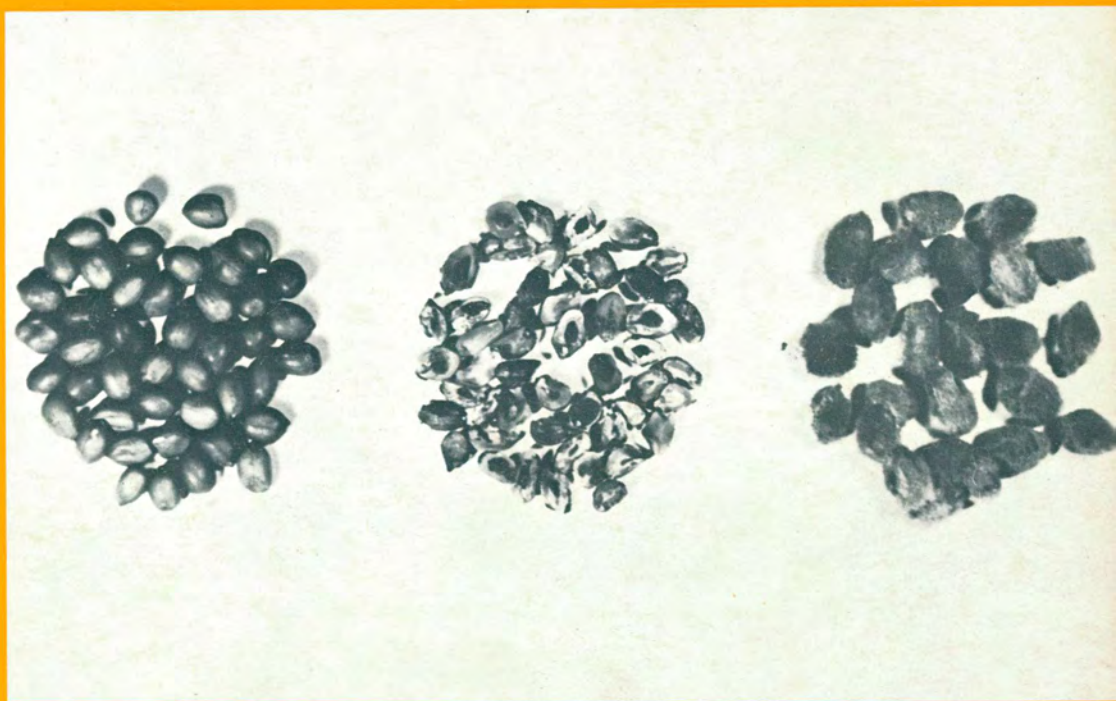


perspective on mycotoxins



FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS ROME

perspective
on
mycotoxins

selected documents
of the
joint fao/who/unep conference on mycotoxins

held in nairobi,
19-27 september 1977

under the sponsorship of
the food and agriculture organization of the united nations
the world health organization
and
the united nations environment programme

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F O R E W O R D

This publication is issued by FAO under FAO/UNEP Project 0107-75-01, "Control of Environmental Contaminants in Food: MYCOTOXINS." It contains The four major background documents prepared for discussion of agenda item 4, "Problems of Mycotoxins", at the Joint FAO/WHO/UNEP Conference on Mycotoxins, held in Nairobi, Kenya, 19-27 September 1977. The information contained in these papers is considered to be a valuable contribution to a better, and eventually more complete, perspective on the manifold problems posed by the various mycotoxins and on some approaches to the control of these problems.

The documents were prepared based on the thorough and dedicated work of four knowledgeable authorities on the topics:

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In addition, the invaluable efforts of Mrs. Monica Rose in the finalization of much of the documentation for the Conference, particularly MYC-4a, are gratefully acknowledged.

A list of publications and papers on mycotoxins published by FAO is given at the end of this publication. Comments or suggestions for possible future editions of this publication should be sent to:

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FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS - Rome



WORLD HEALTH ORGANIZATION
Geneva



UNITED NATIONS ENVIRONMENT
PROGRAMME - Nairobi

Agenda Item 4

MYC-3

JOINT FAO/WHO/UNEP CONFERENCE OF MYCOTOXINS

Nairobi, Kenya, 19-27 September 1977

ASSESSMENT AND CONTROL OF ENVIRONMENTAL QUALITY: MYCOTOXINS

S U M M A R Y

Assessment of the environmental impact of pollutants is a major tool for sound environmental management. Information required for assessment is knowledge of pathways of pollutants in the environment including the food chain, of human and non-human exposures, of dose/effect relationships, and of social and economic effects. In the case of aflatoxins, enough information is available for moving to control of the problems, with special attention to preventive measures applied at the source of contamination, and to training at all levels.

This paper outlines the place of food contamination control in UNEP's concern over protection of the environment and natural resources, using mycotoxins as a case study.

This paper is based on text prepared by
Dr. H.P. Mollenhauer, at the time Director,
Division of Environmental Assessment for the
Secretariat of the United Nations Environment
Programme (UNEP).

- This at least we know: the earth does not belong to man; man belongs to the earth.

Letter of Indian Chief Seattle to Franklin Pierce, President of the USA, 1854.

I. INTRODUCTION

1.1 Ever since his appearance on earth, man has struggled within his varied environments to produce food and preserve it against destruction. Already in antiquity he had learned how to store and retain durable foods such as grain, oil and wine in suitable buildings and containers so that they would not spoil. In these early periods, spoilage by microbial contamination must have been the major cause of food losses and subsequent hunger in areas where distinct seasons reduced the productive period of the year. The Prefect of Food Distribution (praefectus annonae) in the Roman Empire was evidently familiar with the biological requirements for transporting and storing huge quantities of grain for feeding the population of the City.

1.2 It is also recorded from those days that contamination of foods and of drinking water with chemical pollutants was felt, although not recognized as such - we know for instance of lead contamination of water. Over the following millennia various sources of pollution developed; we know of a complaint about air pollution in London, caused by soap-boilers in 1666. The slow development of industry gradually brought about pollution and, in its wake, health risk. One of the oldest examples must be water pollution by tanners and other small industries (1).

1.3 The population accepted such local environmental pollution as unavoidably connected with industrial progress. There was still enough room in the environment. In the beginning of the 20th Century, pollution began to be understood as a danger to man and his environment. Chemical pollution of all the media of the environment increased rapidly and with industrialization reached proportions which at last were recognized as dangerous - possibly destructive of the human environment. It was recognized that chemical pollution also comes from non-industrial sources.

1.4 Because pollution appeared to be related directly to industrial development, very little alarm was felt in less developed countries, except perhaps in some large urban settlements. As knowledge expanded during the 1960's it became clear that pollution would affect and indeed had already affected all countries, industrialized and non-industrialized. This danger was one of the major concerns that led to the UN Conference of the Human Environment (Stockholm 1972).

1.5 As an expression of global concern the United Nations Environment Programme was established after the Stockholm Conference and has since engaged in a broad programme covering many aspects of environmental pollution and management which are of concern to developed and to developing countries. The United Nations Environment Programme's basic approach moves from assessment to management. It is in this context that the Mycotoxin Conference convened by FAO, WHO and UNEP in 1977 in Nairobi appears to be well timed. Previous meetings ^{1/} have assessed the significance of mycotoxins to a fair degree so that the time has come to translate the results of epidemiological, toxicological, biological, chemical and technological studies into practical measures to cope with the situation.

1/ Third International IUPAC Symposium on Mycotoxins in Foodstuffs, Paris, 16-18 September 1976; UJNR Conference on Mycotoxins in Human and Animal Health, College Park, Maryland, USA, 4-8 October 1976.

1.6 The Mycotoxin Conference is also a clear indication of the growing concern of less developed countries for environmental pollution. The knowledge of mycotoxin pollution to be reviewed and described here is not due to environmental recent events but to progress in scientific research, rising awareness of environmental quality and concern about the fulfilment of basic human needs and protection of human health.

II. POLLUTANTS - Some Definitions

Under this term a broad spectrum of damaging environmental factors is understood.

In view of the variety of pollutants no one method or model can be used to assess their impact and take management actions. The major types of pollutants requiring different treatment are chemical and microbial pollutants, in addition to physical pollution by radiation, heat, noise, etc. Chemical pollutants occur in the environment naturally, by human activity and unintentionally. For their assessment, no distinction can be made between natural pollutants and others.

2.1 Chemical Pollutants

2.1.1 The major contribution of chemical pollution stems from industry and from the widespread use of chemicals. As mentioned in the introduction, the explosive development of chemical industry led to an alarming situation. This is illustrated by the fact that the production of plastic materials, for instance, has risen from 100 000 tons in 1930 to 30 million tons in 1971 (2).

2.1.2 Chemical pollution stems from the immediate use of chemicals in industry, from use of pesticides and from unintended emission of air and water pollutants, from industrial and domestic waste and from the production and use of energy, be it coal, oil or gas.

2.2 Biological Pollutants

2.2.1 Biological pollutants have been with us since time immemorial and possibly to a greater degree than today. Recent scientific studies have led to our understanding of the seriousness of the situation and to the possibility of curing it.

2.2.2 Examples of microbial environmental hazards are a variety of food spoilage bacteria and fungi (Salmonella, Staphylococcus aureus, Escherichia coli, Clostridium botulinum and welchii, Aspergillus flavus, Fusarium spp.) and parasitic diseases. At the same time, there is an equally large variety of beneficial microbes living in foods which are mostly responsible for maturing and curing processes, such as fermentation of bakery goods, acidification of sour foods, maturation of cheese, of cream for butter making, for yoghurt and so on.

2.3 Mycotoxins

2.3.1 Mycotoxins are toxic chemical substances elaborated by fungi. As such they are chemical pollutants of biological origin. They can occur wherever fungi proliferate, but present the most serious hazards in foods and animal feeds.

2.3.2 Outbreaks of mycotoxins have been recorded sporadically, a prominent example being St. Anthony's Fire caused by ergot, or more recent outbreaks of certain mycotoxicoses which are well documented (3).

2.4 Physical Pollution

This term covers mainly radiation pollution of various kinds, including ionizing radiation. Also mentioned for completeness must be thermal pollution as a result of energy production and noise, which ranks rather high in popular opinion as a disturbing factor.

