage of aflatxoin-free samples were : China, 98 % of 77 samples: Gambia, 50 % of 6 samples: India, 50 % of 16 samples: Sudan, 16 % of 104 samples: South Africa, 98 % of 399 samples: and USA, 90 % of 102 samples, Aflatoxin concentrations were highest in the Sudanese samples (<300 µg kg^{-1}). A description is given of an optical/electronic sorting system for separation of discolored kernels. The kernels pass in front of a screen coloured to match normal kernels : an optical system detects kernels which differ in color from the screen. These kernels (which have a much higher than normal likelihood of aflatoxin contamination) arc then automatically separated. Throughput of a 12-channel apparatus is 2 t h^{-1}: mean aflatoxin content of the selected normal kernels is reduced by 90-95 % as compared to the unsorted material.

1639. Henderson, J.C., and Hagan, W. 1989. Potential for aflatoxin removal by density segregation. Proceedings of the American Peanut Research and Education Society 21:64.

A patented process to separate aflatoxin-contaminatcd groundnuts from uncontaminated groundnuts is discussed. Contaminated groundnut lots taken through the process, from incoming raw groundnuts to peanut butter in the jar, are analytically profiled by data from three analytical procedures : (1) aflatest affinity column chromatography, (2) thin-layer chromatography (TLC), and (3) high performance liquid chromatography (HPLC) with post- column iodine derivatization.

1640. Henderson, J.C., Kreutzer, S.H., Schmidt, A.A., Smith, C.A., and Hagen, W.R. 1989. Flotation separation of aflatoxin-contaminated grain or nuts. United States Patent 4, 795, 651.

Disclosed is a method for separating mycotoxin-contaminated grains, kernels, seeds and nuts from uncontaminated whole grain seeds, whole or split kernel nuts and seeds to obtain a substantially uncontaminated supply of these foods. The separate contaminated source can be further processed to lower the mycotoxin contamination. The process involves the separation of the mycotoxin or aflatoxin-containing materials by floating the aflatoxin-contaminated foods in a liquid having a specific gravity of from about 0.9 to about 1.2. A highly preferred process uses dynamic llotation.

1641. Kirksey, J.W., Cole, R.J., and Dorner, J.W. 1989. Relationship between aflatoxin content and buoyancy of florunner peanut kernels. Peanut Science 16: 48-51.

A water floatation method was used to study the distribution of aflatoxin relative to kernel density in naturally contaminated samples of shelled fanners' stock groundnuts. Five-hundred gram samples of visibly undamaged, contaminated groundnuts were added to 2000 mL of tapwateT, and approximately 15-30 % of the kernels rose to the surface as buoyant kernels. These buoyant kernels contained an average of 95 plus % of the total sample aflatoxin content. Buoyant kernels, when examined internally, all had a hollow space or "lumen" inside the kernel between the two cotyledons. Data showed an association between aflatoxin content, kernel lumen volume, and the propensity of kernels to float. The lumen may provide a reservoir of air for flotation, fungal growth, and aflatoxin production. The positive association between the presence of a lumen and aflatoxin contamination may provide a possible resistance strategy, if the presence or absence of a lumen is genetically controlled or if it can be manipulated physiologically.

1642. Kirksey, J.W., Cole, R.J., Dorner, J.W., and Henning, R.J. 1987. Density segregation of peanuts naturally contaminated with aflatoxin. Proceedings of the American Peanut Research and Education Society 19: 36.

A water flotation method was tried to reduce aflatoxin concentrations in naturally

contaminated samples of shelled farmers' stock groundnuts. Five-hundred-gram samples of contaminated groundnuts were added to 2000 mL of tap water with approximately 55-15 % of the kernels rising to the surface as "floaters". These floaters, when analyzed, contained 80-90 % of the total sample aflatoxin content and their removal resulted in considerable cleanup of the samples. However, there were some limitations. Splits, small whole kernels (< 16/64 in.), and balds did not follow the same "float-sink" pattern of aflatoxin reduction. The method seems best suited for aflatoxin reduction in jumbo, medium, and No. 1 sized kernels.

1643. Leek, J.M. 1989. Aflatoxin management at the manufacturing level. Proceedings of the American Peanut Research and Education Society 21: 64.

The role of the manufacturer is to assure that groundnut products meet consumer requirements for both product performance and product wholesomeness. Aflatoxin measurements on shelled groundnut lots do not correlate well with aflatoxin levels in peanut butter made from the lots. However, removing defects (loose shelled kernels) can reduce aflatoxin levels in peanut butter and improves the correlation between aflatoxin levels in raw groundnuts and in peanut butter. Most of the defects identified are best isolated at the farmers' stock and shelling plant levels of the production chains, although manufacturers have influence on many sources of aflatoxin through blanching and related systems.

1644. Marion, J.E., Ayres, J.L., and Steele, B. 1978. Non destructive quality evaluation of agricultural products - industrial application. Journal of Food Protection 41: 54-56.

A detailed discussion of electronic color sorting of groundnuts, pecans and almonds is presented, particularly in relation to aflatoxin sampling and reduction, preparing for further processing, and evaluating commercial sorters for speed and accuracy.

1645. Maselli, J.A. 1977. Controlling aflatoxin in your plant. Manufacturing Confectioner 57: 35-38,40-41.

The clinical effects of aflatoxin exposure are briefly described, and the current US FDA regulations and sampling procedure for control of aflatoxins in groundnuts are discussed. Some quality control procedures to ensure that only minimal quantities of toxin persist into the finished product are outlined.

1646. Morgan-Grampian Ltd. 1980. Colour sorting of African groundnuts. Control and Instrumentation 12(5): 27.

Optical electronic sorters are being used in Senegal to reduce the high incidence of aflatoxin in groundnuts, and thereby raise the quality and value of the residual cake following oil extraction. Tests set up to discriminate between acceptable nuts and those containing *Aspergillus flavus* confirmed that the machine could achieve the critical level oil" 50 µg kg^{-1} aflatoxin B_1. Once the oil has been extracted from the nuts, the remaining meal represents 50% of the input and contains virtually all the contaminants, raising its aflatoxin content to 100 µg kg^{-1}. This level is considered safe for mixing with other ingredients to form cattle cake containing a maximum of 20 µg kg^{-1} aflatoxin. The machine has been developed in order to recognize not only dark nuts but also unwholesome shades of yellow, pink, brown and green from areas of acceptable white meat. Provision is also made for removing dust produced during shelling.

1647. Parker, W. 1989. Aflatoxin removal by blanching. Proceedings of the American Peanut Research and Education Society 21: 64.

Removal of the skin or testa from dried groundnuts provides a superior color contrast when sorting a white, dried kernel in comparison to redskin or roasted groundnuts. The low temperature heat from blanching produces a bloom effect on the major and minor damage resulting in a significant increase of me damaged kernels that can be rejected and removed by electronic sorting. Groundnut lots designated for blanching by PAC-USDA must not exceed an average of 10 µg kg^{-1} aflatoxin alter blanching. Shelf life is not changed as a result of the blanching process.

1648. Pattinson, I., Crowther, P., and Noubey, H.E. 1975. The separation of aflatoxin infected groundnut kernels. Tropical Science X(4): 212-221.

A field trial was conducted into the separation of aflatoxin- contaminated groundnuts from sound kernels using air separation, size grading and color sorting equipment. With the use of air separation and color sorting equipment, it was possible to remove infected from non-infected kernels almost completely.

1649. Pettit, R.E., and Chan, A.K. 1980. Detection of mold and mycotoxin damaged peanut kernels with helium-neon laser reflected energies. Proceedings of the American Peanul Research and Education Society 12: 43.

An improved nondestructive technique for accurately detecting the extent to which kernels are damaged by molds and aflatoxin has been under study. A helium-neon laser emitting light at 6550 A wavelength with horizontal and vertical polarized components was first standarized on a known background target. The laser was then directed on the groundnut kernel surface and the back scattering amplitude of both polarizations recorded along with a computer calculated ration of the amplitudes. Preliminary results indicate that the amplitudes detected for pickout kernels are 30 to 40 % below the amplitude recorded for sound mature healthy kernels. The

amplitudes for *Aspergillus flavus* inoculated kernels were 15 to 20 % below those recorded for the sound mature kernels. The polarization ratio for sound mature healthy groundnut kernels deviated only slightly from unity. With mold damaged kernels examined along the long axis the horizontal wave component decreased so that the polarization ratio ranged from 0.60 to 0.80. Evidence indicates that the amplitude of the reflected laser beam correlates with the surface features of the kernels while the polarization ratio provides information on the internal composition of the kernels.

1650. Pettil, R.E., and Geiger, R.L. 1981. Dielectric properties of mold and mycotoxin damaged peanuts. Phytopathology 71: 249.

Dielectric characteristics of good sound mature kernels and damaged kernels of groundnut were investigated over a frequency range of 20 KHz to 20 MHz. Groundnuts were placed in a test capacitor and measurements were made on a HP 4342A Q meter. Equivalent moisture contents were maintained using humidity chambers as determined by the oven dry method. Preliminary results indicate that the real part of the dielectric constant (permittivity) is essentially independent of mold contamination level whereas the imaginary part (all. loss tangent) is strongly dependent upon contamination level. The simultaneous determination based upon these dielectric characteristics, provides a means of detecting varying degrees of mold and mycotoxin damage.

1651. Prevot, A. 1974. Evaluation of methods for elimination of aflatoxins in groundnut products. [Evolution et methodes d'elimination des aflatoxines dans les produits oleagineux (Huiles et tourteaux)]. Revue francaise des Corps Gras 21(2): 35-47.

Several methods of decontamination and detoxification of groundnuts and groundnut cake are reviewed. Aflatoxin levels can be reduced by segregation and decontamination of groundnuts at shelling and alter shelling. Influences of different stages in refining of oil on elimination of aflatoxin are described. Ammoniation of contaminated cake seems to be appropriate at industrial level.

1652. Read, M. 1989. Removal of aflatoxin contamination from the Australian groundnut crop. Pages 133-140 in Aflatoxin contamination of groundnut : proceedings of the International Workshop. 6-9 October, 1987, ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

The Australian groundnut crop is significantly affected by aflatoxin contamination in some years because of preharvest drought stress. By a process of selective segregation and sorting, aflatoxin-contaminated kernels are removed from the contaminated lots to reduce aflatoxin levels to an acceptable regulatory limit (15 µg kg^{-1}). 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.

1653. Telford, A.P. 1982. Control of aflatoxins in peanuts by segregation and sorting. Food Technology in Australia 34(5): 214-215.

Groundnuts are sampled by shellers when delivered by the grower and tested far moisture and aflatoxin content. Aflatoxin positive loads are processed separately by procedures which include color sorting and hand picking to reach the required 15 µg kg^{-1} limit. Moisture is controlled below the level which allows aflatoxin production.

1654. Tiemstra, P.J. 1977. Aflatoxin control during food processing of peanuts. Pages 121-137 in Mycotoxins in human and animal health. (Rodricks, J.V., Hesseltine, C.W.. and Mchlman, M.A., eds.). Illinois. USA : Pathotox Publishers, Inc. 807 pp.

Testing, removal, and segregation of aflatoxin-contaminated groundnuts is described in relation to the USA groundnut industry. Checking at all stages from growers to consumers is necessary. Sorting and removal of discolored groundnuts can reduce aflatoxin levels in raw groundnuts by 16 %. Reduction may be improved if sorting is done after roasting and blanching. About one third of the aflatoxin is destroyed during roasting. Raw groundnuts containing 25 µg kg^{-1} of aflatoxin will, as a finished product, contain approximately 8 µg kg^{-1} of aflatoxin. Selective procedures in the USA have been very effective so that few lots exceed the acceptance level of 25 µg kg^{-1}. The average aflatoxin content of groundnut products sold in the USA is less than 2 µg kg^{-1}.

8.2.10 Chemical Detoxification

1655. Achaya, K.T. 1975. A proposal to reduce aflatoxin levels in groundnut meal during solvent extraction. Proceedings of the Nutrition Society of India No. 19: 57-59.

Present measures for prevention or reduction of aflatoxin levels in groundnuts are described and a suggestion is made that during solvent extraction of groundnut meal, the solvent, hexane, should carry dissolved ammonia gas, which is known to destroy

aflatoxin. Further work will be required to determine the ammonia concentration needed, effect on meal and oil quality, and costs.

1656. Adrian, J. 1976. [Evaluation of lysine, methionine and cystine in a groundnut cake treated by ammonia.]. Evolution de la lysine, methionine et cystine dans le tourteau d'arachide traite' a l'ammoniac. Revue francaise Corps Gras 23(4): 209-212.

A treatment of groundnut cake by ammonia under pressure modified the equilibrium of its amino acids. Lysine did not change, cystine was destroyed to a large extent, and methionine increased significantly. The digestibility of amino acids measured in vitro was greatly improved due to a higher proportion of solubilized or 'free' amino acids. Eventhough the cystine is being destroyed in this process of detoxification, this method is preferable in-terms of protein efficiency of the groundnut cake.

1657. Ahmed, E.M., and Wei, C.I. 1987. Destruction of aflatoxin by microwave oven and chlorine gas. Proceedings of the American Peanut Research and Education Society 19: 41.

The effects of oven and microwave roasting on aflatoxin contaminated groundnuts and the effect of chlorine treatment on aflatoxin B_1 detoxification were studied. In artificially contaminated groundnuts, oven roasting for 30 min at 150°C or microwave roasting for 8.5 min at 0.7 KW were equally effective in destroying 30 to 45 % of aflatoxin B_1. In naturally contaminated groundnuts, both oven and microwave roastings were equally effective in destroying 48 to 61 % of aflatoxin B_1 and 32 to 40 % of aflatoxin G_1. Chlorine gas treatment was very effective in destroying aflatoxin B_1. Time course study of this treatment (100 µg aflatoxin B_1 with 15 mg chlorine gas at standard temperature and pressure) showed that about 60 to 75 % of the toxin was destroyed within 10 min of exposure. During the treatment process, at least three new fluorescent reaction products were produced and two of them were identified as 2,3-dichloro aflatoxin B_1 and 2.3-dihydroxy aflatoxin B_1 (diol). Use of radio-labelled aflatoxin B_1 confirmed these results. Chlorine- dose related study at 10 min exposure indicated that even the treatment of 100 µg of aflatoxin B_1 with 7.5 mg of chlorine caused about 75 % destruction. Preliminary mutagenicity study using the Ames Salmonella assay indicated that the mutagenic activity of the 10-min treated sample in the presence of rat liver S-9 mix can be reduced to about 10 % of that of the untreated control. The results indicated that low energy microwave roasting is not an efficient method to remove aflatoxin B_1 from contaminated groundnut samples, while chlorine gas could be an effective agent in reducing aflatoxin toxicity.

1658. Aibara, K., and Yano, N. 1977. New approach to aflatoxin removal. Pages 151-161 in Mycotoxins in human and animal health. (Rodricks, J.V., Hesseltine, C.W., and Mehlman, M.A., eds.). Illinois, USA : Pathotox Publishers, Inc. 807 pp.

A method is described for removal of aflatoxin from groundnuts by extraction with methoxymethane (dimethylether) at room temperature in an autoclave or in pressure-tight columns. Contaminated groundnuts are pulverized to pass through a 12 mesh screen, and extracted in stainless steel columns at approximately 6 kg cm^2 pressure. The extract can be used for preparation of groundnut oil, as aflatoxin is inactivated during refining, and methoxymethane is easily removed from the defatted product because of its low boiling point. The following compositions were obtained for raw and methoxymethane-treated groundnut samples, respectively : crude fat 54.8 and 0.1 % : crude protein 19.7 and 54.7 % : and total aflatoxins 1000 and 2.25-3.25 ppb (2.25-3.25 µg kg^{-1}). Duckling feeding tests showed no toxic effects of methoxymethane-treated groundnut powder.

1659. A pelt, J. 1989. Chemical disposal of undesired substances. Muhle + Mischfuttertechnik 126(30): 435-436.

Detoxification methods were developed for the removal of aflatoxin B_1 from groundnut, coconut, babassu seed and maize compounds imported into the German Federal Republic, and also for the removal of zearalenone, trichothecene and ochratoxin A in homegrown oats, barley, rye and maize. The use of heal treatment and/or alkali treatment was evaluated using skin toxicity and chicken embryo tests and in sow feeding trials.

1660. Asahi Kasei Kogyo Co. Ltd. 1978. Aflatoxin removal. British Patent 1 522 232.

A process is described for removal of aflatoxin from groundnuts, cereals, and oilseeds by extraction with methoxymethane (dimethylether) which may optionally be mixed with water, avoiding denaturation of proteins.

1661. Beckwith, A.C., Vesonder, R.F., and Ciegler, C. 1976. Chemical methods investigated for detoxifying aflatoxins in foods and feeds. Pages 58-67 in Mycotoxins and other Fungal Related Food Problems (Rodricks, J.V., ed.) Advances in Chemistry Series No. 149, American Chemical Society, Washington, DC, USA.

Refined edible vegetable oils are free of aflatoxins because the alkaline washes and bleeching agents used in the oil processing are among the chemical systems that remove or destroy aflatoxin. Currently, ammonia in conjunction with elevated temperatures or pressures, or both, as well as elevated moisture levels offers the best way to detoxify agricultural seed commodities for feed. Research at the Northern Regional Research Laboratory, Illinois, has shown that ammoniation of contaminated

whole corn reduces aflatoxin B_1 to a chemically nondetectable level and that the ammoniation products arc nontoxic to ducklings and chickens. Using radio labelled aflatoxin B_1 to spike white corn meals it was shown that ammoniation at ambient temperature induced the covalent binding of aflatoxin B_1 or B_1 degradative products primarily to corn proteins and water-soluble components.

1662. Belebeau, M.J., Gousse, R., and Weil, A. 1974. [Nutritional value of groundnut cuke detoxified by ammoniation for a developing rat.]. Valeur nutrionelle pour le rat en croissance d'un tourteau d'arachide detoxifie par ammoniation. Revue francaise Corps Gras 21: 469-473.