III. ASSESSMENT OF ENVIRONMENTAL IMPACT OF POLLUTANTS

On the assessment side UNEP is actively engaged in producing and putting together the criteria and machinery for a "Total assessment". Diagram I gives a simplified overview of what is involved in such an exercise (4). The following considerations will be along the lines of this plan.

3.1 Pathways

3.1.1 Assessment requires the study of "pathways" of pollutants in the various media of the environment including food stuffs. During their movements through the environment, pollutants may undergo changes, breakdown of the original molecule and disintegration, sometimes into harmless derivatives. The Monitoring and Assessment Research Centre at the Chelsea University College is carrying out scientific studies on pathways of sulphur oxides in the environment in cooperation with UNEP.

3.1.2 At the same time UNEP's Global Environmental Monitoring Service (GEMS) is being set up to collect, evaluate and monitor data on the occurrence of pollutants and determine trends of environmental changes.

3.1.3 The main objectives of a Joint FAO/WHO food and animal feed contamination monitoring programme (which includes aflatoxins) under development with the support of UNEP are the following:

- (i) to determine global trends in food contamination;
- (ii) to determine the geographical spread of specific toxic substances;
- (iii) to identify groups within a population that may be at a high risk;
- (iv) to determine the total intake of the pollutants under consideration (5).

3.2 Health Criteria, human

3.2.1 For evaluation of toxicity to humans, Health Criteria Documents have been prepared by WHO in cooperation with UNEP on the dose/effect relationship of certain pollutants. They cover such factors as:

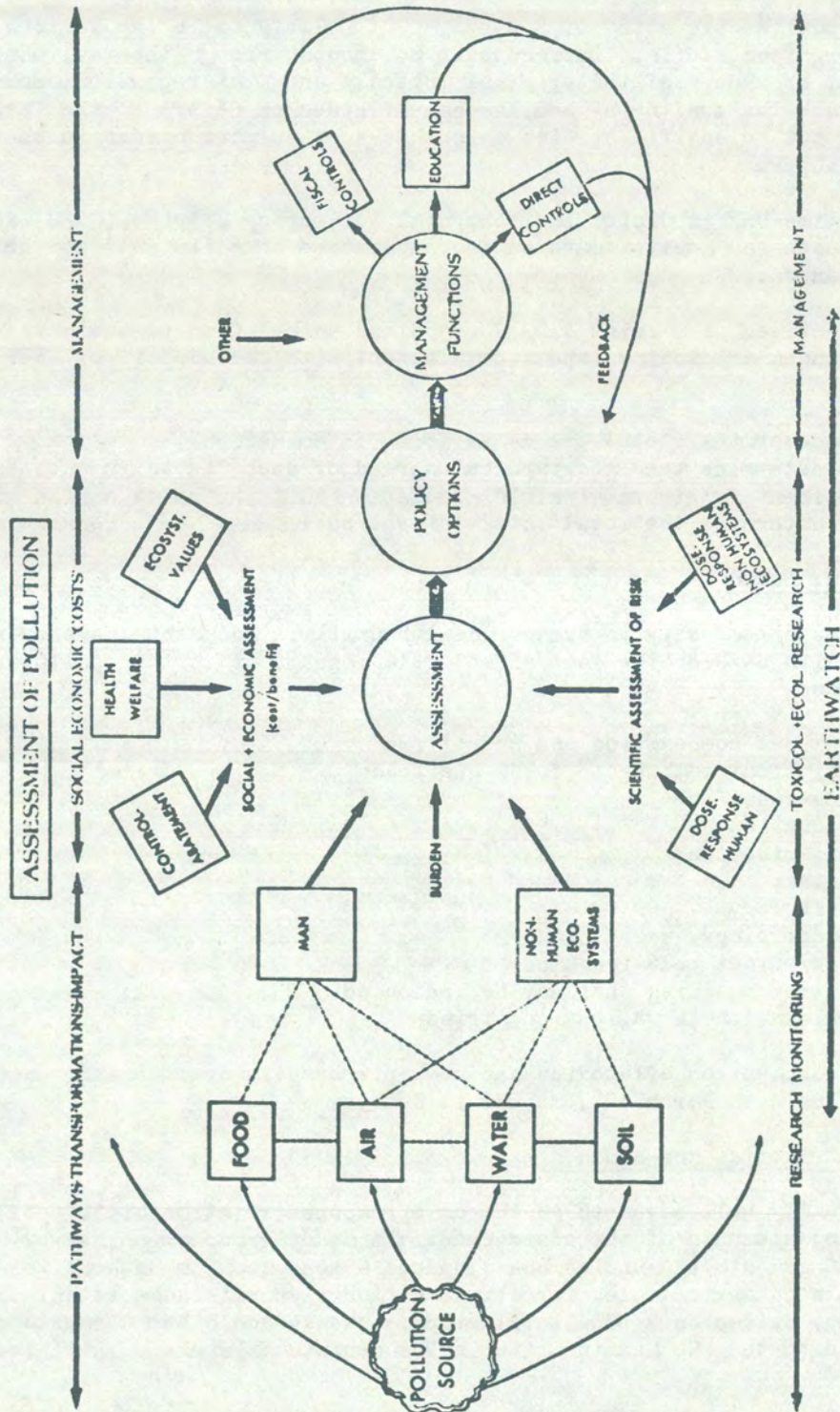
- chemical composition and properties
- analytical methods in foods and feeds
- occurrence
- production
- contamination
- up-take
- toxicology
- epidemiology
- dose/effect relationships
- control measures that may be indicated
- legislation in various countriss

A criteria document on aflatoxins has been prepared in draft and discussed at an expert consultation held in March 1977 at WHO in Geneva.

3.3 Environmental Criteria, non-human

Work is not equally well advanced on the dose/response relationship of pollutants for the other (non-human) elements of the environment. A study group organized by UNEP in cooperation with FAO and other agencies has produced a monograph on methods to determine the effects of pollutants on agriculture, forestry and fisheries with some technical annexes on the effect of sulphur oxides on agricultural crops, forests and other elements of the ecosphere comparable to the WHO Health Criteria Documents. This action will lead to

Diagram I



individual documents on the effects of pollutants on "non-human targets" ^{1/} in the environment. UNEP's International Register of Potentially Toxic Chemicals will supply such information in due course.

3.4 Social and Economic Effects: "cost/benefit"

3.4.1 A further element of assessment is the evaluation of social and economic effects of pollution and of abatement. There is the benefit of industrial chemicals, of drugs, pesticides and so on (in some cases there may be none!) to be weighed against their human health hazard and risk of damage to the environment. On the benefit side, economic gain, employment and vested interest play a major role when making policy decisions on some pollutant chemicals.

3.4.2 In the case of mycotoxins this part of the assessment amounts mainly to the cost of prevention and detoxification as against health risk, in other cases - as with DDT - it is rather difficult to estimate the health risk connected with accumulation of the chemical in the fatty tissue of the human body and other possible health hazards against the health risk connected with the ban on a very effective and cheap insecticide and the economic and social disadvantages of such action.

3.4.3 In the area of evaluation of "cost/benefit" in its widest sense, more work is required. UNEP is about to organize meetings of economists and environmental scientists in order to draw up action plans on how to approach this area. The groups will collect all available data on human and "non-human" toxicology, on pathways and on monitored data of occurrence of the pollutants and put this within the context of economic and social thinking.

3.4.4 It appears to be quite clear already that a "total assessment" of pollutants will limit itself to certain geographical regions, social groups or strata, or industries.

3.4.5 In the international field, some of this work has already been initiated by the OECD - Environment Committee and its Sector Groups and by the UN Economic Commission for Europe (ECE) in Geneva. At present the interest is focussed on sulphur oxides although other pollutants may be more easily attacked. In May 1977, the Fifth Session of the United Nations Environment Programme Governing Council has reemphasized the urgency to make early use of the environmental data which have been and are being accumulated, and not to wait until the last datum has been gathered. The results of assessment of pollutants will lead to policy options to be decided upon by environmental management.

3.5 Environmental Health Education and Training

In order to combat pollution successfully and for all times, education is required in every aspect of environment conservation, protection and development; in fact, it is the major condition without which there is little hope for improvement and progress. Government and other action in combating pollution will remain palliative even if the obvious damage to the environment may be cured for the moment, if it is not followed up by education and training (7). UNESCO, in cooperation with UNEP, have organized the Inter-Governmental Conference on Environmental Education from 14 to 26 October 1977 in Tbilisi.

IV ASSESSMENT OF FOOD CONTAMINATION AND ITS MANAGEMENT

4.1 General

4.1.1 Environmental contamination of foods cannot be seen in isolation, but as part of the whole environment in which their production and processing take place (8).

^{1/} This term was used by the Stockholm Conference. The term "target" should be replaced by "Receptor" as nobody is deliberately aiming at those "targets".

4.1.2 Food, air and drinking water are the connecting links between man and environment. Within the environment, foods appear to lie at the end of an imaginary funnel into which environmental pollution is put, through air, water and soil.

4.1.3 The elements of assessment listed in Diagram I apply to the assessment of food contamination. Sources, pathways, dose/response relationships, social and economic factors are put together for an overall assessment.

4.2 Food Control Measures

4.2.1 According to the Declaration of Human Rights and the Declaration of Stockholm, everybody has a right to an environment, and to foods which are reasonably free from substances creating health hazards (9, 10).

4.2.2 In Article 25 of the Human Rights Declaration, it is stated that everybody has a right to a standard of living securing his health, including food and the necessary social services as far as this is beyond his influence. In this context, it is the duty of the government or other appropriate authorities to protect the health of the population (11).

4.2.3 The task of international authorities and organizations would be to provide these authorities with necessary tools for such measures as may be required for the protection of the populations and their environment besides preparing the ground for international agreements and conventions. (Codex Standards, Code of Ethics for the International Trade in Food (12)).

4.3 Control Measures at Three Levels

4.3.1 The control of environmental pollution and especially that of foods should advance on three levels, preferably concurrently:

- cleaning up the environment
- appropriate technology
- health protective measures such as setting permissible levels of pollutants; registration of chemicals

4.3.2 It would be leading too far to enumerate and deal with all possible and necessary control measures which are being carried out or planned at present, as for instance, control of water and air pollution, waste water treatment and waste disposal.

4.3.3 The aim of measures for maintaining pure food is to protect the consumer immediately by setting up tolerable maximum levels to stop the original sources of contamination in the environment; and to adapt technology including agriculture to avoid contamination of foods. Examples of adapted technology in agriculture would be support of biological plant protection in combination with a minimum of chemical pesticides in an integrated system of plant protection; or development of better, more specific, less persistent pesticides of low mammalian toxicity by the chemical industry (13).

4.3.4 Agriculture is not the only human activity responsible for food contamination. Air, water and soil are contaminated with pollutants from industrial and domestic sources, which find their way into the food chain and foodstuffs for human consumption. There is room for improvement also in the handling of foods from harvest, storage, processing, packaging and distribution through to the preparation and storage in the domestic sphere.

4.3.5 Besides improvement of environmental quality and setting of tolerable levels of contaminants in foods, there are technical possibilities for reducing the contamination of foods. Production and processing of foods can be adapted to prevent possible contamination more than is being done presently. In this field of applied food science and technology much remains to be done if one assumes that a certain level of environmental pollution will remain unavoidable for technical and economic reasons.

4.3.6 New prevention and decontamination techniques are required to stop the necessity of destroying large quantities of contaminated foods.

4.3.7 As soon as methods for decontamination of foods are available, the law makers should be prepared to approve respective regulations for handling of such foods before or after treatment if legal arrangements do not yet exist. Related to the subject of adapted technology are also considerations of choosing the location for food growing and production and development of disease and contamination (e.g. mycotoxin) resistant varieties of food plants.

4.3.8 The closing link in the chain of protection against contaminated foods is the concern about the foodstuffs offered for sale to the consumer. At this point joint international efforts have set in rather early long before environmental contamination had become a widely recognized problem. In 1962 a Joint FAO/WHO Codex Alimentarius Commission was founded which has now a membership of over 115 countries; the Commission occupies numerous technical committees of government experts with approving international standards for a large number of food items, laying down a minimum quality. The quality criteria include permissible levels for a number of contaminants (not, however, for mycotoxins so far, except in the case of enzyme preparations for use in food processing).

4.3.9 In addition to the Codex programme UNEP in cooperation with FAO and WHO has developed a programme for the control of contaminants which has led already to two publications:

- Guidelines for establishment of an effective national food control system
FAO Food Control series No. 1 (WHO No. 1), Rome 1976, and
- Methods of sampling and analysis of contaminants in food,
EEN: FC/76/3 - FAO Food Control series No. 3: Food Control
(WHO No. 3) Rome, 1976

4.3.10 In the case of food contamination with mycotoxins, at least with aflatoxin, it is suggested that enough reliable information is available for environmental management action by the appropriate authorities. This contribution is a further attempt to draw attention of authorities and policy-makers to action which is urgently required.

V THE CASE OF MYCOTOXINS

5.1 General

5.1.1 The term mycotoxins covers a broad range of toxins which are produced by a variety of fungi under certain favourable environmental conditions.

5.1.2 In speaking of mycotoxin contamination of foods, reference is almost exclusively made to the occurrence of aflatoxins.

5.1.3 Very recently a number of other mycotoxins has been described, such as Zearalenone (14, 15) a phytoestrogen produced by Fusarium and Trichothecenes, also produced by Fusarium, Stachybotris spp. and Dendrodochium (16, 17). Citrinin found in wheat, barley and rye, and Patulin found in apple juice are toxins produced by Penicillium. Ochratoxin is produced by Aspergillus ochraceus and Penicillium iridicatum on maize; Trichothecenes are produced by Fusarium on maize (5).

5.1 The toxic effect of aflatoxin had been described already by Plaut (18) in 1913, but he did not isolate the toxin and the publication was soon forgotten. The modern discovery of aflatoxin is mostly referred back to an outbreak in England in 1960 where within a few months 100 000 young turkeys and 14 000 ducklings died. At the same time a massive occurrence of hepatoma in trout was diagnosed in California. About 1961 a substance was isolated from the feed which caused what was called turkey X disease. The substance was found to be produced by Aspergillus flavus and called aflatoxin. The various isolated components were called B₁ and B₂ (for blue) and G₁ and G₂ (for green) according to the colour of their fluorescence in U.V.-light. Two more isolates were termed aflatoxin M₁ and M₂ after their occurrence in milk, having been metabolized by cows after feeding with contaminated feeds.

5.2 Assessment of the Impact of Mycotoxins

The assessment of the impact of mycotoxins is in a way much easier than with chemical pollutants because the pollutant is produced and active in situ, it does not move through the environment in search for a "target", with the exception of contaminated feedstuffs having passed through farm animals. All that is required is determination and monitoring of occurrence in foods, study of toxic effects on human health, health risk assessment and cost of prevention and detoxification. By contrast to chemicals, there is no benefit derived from the production of the pollutant, which simplifies matters. There is one additional part to environmental impact assessment and that is the effect on non-human recipients. The health of farm animals is affected in that fertility of pigs may be impaired, hens may stop laying and so on. This would, however, form part of the human food component in the cost benefit assessment.

5.3 Occurrence

Another document in this publication describes in detail the occurrence of mycotoxins in different foods and agricultural products, the conditions involved and the countries which have reported incidence data.

5.4 Source of Contamination

5.4.1 The toxins are produced by various fungi which have been described elsewhere (3, 14, 19). There are certain *A. flavus* strains that do not produce toxin and there are no methods available yet to differentiate the toxin-forming from the non-toxin-forming strains. There has also been no proof of any preference of non-toxin-forming species for certain foods, nor for a geographical distribution according to climate. In order to bring some light on this question, ecological monitoring over large parts of the globe may be required (20).

5.4.2 Although the fungi will grow a mycelium where and whenever the milieu is suitable they do not always produce toxin nor do they invade all foods equally.

5.5 Pathways in the Environment

5.5.1 The pathways through the environment of the pollutant aflatoxin are comparatively easy to follow. It reaches the human receptor almost exclusively through food and beverages. One important side track is the use of contaminated feedstuffs for raising farm animals. It is here that the toxic effects of aflatoxin have been noticed most prominently. Cows excreted less than 1% of B_1 and B_2 as M_1 and M_2 with the milk 12 to 14 hours after dosage with contaminated feed. After discontinuing feeding of contaminated feed, the milk remained positive for 14 days (21).

5.5.2 Milk has been found to contain low levels of aflatoxin during the winter feeding period whereas the values are reduced when cattle is taken to pasture (22, 23). Consequently aflatoxin has also been found in milk products, such as skim milk, which in turn may be fed to other farm animals.

5.5.3 Careful investigations (24, 25, 26) have shown that aflatoxin is retained in meat and meat products. The highest concentration found was 137 ppb in pig liver and 54 ppb in kidney. No macroscopic changes of these organs were noticed so that such meat would pass meat inspection.

5.6 Toxicity

5.6.1 The toxicological effects of mycotoxins and especially of aflatoxin have been studied in great depth and are well documented (6). Another Conference document in this publication summarizes the present position on health and toxicological aspects. By 1969, 500 publications had come out including 100 on poultry, 70 on rodents, 40 on pigs, 30 on cattle, sheep, horses, 20 on fish, 30 on arthropods and micro-organisms and 20 on primates and man (27). According to Frank (19) this figure had risen to more than 1,000 publications by 1977.

5.6.2 Acute and chronic toxicity of various aflatoxins have been studied in mammalian avian and fish species. Carcinogenicity and mutagenicity are known to occur amongst some of these species. It is most probable to assume that man may respond to either acute or chronic effects of these toxins when he eats contaminated food. Therefore, it would be of practical interest to assess more fully the human, animal and plant exposure to aflatoxin. In humans, the approach may be to devise reliable and specific assay methods for quantitative screening of aflatoxin P₁ in urine. (Aflatoxin P₁ is now known to be a major urinary metabolite of Aflatoxin B₁ in monkeys.)

5.7 Some Social and Economic Considerations for Management

5.7.1 It is generally accepted that the protection of human health and well-being is the primary purpose of environmental pollution control.

5.7.2 When it comes to policy decisions on control measures, one cannot simply demand absolute protection against any health risk at all costs. There is not only the need for weighing actual economic cost against health risk but there is also one element of health risk to be weighed against another, as in the case of banning DDT.

5.7.3 Another difficulty lies in the often persistent residual of uncertainty in evaluating health risk. Policy-makers are now mostly prepared to take protective measures against demonstrated health risks, but many still tend to neglect adverse effects of pollutants where the experts cannot provide satisfactory and convincing evidence.

5.7.4 At present, the insidious hazards - such as carcinogenic, teratogenic and mutagenic effects that may result from long-term exposure - are of greater social concern than the acute or subacute hazards. Public opinion provides strong backing for a preventive approach, through prediction and evaluation to such adverse effects of environmental pollutants (28).

5.7.5 As much as people cherish their health, it is difficult for the economist to put a value on the benefit of health protection. Some other economic parameters, including international trade, are discussed in another document in this publication.

5.7.6 The existence of health is mostly considered as something given; its importance is not adequately appreciated until it is absent. That is, it is evaluated not when present, but rather when absent. Nevertheless, the existence of health in a population should be a basic factor in social and economic development as regards:

- the capacity to work (normal productivity is considered in relation to a healthy population);
- capacity for education; healthy populations have at least a greater access to the means of education (e.g. schools, training at work).

Damage to health can be evaluated in economic terms as far as medical care and hospital costs are involved; the loss of working capacity per person can also be estimated. However, this will remain only one part of the sum total of the value of health. In the case of mycotoxins, one of the arguments in favour of allowing a certain amount of contamination is the danger of hunger if stringent standards were applied (3). It should be recapitulated, however, that people under malnutrition would suffer ever so much more from mycotoxins as the well cared for, and that as soon as there is no lack of food any risk of cancer should be avoided.

5.7.7 Most diseases may be regarded as a disturbance of the dynamic equilibrium between man and his environment which affects individuals or groups of people or animals (7). In this context it might be mentioned that people are occasionally prepared to accept a health risk if it is counterbalanced by some benefit or pleasure, as for instance participation in motor traffic, or cigarette smoking.