The nutritional value of a groundnut cake detoxified by ammoniation (25 μg kg^{-1} of aflatoxin B1 after treatment compared to 600 μg kg^{-1} prior to it) was estimated in developing rat. The cake included at the rate of 20 % in the diet had about 63 % nitrogen. The control diet consisted of a groundnut cake having a standard nutritional value with the aflatoxin B_1 content less than 25 μg kg^{-1}. In ad-libitum feeding, no significant differences were found between the cummulative weights obtained during 4 weeks. In case of weekly weights, the detoxified cake caused a reduction of 17 % in the fourth week. No differences were obtained in consumption indexes during the experimental period. The urea and alkaline phosphatases content in the blood plasmas did not vary during the fourth week, while the weekly and cummulative protein efficiency coefficient (PEC) was reduced from 15 and 6 % respectively in the detoxified cake.

1663. Briantais, M.M.G, Calet, C., Le Lay, Y., and Viroben, G. 1982. "Rapport de mission au Senegal sur la detoxification du tourteau d'arachide.". Rev. Aliment. Anim. 2: 9-14.

It is a report of a study group that went to Senegal to examine possibilities of detoxifying groundnut cake. Detoxification of aflatoxin-contaminated groundnut cake is feasible. It is suggested that chemical treatment (formaldehyde + ammonia) should be used on all groundnut cakes to ensure that aflatoxin levels in the cake are below 100 μg kg^{-1}. Cultural and storage practices to minimize aflatoxin contamination are also suggested.

1664. Ciegler, A. 1978. Detoxification of aflatoxin-contaminated agricultural commodities. Pages 729-738 in Toxins : Animal. Plant and Microbial. (Rosenberg, E.P.. ed.). Pergamon Press, New York, N.Y.

Methods of decontamination and detoxificationof aflatoxin- contaminated agricultural commodities are reviewed. Aspectsdiscussed include physical removal/segregation of contaminated materials,inactivation, and chemical detoxification.

1665. Codifer, L.P.Jr., Mann, G.E., and Dollear, F.G. 1976. Aflatoxin inactivation : treatment of peanut meal with formaldehyde and calcium hydroxide. Journal of the American Oil Chemists' Society 53(5): 204-206.

Groundnut meal contaminated withh aflatoxins (600 μg kg^{-1}) was treated with formaldehyde alone and in combination with calcium hydroxide in a bench-scale reactor, operated both sealed and at atmospheric pressure. In general, addition of calcium hydroxide to formaldehyde caused greater inactivation of the aflatoxins than did formaldehyde alone. With the reactor sealed and 25 % moisture in the meal, treatments for 1 h with 0.5 % and 1 % formaldehyde plus 2 % calcium hydroxide yielded products having 3 and 1 μg kg^{-1} aflatoxins, respectively, whereas reflux at atmospheric pressure with 20 % meal moisture and 1 h treatment with 1 % calcium hydroxide yielded a product with 5 μg kg^{-1} aflatoxins.

1666. Coker, R.D. 1989. Control of aflatoxin in groundnut products with emphasis on sampling, analysis, and detoxification. Pages 123-132 in Aflatoxin contamination of groundnut: Proceedings of the International Workshop, 6-9 Oct 1987, ICRISAT Center, Patancheru A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

This paper reviews the work carried out at the Overseas Development Natural Resources Institute (ODNR1), London, on the development of efficient sampling, sample preparation, aflatoxin analysis, and chemical detoxification procedures. The control of aflatoxin in groundnut products requires a combination of quality control and decontamination/detoxification procedures. The use of selected mathematical models to describe the distribution of aflatoxin in groundnut kernels, roasted groundnuts, peanut butter, and groundnut cake has been investigated for developing 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 developed for the accurate analysis of the aflatoxin content of groundnut products using bonded-phase clean-up 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 aflatoxin in 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.

1667. Coker, R.D., Jewers, K., and Jones, B.D. 1985. The destruction of aflatoxin by ammonia : practical possibilities. Tropical Science 25: 139-154.

This paper reviews the effects on trade of legislation restricting the levels of aflatoxin in cereals and oilseed cakes used in the preparation of animal feeds, and

the processes which have been developed to destroy the aflatoxin in situ by means of ammonia gas. The acute and chronic effects of aflatoxin in animals are outlined, and the way in which these data have been used to set upper limits for the level of aflatoxin in commodities is described. Methods available for controlling the amounts of aflatoxin in raw materials are briefly discussed, and the processes for the ammoniation of maize, cottonseed and groundnut cake are summarized. The toxicological data available for the various ammoniated products is considered. It is concluded that ammoniation provides an effective way to reduce the aflatoxin content of contaminated raw materials, but that universal acceptance of the products will only be forthcoming when adequate toxicological data have been obtained to show that the transformation products of aflatoxin present no hazard to animals or man.

1668. Coker, R.D., Jewers, K., and Jones, B.D. 1986. The treatment of aflatoxin contaminated commodities. International Biodeterioration Supplement 22: 103-108.

This paper very briefly reviews several methods for decontamination and detoxification of aflatoxin-contaminated foods and feeds. The utilization of ammonia gas has been extensively investigated and commercial facilities exist for the detoxification of groundnut cake by ammoniation.

1669. Coker, R.D., Jewers, K., Jones, N.R., Nabney, J., and Watson, D.H. 1985. Process for the destruction of aflatoxin in agricultural products. UK Patent GB 2108365 B. 7 pp.

A process for detoxifying an agricultural product, particularly groundnut cake, contaminated with aflatoxin is outlined. The process comprises treating, in an insulated reaction vessel, the contaminated product with steam until the product is moistened to a level of from 12 to 18 %, especially from 15 to 17 % (by weight) of water, treating the moistened product with gaseous ammonia until the level of ammonia in the mixture is from 2 to 5 %, especially about 3 % (by weight) and the temperature of the mixture in the reaction vessel is between 110 to 150°C, especially in the range 115 to 125°C. This process leads to about 90 % destruction of aflatoxin in groundnut cake.

1670. Conkerton, E.J., Chapital, D.C., Lee, L.S., and Ory, R.L. 1980. Effect of ammoniation on the physiocochemical properties of peanut and cottonseed meals. Journal of Food Science 45(3): 564- 566.

Groundnut and cottonseed meals prepared from seeds inoculated with aflatoxin-producing *Aspergillus parasiticus* were treated in a laboratory ammoniator under three conditions of heat and pressure. Detoxification of the meal was similar for all treatments. Differences between the nonammoniated meals and those treated under the mild conditions of heat and pressure were negligible in respect of nitrogen solubility, methionine, lysine, available lysine, sugars, and immunochemical and gel-electrophorelic protein patterns. Meals ammoniated under the harsher conditions of heat and pressure showed reductions in methionine, lysine and available lysine contents and in free sugars as well as changes in nitrogen solubility and gel-electrophoretic patterns.

1671. Cucullu, A.F., Lee, L.S., Pons, W.A.,Jr., and Stanley, J.B. 1976. Ammoniation of aflatoxin B_1 : Isolation and characterisation of a product with molecular weight 206. Journal of Agricultural and Food Chemistry 24: 408-410.

This paper describes the isolation and characterization of a second major ammoniation product from a model system in which aflatoxin B_1 reacted with ammonium hydroxide under elevated temperature and pressure. The compound (product) was identified as dihydro-4-hydroxy-6 methoxyfurol2,3-b]benzofuran, molecular weight 206, melting point 145-146 C, Y max (MeOH) 205, 227 (sh), 269, and 278 (sh) nm with 35000. 8130, 730, and 560. respectively. It is a nonfluorescent phenol which is similar in structure to aflatoxin D,, but lacks the cyclopentenone ring.

1672. Degesch GMBH 1978. Toxin removal. British Patent 1 529 027

A process is described for treatment of moldy foods (groundnut) to neutralize toxic-products, such as aflatoxins, which involves treatment with ethylene oxide and/or methyl formate in a moist medium.

1673. DeLort-Laval, J., Viroben, G., and Borgida, L.P. 1980. [Inactivation of aflatoxins in groundnut meal for broilers by treatment with ammonia or monomethylaminc.|. Efficacite biologique pour le poulet de chair du tourteau d'arachide traite a l'ammoniac ou a la monoethylamine en vue de l'inactivation des aflatoxines. Annales de Zootechnie 29(4): 387-400.

It has been reported that gaseous ammonia at less than 100°C (Viroben et al. 1978) or monomethylamine (Giddey et al. 1977) reduces the level of aflatoxin in contaminated groundnut oilmeal. To test this a 22 % protein diet of maize and groundnut oilmeal with added lysine and threonine was given to chickens in an experiment similar to the protein efficiency test. The ammonia treatment at about 1, 2, or 3 atmospheres pressure did not have any significant effect on feed intake of 22-49 day-old chickens, daily gain or efficiency of feed conversion (FCE) for chickens given groundnut oilmeal with aflatoxin B, at 0.98 or 1.14 mg kg^{-1}. Mono-methylamine treatment of the diet with aflatoxin at 0.98 mg kg^{-1} given to chickens from 10 to 24 days old, significantly decreased average daily gain from 13.2 to 7.8 g, daily DM intake from 38.2 to 27.7 g, and gain g^{-1} feed intake from 0.35 to 0.28 g. In another experiment chickens were given a semi-purified diet with

groundnut oilmeal as the only source of protein, plus amino acids (including methionine at 0. 0.1, 0.2, or 0.3 % of diet). Addition of methionine increased the daily gain and FCE; FCE was highly correlated with the daily intake of sulphur amino acids when plotted on a logarithmic scale (r = 0.983). There was no decrease in availability of amino acids from the treatments under comparison, all differences being explained by the lower cystine content and the lower acceptance of the treated products. It is conlcuded that rations should be formulated after allowance is made for the non-protein nitrogen added during the treatment of the groundnut oilmeal and that allowance should also be made for cystine present in the groundnut oilmeal. It is recommended that the levels of ammonia nitrogen in diets for broilers, laying hens and fattening pigs should not exceed 0.15, 0.3 and 0.1 %, respectively. After treatment with monoethylamine, the maximum levels should be one-third of these values.

1674. Dollear, F.G. 1969. Detoxification of aflatoxins in foods and feeds. Pages 359-391 in Aflatoxin : Scientific Background, Control , and Implications (Goldblatt, L.A., ed.). New York, USA : Academic Press. 472 pp.

Methods of decontamination and detoxification of aflatoxin- contaminated groundnuts, cottonseed and their products arc reviewed. Topics discussed include physical separation of contaminated nuts and seeds, removal of aflatoxin by solvent extraction, heat inactivation, microbial degradation, and chemical detoxification of contaminated commodities. Criteria for detoxification are also reviewed.

1675. Dollear, E.G., and Gardner, H.K. 1966. Inactivation and removal of aflatoxin. Pages 72-81 in Proceedings of the Fourth National Peanut Research Conference, 14-15 July, Tifton, Georgia, USA.

Several methods of decontamination and detoxification of agricultural commodities including groundnuts and groundnut meal are reviewed. Research results on detoxification of aflatoxin- contaminated groundnut and cottonseed meals with ammonia are presented. Ammoniation of contaminated meal appears to be effective in greatly reducing aflatoxin levels. Aflatoxin removal by solvent extraction is also discussed.

1676. Dollear, F.G., Mann, G.E., Codifcr, L.P.Jr., Gardner, H.K.Jr., Koltun, S.P., and Vix, H.L.E. 1968. Elimination of aflatoxins from peanut meal. Journal of the American Oil Chemists' Society 45(12): 862-865.

Treatments of aflatoxin-contaminated groundnut meal with ammonia, methylamine, sodium hydroxide and ozone were effective in either destroying aflatoxins or greatly reducing aflatoxin levels as indicated both by aflatoxin analysis, by TLC, and feeding experiments with ducklings and rats. Extraction with acetone- water (90:10, v/v) proved most effective in removing all aflatoxins from the contaminated meal. The treated groundnut meals had lower protein efficiency ratios than the original meals.

1677. Doyle, M.P., Applebaum, R.S., Brackett, R.E., and Marth, E.H. 1982. Physical, chemical and biological degradation of mycotoxins in foods and agricultural commodities. Journal of Food Protection 45(10): 964-971.

Aflatoxin is partially or completely degraded by irradiation, heat, or treatment with strong acids or bases, oxidizing agents or bisulfite. Hydrogen peroxide plus riboflavin denature aflatoxin in milk. Mycelia of *Aspergillus parasiticus* can degrade aflatoxin, possibly via fungal peroxidase. Such degradation is affected by strain of *A. parasiticus,* amount of mycelium, temperature, pH, and concentration of aflatoxin. Adsorbants, including bentonite and activated charcoal, can physically remove aflatoxin and patulin from liquid foods. Patulin is stable at low pH values but not in the presence of large amounts of vitamin C or bisulfite. Patulin can be degraded by actively fermenting yeasts and rubratoxin can be degraded by the mycelium of Penicillium rubrum.

1678. Draughon, F.A., and Childs, E.A. 1982. Chemical and biological evaluation of aflatoxin after treatment with sodium hypochlorite, sodium hydroxide, and ammonium hydroxide. Journal of Food Protection 45(8): 703-706.

Aflatoxin B, was mixed with eleven concentrations of sodium hydroxide, sodium hypochlorite and ammonium hydroxide. Aflatoxin was quantified by fluorometric determination and toxicity of aflatoxin treated with NaOH and NH_4OH was evaluated by the brine shrimp assay. Detoxified aflatoxin B_1 was then screened for mutagenicity using the Salmonellaa/mammalian microscope mutagenicity test (Ames test). Sodium hydroxide, sodium hypochlorite and ammonium hydroxide reduced fluorescence by 92, 96, and 94 %, respectively, at concentrations of 25, 11, and 875 mg 50 g^{-1}. A high negative correlation was observed between decrease in fluorescence and increase in survival of brine shrimp (r = 0.88) for aflatoxin treated with NaOH and NH_4OH. Equivalent amounts of aflatoxin B_1 (0.05 µg) and aflatoxin B_1 + detoxified B_1 (0.05 µg + 0.05 µg, respectively) were not significantly different ($P > 0.05$) in the number of revertants resulting in the Ames test. Therefore, aflatoxin B_1 in the presence of detoxified aflatoxin did not increase in mutagenicity.

1679. Dwarakanath, C.T., Rayner, E.T., Mann, G.E., and Dollear, F.G. 1967. Reduction of aflatoxin levels in cottonseed and peanut meals by ozonization. Journal of the American Oil Chemists' Society 45(2): 93-95.

Cottonseed and groundnut meals were treated with ozone to destroy or eliminate aflatoxins. High meal moisture contents (cottonseed meal 22 %, groundnut meal 30

%), high temperature (100°C). and longer treatment (2 h) favored inactivation of aflatoxins. Aflatoxins B_1 and G_1 were readily destroyed by the ozone processes whereas aflatoxin B_2 was not affected. In cottonseed meal, 91 % of the total aflatoxins was inactivated in 2 h, a decrease from 214 to 20 µg kg^{-1}; in groundnut meal, 78 % was destroyed in 1 h, a decrease from 82 to 18 µg kg^{-1}. In both meals, aflatoxin B_1 was totally inactivated within the times specified.

1680. Farah, Z., Martins, M.J.R., and Bachmann, MR. 1983. Removal of aflatoxin in raw unshelled peanuts by a traditional salt boiling process practised in the North East of Brazil. Lebensmittel- Wissenschaft und Technologic 16(2) : 122-124.

No aflatoxins could be detected in salt-boiled groundnuts in contrast to raw groundnut samples which were all contaminated. The efficiency of sodium chloride in removing aflatoxins was confirmed by cooking laboratory-inoculated batches of raw, unshelled groundnuts in a 5 % aqueous sodium chloride solution at 116°C and 0.7 bar for 30 min. Results of 5 batches of groundnuts containing aflatoxin at 19,992-66,320 µg kg^{-1} showed a removal of aflatoxins from 80 % to near 100%.

1681. Ferrando, R., Parodi, A.L., Henry, N., N'Diaye, A.L., Fourlon, C., and Oelort-Laval, J. 1975. Ilnfluence of ammonia treatment of A. flavus-contaminated groundnut meal on its feed efficiency in the duckling.]. Influence d'un traitement par rammoniaque du tourtcau d'arachide contamine par *A. flavus* sur son efficacite alimentaire chez le caneton. Annales de la Nutrition et de l'Alimentation 29(1). 61-66.

There was no significant difference between detoxified (with 25 % ammonia) groundnut meal and soybean meal with respect to growth and food efficiency. Ducklings fed a diet containing nontreated groundnut meal showed a decrease in body weight compared with the detoxified meal and soybean meal. However, the liver weight increased in that group. Characteristic lesions of aflatoxicosis developed in ducklings fed nontreated groundnut meal which contained 285 µg kg^{-1} aflatoxin B_1. Lesions were very slight, but present in the liver and kidneys of ducklings fed detoxified groundnut meal, and there were no differences in kidney lesions in the groups fed detoxified and nontreated groundnut meal. Ducklings fed detoxified groundnut meal absorbed 0.4 µg of aflatoxin B_1 during the first 8 days of the experiment, and 8-10 µg over the 4-weck period.

1682. Feuell, A.J. 1966. Aflatoxin in groundnuts. Part IX - Problems of detoxification. Tropical Science 8(2): 61-70.

Oil extracted from aflatoxin-contaitung groundnut kernels by crushers or expellers is toxin-free; extraction of residual oil from the press-cake by the usual type of solvent does not remove aflatoxin either. Consequently, press-cake and meal contain practically all the aflatoxin originally present in kernels. Trials to detoxify groundnut meal by radiation, heat, solvents, or reactive chemicals, conducted at the Tropical Products Institute in London, UK, were not very successful. The only treatments that destroyed or removed the toxin - i.e., prolonged wet heating at 120°C, extraction with methanol, and treatment with 10 % HCl, SO_2, or Cl_2, are expensive and cause deterioration of the meal. It is concluded that detoxification is not a practical possibility and that the emphasis must be on the prevention of infection by the aflatoxigenic fungus *Aspergillus flavus.*

1683. Fischbach, H., and Campbell, A.D. 1965. Note on the detoxification of aflatoxins. Journal of the Association of Official Agricultural Chemists 48: 28.