5.7.8 The valuation of health protection will remain a political decision.

5.8 Assessment for Management of the Problem

5.8.1 In the case of mycotoxin pollution, the following items can be put down on the credit and on the debit sides:

Table 1

<u>benefits by avoidance of:</u>	<u>Costs:</u>
human illness	decontamination
reduced productivity	prevention equipment and efforts
loss of food or of food quality	monitoring
livestock mortality	sampling and analysis
livestock lower growth rate	epidemiology
livestock lower fertility	agricultural extension services
reduced market price of commodities	education and training (adapted technology)

5.8.2 Scientific and technological knowledge and data for curing the situation are available and a sound basis for management to go ahead with the measures listed on the right hand side of Table 1.

5.8.3 The Mycotoxin Conference will address itself to recommending suitable steps for efforts in fields of prevention, decontamination, surveillance and control, and extension; documentation has been provided to assist the deliberations.

5.8.4 It is suggested that at present much effort is spent on the contaminated end product, but not enough on prevention at the source. Especially the developing countries should take immediate policy decisions to strengthen agricultural extension efforts and ensure their impact as a matter of high priority. One crucial factor in this is the availability of well trained and supported personnel to help farmers and others avoid the economic and health hazards of pollution e.g. by mycotoxins.

5.8.5 As part of the environmental management, surveillance and control measures are important as an immediate measure before prevention has taken effect.

5.8.6 UNEP considers the role of fostering availability of the required training, education and information as of special importance. To these ends, projects have been mounted with FAO and WHO to prepare criteria documents; to train analysts, inspectors, researchers and contamination control managers; to establish provisions (limits, methods of analysis) for contaminants in food standards elaborated by the Codex Alimentarius Commission and to publish guidelines assisting especially the developing countries in their efforts at prevention and control of food and feed contamination by mycotoxins.

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JOINT FAO/WHO/UNEP CONFERENCE ON MYCOTOXINS
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GLOBAL PERSPECTIVE ON MYCOTOXINS

S U M M A R Y

This document is divided into two parts. The first part presents a general overview of the more important mycotoxins, their occurrence in agricultural commodities their significance for human and animal health and productivity, and economic and regulatory aspects of the problems and of their control. Emphasis is placed on aflatoxins, zearalenone, ochratoxins and trichothecenes.

Part II gives a global review of information on mycotoxins incidence and problems particularly with respect to cereal grains, oilseeds, vegetable oils, pulses, root crops, tree nuts, animal products and other miscellaneous commodities.

I. GENERAL OVERVIEW

1. RECOGNITION OF THE MYCOTOXIN PROBLEM

1.1 For countless centuries, changes in the flavour and quality of foods due to mould growth have been recognized. Some of these changes are desirable in that a pleasing flavour is imparted to the food as in certain cheeses. In most cases, however, moulds cause unwanted changes in foods, producing unpleasant flavours and odours and decomposition in varying degrees. Although unpalatable to man and animals and undesirable for this reason, the possible toxic effects from ingestion of mouldy products were not widely appreciated until recently despite disease outbreaks that were associated with mouldy feeds or foods. Ergot poisoning from eating cereal grains infected with the parasitic fungus Claviceps purpurea has occurred sporadically over the centuries, causing suffering and death in many people. The toxic components of ergot responsible for ergotism disease were identified as alkaloids in 1875, over 100 years ago. Another dramatic disease outbreak attributed to mould contaminated cereal grains occurred in certain areas of Russia in 1942-1947. The disease, alimentary toxic aleukia, was caused by eating cereal grains, particularly millet, that had overwintered in the field. Ten percent of the population of certain communities were reported to have died of the disease. Although Russian scientists published numerous papers dealing with alimentary toxic aleukia and the identification of the responsible fungi, the implications for potential health hazard from these and other moulds under less drastic conditions went generally unheeded. (1)

1.2 A combination of circumstances in the early 1960's changed the attitude towards moulds in foods and feed. The focal point was the outbreak of turkey disease in England which resulted in the death of thousands of turkey poults. The disease was traced to a mouldy peanut cake in the ration and in a remarkably short time the responsible mould, Aspergillus flavus, and its toxic metabolites, the aflatoxins,* were isolated. Impetus for this work and that which followed was the finding that the toxic cakes caused liver cancer in rats; this was confirmed by feeding studies that established aflatoxin as one of the most potent carcinogens known, causing hepatomas when fed to rats at levels as low as 1 part per billion in the diet. This finding caused great concern for the possible hazard to human health from the ingestion of groundnuts or groundnut products that could not be recognized as mould damaged, yet carried a low but potentially toxic level of aflatoxin. That the aflatoxin hazard was not limited to a single commodity was demonstrated by concurrent outbreaks of liver cancer in commercial trout hatcheries that were traced to cottonseed cake as the offending dietary component. Again, aflatoxins were isolated from the cake and shown to be the causative agent. Surveys for aflatoxin in other agricultural products were soon initiated. (2)

1.3 The development of analytical methodology suitable for routine application was an important step that made surveillance activities feasible. Identification of the aflatoxins as mould metabolites that were responsible for disease outbreaks in farm animals, and that also presented potential health hazard to man, led to an immediate expansion of scientific effort on the identification of the moulds and their metabolites (mycotoxins) that might be causative agents for other mycotoxicoses in farm animals.

2. MYCOTOXINS CURRENTLY IDENTIFIED AS HAVING SIGNIFICANT OCCURRENCE IN FOODS OR FEEDS

Although many toxic mould metabolites have been isolated from laboratory cultures of moulds that occur on agricultural products, so far only seven have been found to have possible significant occurrence in naturally contaminated foods and feeds. These are aflatoxins, zearalenone, ochratoxin A, citrinin, trichothecenes, patulin, penicillic acid and the ergot alkaloids. Further research may add others to this list or delete one or two on the basis of ongoing long-term chronic toxicity studies.

3. AFLATOXINS

3.1 Aflatoxin-producing moulds and aflatoxins produced

The term aflatoxin as used in discussing mould contaminated commodities is a generic term that refers to one or more of 4 principal metabolites produced by certain strains of fungi of the Aspergillus flavus group. These are aflatoxins B₁, B₂, G₁ and G₂. Aflatoxin B₁ is generally found in greatest concentration; B₂ and G₂ in least concentration and G₁ at intermediate levels. The relative concentrations, however, vary greatly depending on the fungal strain, substrate and conditions for growth. Concentrations of B₁ or total aflatoxins are commonly reported in analytical determinations. (3)

3.2 Natural occurrence in agricultural commodities

3.2.1 Extensive investigations on the occurrence of aflatoxin have been made, far more than any other mycotoxin. Aspergillus flavus is universally distributed in the environment and aflatoxin contamination in foods and feeds has been detected in all parts of the world.

3.2.2 Aflatoxin has been found in the following commodities:

- (a) Oilseeds: groundnuts, cottonseed and copra.
- (b) Oilseed meals: groundnut, cottonseed, copra, sunflower and soybean.
- (c) Crude vegetable oils: groundnut, olive and coconut.
- (d) Cereal grains: maize, sorghum, rice, wheat, barley, millet and oats.
- (e) Cereal meals: maize and sorghum.
- (f) Tree nuts: pistachio and Brazil nuts, almonds, walnuts, pecans and filberts.
- (g) Fruits: figs

3.2.3 Aflatoxin M₁, an animal metabolite of aflatoxin B₁, has been found in the milk of dairy animals. Incidence of aflatoxin in some of these commodities, e.g. oats, barley and wheat, has been limited to a few occurrences at low levels. Even though the hazard appears small in these cases, it must be recognized that only one or two countries may have reported analyses and the results may not be applicable to the crop grown and harvested under climatic conditions elsewhere.

3.3 Conditions for mould growth and aflatoxin production

3.3.1 Laboratory studies show that a moisture content in equilibrium with a relative humidity of about 85(±) percent is the lower limit for growth of A. flavus and production of aflatoxin on natural substrates. This corresponds to a moisture content of 18 to 18.5% in wheat, corn and sorghum grains; 16.5% in paddy and 17.5% in polished rice; 17-18% in soybeans and 9-10% in peanuts, Brazil nuts, other nuts and copra. A. flavus produces aflatoxin over a temperature range from about 12 to 42°C with optimum production at 25 to 32°C, the limits depending on the substrate and specific experimental conditions. Under laboratory conditions at 25 to 30°C, aflatoxin has developed within 48 hours on moistened groundnuts, rice and cottonseed, whereas a minimum period of 4 to 5 days on wheat has been reported. (4,5,6,7,8,9)

3.3.2 The moisture limits stated above do not represent safe storage conditions for the commodities listed. Other fungi will invade and damage these products at lower moisture levels. Cereal grains should be dried to a moisture content of 13% or less, for storage soybeans to 12%, groundnuts to 8% and cottonseed to 10%. Safe moisture levels,

however, vary with the temperature at which the commodity is stored and the duration of storage. Moisture content of cereal grains, for example, should be 11-12% for prolonged storage in hot climates. (10, 11)

3.3.3 The presence of Aspergillus flavus mould growth on a commodity is not necessarily indicative of aflatoxin contamination. Many, but not all, strains of A. flavus are toxin producers. Studies of nearly 1400 strains isolated from different sources showed that 58% produced aflatoxin. In another study, A. flavus isolated from maize displayed a broad spectrum of aflatoxin production ranging from no detectable yields to high levels. (>100 ppm). (12, 13)

3.3.4 It is also true that absence of visible mould growth does not assure freedom from mycotoxin contamination. For example, grains that appear sound and fit for human consumption may contain significant levels of mycotoxins.

3.3.5 Production of aflatoxins by A. flavus may be affected by the presence of other fungi which is the usual situation in nature for all agricultural commodities. Little or no aflatoxin was found when an aflatoxin-producing strain of A. flavus was inoculated onto moist maize and allowed to grow along with other fungi naturally present on the grain. Similarly, rice inoculated with a combination of A. flavus and another Aspergillus species commonly found in stored grains produced little aflatoxin when incubated at moisture levels suitable for mould growth. In contrast to these findings, blight damaged maize seed (Helminthosporium maydis blight) was invaded to a greater extent by A. flavus and other fungi than non-blight damaged seed. (13, 14, 15)

3.3.6 It may be concluded that the finding of A. flavus in a commodity can only be a presumptive indication of aflatoxin contamination. Analytical determinations must be undertaken to establish the presence of toxins.

3.4 Sites of aflatoxin contamination

3.4.1 Aflatoxin contamination may occur in the growing, harvesting, storage, transporting or processing of an agricultural commodity. It may also occur in the home of the consumer if adequate protection is not given to left-over prepared foods.

3.4.2 Although the aflatoxin-producing mould, Aspergillus flavus has been traditionally considered to be a storage mould, it has been recently established that infection and aflatoxin formation may occur in several crops in the field. Crops in which field infection with A. flavus and aflatoxin contamination have been found include cottonseed, maize, groundnuts, pistachio nuts, almonds, filberts and figs. Environmental conditions conducive to A. flavus infection are a warm and humid atmosphere. High temperatures favour competition of A. flavus with other moulds. Insect damage, providing an opening for entry of the mould, is an important contributing factor. Insects are carriers of A. flavus and may carry the mould directly into the seed. But main access to the seed may be provided by the natural opening of the boll in the case of cottonseed and the hard shell in the case of pistachio nuts. Both occur before the seeds are mature and when they have a moisture content favourable for A. flavus growth. Stress conditions, such as drought in the case of groundnuts, encourage insect attack and lower the resistance of the growing plant to mould invasion and subsequent development of aflatoxins. In-field contamination appears to be a major site for contamination of cottonseed and maize in the United States and of pistachio nuts in Iran and Turkey.

3.4.3 The post-harvest drying operation is a frequent site of aflatoxin contamination in many crops. Delay in drying to safe moisture levels increases risks of mould growth and mycotoxin formation. Groundnuts are particularly vulnerable since they are lifted at high seed moisture levels and usually carry large A. flavus inocula from contact with the soil. Pod or seed damage permits mould entry and further increases risk of aflatoxin contamination in the drying process.

3.4.4 Insect attack and mould growth are major causes of deterioration of the quality of stored seeds. Insects can be controlled by fumigation and good sanitation practices. Insect control is essential not only to prevent direct insect damage but also because insects generate moisture and can raise the moisture level of dry grain sufficiently for moulds to grow and produce toxins.

3.4.5 Mould growth is best prevented by controlling the moisture level of the stored seed. Placing only dry, cleaned products in storage and protecting them from rain or ground water are first requirements for safe storage. Less obvious, but equally important, is protection against, or provision for eliminating, local moisture accumulations that result from temperature gradients set up between the interior and the exterior of the seed mass in response to external temperature changes. Moisture may also be absorbed by the stored seed from the atmosphere in high humidity climates. Regardless of cause, local moisture accumulations above the safe level engenders high risk of mould growth and mycotoxin contamination.

3.5 Adverse effects of aflatoxin-contaminated feeds on livestock and poultry production.

3.5.1 The deaths of turkey poults, ducklings and other fowl that led to the discovery of aflatoxin as the causative agent attest to the acute toxicity of this mycotoxin. Other incidents of disease outbreaks in farm animals associated with feed contaminated with aflatoxin or infected with the *A. flavus* mould have been reported from England, South Africa, U.S.A., India, Germany, Latvia and Denmark. (16, 17)

3.5.2 Although acute aflatoxin poisoning may also cause economic loss, the effects of ingestion of levels that do not produce overt signs of illness very likely cause greater losses because they can continue over longer periods of time before their cause is recognized. The effects of feeding various levels of aflatoxin to swine, cattle and poultry have been investigated by several workers. In growing-fattening swine no evidence of toxic effects was observed when the aflatoxin level fed was 233 $\mu\text{g/kg}$ or less. At higher levels (465, 600 and 810 $\mu\text{g/kg}$) (Table I/1) significant differences were noted at one or more concentration with respect to weight gains, feed efficiency, organ weights, liver histopathology and in levels of certain blood enzymes. (18)

3.5.3 Cross-bred steers, 6 to 8 months old, showed no toxic effects when fed aflatoxin at levels of 100 and 300 $\mu\text{g/kg}$ in the ration but both weight gain and feed efficiency were adversely affected when the level of aflatoxin was increased to 700 and 1000 $\mu\text{g/kg}$ (Table I/2). Autopsy of the animals after feeding for 133 to 196 days showed an increase in liver weights when aflatoxin in the ration was 700 $\mu\text{g/kg}$ or higher. At 1000 $\mu\text{g/kg}$ in the ration, the livers from some animals were greatly abnormal. (18)

3.5.4 Calves are more susceptible to aflatoxin than are older bovines. Calves fed rations containing 2, 220, or 440 $\mu\text{g/kg}$ aflatoxin from 4 days to 4 months of age showed significantly reduced growth rates and food intakes over the first three months. In the fourth month there was no difference in performance between any of the groups. (18)

3.5.5 Dairy cows fed moderate to high levels of aflatoxin in their rations excreted detectable amounts of an aflatoxin metabolite, aflatoxin M_1 , in their milk. Aflatoxin M_1 , like aflatoxin B_1 , causes liver lesions in experimental animals. The presence of M_1 in milk presents a hazard to humans who drink the milk and particularly to infants since the young of most species have the greatest susceptibility to aflatoxins. The concentration of aflatoxin in the milk depends on the quantity of aflatoxin ingested and the time elapsing between ingestion and milk collection as shown in Table I/3. (19, 18)

3.5.6 Ducklings are extremely sensitive to aflatoxin; the oral 7-day LD_{50} is reported as 18.2 μg aflatoxin B_1 and 16 μg aflatoxin M_1 in the day-old duckling. Deaths and depression in weight gain during the first 5 weeks were observed in ducklings fed a diet containing 30 $\mu\text{g/kg}$ aflatoxin from 7 days of age. Eight of eleven birds which survived this period had hepatic tumours at 14 months. Chickens are more resistant to aflatoxins than

ducks and turkey poults, but some breeds are less resistant than others. Arbor-Acres hybrid broiler type chicks were resistant to at least 1600 $\mu\text{g/kg}$ aflatoxin in their feed since only minor liver lesions occurred at this level. The New Hampshire breed was susceptible to aflatoxin levels of 500 $\mu\text{g/kg}$ but they recovered quickly when toxin was removed from the diet. No effect on egg production was observed in White Leghorn hens fed rations containing 2700 $\mu\text{g/kg}$ aflatoxin. (20, 21, 22, 23, 24, 18)

3.5.7 Table I/4, reproduced from a literature report, is a compilation showing aflatoxin levels in feed that produce adverse effects in various farm animals. Susceptibility is greatest in the young, generally decreasing with the age of the animal. (25)

3.5.8 In addition to the chronic effects of aflatoxin ingestion just discussed, ingestion of aflatoxin may have secondary effects such as the enhancement of susceptibility to disease. Among the infectious processes enhanced by aflatoxin consumption are candidiasis, cecal coccidiosis and salmonellosis (paratyphoid) in chickens. Impairment of acquired immunity to fowl cholera vaccination has been demonstrated in turkeys fed rations containing 250 $\mu\text{g/kg}$ aflatoxin. (26)

3.5.9 Thus, depressed growth rates, decreased efficiency in the utilization of feed and higher incidence of infectious disease may result from aflatoxin contaminated feeds and may be the greatest contributors to economic loss in livestock production caused by aflatoxin.

Table I/1. EFFECT OF AFLATOXIN ON RATE OF GAIN AND FEED CONVERSION IN SWINE
(from: Keyl, A.C. and Booth, A.N. 1971. Aflatoxin effects on livestock.
J. Amer. Oil Chem. Soc. 48: 599-604)

Ration	Aflatoxin content of ration, $\mu\text{g/kg}$			Average daily gain* kg.	Gain/feed
	B ₁	B ₂	G ₁		
Control	6	-	-	0.71 ^{c, d}	0.26
Test 1	450	-	30	0.68 ^{c, d}	0.27
Test 2	615	105	45	0.60 ^d	0.27
Test 3	810	135	60	0.47 ^e	0.23

* Values with unlike superscripts are significantly different.

Table I/2. EFFECT OF AFLATOXIN ON RATE OF GAIN AND FEED CONVERSION IN BEEF CATTLE FED COTTONSEED MEAL CONTAINING GRADED LEVELS OF AFLATOXIN B₁ FOR 133 DAYS
(from: Keyl, A.C. and Booth, A.N. 1971. Aflatoxin effects in livestock. J. Amer. Oil Chem. Soc. 48: 599-604)

Aflatoxin in ration µg/kg	Mean body weights		Gain/ feed
	Initial (lb)	Daily gain* (lb)	
Control	401	2.51 ^b	0.175
100	427	2.63 ^b	0.164
300	417	2.40 ^b	0.159
700	406	1.90	0.155
1,000	433	1.76 ^c	0.152

* Values with unlike superscripts are significantly different

Table I/3. AFLATOXIN M₁ IN MILK FROM COWS FOLLOWING WITHDRAWAL OF AFLATOXIN B₁
(from: Keyl, A.C. and Booth, A.N. 1971. Aflatoxin effects in livestock. J. Amer. Oil Chem. Soc. 48: 599-604)

Cow	Aflatoxin fed in week before withdrawal, mg	Aflatoxin in dry milk, µg/kg				
		Days after withdrawal				
		1	2	3	4	7
1	82	44	17	5	*	5
2	96	55	27	*	3	1
3	1500	718	434	285	160	*

* Not analyzed

Table I/4.