This paper briefly presents some findings with regard to the detoxification of aflatoxins. A highly contaminated groundnut meal (containing about 1000 µg kg^{-1} aflatoxin), when exposed overnight to a 10 per cent chlorine gas atmosphere, was found to have lost about 90 % of its initial fluorescence and to have become nontoxic to the chick embryo. NaOCl (5 %) solutions are used in disposing of aflatoxin-contaminated materials in laboratories.

1684. Fonseca, H. 1988. Aflatoxin removal from peanut meals with aqueous ethanol, in a commercial scale. Proceedings of the Japanese Association of Mycotoxicology Supplement No. 1: 81-82.

Aflatoxin removal from groundnut meal using aqueous ethanol was attempted in a commercial scale experiment. Results showed that 90"GL alcohol removed 87.4% aflatoxin from contaminated groundnut meal after 3 hours of treatment. It was also found that coarse ground meal permitted better aflatoxin extraction than thick pieces and that samples taken from the lower part of the extraction vessel showed better aflatoxin removal than those from the upper part.

1685. Food and Agriculture Organization. 1979. Prevention of mycotoxins. FAO Food and Nutrition Paper No. 10, pp. 71, FAO, Rome.Italy.

This publication is in three parts. Part I gives a schedule of practices recommended for standing crops, for harvesting and drying, for storage of crops, for transportation, for processing (postharvest, including decontamination), for prevention, and for control/detoxification of aflatoxins and other mycotoxins. Part II is a discussion of factors relating to the recommended practices. Part III focuses on needs and aids for implementation of the recommendations. The commodities mentioned in the discussion are those that form the bulk ot the food and feedingstuffs in some countries. References are also given to existing practices, current trends in research, and their likely outcome in relation to the problem posed by mycotoxins.

1686. Frayssinet, C, and Lafarge-Frayssinet, C. 1990. Effect of ammoniation on the carcinogenicity of aflatoxin-contaminated groundnut oil cakes : Long-term feeding study in the rat. Food Additives and Contaminants 7(1): 63-68.

The efficacy of detoxification by ammoniation of aflatoxin- contaminated groundnut oil cakes was determined in long-term (18 months) feeding experiments with *rats.* The aflatoxin content of the cake was reduced very considerably by the pressurized application of ammonia, dropping from 1000 to 140 µg kg^{-1} at a gas pressure of 2 bar and to 60 µg kg^{-1} at 3 bar. No reversion was noted during the experiment. The percentage of hepatic tumors obtained was very high for the untreated cakes, but fell sharply with medium treatment and was reduced to zero by the treatment at 3 bar. A satisfactory dose-effect relationship was shown between the residual aflatoxin content of the cakes and the observed incidence of tumours. It is concluded that ammonia treatment is a practical solution to the problem of the carcinogenic potency of contaminated oil cakes.

1687. Fremy, J.M, Gautier, J.P., Herry, M.P., Terrier, C, and Calet, C. 1988. Effects of ammoniation on the 'carry-over' of aflatoxins into bovine milk. Food Additives and Contaminants 5(1) : 39-44.

Lactating cows were fed a diet containing aflatoxin B1-contaminated groundnut cakes, and then on cakes treated with ammonia gas. Between the two periods the diet contained noncontaminated soyabean meals. With the nontreated groundnut meal containing aflatoxin B_1 at 1100 µg kg^{-1}, total excretion of aflatoxin M, was 2.6 % of total ingested aflatoxin B_1. With the treated groundnut meal, aflatoxin M, content of milk was below 0.1 µg $litre^{-1}$. In a second experiment 50 lactating cows were fed for 16 months on mixed feeds containing 30 % ammoniated groundnut cake containing aflatoxin B_1 less than 10 µg kg^{-1}. Aflatoxin M_1 residue in milk was not above 0.1 µg $litre^{-1}$.

1688. Friot, D., Calvef, H., Diallo, S., and Wane, M. 1975. [Detoxified groundnut meal in feeds for poultry.]. Tourteau d'arachide detoxifie dans l'alimentation des volaillies. Revue d'Elevage et de Medecine Veterinaire des pays Tropicaux 28: 419-425.

Two groups of 50 pullets, each with 5 cockerels, had feed containing 15 % groundnut oilmeal detoxified by the use of alkali. The normal meal contained aflatoxin 1180 µg kg^{-1}, the detoxified meal 120 µg kg^{-1}. The detoxified meal had slightly lower mineral content. During 21 weeks there was no difference in weight or feed intake between the groups on detoxified or nontreated meal. The group having detoxified meal laid more eggs and a greater weight of eggs; the eggs also had a significantly higher hatchability. Chickens produced by pullets given the detoxified meal had a slightly lower growm rate than those from pullets given the contaminated meal.

1689. Gardner, H.K.,Jr., Koltun, S.P., Dollear, F.G., and Rayner, E.T. 1971. Inactivation of aflatoxins in peanut and cottonseed meals by ammoniation. Journal of the American Oil Chemists' Society 48(2): 70-73.

In pilot plant scale tests, treatment with gaseous ammonia at 2 levels each of moisture content, reaction time, temperature, and ammonia pressure inactivated the aflatoxins (121 µg kg^{-1}) in groundnut meal to a non-detectable level. With a similar treatment, aflatoxins (350 µg kg^{-1}) in cottonseed meal were reduced to 4 µg kg^{-1}. Using large scale equipment the aflatoxin content of cottonseed meal was reduced from 519 µg kg^{-1} on average to < 5 µg kg^{-1} under optimum processing conditions. The ammoniation treatment lowered the nitrogen solubility and epsilon amino-free lysine levels of the meal.

1690. Gardner, H.K.Jr., Koltun, S.P., and Vix, H.L.E. 1968. Solvent extraction of aflatoxins from oilseed meals. Journal of Agricultural and Food Chemistry 16(6): 990-993.

Aflatoxin was removed or significantly reduced in cottonseed and groundnut meals by extracting with a tertiary solvent system of 54 % acetone, 44 % hexane, and 2 % water (by weight) or a binary solvent system of 90 % acetone and 10 % water (by weight). The tertiary solvent system simultaneously removed oil and aflatoxin from prepressed cake containing 12 to 15 % oil, resulting in residual lipids content of approximately 1 % and aflatoxin levels of < 40 fig kg^{-1}. The binary solvent system reduced the aflatoxin content of prepressed cottonseed and groundnut meals to < 10 µg kg^{-1} in small scale batch extractions and < 40 µg kg^{-1} in continuous pilot plant extractions. Both solvent systems definitely offer economically feasible methods for reducing aflatoxins in contaminated cottonseed and groundnut meals to a level of 30 µg kg^{-1} or below.

1691. Ghewande, M.P., Nagaraj, G., and Raj Jhala. 1986. Aflatoxin production and detoxification in groundnut. Pages 15-16 in Proceedings of the National Seminar on Plant Protection in Field Crops, 29-31 January 1986, Central Plant Protection Training Institute, Hyderabad 500 030, India.

Twenty-eight groundnut genotypes were tested for resistance to seed colonization by *Aspergillus flavus* and for aflatoxin content. Of these, J 11, TMV 12, and Ah 7223, in general, were found to have less seed colonization. Ten cross derivatives, involving J 11 as pollen parent in some cases, were also tested for seed colonization. Genotypes OGO-2 and 1-4 had seed colonization of 8-9 %. Seed treatment with solid sodium chloride at 2 % level inhibited seed colonization considerably.

Aflatoxin content was found to vary among genotypes. Those which had more than 3 % phenols were found to contain less aflatoxins. Fumigation with ammonia was found to be highly effective, reducing aflatoxins by 68 %. NaOH spray (2 %) also reduced aflatoxins by about 70 %. Fumigation with burning cowdung fumes and sun drying for one day were found effective in reducing aflatoxin (16-25 %).

1692. Giddey, C, Brandt, J., and Bunter, G. 1977. [The detoxification of oil-seed cakes polluted by aflatoxin, research and development of an industrial process.]. Recherches sur la detoxification des tourteaux oleagineux contamines par l'aflatoxine et developpement d'un procede' industriel. Archives de l'Institut Pasteur de Tunis 54: 527-535.

A method was developed for detoxification of highly aflatoxin-contaminated groundnut meal using the calcium hydroxide/monomethylamine alkaline degradation of aflatoxin.

1693. Giddey, C., Brandt, J., and Bunter, G. 1977. The detoxification of oil-seed cakes polluted by aflatoxins. Research and development of an industrial process. Ann. Technol. Agric. 27: 331-338.

A method was developed for detoxification of highly aflatoxin-contaminated groundnut meal using the calcium hydroxide/monomethylamine alkaline degradation of aflatoxin.

1694. Goldblatt, L.A. 1965. Removal of aflatoxin from peanut products with acetone-hexane-water solvent. Pages 261-263 in Mycotoxins in Foodstuffs. (Wogan, G.N., ed.). Cambridge, Massachusetts, USA : M.I.T. Press.

An azeotrope of acetone, hexane and water was found as a suitable solvent for removal of aflatoxin from contaminated groundnut meal.

1695. Goldblatt, L.A. 1966. Some approaches to the elimination of aflatoxins from protein concentrates. Advances in Chemistry Series 57: 216-226.

Prevention, removal and inactivation of aflatoxin in groundnut protein concentrates is considered. Prevention is best and may be achieved by careful harvesting, handling, drying and storage of the crop produce. Removal of toxic kernels is also possible and this can be done by handpicking of discolored and moldy kernels. Aflatoxin may also be removed by polar solvents, by exposure to moist heat, and by treatment with ammonia.

1696. Goldblatt, L.A. 1967. Detection and elimination of aflatoxin. Pages 160-174 in Trout hepatoma research conference papers (Halver, J.E., and Mitchell, L.A., eds.). Bureau of sport Fisheries and Wildlife Pub., Washington.

Methods of detection and elimination of aflatoxins in agricultural commodities are reviewed.

1697. Goldblatt, L.A. 1968. Critical evaluation of aflatoxin detoxification in oilseeds, in Proceedings of the Conference on Protein-Rich Food Products from Oilseeds, 15-16 May 1968, USDA, ARS, New Orleans, Louisiana, USA.

Methods of decontamination and detoxification of aflatoxin-contaminated oilseeds are critically reviewed.

1698. Goldblatt, L.A., and Dollear, F.G. 1977a. Review of prevention, elimination, and detoxification of aflatoxins. Pure and Applied Chemistry 49: 1759-1764.

The best approach to contain aflatoxin contamination is prevention. Good farm management practices are essential. This includes use of sound, fungus-free, viable seed, proper fertilization, control of insects and diseases, prevention of lodging, and harvesting practices that avoid damaging the crop and picking up excessive leaves, trash, and dirt. Special attention should be given to diverting aflatoxin-containing lots from food and feed channels as early as possible in the marketing chain. Clean, dry, adequately cooled and ventilated storage and good sanitation are essential to minimize mold contamination. Genetic approaches and use of mold inhibitors may be helpful. When prevention fails, hand or electronic sorting can remove contaminated seeds. Aflatoxin may be removed during processing by alkali refining of crude oils or by extraction of contaminated oilseed meals with polar organic-solvents. Some aflatoxin is destroyed or degraded during normal preparation of some foods. Many chemicals, especially oxidizing and reducing agents and acids and bases, have been screened as reagents for destroying aflatoxin in contaminated feeds. Several processes have been patented. In the USA aflatoxin in copra has been destroyed on a commercial scale by hot aqueous calcium hydroxide and in cottonseed meal by treatment with ammonia under pressure.

1699. Goldblatt, L.A., and Dollear, F.G. 1977b. Detoxification of contaminated crops. Pages 139-150 in Mycotoxins in Human and Animal Health (Rodricks, J.V.. Hesselline. C.W., and Mehlman, M.A., eds.). Illinois, USA : Pathotox Publishers, Inc. 807 pp.

Methods of decontamination and detoxification of aflatoxin- contaminated agricultural products are reviewed. Topics discussed include physical removal/segregation of contaminated nuts and seeds, inactivation, and chemical detoxification.

1700. Goldblatt, L.A., and Dollear, F.G. 1979. Modifying mycotoxins contamination in feeds - Use of mold inhibitors, ammoniation, roasting. Pages 167-184 in Proceedings of the Symposium on Interactions of mycotoxins in animal production, 13 July 1978, Michigan State University, Washington DC, USA; National Academy of Sciences 1979.

Methods of prevention, elimination and detoxification of ergot, aflatoxin, ochratoxin, zearalenone and T-2 toxin are reviewed. Prevention is the best approach, but when contamination occurs the detection and physical separation of contaminated lots may prevent contamination of larger supplies. Physical separation of ergot-contaminated grain and some aflatoxin-contaminated groundnuts and Brazil nuts was successful. Detoxification of aflatoxin has been studied most extensively; ammonia appears to be the most promising reagent, but processing conditions differ markedly with commodities such as maize, cottonseed and groundnut. Ammonia also appears to be effective for detoxification of zearalenone in maize.

1701. Goldblatt, L.A., and Robertson, J.A.Jr. 1965. Extraction of aflatoxin from groundnut meal with acetone-hexane-water azeotrope. International Biodeterioration Bulletin 1:41-42.

An azeotrope of acetone, hexane and water was found as a good solvent for removal of aflatoxin from contaminated groundnut meal.

1702. Grehaigne, B., Chouvel, H., Plna, M., Graille, J., and Cheftel, J.C. 1983. Extrusion-cooking of aflatoxin-containing peanut meal with and without addition of ammonium hydroxide. Lebensmittel- Wissenschaft & Technologie 16(6): 317-322.

Groundnut meals containing an average of 250 µg kg^{-1} aflatoxin B_1 were processed in a twin-screw extruder in the presence of 0-2.5 % ammonium hydroxide. The aflatoxin B_1 content determined after extrusion was reduced by 23-66 % in the absence, and by 40-87 % in the presence of ammonium hydroxide. When non-extruded groundnut meals were incubated with proteolytic enzymes at acid and/or alkaline pH, and at 37 or 50°C, the aflatoxin content determined by extraction from the dried-enzyme-treated meals was lower than that of the initial meals. It is suggested that extrusion could either cause destruction of aflatoxin molecules or disruption of their binding to groundnut meal constituents.

1703. Helme, J.P., and Prevot, A. 1973. Pilot plant and commercial scale removal of aflatoxins. Journal of the American Oil Chemists' Society 50: 306A.

Aflatoxins in groundnut meals can be removed by treatment with polar solvents or gaseous ammonia. Among several possibilities, economically interesting solutions were chosen in order to propose a commercial plant. Extractions were carried out in a stainless steel apparatus allowing direct extrapolation into a commercial scale de Smet extractor. Numerous extraction series were made with acetone-hexane-water azeotrope and addition of some alkalies. The effect of the number and duration of passes, and the granulometry and the meal-solvent residence time were studied. Commercial conditions resulted in a 50 µg kg^{-1} level of aflatoxin content. Increase of water and acetone content and introduction of ammonia in the azeotropic mixture allowed reduction of aflatoxin content to 8 µg kg^{-1}. Unfortunately, with a 60 ton day^{-1} French extractor in a Lesieur plant, the azeotropic mixture was not active enough for removing the toxin to acceptably low levels. Ammoniations were carried out in a Speichim reactor (25 kg $Batch^{-1}$) in an industrial plant. Hundreds of tests were run with expeller and extraction meals in variable conditions of ammonia pressure, water content, reaction temperature and reaction lime. Ammoniation easily allowed reduction to below 50 μg kg^{-1}. The soluble nitrogen content decreased with rigor of working conditions. Amino acid determination showed that lysine degraded by treatment with gaseous ammonia. Water content of meals was the limiting factor. Nutritional tests on rats showed that the food value of the meals was not affected by treatment with polar solvents. Ammoniation, although it gave less rapid growth curves, was judged to be satisfying after zootechnical tests. Examination of organs showed few noxious effects on liver and kidney cells after solvent treatment or ammoniation. In the present state of research, both processes remain possible, ammoniation having a slight advance in technological implications.

1704. Jetro. 1976. Establishment of technique for the removal of "Aflatoxin" - A carcinogen in peanuts. Japan's Industrial and Technical Information 4(8):23-25.

A process for detoxifying aflatoxin-contaminated groundnuts is outlined. This process uses the solvent methoxymcthane as the removal agent and oil extraction can be carried out simultaneously with the aflatoxin removal process. The solvent used can be removed easily and thoroughly from defatted products because of its low boiling temperature.

1705. Jordy, A. 1978. Process for neutralizing the poisonous metabolism products of mould fungi. FRG Patent 1529027. 2 pp.

A process is given for removing aflatoxins from groundnuts by extraction with ethylene oxide and/or methyl formate. The moisture content of the product to be treated is preferably kept at a maximum of about 16 % by weight. The gas or gas mixture is preferably used in a density of from 1000 to 1500 mg L^{-1}.

1706. Lee, L.S., Conkerton, E.J., Ory, R.L., and Bennett, J.W. 1979. [^{14}C] Aflatoxin B_1 as an indicator of toxin destruction during ammoniation of contaminated peanut meal. Journal of Agricultural and Food Chemistry 27(3): 598-602.

Groundnut meal which was spiked with ^{14}C aflatoxin B_1 was ammoniated at 75°C, 35 psig for 30 min in a laboratory reactor, and the distribution of label was measured in subsequent fractions. Only 45-50 % activity was detected in the ammoniated meal after initial air-drying, 8 % was detected in humins remaining after acid hydrolysis, 4-6 % was associated with protein, and 33-36 % was associated with nonprotein residue. This residue had all of the nonreacted aflatoxin B_1.

1707. Lee, L.S., and Cucullu, A.F. 1978. Conversion of aflatoxin B_1 to aflatoxin D, in ammoniated peanut and cottonseed meals. Journal of Agricultural and Food Chemistry 26(4): 881-884.

Samples of groundnut and cottonseed meals spiked with pure aflatoxin B_1 or inoculated with aflatoxigenic cultures of *Aspergillus parasiticus* or *A.flavus* were ammoniated under laboratory conditions closely simulating those used in commercial detoxification. In all experiments, the average content of aflatoxin B_1 after processing was similar to that of aflatoxin D_1, to which some of the original aflatoxin B_1 was converted by ammoniation.