DIETARY AFLATOXIN CONCENTRATION CAUSING TOXICOSIS

(from: Edds, G.T. 1973. Acute aflatoxicosis. A review. J. Amer. Vet. Med. Assoc. 162: 304-309)

Species	Age	Aflatoxin (ppm)	Duration of Feeding	Effects
Calves	Weanling	0.22 - 2.2	16 weeks	Stunting, death, liver damage
Steers	2 years	0.22 - 0.66	20 weeks	Liver damage
Cows	2 years	2.4	7 months	Liver damage, clinical illness
Pigs	Newborn	0.234	4 days (to sow)	Stunting
Pigs	2 weeks	0.17	23 days	Anorexia, depression, icterus, ascites, stunting
Pigs	4-6 weeks	0.41 - 0.69	3-6 months	Stunting, liver damage
Chickens	1 week	0.84	10 weeks	Stunting, liver damage
Chickens, New Hampshire	2 days	0.2	40 days	Increased growth
Ducks	Unknown	0.3	6 weeks	Liver damage, death

3.6 Association of aflatoxin-contaminated foods with disease in humans

3.6.1 Acute toxicity data are scarce in relation to human exposure, but there are reported epizootics of apparent aflatoxicoses, the primary manifestation of which is the acutely diseased liver. A recent case was an epidemic of fatal hepatitis that broke out in several tribal villages in the states of Gujarat and Rajasthan in western India in the autumn of 1974. Nearly 400 people were affected with a mortality rate of 20%. There was a double peak in mortality - one in the age group 5 to 15 years and the other in the age group over 30 years. Maize, a principal item in the diet, was heavily contaminated with aflatoxin, ranging in concentrations from 0.25 to 15.6 mg/kg (mean 6.0 mg/kg). The epidemic broke out in October after the harvest of maize; reached a peak in November and December and declined in January when the stock of maize was depleted. (27)

3.6.2 Aflatoxin may be associated with an acute disease involving encephalopathy and fatty degeneration of the viscera (EFDV) that caused the deaths of several hundred children in Thailand per year. Analyses of foods from local markets showed that aflatoxin contamination paralleled incidence of the disease on both a geographic and a seasonal basis. Chemical analysis of autopsy specimens revealed aflatoxin in one or more specimens from 22 of 23 cases of EFDV at levels up to 93 $\mu\text{g/kg}$ in liver, 123 $\mu\text{g/kg}$ in stool, 127 $\mu\text{g/kg}$ in stomach and intestinal content and 8 $\mu\text{g/kg}$ in bile. Only trace amounts of aflatoxin could be demonstrated in some autopsy specimens of 11 of 15 children who died from causes other than EFDV. Monkeys given a single large dose of aflatoxin developed a pathology similar to EFDV in children. Deaths of children with the EFDV syndrome also have been reported in Czechoslovakia. Over a period of 5 years, deaths of 27 children occurred at ages 3 days to 8 years. Twenty children died within 2-7 days after the first symptoms of the disease,

three within 1-2 months after acute onset, and four children 2-4 months after the first symptoms. Aflatoxin was demonstrated in liver specimens of all groups. The source of aflatoxin in three cases was aflatoxin-contaminated milk food. (28, 29)

3.6.3 Two other cases of acute poisoning, both resulting in deaths of children, have been reported as being possibly due to aflatoxin. One was a fatal hepatitis in a 15-year old African boy. After the boy died, a sample of cassava obtained from his home was found to contain 1.7 mg aflatoxin per kg. In the other fatality, a 10-kg child died after consuming aflatoxin contaminated steamed rice for two days. The level of aflatoxin in the rice was 6 mg/kg. (28, 30)

3.6.4 The possible effect of chronic ingestion of low levels of aflatoxin in contaminated foods has been investigated in epidemiological studies carried out in five countries - Thailand in southeast Asia and Swaziland, Mozambique, Uganda and Kenya in Africa. The results show a highly significant positive correlation between the level of intake of aflatoxin and the incidence rate of primary liver cancer. Aflatoxin intake was determined by the collection and analysis of "plate" samples of foods as prepared for the table from homes in four of the studies and by analysis of home-stored and market foods in the other. Variations in aflatoxin intake and cancer rate were found in different regions within countries that were primarily related to climatic differences. Although the studies show a relationship between aflatoxin ingestion and primary liver cancer, there are a number of factors which complicate its interpretation as cause and effect. Despite the lack of definite proof for this, it seems prudent to avoid aflatoxin contaminated foods in the diet. (31-38)

3.6.5 For a more in-depth study and authoritative interpretation of human health hazard associated with aflatoxin contaminated food, reference is invited to document MYC-4b, "Health and Toxicological Aspects of Mycotoxins" and to the WHO/UNEP Environmental Health Criteria Document on Mycotoxins in press. (See following article in this publication.)

3.7 Regulatory aspects

3.7.1 Eighteen countries now have regulations or guidelines which prescribe maximum acceptable limits for aflatoxin in foods and/or feeds. Limits prescribed vary from 0 to 50 $\mu\text{g/kg}$ for foods and from 0 to 1000 $\mu\text{g/kg}$ for feeds. The lower limit of aflatoxin concentration actually depends on the sensitivity of the analytical method designated as the official method. In the case of foods, this usually sets 5 $\mu\text{g/kg}$ aflatoxin B_1 as the "zero" limit, and 10 to 100 $\mu\text{g/kg}$ in the case of feeds. The commodities over which control is exercised vary from country to country ranging from all foods and feeds to an individual commodity. Groundnuts and groundnut cakes are the most frequent individual commodities specified. Limits are prescribed for finished feeds in several countries, the limit varying with the age and species of the animal for which the feed is intended. The approach in setting these limits has generally been conservative. (39)

3.7.2 Some guidance in setting tolerance levels for aflatoxin in feeds is given by data on tissue levels found in animals after feeding various levels of aflatoxin. Tissue residues have been observed to vary with the species and breed of animal, level and duration of exposure, and time after cessation of exposure that the tissue specimen was collected. Residue levels also differ in different tissues. In meat animals, aflatoxin B_1 is likely to be found at highest levels in the liver, together with approximately equal concentrations of aflatoxin M_1 . In chickens, levels of B_1 in the liver are higher than those found in the egg. A number of studies have been undertaken to determine the ratio of aflatoxin in the feed to that in the muscle, liver, kidney, milk and eggs. A range of values has been found, varying with the factors mentioned above and the specific conditions of the study. The results of these studies have been compiled and average values calculated for the feed/tissue ratios. Average values for the ratio of aflatoxin B_1 concentration in the feed to aflatoxin B_1 concentration in the liver were: beef - 14000/1; swine 800/1; broiler chickens - 1200/1. For laying hens, the ratio of aflatoxin B_1 in the feed to that in the eggs was 2200/1. In milk, aflatoxin M_1 , the animal metabolite of B_1 is formed; for dairy cows the average ratio of aflatoxin B_1 in the feed to M_1 in the milk was 300/1. It is evident that the feed/milk ratio is relatively low and that caution must be exercised in

selecting feed for dairy animals. It should also be stressed that the values cited for feed/tissue ratios are averages and may not be applicable to a particular feeding situation. Further information on the subject, including other factors to be considered in setting regulatory limits for aflatoxin in foods and feeds are discussed in the FAO/UNEP document, "Mycotoxin Surveillance - A Guideline". Reference is also invited to document MYC-7, "Surveillance and Control Measures to Reduce Contamination by Mycotoxins". (39, 40, 41) (See FAO Food and Nutrition Paper No. 2, "Mycotoxins" Annex VII.)

3.8 Economic aspects

3.8.1 The economic loss resulting from aflatoxin-contaminated foods and feeds is difficult to estimate but undoubtedly is large, judging from the widespread occurrence of aflatoxin contamination and the large number of commodities affected. Losses come about in many ways: from direct food losses; from human illness and reduced productivity; from livestock losses from deaths and lower growth rates and feed efficiency; in direct costs of systems for control of aflatoxin in foods and feeds; in the reduced value of rejected commodities, and in the costs of detoxification to recover an acceptable product, and, in some cases, from loss of export markets.

3.8.2 Human health has been dramatically affected in outbreaks of acute aflatoxicosis, but these tragic events may be only a part of the cost to society in terms of impaired health and productivity from the ingestion of sub-clinical levels of aflatoxin. Epidemiological studies have indicated a relationship between aflatoxin intake and incidence of liver cancer in several of the developing countries. This disease, of particularly high incidence in males, has a peak frequency at age 30-45 years, shortening the life of the labour force as well as entailing costs of hospitalization and medical treatment.

3.8.3 No monetary values have been estimated for losses in livestock production from aflatoxin-contaminated feeds. However, sickness and deaths in livestock associated with contaminated feeds have been documented in many countries and it is likely that the number of such cases reported will increase as analytical facilities become available to veterinarians as a diagnostic procedure. The economic loss of these cases of overt manifestation of aflatoxin poisoning is probably small compared to losses from reduced growth rate, lower feed efficiency and increased susceptibility to infectious disease that results from the presence of low levels of aflatoxin in livestock feeds. These losses can continue over long periods before their cause is recognized and, providing other sources of feed are available, remedied.

3.8.4 Some data are available on the cost of control of the aflatoxin content in groundnuts as practiced in the United States. The system provides three control points: (1) at the first buying point for farmers' stock (in-shell) groundnuts; (2) at the sheller, and (3) at the manufacturer of consumer products. Loss in value of farmers' stock groundnuts resulting from rejection at point (1) averaged about half a million dollars for 1974 and 1975. This was approximately 0.15% of the total farm value of the groundnuts. Sampling and analyses of the raw, shelled groundnuts at control point (2) and indemnification losses for payments to shellers for lots that did not meet permitted aflatoxin levels, averaged about \$1.80 per 1,000 lbs of shelled groundnuts for crop years 1974 and 1975. This cost represented about 0.6% of the estimated value of the edible crop as raw, shelled nuts. These costs do not include the cost of sampling and analysis carried out by manufacturers of consumer products in their internal quality control system, nor certain other inspection costs borne by the Government. (42)

3.8.5 Estimates also have been made of costs to importers for control of aflatoxin in Brazil nuts and pistachio nuts marketed in the United States. For Brazil nuts, the average cost per year for the 1970-1976 period was \$2.73 per 1,000 lbs of nuts or 1.23% of the value of the nuts imported. Costs included those for analyses, insurance premiums and reconditioning. For pistachio nuts, the average of the costs for 1975 and 1976 was \$5.30 per 1,000 lbs of nuts. This represented 0.5% of the value of the imported pistachio nuts. (43,44)

3.8.6 Another economic loss resulting from aflatoxin contamination is the cost of detoxification of the commodity to make it acceptable for food or feed use. A few cases have been reported in which a shipment of a commodity has been prohibited from use by regulatory action and has resulted in total loss of the commodity. However, these incidents have been exceptional. Detoxification procedures are being applied to at least three commodities: groundnuts and cottonseed and cottonseed meals. The method applied to groundnuts is physical removal of contaminated kernels, accomplished by screening to remove shrivelled and broken kernels and by electronic sorting or hand-picking to remove discoloured kernels. Such methods are widely practised but no estimates are available on costs of removal or losses suffered from the reduced value of the rejected groundnuts.