1708. Lee, L.S., Cucullu, A.F., Franz, A.O., and Pons, W.A. 1969. Destruction of aflatoxins in peanuts during dry and oil roasting. Journal of Agricultural and Food Chemistry 17(3): 451-453.

Blanched whole groundnut kernels inoculated with an aflatoxigenic strain of *Aspergillus flavus* and subjected to continuous shaking during incubation for 40, 46, 64, and 72 h produced groundnuts virtually clear of visible mold and containing four graded levels of total aflatoxins - 130, 260, 2560, and 6300 µg kg^{-1}. Although individual kernels differed in aflatoxin content, replicate assays of 50 g aliquots of contaminated whole kernels were subjected to oil and dry roasting under live time-temperature conditions simulating those used in industry to effect a normal roast for high quality groundnuts. Average reduction in aflatoxin content ranged from 45 to 83 %. depending on roasting conditions and the level of aflatoxins in the raw kernels. There was an over-all reduction of 65 % in B_1 and 62 % in G_1 for oil roasting, and 69 % in B_1 and 67 % in G_1 for dry roasting. The degree of reduction in aflatoxin conetent was greatest at the highest aflatoxin contamination levels, for both oil- and dry-roasted groundnuts.

1709. Lee, L.S., Stanley, J.B., Cucullu, A.F., Pons, W.A.Jr., and Goldblatt, L.A. 1974. Ammoniation of aflatoxin B_1 : isolation and identification of the major reaction product. Journal of the Association of Official Analytical Chemists 57: 626-631.

The major product formed from reacting pure aflatoxin B_1 with ammonium hydroxide at 100°C under pressure was isolated in crystalline form. The compound, molecular weight 286, ultraviolet absorption, Y max MeOH 227, 324 nm (E 15920. 12440), is nonfluorescent, exhibits phenolic properties, and lacks the lactone group characteristic of aflatoxin B_1. Acidification of the compound did not regenerate aflatoxin B_1. Calculations made from ultraviolet absorption spectra indicated that the compound comprises about 30 % of the crude ammoniation product. It is postulated that the new product, $C_{16}H_{14}O_5$, arises from opening the lactone ring of aflatoxin B^1 during ammoniation, formation of the ammonium salt of the resultant hydroxy acid, and loss of carbon-dioxide from this B-keto acid. Since this compound arises from decarboxylation the trivial name aflatoxin D_1 is proposed.

1710. Lesieur, B. 1977. [Development of an industrial process for the treatment of groundnut cake contaminated by aflatoxin|. Mise au point d'un procede' industriel pour le traitement des tourteaux d'arachide contamine's par l'aflatoxine. Archives de l'Institut Pasteur de Tunis 54: 521-526.

An industrial process to remove aflatoxin from contaminated groundnut cake using gaseous ammonia is described. Safety and pollution problems are covered along with data on capital investment and running costs.

1711. Lingquist, R.H. 1977. Removal of aflatoxin from peanuts. United States Patent 4 062 984. 4 pp.

A process is given for removing aflatoxin from seeds or nuts such as groundnuts by extraction with methoxymethane.

1712. Manabe, M., Minamisawa, ML, and Matsuura, S. 1978. Detoxification of aflatoxin and sterlization of mycotoxin- producing fungi in peanut meal using ammonia gas on laboratory scale. Report of National Food Research Institute (Japan) No. 33 : 65-69.

Aflatoxin-contaminated groundnut meal (127 µg kg^{-1}) was detoxified by treatment with ammonia gas in a glass desiccator (4L capacity). Aflatoxin B_1 concentration was reduced by half by treatment with 13.1 % (w/w) ammonia gas at 20°C for 24 h, and after 144 h the concentration was reduced to 19 µg kg^{-1} (85 % reduction). Chicken embryo bioassay revealed decreasing toxicity with longer treatment time. Treatment with 0.7 % ammonia gas at 20"C for 24 h was sufficient to kill conidia of *Aspergillus flavus* NRRL 3000. A. ochraceus 0458, and A. versicolor 0056.

1713. Mann, G.E., Codifer, L.P.Jr., Gardner, H.K.Jr., Koltun, S.P., and Dollcar, F.G. 1969. Chemical inactivation of aflatoxins in peanut and cottonseed meals. Journal of the American Oil Chemists' Society 47(5): 173-176.

Several organic and inorganic reagents were tested for destruction or inactivaiion of aflatoxins present in groundnut and cottonseed meals. The meals were treated with chemicals in a special laboratory-scale reactor, and were evaluated for aflatoxin contents. In some instances, a larger pilot-plant scale reactor was used for treatments. Ammonia, methylamine, sodium hydroxide and formaldehyde considerably reduced aflatoxin levels in the contaminated meals. Effects of various reaction parameters, including time, temperature and moisture content, on the efficiency of these chemicals are discussed.

1714. Morcau, C. 1976. (Variations of fungal pollution in peanuts and their cakes from harvest to consumption.]. Variations de la pollution fongique des arachides et de leur tourteaux de la recolte a la consommation. Revue de Mycologie 40: 97-115.

Mycological analysis of postharvest groundnut samples was carried out Various soil-fungi were found to infect the pods; most of them grew through the shell, but not all reached the kernels. The most common were *Aspergillus flavus* and A. niger. Where they arc treated in oil frabics, the originally heavily contaminated kernels are processed through some thermic treatments which progressively eliminate all fungal contamination. Nevertheless, a recontamination occurs as soon as the pellets are cooled, and a large mold development occurs in the usual conditions of storage. The cakes used to feed cattle arc often highly contaminated. Detoxification of cakes by ammoniation destroys the aflatoxin and also has a fungicidal action against many molds, especially *A. flavus*.

1715. Morcau, C. 1977. [Fungal pollution in peanuts and their cakes.]. Contamination fongique des arachides et de leurs tourteaux. Revue francaise des Corps Gras 24(1): 27-34.

Mycological analysis of postharvest groundnut samples was carried out. Various soil-fungi were found to infect the pods; most of them grew through the shell, but not all reached the kernels. The most common were *Aspergillus flavus* and A. niger. Where they are treated in oil fabrics, the originally heavily contaminated kernels are processed through some thermic treatments which progressively eliminate all fungal contamination. Nevertheless, a recontamination occurs as soon as the pellets are cooled, and a large mold development occurs in the usual conditions of storage. The cakes used to feed cattle are often highly contaminated. Detoxification of cakes by ammoniation destroys the aflatoxin but also has a fungicidal action against many molds, especially *A. flavus*.

1716. Natarajan, K.R. 1975. A study of the chemical inactivation of aflatoxins in peanut protein isolates and some of their physiochemical properties. Dissertation Abstracts International B 36 : 211-212.

81-89 % and 51-56 % of aflatoxins originally present in raw groundnuts were retained in protein concentrate and isolate, respectively. When prepared by acqueous extraction 55-65 % of aflatoxins present in defatted groundnut meal were retained in protein isolate prepared from it. NaOCl was effective in destroying aflatoxins B, and B_2 in the protein isolates. NaOCl treated isolates were darker and slightly less soluble at pH 5.5- 7.5 than nontreated isolates; high concentrations of NaOCl also reduced tryptophan and tyrosine contents. Polyacrylamide gel electrophoresis showed that NaOCl may destroy x-conarachin, a major globular protein in the conarachin fraction, a major subunit of conarachin with mol. wt. of approx. 62 000 (probably from x-conarachin). Tryptophan content of the conarachin fraction was reduced by NaOCl treatment.

1717. Natarajan, K.R., Rhee, K.C., Cater, C.M., and Mattil, K.F. 1975. Destruction of aflatoxins in peanut protein isolates by sodium hypochlorite. Journal ol the American Oil Chemists' Society 52(5): 160-163.

The efficacy of sodium hypochlorite in destroying aflatoxins in contaminated raw groundnuts and groundnut meal undergoing processing for the preparation of protein isolates was tested. Effects of sodium hypochlorite concentration, reaction pH, temperature, and time were investigated. Treatments with 0.4 % sodium hypochlorite at pH 8 produced groundnut protein isolates with trace amounts of aflatoxins B, and B_2 from ground raw groundnuts containing 725 μg kg^{-1} and 148 μg kg^{-1} anatoxins B_1 and B_2 whereas nontreated protein isolates contained 384 μg kg^{-1} aflatoxin B_1 and 76 μg kg^{-1} aflatoxin B_2. At pH 9 or 10, 0.3 % sodium hypochlorite reduced aflatoxin B_1 content from 380 μg kg^{-1} to below detectable levels and aflatoxin B_2 content from 52 μg kg^{-1} to 2 *μg* kg^{-1}. In defatted groundnut meal containing 136 *μg* kg^{-1} aflatoxin B_1 and 36 μg kg^{-1} aflatoxin B_2, 0.25 % sodium hypochlorite at pH 8 (0.20 % at pH 9 and 0.15 % at pH 10) reduced both aflatoxins to below delectable levels in protein isolates compared with 75 ug kg^{-1} aflatoxin B, and 17 μg kg^{-1} aflatoxin B_2 in nontreated protein isolates. Results indicated that both sodium hypochlorite concentration and pH are important factors in inactivation of aflatoxin.

1718. Ochomogo, M.G. 1979. Detection and detoxification of aflatoxin in corn and peanuts. Disseration Abstracts International 39(8): 3742.

The incidence of aflatoxin in maize and groundnuts in South East Brazil and South East USA was compared. Aflatoxin was found in 45 % of samples in Brazil and in 77 % of samples in the USA, the range of contaminauon in both countries being >20 - >100 μg kg^{-1} . The Brazilian foods were sold for milling whereas the US ones were not. Attempts to chemically detoxify aflatoxin-contaminated samples were made using hydrogen peroxide, ammonium hydroxide, formaldehyde, sodium hypochlorite and isopropyl alcohol. Results indicated that 1.5 % hydrogen peroxide,

1 % sodium hypochlorite and 75 % isopropyl alcohol significantly reduced aflatoxin contamination.

1719. Okonkwo, P.O., Umerah, G., and Nwokolo, C. 1977. Procedures to reduce aflatoxin levels in common foods. West African Journal of Pharmacology and Drug Research 4(1): 62-63.

A study of major foods in Nigeria showed that groundnuts and dried fish were highly contaminated with aflatoxins (900 µg kg^{-1} in groundnuts; 600-700 µg kg^{-1} in dried fish) whereas various grains, millets, guinea corn and rice were moderately contaminated (300-150 µg kg^{-1}). Low risk foods such as yams became contaminated with aflatoxin after storage, particularly when wrapped in cellophane or even in newspaper. Immersion of foods in a dilute (1 %) solution of NaCl 16.5 % and sodium hypochlorite 1 % (Milton) reduced the level of aflatoxin by almost 50 %. Exposure to direct sunlight caused a marked decline (30-16 %) in aflatoxin levels of foods.

1720. Park, D.L., Lee, L.S., Price, R.L., and Pohland, A.E. 1988. Review of the decontamination of aflatoxins by ammoniation : Current status and regulation. Journal of the Association of Official Analytical Chemists 71(4): 685-703.

This paper reviews various studies on ammoniation of aflatoxin- contaminated corn, groundnut and cottonseed meals, and outlines current applications and regulatory status of ammonia for reducing aflatoxin levels in animal feeds. Results demonstrate overwhelming support for the efficacy and safety of the ammoniation as a practical solution to aflatoxin detoxification in animal feeds.

1721. Parker, N.A., and Melnick, D. 1966. Absence of aflatoxin from refined vegetable oils. Journal of the American Oil Chemists Society 43: 635-638.

The present investigation is the first definitive study of the fate of the aflatoxins in vegetable oils undergoing processing. Crude oils, obtained by solvent extraction or by hydraulic pressing of ground moldy groundnuts (not suitable for human consumption), contained only small fractions of the aflatoxin originally present in the groundnuts; the meals retained the bulk of the aflatoxin. Conventional alkali refining and washing of the oils reduced aflatoxin content to a range of 10 to 14 µg kg^{-1}. The subsequent bleaching operations essentially eliminated aflatoxin froom the oils; the concentrations after this process being less than 1 µg kg^{-1}. The above results were confirmed using corn oils obtained from corn germ deliberately contaminated in the laboratory with *Aspergillus flavus.* The nonfluoreseing forms of aflatoxins, capable of being produced during the alkali refining operations, are also absent from the refined vegetable oils; these aflatoxin derivatives are readily converted to their original form on acidification and are thereby measurable by fluorescence, if present.

1722. Parpia, H.A.B. 1965. Report of the work on aflatoxin carried out at Central Food Technological Research Institute (CFTRI), Mysore, India. Nutrition Document : Aflatoxin/11, P.A.G. (WHO/FAO/UNICEF), July 1965 Meeting - Rome. 5 pp.

Research on aflatoxins carried out at the Central Food Technological Research Institute, Mysore (India), is presented. Calcium chloride solution (1 %) removed about 80 % of aflatoxin from contaminated groundnut cake flour. Heating toxic groundnut kernels in water or 0.5 % $CaCl_2$ solution at 90°C for half an hour removed 90-95 % of the toxin. The results showed that precipitation of proteins with calcium chloride at 6.8 pH helped in avoiding most of the toxin going with the protein fraction. A paper chromatographic method for aflatoxin analysis is reported. Aflatoxin B_1 was found in 20 % samples of breast milk from mothers and in 60 % samples of urine of cirrhotic children.

1723. Parpia, H.A.B. 1966. Report of work carried out at the Central Food Technological Research Institute, Mysore, India on aflatoxin. Nutrition Document : Aflatoxin/18, P.A.G. (WHO/FAO/UNICEF), August 1966 Meeting - Geneva. 7 pp.

A method for reclamation of toxic groundnut meal for edible purposes is reported. The method is based on the oxidation of aflatoxin by a suitable oxidising agent. Treated meal has a good appearance with good organoleptic quality. Nutritive value of the protein is not altered. Some extraction procedures and effects of aflatoxin on rats are reported.

1724. Patel, U.D., Govindarajan, P., and Dave, P.J. 1989. Inactivation of aflatoxin B_1 by using the synergistic effect of hydrogen peroxide and gamma radiation. Applied and Environmental Microbiology 55(2): 465-467.

Inactivation of aflatoxin B_1 was studied by using gamma radiation and hydrogen peroxide. A 100-krad dose of gamma radiation was sufficient to inactivate 50 µg of aflatoxin B_1 in the presence of 5 % hydrogen peroxide ; 400 krad was required for total degradation of 100 µg of aflatoxin in the same system. Degradation of aflatoxin B_1 was confirmed by high pressure liquid chromatography (HPLC) and thin-layer chromatography (TLC). Ames microsomal mutagenicity tests showed loss of aflatoxin activity. This method of inactivation also reduced the toxin levels in artificially contaminated groundnuts.

1725. Paulsen, M.R., Brusewitz, G.H., Clary, B.L., Odell, G.V., and Pominski, J. 1976. Aflatoxin content and skin removal of Spanish peanuts as affected by

treatments with chemicals, water spray, heated air, and liquid nitrogen. Journal of Food Science 41(3): 667-671.

Unshelled Spanish groundnuts contaminated with aflatoxin were shelled and treated with forced heated air, liquid nitrogen. H_2O_2, HCl. sodium oleate and water spray. After passing through a whole nut blancher the aflatoxin content and percentages of blanched and whole kernels were determined. Blanching percentages were significantly higher for sodium oleate, water spray, liquid nitrogen and H_2O_2 than for HC1 or heat treatments. The heat treatments produced the highest percentage of whole kernels. In most tests, the non-blanched kernels had higher levels of aflatoxin than had those that blanched fully. The treatments which were most effective in producing low levels of aflatoxin in the blanched kernels were H_2O_2, water spray and HCl.

1726. Piva, G., Pietri, A., and Carini, E. 1985. [Use of calcium hydroxide and paraformaldehyde for detoxification of groundnut meal contaminated with aflatoxin $B_{,}$, and some observations on aflatoxin M_1 in milk.]. Detossificazione di farina di estrazione di arachide contaminata da aflatossina B_1 con idrossido di calcio e paraformaldeide e riflessi sul tenore in aflatossina M_1 del latte. Zootecnica e Nutrizione Animale 11(4): 303-310.

In groundnut meal treated with 4 % calcium hydroxide and 0.5 % paraformaldehyde in an autoclave at 2 atmospheres for 20 min, aflatoxin B_1 decreased from 401 to 29.5 10 µg kg^{-1}. In two trials lasting 10 and 22 days, aflatoxin M_1 was less in milk of two dairy cows given diets containing detoxified meal than in milk from cows given aflatoxin B_1 contaminated nontreated meal. The percentage of aflatoxin M_1 in milk compared with aflatoxin B_1 intake was similar with both treated and nontreated feeds.

1727. Poivre, R., Flament, C., and Sautier, C. 1986. [Partly defatted groundnuts, a nontoxic protein-energy food.]. L'arachide deshuilee partiellcment, aliment proteino-energetique detoxique. Pages 551-555 in Les malnutritions dans le pays du Tiers-Monde. Paris, France : Editions INSERM Vol. 136.

A method to remove aflatoxins from contaminated groundnuts is described. Partly defatted groundnuts were used to prepare a flour which contained per 100 g protein 45.5 g, fat 25 g and 500 Kcal. The use of the groundnut flour in diets for man is discussed.

1728. Prevot, A. 1974. Evaluation of methods for elimination of aflatoxins in groundnut products. [Evolution et methodes d'elimination des aflatoxines dans les produits ole'agineux (Huiles et tourteaux).] Revue francaise des Corps Gras 21(2): 35-47.

Several methods at decontamination and detoxification of groundnuts and groundnut cake are reviewed. Aflatoxin levels can be reduced by segregation and decontamination of groundnuts at shelling and after shelling. Influences of different stages in refining of oil on elimination of aflatoxin are described. Ammoniation of contaminated cake seems to be appropriate at industrial level.

1729. Prevot, A. 1986. Commercial detoxification of aflatoxin- contaminated peanut meal. Pages 341-351 in Mycotoxins and Phycotoxins (Steyn, P.S., and Vleggaar. R., eds.). A Collection of Invited Papers Presented at the Sixth International IUPAC Symposium on Mycotoxins and Phycotoxins, Pretoria, Republic of South Africa, 22-25 July 1985. Elsevier : Amsterdam. 545 pp.

In spite of preventative efforts, aflatoxin contamination of groundnuts is often unavoidable. Removal by extraction and by many chemicals has been tried. In 1979, an ammoniation semi- continuous plant (100 t day^{-1}) was installed by the French Lesieur Co. at the oil mill of Dakar (Senegal) with the financial support of the European Fund of Development (FED). Improvements to the detoxification process have been made by Sonacos combining formaldehyde as antibinding agent and ammonia. The capacity is now 600 t day^{-1}, and 3 other plants have been built. The treated groundnut meal has good nutritional quality. In France, a detoxification plant built in Brittany using a similar process has a capacity of 500 t day^{-1} and gives similar results.

1730. Kayner, E.T., and Dollear, F.G. 1968. Removal of aflatoxins from oilseed meals by extraction with aqueous isopropanol. Journal of the American Oil Chemists' Society 45: 622-624.

Aqueous isopropanol was found to be an effective solvent for removal of aflatoxins from contaminated cottonseed and groundnut meals. Extraction with six passes of 80 % aqueous isopropanol at 60° C resulted in complete removal of aflatoxins in both meals, as analysed by thin-layer chromatography. Under similar extraction conditions, the isopropanol-water azeotrope, 88 % isopropanol by weight, removed 88 % of the total aflatoxins in groundnut meal, a reduction from 82 to 10 µg kg^{-1} and 79 % of the total aflatoxins in cottonseed meal, a reduction from 214 to 46 µg kg^{-1}. Lower temperatures were less effective with both solvent systems.

1731. Rayner, E.T., Dollear, F.G., and Codifer, L.P.Jr. 1970. Extraction of aflatoxins from cottonseed and peanut meals with ethanol. Journal of the American Oil Chemists' Society 47(1): 26.

Cottonseed and groundnut meals containing aflatoxins were extracted with 95.6 % and 90 % aqueous ethanol at 75°C to lower the level of aflatoxins. These solvents

removed 93-96 % of the aflatoxins in the cottonseed meal and 96-98 % of the aflatoxins in groundnut meal.

1732. Knee, K.C., Natarajan, K.R., Cater, C.M., and Mattil, K.F. 1977. Processing edible peanut protein concentrates and isolates to inactivate aflatoxins. Journal of the American Oil Chemists' Society 54: 245A-249A.

Experiments were conducted to study the efficacy of some oxidizing or other reactive chemicals for destruction of aflatoxins in conjuction with the aqueous extraction process for the production of groundnut protein concentrates and/or isolates directly from aflatoxin-contaminatcd raw groundnuts. The chemicals tested included acetone, isopropyl alcohol, methylaminc, hydrogen peroxide, benzoyl peroxide, ammonia gas, and sodium hypochlorite. Among these chemicals, hydrogen peroxide, benzoyl peroxide, and sodium hypochlorite were effective in destruction of aflatoxins during the aqueous extraction process of contaminated groundnuts. However, the use of benzoyl peroxide may pose some difficulties because it is not readily soluble in the aqueous suspensions. It was concluded that aflatoxins can be effectively destroyed during the aqueous processing of groundnuts by properly utilizing either sodium hypochlorite or hydrogen peroxide to produce either groundnut protein concentrates or isolates.

1733. Shantha, T. 1989. Detoxification of groundnut seed and products in India. Pages 153-160 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 October. 1987. ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

This paper very briefly reviews several methods used for destruction of aflatoxin in groundnuts and their products. The exposure of the groundnut oil contaminated with aflatoxin to bright sunlight completely destroys the toxin. Aflatoxin is present in finely suspended solids in the oil and can be removed by filtration or by extraction with 10 % sodium chloride. Aflatoxin-contaminated groundnut kernels (0.5 mm thick flakes) can be partially detoxified if exposed to sunlight for 14 hours. 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 lonn of thin flakes or powder with urea and soyaflour with or without formaldehyde can destroy 90 % of their aflatoxin content. The merits and demerits of these processes are discussed.

1734. Shantha, T., and Sreenivasamurthy, V. 1975. Detoxification of groundnut oil. Journal of Food Science and Technology (Mysore) 12: 20-22.

A method for removing aflatoxin from crude groundnut oil has been developed after studying the efficiency of several extractants such as sodium chloride solution, aqueous acetone, sodium hydroxide and ammonia. Sodium chloride proved to be ideally suitable for removing aflatoxin B_1 from crude groundnut oil.

1735. Sommartya, T., Jatumanusiri, T., Konjing, C, and Maccormac, C. 1988. *Aspergillus flavus* in peanut in Thailand with special reference to aflatoxin contamination, and detoxification. Proceedings of the Japanese Association of Mycotoxicology Supplement No.1: 71-72.

Groundnut kernels collected from north-eastern plantations in Kalasin Province. Thailand, in August 1987 were roasted, split open and the seed coat, cotyledons and embryos were analyzed for aflatoxins by a Velasco aflatoxin meter. Results indicated accumulation of aflatoxin in embryos (274 µg kg^{-1}) and ground cotyledons (275 µg kg^{-1}). Of various food preservatives studied, sodium bisulfite was the best detoxification agent in ground groundnuts, with effective dose as low as 100 mg kg^{-1}.

1736. Sreenivasamurthy, V., Jayaraman, A., and Parpia, H.A.B. 1965. Aflatoxin in Indian peanuts : Analysis and extraction. Pages 251-260 in Mycotoxins in Foodstuffs (Wogan, G.N., ed.). The Masssachusetts Institute of Technology : The M.I.T. Press.

Of the nearly 150 isolates of *Aspergillus flavus* from groundnuts tested, only four produced aflatoxin B_1. These isolates differed from the type strain of *A. flavus* M001 (obtained from me Tropical Products Institute, London) in certain morphological characteristics. Of the several salt solutions tested, 1 % $CaCl_2$ was found most promising in extracting aflatoxin from the contaminated groundnut cake. In the preparation of the protein isolates, precipitation of the protein in $CaCl_2$ solution helped in removing nearly 80 % of the toxin from the protein. Certain apparently healthy groundnuts showed bright bluish-violet fluorescence under ultraviolet light. Extracts of such groundnuts on thin-layer chromatography (TLC) plates showed the fluorophor at the same Rf as that of aflatoxin B_1. A simple solvent system of benzene : cyclohexane : acetic acid (3:5:2, v/v/v) was found to give on paper chromatogram as good resolutions of the aflatoxin complex as found on alumina TLC with chloroform and methanol as solvent.

1737. Sreenivasamurthy, V., Parpia, H.A.B., Srikanta, S., and Shankar Murti, A. 1967. Detoxification of aflatoxin in peanut meal by hydrogen peroxide. Journal of the Association of Official Analytical Chemists 50(2): 350-354.

A new approach to destroy aflatoxin in toxic groundnut meal is described. It involves heat treatment of the meal at 80°C for 30 min with hydrogen peroxide at

a pH of 9.5. The destruction of aflatoxin was confirmed by biological tests, using ducklings and duck embryos.

1738. Srikumlaithong, S., and Munsakul, S. 1983. Detoxification of aflatoxin in peanut oil on laboratory scale. Journal of the National Research Council of Thailand 15(2): 31-38.

Detoxification of groundnut oil was successfully accomplished by lowering the aflatoxin content to less than 20 µg kg^{-1} by treating with Fuller's earth. Stirring the oil with 0.3 % of the earth at 150 rev min^{-1} for 15 min effectively reduced aflatoxin content from 76 to 7.85 µg kg^{-1} which is below the Ministry of Public Health Standard safety value requirement of 20 µg kg^{-1}. Chemical and physical properties of the processed oil comply with the food regulations. Two types of Fuller's earth, viz. AAA and Galleon earth, were used for absorbing aflatoxin ; there were no significant differences between the types of earth in reducing the toxin. The detoxification process was highly effective and involved simple techniques. Equipment used consisted of a mixing tank, locally fabricated and a filter press, commonly available in a groundnut oil factory. The additional cost of processing (i.e.. the cost of Fuller's earth and electricity) was 37.50 baht t^{-1} of oil treated.

1739. Thiesen, J. 1977. Detoxification of aflatoxins in groundnut meal. Animal Feed Science and Technology (Netherlands) 2(1): 67- 75.

An attempt was made to detoxify aflatoxin-contaminatcd groundnut meal for use in compounded rations for ruminants. Detoxification (>99 %) was obtained when the contmainated meal was stored with 5 % ammonia and 20 % water for 10 days in tight plastic bags. Three groups of cows were given, as part of their normal diet, (i) 1kg groundnut meal containing 2500 µg aflatoxin , (ii) 1 kg of 93 % detoxified groundnut meal containing 175 µg aflatoxin, (iii) 3 kg of 93 % detoxified groundnut meal containing 525 µg aflatoxin, respectively. Aflatoxin M_1 excreted by each cow in groups (i) - (iii), respectively, was 94, 20 and 47 µg cow^{-1} day^{-1}. There was a reduction of aflatoxin M_1 in milk, due to detoxification, of 79 % with (ii) and 83 % with (iii). Because of the experimental plan it was not understood why the cows fed on detoxified meal excreted a higher percentage of aflatoxin M_1 relative to ingested aflatoxin than cows fed on the aflatoxin-contaminated meal.

1740. Trager, W., and Stoloff, L. 1967. Possible reactions for aflatoxin detoxification. Journal of Agricultural and Food Chemistry 15(4): 679-681.

The reactions of aflatoxins (B_1, B_2, G_1, and G_2) with a series of selected reagents were observed for possible usefulness in detoxification procedures. Loss of fluorescence and/or change of Rf on TLC were the principal reaction indicators. Chick embryo and tissue culture bioassays were used to provide evidence of detoxification. The reactions appear to be primarily addition and oxidation involving the olefinic double bond of the terminal furan ring and oxidation involving the phenol formed on opening of the lactone ring. Benzoyl peroxide, osmium tetroxide. and 1- /12 react with aflatoxins B_1 and G_1. but not with B_2 and G_2, $Ce(NH_4)_2(SO_4)_3$, NaOCl. $KMnO_4$, $NaBO_3$, and 3 % H_2O_2 + $NaBO_2$ (1+1) react with all four aflatoxins. Detoxification, after contact with gaseous chlorine, chlorine dioxide, and nitrogen dioxide and after treatment with 5 % NaOCl solution, was confirmed by bioassay.

1741. Tsubouchi, H., Yamamoto, K., Hisada, K., Sakabe, Y., and Moriyama, S. 1981. Mycotoxins in foods : 3. Effects of various preservatives on the growth and aflatoxin B_1 production of *Aspergillus flavus* and *Aspergillus parasiticus* in sliced bread. Journal of Food Hygienic Society of Japan 22(2): 142-149.

Various preservatives, such as calcium propionate, sorbic acid, potassium sorbate, dehydroacetic acid, sodium dehydroacetate, benzoic acid, sodium benzoate and isobutyl p-hydroxybenzoatc, were examined for their inhibitory effects on the growth and aflatoxin B_1 production of *Aspergillus flavus* and *A. parasiticus* in sliced bread coated with groundnut butter. Groundnut butter containing a preservative and 100 conidia of each fungus was coated on the upper surface of a slice of bread, and the slice was incubated at 28°C for 10 days. Calcium propionate and sorbic acid showed the most inhibitory effects.

1742. Virobcn, G., DeLort-Laval, J., Colin, J., and Adrian, J. 1978. Aflatoxin inactivation after ammonia treatment. In vitro studies on detoxified peanut meals. Annales de la Nutrition et de l'Alimentation 32(1): 167-185.

Treatment of groundnut oilmcal with gaseous ammonia at 2 to 3 bar is fairly easy and rapid. It reduced aflatoxin content of the meal by up to 95 % ; the content of nitrogen, particularly non- protein nitrogen increased. There was no adverse effect on digestibility in vitro with pepsin, and the treated meal was more sensitive to proteases. Treatment slightly decreased deamination of protein in an artificial rumen and nitrogen solubility in buffer at pH 7.5, which seemed to favour utilization by ruminants. There was no significant effect of ammonia treatment on amino acid composition of the meal, especially of total and available lysine. There was some destruction of cystine, which could be counteracted by a supplement of methionine.

1743. Virobcn, G., Fremy, J.M., and DeLort-Laval, J. 1983. [Treatment of peanut meal with aqueous ammonia at ambient temperature : effect on reduction of aflatoxin M_1 content in milk.]. Traitement a froid des tourteaux d'arachide par une solution aqueuse d'ammoniaque : consequence sur la reduction de la teneur du lait en aflatoxine M_1. Le Lait 63(625/62): 171-179.

Groundnut meal contaminated with aflatoxin B, (at 450 µ00g kg ' DM) was treated with an aqueous ammonia solution (approximately 32 %) at a rate of 20 kg 100 kg ' meal. After homogenization for 5 min. the product was packed in plastic sacks and stored for at least 2 wk before being fed to dairy cows. The treated groundnut meal contained aflatoxin B_1 at 4-13.5 µg kg^{-1} DM. indicating a detoxification of about 98 %. Milk from cows fed the contaminated meal contained aflatoxin M, at 1-1.8 µg kg^{-1} on a DM basis, and milk from cows fed the detoxified meal contained only 0.1-0.22 µg kg^{-1}. The detoxified meal was not as acceptable to the cows as the nontreated meal. The amount of aflatoxin M_1 excreted by cows fed the detoxified meal was higher than would be expected from the aflatoxin B_1 intake. The possibility of the detoxification procedure being partly reversible is discussed.

1744. Vorster, L.J. 1966. [Studies on the detoxification of groundnuts contaminated by aflatoxin.]. Etudes sur la detoxification des arachides contaminees par l'aflatoxine et destinees a l'huilerie. Revue francaise Corps Gras 13(1): 7-12.

This paper presents the results of an investigation into the possibility of the simultaneous removal of oil and aflatoxin from oilcake by the use of suitable solvents.

1745. Wangjaisuk, S., Chokethaworn, N., Vinittketkumnuan, U., and Suttajit, M. 1988. Detoxification of aflatoxin in peanut by ammonium bicarbonate. Proceedings of the Japanese Association of Mycotoxicology Supplement 1: 79-80.

A simple method for the detoxification of aflatoxin B_1 in groundnut with ammonium bicarbonate is presented. The effects of ammonium bicarbonate conentrations, treatment time and temperature on aflatoxin detoxification were investigated. Chloroform extracts from the treated samples were assayed by a modified Ames bacterial test which confirmed that the decrease in mutagenicity was related to aflatoxin detoxification.

1746. Williams, K.R., and Dutton, M.F. 1988. Destruction of aflatoxin during the production of hydrolysed vegetable protein. Journal of Food Protection 51(11): 887-891.

An investigation into the breakdown of atlatoxins during the hydrolysis of artificially contaminated defatted groundnut oilmeal was conducted using a laboratory scale reactor. The conditions selected emulated a commercial process used to produce a protein hydrolysate used in processed food and soup ingredients. Chromatographic analysis showed that aflatoxins at relatively high concentration (aflatoxin B_1, 321 µg kg^{-1}; B_2. 415 µg kg^{-1}; G_1. 161 µg kg^{-1} ; G_2. 15 µg kg^{-1}) were totally destroyed and removed from the product. Residues from extracts were free of mutagenic properties, as monitored by the Ames test.

8.2.11 Microbial Detoxification

1747. Ciegler, A., Lillehoj, E.B., Peterson, R.E., and Hall, H.H. 1966. Microbial detoxification of aflatoxin. Applied Microbiology 14(6): 934-939.

Yeasts, molds, bacteria, actinomycetes, algae, and fungal spores were screened for their ability to degrade aflatoxin. Some molds and mold spores partially transformed aflatoxin B, to new fluorescing compounds. Only one of the bacteria. *Flavobacterium auranliacum* NRRL B-184, removed aflatoxin from solution; both growing and resting cells look up the toxin irreversibly. Toxin- contaminated milk, oil, peanut butter, groundnuts, and corn were completely detoxified, and contaminated soybean was partially detoxified by addition of this organism. Duckling assay showed that detoxification of aflatoxin solution by the bacterium was complete, with no new toxic products being formed.

1748. Cole, R.J., and Kirksey, J.W. 1971. Aflatoxin G_1 metabolism by *Rhizopus* species. Journal of Agricultural and Food Chemistry c19: 222.

Rhizopus stolonifer, R. arrhizus, R. oryzae, and *R.* sp. degraded aflatoxin G_1. An intermediate in the biological degradation was isolated and identified as a previously reported metabolite of *Aspergillus flavus* (aflatoxin B,) and *A. parasiticus* (parasitol). Radioisotope data conclusively demonstrated that this metabolite was derived from aflatoxin G, degradation by the *Rhizopus* spp.

1749. Cole, R.J., Kirksey, J.W., and Blankenship, B.R. 1972. Conversion of aflatoxin B_1 to isomeric hydroxy compounds by *Rhizopus* spp. Journal of Agricultural and Food Chemistry 20: 1100.

An investigation of aflatoxin B_1 degradation by a *Rhizopus arrhizus* isolate from Georgia groundnuts and three knwon *Rhizopus* species revealed that two fluorescent metabolites of aflatoxin B_1 accumulated during degradation. They were identified by physical, chemical, and spectroscopic data as hydroxylated stereo isomers derived from reduction of the ketone function on the cyclopentane ring of aflatoxin B_1. It was conclusively shown with ^{14}C-labelled aflatoxin B_1 that these metabolites were derived from aflatoxin B_1. Two additional fluorescent metabolites appeared during purification of the hydroxy isomers. These were identified as ethyl ether derivatives of the hydroxylated compounds and apparently were formed spontaneously from either one or both hydroxy isomers.

1750. Doyle, MP., Applebaum, R.S., Brackett, R.E., and Marth, E.H. 1982. Physical, chemical and biological degradation of mycotoxins in foods and agricultural commodities. Journal of Food Protection 45(10): 964-971.