3.8.7 Detoxification of aflatoxin-contaminated cottonseed and cottonseed meal is accomplished in the United States by treatment with ammonia at elevated pressure and temperature. The cost of treatment in the United States has been estimated at 8 to 10 dollars per ton. This process also has been adapted to the detoxification of groundnut meals. (45, 46)

3.8.8 Partial or complete loss of export markets is another cost that may result from aflatoxin contamination of a commodity. Even where export markets are maintained by segregation of the more highly contaminated lots, domestic disposal of the segregated lots may, depending on the aflatoxin level, involve additional costs in chemical detoxification or, if fed to farm animals, in reduced livestock productivity. (see last article in this publication and concerning detoxification see FAO/Food and Nutrition Paper No. 2, "Mycotoxins", Annex VI)

4. ZEARALENONE

4.1 Zearalenone-producing moulds

Zearalenone is produced in the laboratory by several species of Fusarium, moulds that are common in nature, occurring as parasites of wild and cultivated plants in which they cause a number of diseases such as wilts, blights and rots. Of the species that have been tested Fusarium roseum (also designated as F. roseum var. graminearum, F. graminearum and as Giberella zeae, the ascospore-producing stage of the species), F. tricinctum and F. moniliforme produce zearalenone in laboratory cultures. F. roseum produces the highest yields and also is the species principally associated with zearalenone in harvested and stored agricultural commodities. In the field, F. roseum infection of maize is often accompanied by a pink discolouration of the kernels. F. moniliforme was associated with zearalenone contamination in fermented porridges and beer made from maize and sorghum and in malted sorghum. (47, 16, 48, 49)

4.2 Natural occurrence in agricultural commodities

Zearalenone has been reported in maize, sorghum, barley, wheat and feeds and in beer and other fermented maize and sorghum products. Zearalenone-contaminated foods or feeds have been detected in North America, Europe and Africa. Incidence appears highest in temperate zones, particularly in seasons of cool, rainy weather.

4.3 Conditions for growth of producing moulds and zearalenone production. Sites of zearalenone contamination.

4.3.1 The Fusarium species generally require moisture levels of 22 to 25% (as is basis) and are able to grow on maize and other crops up to maturation in the field but are limited in the extent they can develop in grain after it has been dried and stored. Highest yields of zearalenone have been produced in laboratory cultures by inoculating maize seeds at moisture contents of 45% and incubating at 24-27°C for 7 days and 12-14°C for 4 to 6 weeks. Although most strains tested have required incubation at a low temperature to obtain high yields, a strain of F. roseum "Gibbsum" isolated from blighted sorghum grain produced higher yields at 25°C than at 10°C when grown on autoclaved sorghum. (16, 48, 50)

4.3.2 Occurrence of zearalenone in maize in the United States has been the highest in years when unusually wet weather has delayed planting in the spring and harvesting in the fall. Ideal conditions for F. roseum infection are 9 days of rain during silking of the ear with mean temperature below 21°C. Zearalenone has been found in Fusarium-infected ears at harvest but only at low concentrations. Storage of high moisture, Fusarium-infected grain at winter temperatures apparently provides conditions for zearalenone production and most disease outbreaks associated with zearalenone-contaminated maize have occurred from midwinter to early spring. In Zambia, zearalenone has been associated with ear rots in maize which have the highest incidence when late rains prevent ears from drying. F. graminearum and F. moniliforme are among the Fusarium species isolated from diseased kernels. (51, 52, 53 48, 54)

4.3.3 Interaction with competing fungi such as Aspergillus and Penicillium species reduced zearalenone production by F. roseum when the competing microorganisms were inoculated on to the substrate at the same time as F. roseum. However, if F. roseum is well established on the substrate, the presence of competing organisms has little effect on zearalenone production. (55)

4.4 Adverse effects of zearalenone-contaminated feeds on livestock and poultry production

4.4.1 Zearalenone and mouldy grains containing zearalenone, produce estrogenic syndrome in farm animals. Swine are the most sensitive of the large farm animals and are most commonly affected. In pre-pubertal gilts the syndrome is characterized by enlarged vulva, mammae and nipples and may be accompanied by prolapse of the vagina. Uteri may be greatly enlarged and ovaries atrophied. Young males may undergo a feminizing effect with atrophy of the testes and enlargement of the mammary glands. It was the search for the causative agent in the rations of swine affected by this syndrome that led to the discovery of zearalenone. Other farm animals affected are dairy cattle, chickens and turkeys. (48)

4.4.2 Stillbirths, neonatal mortality and small litters in pigs were associated with ingestion of zearalenone, among other fusarium toxins, by pregnant sows. Consumption of zearalenone in late pregnancy was related to stillbirths and splayleg while small litter size was correlated with ingestion of zearalenone in early pregnancy. In another study, bred sows fed zearalenone at 100 ppm in their diet produced no pigs; at lower levels the average litter size may have been reduced but the results were inconclusive. Histopathological examination indicates that zearalenone leads to sterility in sows by causing malfunction of the ovary. (56, 57, 58)

4.4.3 Fusarium roseum invaded maize fed at a level of 0.7 to 20% in the diet of pigs depressed growth rate about 40% in both males and females and produced estrogenism in females and testicular atrophy in males. The diets contained about 500 to 600 ppm zearalenone. Pure zearalenone, however, does not cause weight losses in swine and it is probable that other toxins produced by Fusarium roseum are responsible for the reduced growth rates observed. (59)

4.4.4 Turkey poults fed diets containing 10% of maize that was heavily invaded with F. roseum showed an estrogenic response evidenced by swollen vents; those fed 40% of the mouldy maize in their diet showed greatly reduced weight gains and some developed prolapsed cloacae. (60)

4.4.5 Egg shell quality decreased when laying hens were fed rations containing 250 and 500 ppm zearalenone. Other aspects of performance appeared to be unaffected. Male chicks fed diets containing 40 ppm zearalenone showed significant increase in weights of testes and combs but no effect on growth rate. (61, 62)

4.4.6 Zearalenone has been associated with numerous disease outbreaks in farm animals in England, Scotland, France, Finland, Yugoslavia and the United States. A tabulation of such incidents reproduced from the literature appears as Table I/5. (51)

4.4.7 It should be noted that zearalenone may be accompanied by other Fusarium toxins and that these toxins probably contribute to the mycotoxicoses observed in many cases.

Table I/5

TOXICOSES IN FARM ANIMALS ASSOCIATED WITH ZEARALENONE-CONTAMINATED FEED

(from: Shotwell, O.L. 1976. Assay methods for Zearalenone and its natural occurrence. US-Japan Conference. "Mycotoxins in Human and Animal Health." University of Maryland, U.S.A.)

Commodity or Product	Examined because of:	Country	Zearalenone (ppm)	Reference
Hay	Infertility in dairy cattle	England	14.0	(a)
Feed	Infertility in cattle and swine	Finland	25.0	(b)
Corn	Hyperestrogenism in farm animals	France	2.3	(c)
Animal Feed	Hyperestrogenism in cattle and swine	United States	0.1-2,900	(d)
Corn		Yugoslavia	18	(e)
Corn	Poisoning in swine	Yugoslavia	2.5-35.6	(f)
Corn	Severe mould damage and swine refusal	Yugoslavia	0.7-14.5	(g)
Barley	Stillbirths, neonatal mortality and small litters in swine	Scotland	0.5-0.75	(h)
Barley	Death in swine	Scotland	"traces"	(i)
Feed	Field problems in animals	United States	Not stated	(j)
Grain sorghum	Head blight in sorghum	United States	Not stated	(k)
Corn	Swine hyperestrogenism	Yugoslavia	35.6	(1)
Pig feed	Swine hyperestrogenism	United States	50.0	(1)
Corn	Swine hyperestrogenism	United States	2.7	(1)
Sorghum	Cattle abortion	United States	12.0	(1)
Corn	Swine abortion	United States	32.0	(1)
Corn	Swine feed refusal	United States	2.5	(1)
Dairy ration	Cattle feed refusal, lethargy, anaemia	United States	1.0	(1)
Pig feed	Swine internal haemorrhaging	United States	0.1	(1)
Pig feed	Swine hyperestrogenism	Yugoslavia	0.5	(1)
Pig feed	Swine infertility and abortion	United States	0.01	(1)

References - Table I/5

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References - Table I/5 (cont.)

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4.5 Association of zearalenone-contaminated foods with disease in humans.

4.5.1 Zearalenone has been demonstrated to have estrogenic and other effects in animals. Since it has been found in foodstuff destined for human consumption, there is the possibility of it being involved in estrogen-related diseases.

4.6 Regulatory aspects

No countries are reported to have instituted regulatory control on the zearalenone content in foods or feeds.

4.7 Economic aspects

4.7.1 Grain discounted because of mould damage associated with zearalenone contamination may cause considerable loss to the farmer. The National Agricultural Marketing Board in Zambia undertook the task of cleaning grossly infected maize in 1975 in order to recover sound grain suitable for human consumption. Prices paid to the farmer were based on the extent of mould damage. Based on comparison of prices between grades, the farmers

received only about 35% of the average price of acceptable grades. Even so, it appeared doubtful whether the return from sale of the cleaned maize paid costs involved in the installation, maintenance and operation of equipment, handling and storage of grains and other costs involved. (63)

4.7.2 Livestock losses from zearalenone-contaminated feeds represent another cost of this mycotoxin. These losses must be considerable, judging from the numerous disease outbreaks reported in farm animals in which the diagnosis has implicated zearalenone and the probable greater incidence of adverse effects, such as infertility and reduced litter size in swine, where zearalenone has not been recognized as the causal agent. However, no estimates are available on the losses involved.