Aflatoxin is partially or completely degraded by irradiation, heat, or treatment with strong acids or bases, oxidizing agents or bisulfite. Hydrogen peroxide plus riboflavin denature aflatoxin in milk. Mycelia of *Aspergillus parasiticus* can degrade aflatoxin, possibly via fungal peroxidase. Such degradation is affected by strain of *A. parasiticus,* amount of mycelium, temperature, pH, and concentration of aflatoxin. Adsorbants, including bentonite and activated charcoal, can physically remove aflatoxin and patulin from liquid foods. Patulin is stable at low pH values but not in the presence of large amounts of vitamin C or bisulfite. Patulin can be degraded by actively fermenting yeasts and rubratoxin can be degraded by the mycelium of *Penicillium rubrum.*

1751. Hao, Y-Y., and Brackett, R.E. 1988. Removal of aflatoxin B_1 from peanut milk inoculated with *Flavobacterium aurantiacum.* Journal of Food Science 53(5): 1384-1386.

The ability of *Flavobacterium aurantiacum* to reduce aflatoxin B_1 concentration was determined, by inoculating 10 stationary phase cells into aflatoxin B_1-contaminated phosphate buffer (PB). non- defatted peanut milk (NDPM) and partially defatted peanut milk (PDPM). Aflatoxin concentration and cell populations of the bacterium were determined periodically throughout incubation at 30°C. After 24 h, aflatoxin B_1 concentration had decreased about 40 % in PB, 23 % in NDPM and 74 % in PDPM. Viable cell count had generally decreased by < 1 $\log_{10}$ cfu ml^{-1}, but increased about 0.8 log 10 units in control PDPM. Aflatoxin B_1 recovery increased about 30 % on proteolysis of PDPM; proteolysis had no effect on recovery from NDPM.

1752. Hao, Y-Y., Brackett, R.E., and Nakayama, T.O.M. 1989. Removal of aflatoxin B, from peanut milk by *Flavobacterium aurantiacum.* Pages 141-152 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 October 1987. ICRISAT Center, India. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

The potential for using *Flavobacterium aurantiacum* to remove aflatoxin B_1 from peanut milk was evaluated. Preliminary experiments revealed that this bacterium grew in both non- defatted 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 carried out to assess the ability of resting cells of *F. aurantiacum* to remove aflatoxin B_1 from phosphate buffer (PB), NDPM, and PDPM. After 24 hr 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 the bacterium increased recovery of the toxin by about 30 % over non-proteolyzed samples. This increase in recovery of aflatoxin was not observed when NDPM samples were proteolyzed, indicating that some of the toxin may be bound to the peanut protein and not be available for removal by *F. aurantiacum.*

1753. Lillehoj, E.B., Ciegler, A., and Hall, H.H. 1967a. Aflatoxin B_1 uptake by *Flavobacterium aurantiacum* and resulting toxic effects. Journal of Bacteriology 93: 464-471.

Removal of aflatoxin B_1 from liquid cultures by resting and growing cells of *Flavobacterium aurantiacum* NRRL B-184 was studied. Spectrophotometric and thin-layer chromatographic techniques served as aflatoxin assays. Cells grown in the presence of 5 ppm or higher levels of aflatoxin developed aberrant morphological forms. These toxin concentrations partially inhibited growth, and the nature of the inhibition suggested that aflatoxin interfered with cell wall synthesis. Incubation of 1.0 x 10" resting cells per milliliter with 7.0 μg ml^{-1} of aflatoxin B_1 during a 4 h period facilitated complete toxin removal from a buffered aqueous medium. Autoclaved cells and cell wall preparations could remove a fraction of the aflatoxin of a test system. However, the toxin removed by autoclaved cells and cell walls could be extracted by washing with water but the aflatoxin B_1 removed by intact cells could not be extracted into the liquid phase. The uptake of aflatoxin B_1 by resting cells was sensitive to temperature and pH. Ruptured preparations of *F. aurantiacum* were not able to remove or modify the aflatoxin in an aqueous solution.

1754. Lillehoj, E.B., Ciegler, A., and Hall, H.H. 1967b. Aflatoxin G_1 uptake by cells of *Flavobacterium aurantiacum.* Canadian Journal of Microbiology 13(6): 629-633.

Aflatoxin G, was removed from liquid cultures by growing and resting cells of *Flavobacterium aurantiacum* NRRL B-184. In inoculated culture media containing aflatoxin levels of 7500 *μg* L^{-1} and above, there was a protracted growth lag which was subsequently overcome; then occurred toxin removal, concomitant with growth. Only a few cells showed aberrant morphological forms when cultured in the presence of aflatoxin G_1. A comparison of the effects of aflatoxin G_1 with aflatoxin B, on growth and morphology showed that aflatoxin B, was distinctly more toxic. Three hundred and thirty μg of aflatoxin G_1 was removed per 1 x 10^{13} resting cells during a 4-hour incubation period. Preincubation of resting cells with aflatoxin B_1 did not interfere with subsequent uptake of G_1.

1755. Lillehoj, E.B., Stubblefield, R.D., Shanon, G.M., and Shotwell, O.L. 1971. Aflatoxin M_1 removal from aqueous solutions by *Flavobacterium aurantiacum.* Mycopathologia et Mycologia Applicata 45: 259-266.

In liquid cultures growing and stationary phase cells of *Flavobacterium aurantiacum* NRRL B-184 eliminated aflatoxin M_1. Toxin concentrations of 15 μg ml^{-1} and 37.5 μg ml^{-1} interfered with bacterial growth, and at the higher level 4.4 μg M_1 was removed from the growth medium by a milligram (dry weight) of bacteria. Toxin was completely removed from the liquid medium by incubating 5 x 10^{10} resting cells

per milliliter with 8 µg ml^{-1} of allatoxin M_1 for 4 h. Attempted recovery of M_1 from cells following incubation of the bacteria with the toxin demonstrated that the M_1 was essentially nonextructable. Bacterial cells also removed aflatoxin M_1 from toxin-contaminated milk.

1756. Mann, R., and Rehm, H.J. 1976. Degradation products from aflatoxin B_1 by *Corynebacierium rubrum, Aspergillus niger, Trichoderma viride,* and *Mucor ambiguus.* European Journal of Applied Microbiology 2: 297-306.

Degradation of aflatoxin B_1 by *Corynebacierium rubrum* and by *Aspergillus niger* was analysed by adding ^{14}C labelled aflatoxin B_1 to cultures of these microorganisms. Two blue fluorescent compounds, formed by *A. niger* from aflatoxin B_1 with R_1-values 0.42 and 0.48 (R_1 of aflatoxin B_1 = 0.54) were accumulated and characterized by UV-, fluorescence and mass spectrometry. Based on their properties both products were identified to be aflatoxin R_0. Under the same conditions *Mucro ambiguus* and *Trichoderma viride* also produced aflatoxin R_0.

1757. Marth, E.H., and Doyle, M.F. 1979. Update on molds : degradation of aflatoxin. Food Technology 33: 81-87.

This paper briefly reviews chemical, physical, and biological means of degrading aflatoxins. Research needs in these areas are presented.

1758. Mujica, M.T., and Corallini de Bracalenti, B.J. 1985. Inhibition of aflatoxin B_1 'in vitro' by growth of competitive fungi. Revista Latinoamericana de Microbiologia 27(2): 169-173.

Aflatoxin B_1 production by *Aspergillus flavus* NRRL 3251 was inhibited by *Rhizopus* sp or *Aspergillus niger* when grown in culture medium for up to 9 days at 28°C.

1759. Nout, M.J.R. 1989. Effect of *Rhizopus* and *Neurospora* spp. on growth of *Aspergillus flavus* and *A. parasiticus* and accumulation of aflatoxin in groundnut. Mycological Research 93(4): 518-523.

The influence of *Rhizopus* and *Neurospora* spp. on growth and aflatoxin B, accumulation of *Aspergillus flavus* and *A. parasiticus* was investigated. When inoculated simultaneously with *Rhizopus* or *Neurospora* spp. on mashed groundnuts, *Aspergillus* spp. were able to grow, but to a lesser extent and with visually different mycelial development and sporulation behavior. Accumulation of aflatoxin B_1 observed during a 6 wk incubation, averaged 34 % in mixed cultures with *Rhizopus* spp. and 1.7 % in mixtures with *Neurospora* spp., as compared with pure cultures *of Aspergillus* spp. On aqueous groundnut extracts, selected strains *of Rhizopus* and *Neurospora* showed the ability to cause an exhaustion, particularly of C compounds, inhibiting *Aspergillus* growth and aflatoxin B_1 accumulation. There was also evidence for the formation of *Rhizopus* and *Neurospora* metabolites inhibiting growth and/or aflatoxin B_1 production. In addition *Rhizopus* and *Neurospora* strains degraded aflatoxin B_1.

1760. Teunisson, D.J., and Robertson, J.A. 1967. Degradation of pure aflatoxin by *Tetrahymena pyriformis.* Applied Microbiology 15(5): 1099-1103.

Tetrahymena pyriformis with nutrients, ca. 22 x 10^6 cells, decreased the concentration of aflatoxin B_1 58 % in 24 h and 67 % in 48 h. An unknown, bright-blue fluorescent substance was produced, with intensity about one-half that of the un-changed B_1, with an R_1 of 0.52 compared with 0.59 for B_1 and 0.55 for B2 on a thin-layer chromatography plate, and with an ultraviolet spectrum showing maxima of 253, 261. and 328 mu. In a separate assay, the cells with nutrients did not degrade pure G_1. Starved, washed cells, ca. 11 x 10^6, decreased the concentration of B_1 50 % in 10 h, 70 % in 22 h, and 75 % in 30 h, producing the same unknown component. Ethyl alcohol, 1.96 % (v/v), decreased cell populations and size, but the cells remained actively motile in broth plus alcohol for 96 h. In 72 h, neither toxin (ca. 2 ppm) in combination with ethyl alcohol had more inhibitory effect on cell numbers, with or without nutrients, than was produced by alcohol alone. Aflatoxin B_1 had no observed effect on the viability of the starved cells for 30 h or on the nourished cells for 72 h. There was no noticeable effect of G_1 on the starved cells in 30 h or on the nourished cells in 48 h. After 72 h with G_1 plus nutrients, many of the cells were round with blisters, nonmotile, and apparendy dead or dying.

1761. Tsubouchi, H., Yamamoto, K., Hisada, K., Sakabe, Y., and Tsuchihira, K. 1980. Degradation of aflatoxin B_1 by *Aspergillus niger.* Proceedings of the Japanese Association of Mycotoxicology 12: 33-35.

Aspergillus niger degraded aflatoxin B_1 in a culture medium. Two pathways are indicated for degradation of aflatoxin B_1 in *Aspergillus niger* culture, one directly and another through aflatoxin B_{2a}.

8.2.12 Physical Detoxification

1762. Ahmed, E.M., and Wei, C.I. 1987. Destruction of aflatoxin by microwave oven and chlorine gas. Proceedings of the American Peanut Research and Education Society 19: 41.

The effects of oven and microwave roasting on aflatoxin contaminated groundnuts and the effect of chlorine treatment on aflatoxin B, detoxification were studied. In artificially contaminated groundnuts, oven roasting for 30 min at 150°C or microwave roasting for 8.5 min at 0.7 KW were equally effective in destroying 30 to 45 % of aflatoxin B_1. In naturally contaminated groundnuts, both oven and microwave roastings were equally effective in destroying 48 to 61 % of aflatoxin B_1 and 32 to 40 % of aflatoxin G_1. Chlorine gas treatment was very effective in destroying aflatoxin B_1. Time course study of this treatment (100 jig aflatoxin B_1

with 15 mg chlorine gas at standard temperature and pressure) showed that about 60 to 75 % of the toxin was destroyed within 10 min of exposure. During the treatment process, at least three new fluorescent reaction products were produced and two of them were identified as 2,3-dichloro aflatoxin B_1 and 2,3-dihydroxy aflatoxin B_1 (diol). Use of radio-labelled aflatoxin B_1 confirmed these results. Chlorine- dose related study at 10 min exposure indicated that even the treatment of 100 μg of aflatoxin B_1 with 7.5 mg of chlorine caused about 75 % destruction. Preliminary mutagenicity study using the Ames Salmonella assay indicated that the mutagenic activity of the 10-min treated sample in the presence of rat liver S-9 mix can be reduced to about 10 % of that of the untreated control. The results indicated that low energy microwave roasting is not an efficient method to remove aflatoxin B_1 from contaminated groundnut samples, while chlorine gas could be an effective agent in reducing aflatoxin toxicity.

1763. Andrellos, P.J., Beckwith, A.C., and Eppley, R.M. 1967. Photochemical changes of aflatoxin B_1. Journal of the Association of Official Agricultural Chemists 50: 346.

Irradiation of aflatoxins B_1 and G_1 with UV light (principal wavelength 365 mu) converts both compounds to new fluorescent photoproducts which have much lower R_1 values than aflatoxins B_1 and G_1 when chromatographed on silica gel thin-layer plates. Photoproducts of aflatoxin B_1 form much faster on a silica gel surface than in methanol solution. Photoconversion of aflatoxin B_1 is shown to alter fluorescence comparison assays and identification tests. Studies show that the principal photoproduct developed from aflatoxin B_1 is sufficiently less toxic than the parent aflatoxin.

1764. Applegate, K.L., and Chipley, J.R. 1974. Effects of ^{60}Co gamma irradiation on aflatoxin B_1 and B_2 production by *Aspergillus flavus*. Mycologia LXVI 3: 436-445.

Germination of spores of the aflatoxigenic *Aspergillus flavus* NRRL-3145. previously exposed to specific gamma radiation levels, resulted in a greater production of aflatoxins B_1 and B_2 following inoculation onto a cracked wheat medium or into a synthetic liquid medium than did similarly treated nonirradiated controls. The most notable increases in aflatoxin production occurred from cultures developing from spores having been irradiated with 50, 100, 150, or 200 krad of gamma radiation. Exposure to 400 krad of gamma radiation resulted in complete inhibition of spore germination and consequently negative toxin production. Of the two aflatoxins, the quantities of B_2 produced were significantly lower than that of B_1 except in synthetic medium where B, production was comparable to that of B_1. Analysis of variance revealed that substrate, time of incubation following the respective irradiation doses, as well as radiation levels, all affected the quantities of aflatoxins produced by the fungus.

1765. Baikaloff, A., and Read, M.J. 1985. Aflatoxin - Incidence, segregation and destination in Australia. Proceedings of the American Peanut Research and Education Society 17: 55.

The occurrence of aflatoxin in the Australian groundnut crop is mainly due to late season drought stress. The Australian Peanut Marketing Board uses a minicolumn test to segregate aflatoxin- contaminated (aflatoxin > 16 µg kg^{-1}) farmers'stock groundnuts at the point of delivery. To check the effectiveness of this segregation, an investigation was made to quantify the incidence of aflatoxin in groundnuts at several major points in the deshelling, grading and blanching operations. The aflatoxin "positive" segregation lots contained aflatoxin levels eight times higher than the aflatoxin "negative" lots. Seventy-one % of the aflatoxin through the shellers was concentrated into the oil milling kernels, which comprised 16 % of the total kernels. Oil kernels from "negative" and "positive" stock averaged 48 μg kg^{-1} and 253 µg kg^{-1} aflatoxin, respectively. It appears that in excess of 50 % of the aflatoxin in the product which was roasted and blanched was lost and/or degraded in the operation.

1766. Basappa, S.C. 1983. Physical methods of detoxification of aflatoxin contaminated food materials. Pages 251-273 in Mycotoxins in Food and Feed (Bilgrami, K.S., Prasad, T., and Sinha, K.K., eds.). Bhagalpur, India : Allied Press.

This paper discusses several physical methods for detoxification of aflatoxin-contaminated groundnuts, groundnut oil, maize, cottonseed, and rice. Removing discolored seeds by handpicking or by electronic color sorting devices is efficient for reducing aflatoxin content in groundnuts, maize and cottonseed. The possibility of using air classification to separate aflatoxin- contaminated groundnuts and cottonseed has been explored and found to be a useful tool. Milling of contaminated brown rice has been found to reduce aflatoxin in the polished rice. Roasting, frying, boiling, baking and cooking of contaminated groundnuts have been found to inactivate aflatoxin to a certain extent. Detoxification of groundnut oil by exposure to bright sunlight has proved useful. Decontamination of groundnut oil is also possible by the use of filterpads in the processing line of oil mills. Washing the oil with sodium chloride solution is another promising method for removal of aflatoxin. These detoxification/decontamination procedures are discussed with special reference to their economic and technical feasibility.

1767. Basappa, S.C, and Sreenivasamurthy, V. 1977. State of aflatoxin in groundnut oil. Journal of Food Science and Technology, India 14(2): 57-60.

Centrifuging at 40,000 g removed 65 % of total aflatoxin along with sedimentable cell debris in commercial groundnut oil. Studies suggested that the remainder of the toxin was in a soluble form. Pure aflatoxin added to refined oil was not sedimented. Filtration with membrane filter, sintered glass and filter paper could separate 30-50

% of aflatoxin in the oil. Use of activated Fullers' earth as an adsorbent at *2* % level and centrifugation at 10,000 g sedimented nearly 92.5 % of the aflatoxin.

1768. Basappa, S.C., and Sreenivasamurthy, V. 1979. Decontamination of groundnut oil from aflatoxin by absorption-cum-filtcration. Indian Journal of Technology 17(11): 440-441.

Attempts were made at laboratory and pilot plant level to remove aflatoxin from groundnut oil by the use of filter pads. The filter beds tried were (i) Celite and salt (1:10). (ii) activated fullers' earth and salt (1:2) and (1:10), and (iii) Kaolinite and salt (1:2). Of these, Kaolinite and salt (1:2) proved best for removing aflatoxin but the rate of filtration was slow. The activated fullers' earth and salt (1:2) proved best in efficiency (96 % aflatoxin removed) and speed. These were further used to remove aflatoxin on pilot scale amploying a plate-and-framc filter press, the efficiency ranged from 88-90 % for removal of aflatoxin. The pad could be used to filter 200 kg of oil and the filtration rate was 12 kg oil h^{-1} and the aflatoxin content in filtered oil was 25 µg kg^{-1}.

1769. Chiou, R.Y.Y., Lin, C.M., and Shyu, S.L. 1990. Property characterization of peanut kernels subjected to gamma irradiation and its effect on the outgrowth and aflatoxin production by *Aspergillus parasiticus.* Journal of Food Science 55(1): 210- 213.217.

Groundnut kernels inoculated with *Aspergillus parasiticus* conidia and uninoculated kernels were gamma irradiated with 0-15 KGy using ^{60}Co. Levels of 2.5 and 5.0 KGy were effective in retarding outgrowth of *A. parasiticus* and reducing the population of natural mold contaminants. However, elimination of these molds was not achieved. When irradiated with doses > 10 KGy, seed germination was inhibited, changes in proteins were observed and oil stabilities decreased. After 4 wk incubation of the inoculated kernels in a humidified condition, aflatoxins produced by surviving *A. parasiticus* ranged from 69.12 to 13.48 µg g^{-1} depending upon the original irradiation dose.

1770. Coker, R.D. 1989. Control of aflatoxin in groundnut products with empasis on sampling, analysis, and detoxification. Pages 123-132 in Aflatoxin contamination of groundnut : Proceedings of the International Workshop, 6-9 Oct 1987, ICRISAT Center, Patancheru A.P. 502 324, India : International Crops Research Center for the Semi-Arid Tropics.

This paper reviews the work carried out at the Overseas Development Natural Resources Institute (ODNRI), London, on the development of efficient sampling, sample preparation, aflatoxin analysis, and chemical detoxification procedures. The control of aflatoxin in groundnut products requires a combination of quality control and decontamination/detoxification procedures. The use of selected mathematical models to describe the distribution of aflatoxin in groundnut kernels, roasted groundnuts, peanut butter, and groundnut cake has been investigated for developing 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 developed for the accurate analysis of the aflatoxin content of groundnut products using bonded-phase clean-up 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 aflatoxin in 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.

1771. Coomes, TJ., Crowther, P.C., Feuell, A.J., and Francis, B.J. 1966. Experimental detoxification of groundnut meals containing aflatoxin. Nature 209: 406-407.

A process is given for removing aflatoxin from contaminated groundnut meal by autoclaving. Autoclaving at 15 lb. in^2 (120°C) of wet toxic groundnut meals resulted in a progressive reduction in their aflatoxin B_1 toxicity, with time. Similar experiments involving treatment of pure aflatoxins (either produced by artificial culture or isolated from toxic extracts by preparative-scale, thin-layer chromatography), resulted in the recovery of non-fluorescent products in which the characteristic ultraviolet absorption band of aflatoxin B_1 at 363 mu had disappeared.

1772. Delwichc, S.R., Shupe, W.L., Pearson, J.L., and Sanders, T.H. 1985. The effect of microwave drying of shelled peanuts on energy requirements, physical properties, mycological growth and germination potential. Proceedings of the American Peanut Research and Education Society 17: 53.

Groundnuts (cultivar Florunner) at moisture contents ranging between 8 % and 22 % (wet weight basis) were shelled and microwave vacuum dried at treatment rates of 4, 8, 16 and 32 times the nominal recommended rate for conventional wagon drying. Electrical energy per unit dry mass supplied to the microwave generators was closely equivalent to the energy content of L.P. gas used in wagon drying. Propensity of microwave treated kernels toward splitting and skin slippage was insignificant when compared to conventional within shell deep bed dried control samples. Significant differences occurred for presence of *Aspergillus Jlavus* on surfaces of kernels from the pooled microwave treatment compared to check samples, but differences among the microwave treatments were insignificant in this respect. Aflatoxin was not detectd in any microwave or control treatment. Kernel germination decreased with increasing microwave process rate.

1773. Doyle, M.P., Applcbaum, R.S., Brackett, R.E., and Marth, E.H. 1982. Physical, chemical and biological degradation of mycotoxins in foods and agricultural commodities. Journal of Food Protection 45(10): 964-971.

Aflatoxin is partially or completely degraded by irradiation, heat, or treatment with strong acids or bases, oxidizing agents or bisulfite. Hydrogen peroxide plus riboflavin denature aflatoxin in milk. Mycelia of *Aspergillus parasiticus* can degrade aflatoxin, possibly via fungal peroxidase. Such degradation is affected by strain of *A. parasiticus,* amount of mycelium, temperature, pH, and concentration of aflatoxin. Adsorbants, including bentonite and activated charcoal, can physically remove aflatoxin and patulin from liquid foods. Patulin is stable at low pH values but not in the presence of large amounts of vitamin C or bisulfite. Patulin can be degraded by actively fermenting yeasts and rubratoxin can be degraded by the mycelium of *Penicillium rubrum.*

1774. El-Kady, I.A., and Farghaly, M.S. 1981. Inactivation of aflatoxins in contaminated peanuts. Cryptogamie Mycologie 2(2): 131-136.

A highly aflatoxin-contaminated groundnut sample was treated to inactivate its toxicity by dry heating (roasting) or autoclaving. Aflatoxins in contaminated groundnuts proved resistant to dry heating, while autoclaving was satisfactory for their destruction. Thin-layer chromatography (TLC), toxicity to chick embryo and inhibition of *Bacillus megaterium* growth tests were used to evaluate the inactivation of aflatoxins.

1775. Feuell, A.J. 1966. Aflatoxin in groundnuts. Part IX - Problems of detoxification. Tropical Science 8(2): 61-70.

Oil extracted from aflatoxin-containing groundnut kernels by crushers or expellers is toxin-free; extraction of residual oil from the press-cake by the usual type of solvent does not remove aflatoxin either. Consequently, press-cake and meal contain practically all the aflatoxin originally present in kernels. Trials to detoxify groundnut meal by radiation, heat, solvents, or reactive chemicals, conducted at the Tropical Products Institute in London, UK, were not very successful. The only treatments that destroyed or removed the toxin - i.e.. prolonged wet heating at 120°C, extraction with methanol, and treatment with 10 % HCl, SO_2, or Cl_2, are expensive and cause deterioration of the meal. It is concluded that detoxification is not a practical possibility and that the emphasis must be on the prevention of infection by the aflatoxigenic fungus *Aspergillus Jlavus.*

1776. Food and Agricultural Organization. 1975. information relating to the wholesomeness of irradiated food : effect of r-radiation on the microbiology and chemical properties of peanuts. Food Irradiation Information 4: 70 pp.

Shelled groundnuts packaged in multi-wall paper bags were r- irradiated at 0-150 krad and stored for 10 months at 27-30°C. Aflatoxin contamination during storage was controlled at 100 and 150 krad. Free fatty acid contents changed slightly at < 150 krad, increased 5-6 times at > 150 krad. Thiobarbituric acid values were negative for both irradiated and non-irradiated groundnut samples.

1777. Frank. H.K., and Grunewald, T. 1970. Radiation resistance of aflatoxins. Food Irradiation (Saclay) 11. 15-20.

Destruction of aflatoxins in foodstuffs by irradiation was investigated. No significant destruction of aflatoxin in foodstuffs with irradiation was possible. Dry aflatoxins were highly resistant to radiation.

1778. Harvey, R.B., Kubena, L.F., Phillips, T.D., Huff, W.E., and Corrier, D.E. 1989. Prevention of aflatoxicosis by addition of hydrated sodium calcium aluminosilicate to the diets of growing barrows. American Journal of Veterinary Research 50(3): 416-420.

Hydrated sodium calcium aluminosilicate (HSCAS) was added to the diets of growing barrows in 6 treatments of 5 barrows each at the following concentation : 5 g kg^{-1} HSCAS (0.5 %); 20 g kg^{-1} HSCAS (2 %); 3 mg kg^{-1} aflatoxin (AFT); 5 g HSCAS (0.5 %) plus 3 mg AFT kg^{-1} feed; 20 g HSCAS (2 %) plus 3 mg AFT kg^{-1} feed; and no HSCAS or AFT (control). Barrows were observed twice daily and were weighed weekly, and blood samples were obtained weekly for haemotological and serum biochemical measurements. At the termination of the study, barrows were euthanatized and necropsied. Body weight gains were diminished significantly (P<0.()5) by consumption of 3 mg kg^{-1} AFT. whereas body weight gain in barrows consuming diets containing HSCAS or HSCAS plus AFT did not differ from that in control barrows. Serum enzymatic activities of alkaline phosphatase and Y-glutamyl transferase and prothrombin time were increased in barrows consuming 3 mg AFT kg^{-1} feed, but not in those consuming HSCAS or HSCAS plus AFT. Aflatoxin alone induced decreased serum concentration of urea nitrogen, albumin, total protein, calcium, phosphorus, cholesterol, and glucose, as well as serum total iron-binding capacity, whereas HSCAS or HSCAS plus AFT did not induce such effects. Hepatic lesions in barrows of the AFT-alone treatment group were characterized as perpheral lobular lipidosis accompanied by perportal and interlobular fibrosis and bile duct hyperplasia. Hepatic lesions were not observed in barrows of the 0.5 % HSCAS plus AFT or 2 % HSCAS plus AFT treatment groups. It is suggested that HSCAS can modulate the toxicity of AFT in growing barrows and may offer a novel approach to the preventive management of aflatoxicosis in animals.

1779. Lee, L.S., Cucullu, A.F., and Goldblatl, L.A. 1968. Appearance and

aflatoxm content of raw and dry roasted peanut kernels. Food Technology 22: 1131-1134.

One hundred suspect kernels (slightly discolored, shrivelled, or molded) were hand picked from a sample of domestic groundnuts that had been graded as sound, mature kernels. After roasting half of each kernel, assays for aflatoxins were conducted on both the raw and roasted portion of each individual kernel using the micro technique developed by Cucullu et al. (1966). There was an average reduction of about 80 % in aflatoxin B_1 and about 60 % in aflatoxin B_2 after roasting for one-half h at 150 °C. In most cases the roasted half of each kernel containing aflatoxins, although not deeply colored, was darker than the roasted controls. However, many of the raw halves appeared almost identical to normal groundnuts. Generally, when the level of the toxins was high in the raw kernels, some toxins were detected in the roasted portions. When the levels were low in the raw half, toxins were generally not detectable after roasting.

1780. Lee, L.S., Cucullu, A.F., Franz, A.O., and Pons, W.A. 1969. Destruction of aflatoxins in peanuts during dry and oil roasting. Journal of Agricultural and Food Chemistry 17(3): 451-453.

Blanched whole groundnut kernels inoculated with an aflatoxigenic strain of *Aspergillus flavus* and subjected to continuous shaking during incubation for 40, 46, 64, and 72 h produced groundnuts virtually clear of visible mold and containing four graded levels of total aflatoxins - 130, 260, 2560, and 6300 µg kg^{-1}. Although individual kernels differed in aflatoxin content, replicate assays of 50 g aliquots of contaminated whole kernels were subjected to oil and dry roasting under five time-temperature conditions simulating those used in industry to effect a normal roast for high quality groundnuts. Average reduction in aflatoxin content ranged from 45 to 83 %, depending on roasting conditions and the level of aflatoxins in the raw kernels. There was an over-all reduction of 65 % in B_1 and 62 % in G_1 for oil roasting, and 69 % in B_1 and 67 % in G_1 for dry roasting. The degree of reduction in aflatoxin conetent was greatest at the highest aflatoxin contamination levels, for both oil- and dry-roasted groundnuts.

1781. Lillard, D.A., and Lantin, R.S. 1970. Some chemical characteristics and biological effects of photomodified aflatoxins. Journal of the Association of Official Analytical Chemists 53: 1060.

Changes in UV and infrared absorption spectra were demonstrated upon prolonged exposure of solutions of aflatoxins B_1 and G_1 to long wave UV light. The treated toxins were less toxic to chick embryos and had lower R_1 values on thin-layer chromatograms than the parent compounds. These modified compounds may lack either the double bond of the furan ring or the furan ring itself may be removed from the coumarin portion of the molecule. Furthermore, the lactone ring of the aflatoxins is not opened upon exposure to UV light and is not the reason for decreased toxicity.

1782. Mann, G.E., Codifer, L.P.Jr., and Dollear, F.G. 1967. Effect of heat on aflatoxins in oilseed meals. Journal of Agricultural and Food Chemistry 15: 1090-1092.

Effects of heat treatments on aflatoxins present in a contaminated cottonseed meal were investigated. Temperature, period of heating, and moisture content of the meal were varied in these experiments. Heating at 60° C and 80° C did not lead to marked reductions in aflatoxin levels. Definite reductions were obtained at 100°C, greater decreases taking place with increasing periods of heating and increasing moisture content. The lowest level of aflatoxins B_1 and B_2 achieved practically was about 44 µg kg^{-1} obtained by heating for 120 min at 100°C with a moisture content of 20 %. This represented about 80 % reduction in the 214 µg kg^{-1} aflatoxins present in the nontreated cottonseed meal. About 34 % reduction in aflatoxins (111 to 73 µg kg^{-1}) was obtained when a contaminated groundnut meal was heated in a similar fashion.

1783. Marth, E.H., and Doyle, M.P. 1979. Update on molds : degradation of aflatoxin. Food Technology 33(1): 81-87.

This paper briefly reviews chemical, physical, and biological means of degrading aflatoxins. Research needs in these areas are presented.

1784. Masimango, N., Kcmaclc, J., and Ramaut, J.L. 1978. The role of adsorption in the elimination of aflatoxin B_1 from contaminated media. European Journal of Applied Microbiology and Biotechnology 6(1): 101-105.

Studies on the potential for removal of aflatoxin B_1 from aqueous buffer model solutions by adsorption on fungal mycelium or various clays are described. Treatment with coarse, wet mycelium of non-toxigenic strains of *Aspergillus flavus* reduced the aflatoxin concentration in the medium to 30-46 % of the initial value; disintegrated mycelium had a greater adsorption effect, reducing aflatoxin concentration to 10-20 % of the initial value. Treatment with clays (18 types tested) considerably reduced the aflatoxin concentration in the supernatant, residual aflatoxin concentration ranging from traces to 30 %: bentonite showed the greatest aflatoxin adsorption capacity of the clays studied. Vermiculite had a greater aflatoxin adsorption capacity in the finely-powdered than the coarse form; particle size had little effect on the adsorption capacity of montmorillonite. Heating at 65°C increased the aflatoxin adsorption capacity of bentonite, montmorillonite and sepiolite, the increase being greatest for sepiolite. It is suggested that it might be possible to remove aflatoxin from contaminated liquid foods by an adsorption process.

1785. Miller, N., Villiers, J.B.M.de.. and Pretorius, H.E. 1985. Detoxification of aflatoxin-containing crude groundnut oil. Lebensmittel-Wissenschaft. & Technologie 18: 201-202.

Groundnut oil contaminated with aflatoxins was treated with activated or inactivated kaolin at 1, 1.5 and 3 % doses for 15 to 30 min. Satisfactory results were obtained with activated kaolin at 3 % for 15 min at 80°C.

1786. Padwal-Deasi, S.R., Ghanekar, A.S., and Sreenivasan. 1976. Studies on *Aspergillus flavus* - 1. Factors influencing radiation resistance of non-germinating conidia. Environmental and Experimental Botany 16 : 45-51.

In vitro studies were conducted on conidia of *Aspergillus flavus* (aflatoxin-producing) and *Aspergillus flavus-oryzae* (non-toxigenic) strains. These strains differed in resistance to heat and gamma radiation, the toxigenic strain being more resistant to both treatments. Results of tests on dose-modifying factors indicated that composition, temperature and pH of suspending media affected radiation resistance. The size of the initial population and the age of the conidia did not influence the radiation resistance of either strain. Studies on thermal inactivation of the conidia suggested that the temperature employed was more important than the duration of heat treatment. Conidia of both strains showed a synergistic effect of combined heat and radiation treatments, although a heat-radiation sequence was more effective than a radiation-heat sequence.

1787. Patel, U.D., Govindarajan, P., and Dave, P.J. 1989. Inactivation of aflatoxin B_1 by using the synergistic effect of hydrogen peroxide and gamma radiation. Applied and Environmental Microbiology 55(2): 465-467.

Inactivation of aflatoxin B_1 was studied by using gamma radiation and hydrogen peroxide. A 100-krad dose of gamma radiation was sufficient to inactivate 50 µg of aflatoxin B_1 in the presence of 5 % hydrogen peroxide; 400 krad was required for total degradation of 100 µg of aflatoxin in the same system. Degradation of aflatoxin B_1 was confirmed by high pressure liquid chromatography (HPLC) and thin-layer chromatography (TLC). Ames microsomal mutagenicity tests showed loss of aflatoxin activity. This method of inactivation also reduced the toxin levels in artificially contaminated groundnuts.

1788. Paulsen, M.R., Brusewitz, G.H., Clary, B.L., Odell, G.V., and Pominski, J. 1976. Aflatoxin content and skin removal of Spanish peanuts as affected by treatments with chemicals, water spray, heated air, and liquid nitrogen. Journal of Food Science 41(3): 667-671.

Unshelled Spanish groundnuts contaminated with aflatoxin were shelled and treated with forced heated air, liquid nitrogen, H_2O_2, HCl, sodium oleate and water spray. After passing through a whole nut blancher the aflatoxin content and percentages of blanched and whole kernels were determined. Blanching percentages were significantly higher for sodium oleate, water spray, liquid nitrogen and H_2O_2 than for HCl or heal treatments. The heat treatments produced the highest percentage of whole kernels. In most tests, the non-blanched kernels had higher levels of aflatoxin than had those that blanched fully. The treatments which were most effective in producing low levels of aflatoxin in the blanched kernels were H_2O_2, water spray and HCl.

1789. Peers, F.G., and Linsell, C.A. 1975. Aflatoxin contamination and its heat stability in Indian cooking oils. Tropical Science 17(4): 229-232.

Sixty-nine groundnut oils and 16 other cooking oils on sale in Indian villages were analyzed for aflatoxin contamination. Groundnut and groundnut meal samples (24) were also analyzed and three were positive for aflatoxin B_1 at 2-270 µg kg^{-1}. Of the 69 groundnut oils, 15 were contaminated with aflatoxin B_1 at 3-175 µg kg^{-1}, of which seven also contained aflatoxin B_2. Of the 16 other oils, only one, a coconut oil, contained 3 *µg* kg^{-1} aflatoxin B_1. Destruction of aflatoxin B_1 in artificially contaminated maize oil was only apparent at 250°C and above. Using naturally contaminated groundnut oil (175 *µg* kg^{-1} aflatoxin) the results were essentially the same and confirmed that destruction of aflatoxin B_1 is likely to occur at normal frying temperature if cooking is done indoors.

1790. Pettit, R.E., Sarr, B.A., Machen, M.D., and Phillips, T.D. 1990. Detection and detoxification of aflatoxin-contaminated groundnut products in West Africa. Page 97 in Summary Proceedings of the First ICR1SAT Regional Groundnut Meeting for West Africa, 13-16 Sep 1988, Niamey. Niger. Patancheru, A.P. 502 324, India : International Crops Research Institute for the Semi-Arid Tropics.

A new mycotoxin detection procedure termed "Selectively Absorbed Mycotoxins (SAM)", has been developed. It is a modification of the Holaday-Velasco Minicolumn procedure for aflatoxin screening. With the SAM assay procedure, mycotoxins are extracted into a methanol-water solvent. Toulene is mixed with the extract to form a two-phase solution where aflatoxins and zcaralenone partition into the upper toulene phase, thus separating them from potential interfering compounds. The toulene sample is passed through a SAM-Aflatoxin Zcaralenone (SAM-AZ) tube which removes other interfering compounds in a pre-absroption layer, and selectively absorbs any aflatoxin and/or zcaralenone at specific bands in the tip of the tube. Each positive band glows with an obvious blue color under long wave ultraviolet light. The sensitivity of the assay is designed to provide a YES/No screen at designated levels of aflatoxin and zcaralenone. Detoxification of aflatoxin-contaminated groundnut products has been accomplished by the addition of a high-alfinity sorbent (hydraied sodium calcium aluminosilicate) when added at 0.5 % by weight. Aluminas, silicas, and aluminosilicates were evaluated for their ability

to absorb aflatoxin from groundnut oil and aqueous solution. Sorbents such as bcohmitc alumina, synthetic xeolite and muscovite silica sorbed less than 50 % of the aflatoxin present. Novo Sil (hydrated sodium calcium aluminosilicate, HSCAS), Pyran RG 140 pyrophyllile, and Filtrol (acid-activated bleaching earth), sorbed 85 % or more of the aflatoxin. When HSCAS was added to chicken feed containing 7.5 mg aflatoxin, the toxicity was reduced so that broiler and leghorn chicks appeared normal in their growth and their livers were normal. The safety and effectiveness of the detoxification procedure was measured with the Ames assay.

1791. Phillips, T.D., Kubena, L.F., Harvey, R.B., Taylor, D.R., and Heidelbaugh, N.I). 1988. Hydrated sodium calcium aluminosilicate : A high affinity sorbent for aflatoxin. Poultry Science 67: 243-247.

Aluminas, silicas and aluminosilicates were evaluated for their ability to sorb radiolabeled aflatoxin B_1 from aqueous solution in vitro. Hydrated sodium calcium aluminosilicate (HSCAS) was selected for testing in vivo due to its high affinity for aflatoxin B_1, because of its stable association with the toxin, and its GRAS (generally recognized as safe) status as an anticaking agent. The HSCAS, when added to the diet of Leghorn and broiler chicks at a level of 0.5 %, significantly diminished the adverse effects of feeding 7.5 mg of aflatoxin B_1 kg^{-1} of feed. Thus, this agent and other aluminosilicate congeners may prove effective in the management of aflatoxicosis.

1792. Pluyer, H.R., Ahmed, E.M., and Wei, C.I. 1987. Destruction of aflatoxins in peanuts by oven- and microwave-roasting. Journal of Food Protection 50(6): 504-508.

Effects of oven and microwave roasting on aflatoxin-contaminatcd groundnuts were studied. In artificially contaminated groundnuts, oven-roasting for 30 min at 150° C or microwave-roasting for 8.5 min at 0.7 KW were equally effective in destroying 30-45 % of aflatoxin B_1. In naturally contaminated groundnuts, both oven- and microwave-roasting were equally effective in destroying 48-61 % of aflatoxin B_1 and 32-40 % of aflatoxin G_1.

1793. Priyadarshini, E., and Tulpule, P.G. 1976. Aflatoxin production on irradiated foods. Food and Cosmetics Toxicology 14: 293-295.

The effect of irradiation on the ability of some commonly used foods to support aflatoxin production was studied under laboratory conditions. Levels of aflatoxin were significantly higher in some irradiated cereals, millets and root vegetables than in non-irradiated samples of the same commodities.

1794. Rodriguez, M, and Rodriguez, A. 1983. [Reduction of anatoxin production in groundnut by gamma irradiation.]. Reduccion de la produccion de allatoxinas en mani por radiaciones gamma. Ciencia Y Tecnica en la Agriculture 5(2): 103-110.

Groundnuts were inoculated with spore suspension of *Aspergillus parasiticus* and irradiated with gamma rays from a Co 60 source at 50, 100, 150 and 200 krad. Aflatoxins were detected by thin-layer chromatography. Concentrations of aflatoxins were lowest on groundnuts irradiated at 200 krad.

1795. Samarajeewa, U. 1988. Solar degradation of aflatoxin B_1 in foods. Proceedings of the Japanese Association of Mycotoxicology Supplement No. 1: 91-92.

The solar degradation of anatoxin B_1 in foods is discussed. The combination of solvent and radiation, the design of a pilot plant for detoxification of aflatoxin B_1 contaminated coconut oil by solar irradiation, and the non-toxicity of solar irradiated coconut oil to ducklings are considered.

1796. Samarajeewa, U., Gamage, T.V., and Arseculeratne, S.N. 1988. Non-toxicity of solar-irradiated edible oils contaminated with aflatoxin B_1. Toxicon 26(1): 38.

Edible oils contaminated with aflatoxin B_1 were examined for loss of toxicity on being subjected to solar radiation. Coconut, groundnut, soybean and sesame oils and chloroform and ethyl acetate contaminated with aflatoxin B_1 (1µg ml^{-1}) were exposed to radiation from the sun at midday for 30 min at an oil layer thickness of 1.6 mm. The oils, after exposure, were examined by thin-layer chromatography (TLC) for residual aflatoxin B_1 and any new fluorescent compounds. One day old 'veluvi' ducklings were fed with pure aflatoxin B, (4.7 µg/d) for 7 days. The same amount of aflatoxin B_1 in coconut oil was solar-irradiated in a pilot plant and the extract were led to a second group of ducklings. Control groups of ducklings were fed with extracts from non toxic oil after solar irradiation and the vehicle, propylene glycol, alone. No residual aflatoxin B_1 or new fluorescent compounds were observed on TLC alter solar irradiation of the oils, whereas both types of compounds were observed with chloroform and ethyl acetate. The pattern of weight gain in the ducklings fed with extracts of toxic oil after solar irradiation was not significantly different ($P = 0.05$) from those of control groups of ducklings, but was from those fed with pure toxin. The former showed no mortality, as against more than 50 % mortality in the ducklings fed with pure toxin. The ranked mean value for bile duct hyperplasia in the group of ducklings fed with the extracts from toxic oil after irradiation showed no significant differences from that of control groups, but was significantly different from those fed with pure aflatoxin B, ($P = 0.01$). It is suggested that irradiation appears to detoxify edible oils contaminated with aflatoxin B_1.

1797. Shantha, T. 1989. Detoxification of groundnut seed and products in India. Pages 153 160 in Aflatoxin contamination of groundnut : proceedings of the International Workshop, 6-9 October, 1987. ICRISAT Center. India. Patancheru. A.P. 502 324. India : International Crops Research Institute for the Semi-Arid Tropics.

This paper very briefly reviews several methods used for destruction of aflatoxin in groundnuts and their products. The exposure of the groundnut oil contaminated with aflatoxin to bright sunlight completely destroys the toxin. Aflatoxin is present in finely suspended solids in the oil and can be removed by filtration or by extraction with 10 % sodium chloride. Aflatoxin-contaminated groundnut kernels (0.5 mm thick flakes) can be partially detoxified if exposed to sunlight for 14 hours. 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. The merits and dements of these processes are discussed.

1798. Shantha, T, and Sreenivasamurthy, V. 1977. Photo-destruction of aflatoxin in groundnut oil. Indian Journal of Technology 15: 453-454.

Unrefined groundnut oil containing aflatoxin (> 100 µg kg^{-1}) was exposed to bright sunlight, gas-filled tungsten lamp, or long wave UV light. Sunlight destroyed 99 % of the aflatoxin present in 15 min, whereas tungsten lamp light and ultraviolet light destroyed 82-85 % of aflatoxin in 18 h and 30-40 % of aflatoxin in 2 h exposure. The photolysed oil was not toxic to albino rats, and its absorption peak shifted from 700 nm to 280 nm alter exposure to sunlight.

1799. Shantha, T., and Sreenivasamurthy, V. 1980. Storage of groundnut oil detoxified by exposure to sunlight. Indian Journal of Technology 18(8): 346-347.

Aflatoxin in groundnut oil, alter being degraded by sunlight, was not regenerated during storage for six months of the oil in dark. There was some increase in peroxide value, but the organoleptic quality of the oil remained unchanged during three months of storage.

1800. Shantha, T., and Sreenivasamurthy, V. 1981. Use of sunlight to partially detoxify groundnut (peanut) cake flour and casein contaminated with aflatoxin B_1. Journal of the Association of Official Analytical Chemists 64(2): 291-293.

Aflatoxin B_1 was added to preparations of groundnut cake flour, groundnut protein isolate or casein at 0.5 µg toxin g^{-1} sample. The preparations were suspended in buffer (pH 5.4 or 7.4) and subjected to sunlight for 6 h ; some samples were dialyzed in light or shade for 6 h. Sunlight destroyed 83-88 % and 23-33 % of aflatoxin added to casein and groundnut cake flour, respectively. It is suggested that the toxin bound to casein is more photo- liable than that bound to groundnut protein.

1801. Shantha, T., Sreenivasamurthy, V., Rati, E.R., and Prema, V. 1986. Detoxification of groundnut seeds by urea and sunlight. Journal of Food Safety 7: 225-231.

A fourteen hour exposure to sunlight destroyed 90 % and 77 % aflatoxin B_1 added to groundnut flakes with and without fat whereas only 50 % of the toxin was destroyed when present as a natural contaminant. Treating the groundnut flakes with 20 % urea and 2 % soybean flour (a source of urease) at 50 % moisture brought about 70 % destruction of aflatoxin B_1. In large scale trials, destruction of aflatoxin was about 85 %. Treatment with urea did not bring down the protein efficiency ratio (PER) value of the material, which was 1.5 after treatment as against 1.6 in the non-treated groundnuts.

1802. Temcharoen, P., and Thilly, W,G. 1982. Removal of aflatoxin B_1 toxicity but not mutagenicity by 1 megarad gamma radiation of peanut meal. Journal of Food Safety 4(4): 199-205.

The toxic and mutagenic effects of gamma-irradiated groundnut meal contaminated with aflatoxin B_1 were studied in *Salmonella typhimurium* strain TM 677. using forward mutation to 8-azaguanine resistance. After treatment with 5-10 megarad gamma-radiation, the contaminated groundnut meal lost its toxic and mutagenic properties. Irradiation at 0.1-1 megarad removed 75-100 % of the toxicity but not mutagenicity.

1803. Waltking, A.E. 1971. Fate of aflatoxin during roasting and storage of contaminated peanut products. Journal of the Association of Official Analytical Chemists 54(3): 533-539.

A study of the effects of the pilot roasting of aflatoxin- contaminated groundnuts showed an average loss, after roasting, of 40-50 % of the aflatoxins B_1 and G_1 and 20-40 % of the aflatoxins B_2 and G_2. Portions of both raw and roasted groundnuts were stored in paper bags and air-tight glass jars and reassayed for aflatoxins. No significant change in aflatoxin content was found after 3 months storage at 27°C regardless of the container. However, significant reduction of aflatoxins was observed in other aflatoxin-contaminated groundnut products which had been stored at room temperature for > 1 year.

1804. Wei, R.D., and Chu, F.S. 1973. Aflatoxin-solvent interactions induced by ultraviolet light. Journal of the Association of Official Analytical Chemists 56: 1425.

The effect of UV light on the fluorescence intensity of aflatoxins in various solvents and on the photoeatalyzed addition of alcohols and water to the vinyl ether double bond of aflatoxins B1 and G1 was investigated. Four groups of new aflatoxins, designated as B_m. B_c, G_m, and G_c, were found to result from the UV light-catalyzed aflatoxin-solvent interaction. These compounds were less toxic to chicken embryos than the parent toxins.

1805. Worakit Suchada, and Pukrushpan Thanong. 1988. Effects of gamma-rays on the destruction of aflatoxin in peanut. 21: 33-38.

Effects of gamma-ray on the destruction of aflatoxin in groundnuts were studied. Forty groundnut samples, collected from markets in different pans of Thailand, were inoculated with a toxigenic strain of *Aspergillus flavus.* After incubation at room temperature for a certain period of time, the inoculated groundnut samples were irradiated with 6.4 KGy dose. levels of aflatoxin B_1 in irradiated and non-irradiated samples were determined. There were no significant differences in average levels of aflatoxin B_1 between irradiated and non-irradiated samples. Levels of aflatoxin B, in 22 out of 40 samples ranged from 0 to 4834 µg kg^{-1} and most (42.5 %) had levels above the standard value. Maximum microbial load and aflatoxin level were found on the 9th day of incubation, approximately 1.04 x 10^{10} colonies and 1200 *µg* kg^{-1} respectively.

8.2.13 Control In Storage/Transit

1806. Bhat, R.V. 1988. Mould deterioration of agricultural commodities during transit: probelems faced by developing countries. International Journal of Food Microbiology 7(3): 219-225.

The probelem of mold growth and aflatoxin contamination in the commcxiities transported over long distances from the cultivation regions to the consumption centres is discussed. If the contamination occurs during transit, often no insurance coverage for the risk is available. Because of different methods of sampling followed in the exporting and importing countries it is often difficult to define the exact responsibility of the development of aflatoxin as having taken place during transit. The statistics of the export of aflatoxin high-risk commodities like red pepper (chillies), cottonseed and groundnut extractions clearly, for the last decade, demonstrate the extent of loss suffered by the exporting countries because of aflatoxins. The problem of mold damage and mycotoxin contamination can be minimized by improving facilities for storage at port and transit points and on ships.

1807. Dickens, J.W. 1977. Aflatoxin occurrence and control during growth, harvest and storage of peanuts. Pages 99-105 in Mycotoxins in human and aninmal health. (Rodricks, J.V., Hesseltine, C.W., and Mehlman, M.A., eds.). Illinois, USA : Pathotox Publishers, Inc.

This paper discusses causes of aflatoxin contamination and methods of prevention and segregation to control the aflatoxin problem in farmers' stock groundnuts from the time the groundnuts are growing in the field until they are marketed, stored, and shelled.

1808. Holaday, C.E., Williams, E.J., and Pearson, J.L. 1973. Effectiveness of propionic acid and 'Moldstat' as fungicides during peanut storage. Proceedings of the American Peanut Research and Education Association 5: 196.

The molding of farmers' stock groundnuts in storage is a problem in certain locations because of inadequate storage facilities. An experiment was designed to test the effectiveness of propionic acid and 'Moldstat' in preventing molding of farmers' stock groundnuts stored in facilities that do not provide adequate protection from rainy weather. Only the highest concentration of propionic acid prevented aflatoxin build-up. 'Moldstat' provided little or no protection from aflatoxin contamination at any concentration. Results of flavor evaluations on samples from the treatments showed that the groundnuts treated with propionic acid were poor in flavor while those treated with 'Moldstat' had about the same flavor as the controls (groundnuts without any treatment). The free fatty acids were significantly lower in the treated samples than in the controls.

1809. Pollet, A., and Declert, C. 1987. [Condition and extent of aflatoxin contamination of local groundnut stocks in the Ivory Coast. I. - Preliminary data (1985-1986).]. Etat sanitaire et importance des contaminations par aflatoxines des stocks villagcois d'arachide constitue's en Cote d'Ivoire. - Donnees preliminaires (Campagne 1985-1986). 016agineux 42(8-9): 327-336.

Groundnut storage problems were studied in Cote d'Ivoire during 1985 and 1986, from three different aspects; insect pests, fungal infection and aflatoxin contamination. Samples of groundnuts were obtained from 164 farmers' stock three times throughout the storage period : in December 1985, in February and April 1986. Samples were also obtained from different town and village markets. These samples were examined for several parameters such as pest damage (penetrated or scarified pods), fungal infection, aflatoxin contamination, and seed germination. Generally, the market samples were less contaminated with aflatoxin than the samples taken from the farmers' stocks. With a few exceptions the stocks examined were always contaminated with aflatoxin. Nine per cent of the stocks contained aflatoxin levels in excess of 250 µg kg^{-1}. Only four per cent of market samples contained such high levels of aflatoxin. Significant correlations between aflatoxin contamination and scarified/penetrated and broken pods were found.

1810. Smith, J.S. 1989. Aflatoxin management in the Warehouse. Proceedings of the American Peanut Research and Education Society 21: 63.

Aflatoxin contamination of groundnuts during storage can be controlled by good warehousing practices. The most important factor in preventing aflatoxin development in groundnut warehouses is moisture control. Any measure that reduces the risk of rewetting the groundnuts will be effective in aflatoxin control. A properly ventilated structure is needed with a good roof, sidewalls, and floor to prevent water entry. Uniform loading of the warehouse will allow excess heat and moisture to escape and reduce areas favorable for insect infestation which causes heat build-up and moisture accumulation. Frequent checks on warehouse conditions and proper operation of the ventilation system will prevent warehouse conditions from becoming favorable for aflatoxin production.

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SUBJECT INDEX

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Patancheru, Andhra Pradesh 502 324, India

ISBN 92-9066-214-X BOE 018 Printed at ICRISAT Center ICR 91-0074