5. OCHRATOXINS

5.1 Ochratoxin-producing moulds and ochratoxins produced.

Ochratoxins are produced by several species of the fungal general Aspergillus and Penicillium. A. ochraceus, P. viridicatum, P. cyclopium and P. palitans appear to be the main producers. The first three species are commonly found in stored grain. However, P. viridicatum appears to be most frequently associated with ochratoxin in naturally contaminated cereal grains. Although two ochratoxins, designated ochratoxins A and B, have been isolated from laboratory cultures, ochratoxin A alone has been detected in most cases of natural occurrence in agricultural commodities. (64, 65, 66)

5.2 Natural occurrence in agricultural commodities.

Ochratoxin A has been found in barley in Denmark, the United States and Canada; in maize in Yugoslavia and France, and in wheat, oats and dried beans in Canada. All grain samples analyzed in Canada had suffered mould damage and heating in storage.

5.3 Conditions for mould growth and ochratoxin production.

Aspergillus ochraceus requires a minimum relative humidity of 80% for growth at a temperature of 26 to 30°C. This relative humidity corresponds to a moisture content of 15 to 15.5% in cereal grains. However, A. ochraceus is seldom found to be the predominant mould in maize or wheat even in a lot that is undergoing spoilage. In maize inoculated with Penicillium viridicatum and stored at 81% relative humidity and 23°C, sporulation occurred after 27 days; at 86% relative humidity the time was shortened to 7 days. In maize having a moisture content of 22%, P. viridicatum grew well at 4°, 16°, 23° and 30°C. However, at 18% moisture in the maize, there was little growth at any of these temperatures. Compared to A. flavus, a higher moisture level in grain is required for the growth of P. viridicatum. (65, 66)

5.4 Adverse effects of ochratoxin-contaminated feeds on livestock and poultry production.

5.4.1 In experimental studies, nephropathy (kidney damage) has been produced in swine, dogs, ducks, chickens, rats and mice by feeding ochratoxin A in their diets. In swine, this mycotoxicosis is characterized by diuresis and polydypsia; at necropsy gross pathology reveals enlarged kidneys and a colour change from the normal red brown to grey. In field cases, depressed growth rates are also observed. Some of the effects observed may have been caused by citrinin which is frequently found with ochratoxin A in contaminated feeds. (67)

5.4.2 Sexual maturity was delayed in pullets fed 1 ppm ochratoxin A in their rations and egg production was decreased. At levels of 2 and 4 ppm, kidneys and liver were damaged and many deaths resulted. Other studies have confirmed the acute toxicity of ochratoxin A to chickens. Broilers fed diets containing 0.5, 1, 2, 4 or 8 ppm exhibited growth depression at all levels and deaths occurred at the two highest levels. (68, 69)

5.4.3 Residues of ochratoxin A are found at slaughter in the tissues of swine fed levels (1 ppm) of this toxin that have been encountered in contaminated feeds in some regions. Young swine, 8 to 10 weeks old, fed rations containing 1 ppm for 3 months, had residues of 28 mg/kg in kidney, 11 mg/kg in liver, 9 mg/kg in muscle and 3 mg/kg in adipose tissues. The significance of these levels to human health is not known. (70)

5.5 Association of ochratoxin-contaminated foods with disease in humans

5.5.1 It has been suggested that ochratoxin A in the food supply may be a factor in Balkan endemic nephropathy, a fatal renal disorder in humans, occurring in certain areas of Bulgaria, Romania and Yugoslavia. Recent surveys of foodstuffs have shown that ochratoxin A is more frequently present in foods from an endemic area than from non-endemic areas. However, a causal relationship remains to be established. (64)

5.5.2 For further information and authoritative interpretation of human health hazard associated with ochratoxin contaminated food, reference is invited to document MYC-4b, "Health and toxicological aspects of mycotoxins" and the WHO/UNEP Environmental Health Criteria Document on Mycotoxins. (See following article in this publication)

6. TRICOTHECENES

6.1 Trichothecene-producing moulds and trichothecenes produced.

6.1.1 The trichothecenes are a group of related chemical compounds that possess a common structural nucleus. Some 37 different trichothecenes have been produced in laboratory cultures by species of Fusarium, Cephalosporium, Myrothecium, Trichoderma and Stachybotrys. The trichothecenes that have been isolated from naturally contaminated products have been associated with Fusarium or Stachybotrys species. Fusarium species that produce trichothecenes include F. tricinctum (also designated F. sporotrichoides), F. roseum, F. Solani, F. nivale and F. oxysporum. F. tricinctum is one of the fungi most frequently isolated from maize associated with toxicity to farm animals. (71, 72)

6.1.2 Only six of the trichothecenes have been isolated from field infected commodities and/or associated with disease outbreaks in animals or humans. These are T-2 toxin, deoxynivalenol, nivalenol, diacetoxyscirpenol, tetrol and neosolol. T-2 toxin has been most frequently analyzed for and reported in the literature. (71, 72)

6.2 Natural occurrence in agricultural commodities.

Trichothecenes have been reported in maize, barley and in livestock feeds of undefined cereal composition. Reports on occurrence in cereal grains have come from the U.S.A., Japan, Yugoslavia and Hungary (in feeds only). Maize appears to be the cereal most frequently contaminated. Trichothecenes also have been reported to occur in straw and hay in Eastern Europe, Russia and Finland.

6.3 Conditions for mould growth and trichothecene production. Sites of contamination.

6.3.1 The trichothecene producing Fusarium fungi prefer low temperature for growth and toxin production. Toxin formation by strains of F. tricinctum isolated from maize, oats, wheat, sorghum, fescue, hay and some other plant materials was much greater when incubated for 4 weeks at 8°C than at either 14°C or 20°C. In other studies isolates of F. poae and F. sporotrichoides produced the greatest yields of toxin when grown at 15-20°C. Cultured on white corn grits, F. tricinctum produced highest yields of toxin at 15°C; yields declined at higher temperatures and no toxin was produced at 32°C. Others have observed that alternate freezing and thawing of cultures for 9 to 15 days produced the highest yields. (73-76)

6.3.2 The natural occurrence of toxicoses associated with trichothecene-producing Fusarium species is consistent with the laboratory behaviour of these fungi. Disease outbreaks have been limited to temperate climates where the cereal grain or other feedstuffs are subjected to cool temperatures after maturation, either in the field or in storage. A period of cool, rainy weather during the growing period provides favourable conditions for infection and the development of Fusarium diseases in the field.

6.4 Adverse effects of trichothecenes-contaminated feeds on livestock and poultry production.

6.4.1 Trichothecenes depress growth and cause illness and deaths in swine, cattle and poultry. Egg production also is lowered. Trichothecenes cause emesis in swine and they often refuse to eat the contaminated feeds. Prolonged feeding of mouldy maize contaminated with a high proportion of F. tricinatum among other fungi resulted in the death of 7 of 35 cows. The feed consisted of 60% mouldy corn, soybean meal and feed supplements. T-2 toxin was found in the maize at a concentration of 2 ppm (2 mg/kg). The animals at necropsy had extensive haemorrhages on the mucosal surfaces of all viscera. The herd also had a high frequency of abortion. (77)

6.4.2 Swine administered cultures of F. sporotrichoides by stomach tube developed an ulcerous necrotic alteration of the snout, lips, mucous membrane of the mouth, progressive weakness, depression and emaciation accompanied by mucous secretion from the nose. Death ultimately resulted; some animals died within 7½ hours after intubation. (78)

6.4.3 T-2 toxin incorporated in the diets of broiler chicks at levels of 4 ppm and above depressed growth rate. After one week, lesions appeared on the palate and the hard margins of the tongue. After three weeks, the lesions had developed to an extent that the birds could not close their mouths and had great difficulty eating. The same type of lesion has been reported in commercial broiler flocks and attributed to T-2 toxin or related trichothecenes in the feed. The oral lesions reduce feed intake which results in reduced growth rate. Neural toxicity also has been observed in broiler chicks at 4 ppm and above in the feed. (79, 80, 81)

6.4.4 Maize invaded by F. tricinatum fed at 1-5% levels in the diets of laying hens caused a marked decrease in feed intake and a resultant decrease in body weight. Egg production declined or ceased abruptly in Rhode Island Red and New Hampshire breeds, whereas the production of White Leghorn hens was not greatly affected. In another study, T-2 toxin fed to laying hens at 20 ppm in the diet resulted in lowered egg production and thinner egg shells. (61, 82)

6.4.5 The deaths of one-week old turkey poults resulted after being fed a ration containing 1% of maize infected with an isolate of F. tricinatum known to produce trichothecenes. Mouth lesions similar to those observed in chickens developed and growth rate and feed efficiency were reduced. (83)

6.4.6 Mouldy maize infected with Fusarium fungi often causes emesis in swine; swine also refuse to eat some lots of infected maize. Several trichothecenes have been demonstrated to cause emesis in ducklings, cats and other experimental animals. However, the causative factor in maize has been identified as deoxynivalenol. This trichothecene also has been identified as the swine refusal factor. Chickens and cattle are less sensitive to deoxynivalenol and accept maize refused by swine. (84, 85, 86)

6.5 Association of trichothecene-contaminated foods with disease in humans.

6.5.1 Alimentary toxic aleukia (ATA) is a disease of man, acute in appearance, characterized by inflammation of the skin and mucous membrane and vomiting and followed by a subacute phase of which bone marrow depression is the predominant clinical feature. The disease, occurring epidemically in one country during the period 1931-1943, was found to be associated with ingestion of overwintered grains invaded by moulds, in particular Fusarium poae and F. sporotrichoides. Symptoms similar to ATA have been reproduced in cats by feeding cultures of the two Fusarium species. However, the fungal metabolites causally

associated with the effects in the cat where not isolated. In one sample supposed to contain the toxic principle, the following trichothecenes were detected: T-2 toxin, neosolaniol, and T-2 tetraol.

6.5.2 No relevant reproduction of the ATA symptoms in other test animals by trichothecenes has been reported. No current data on human exposure to trichothecenes relating to ATA are available. Thus no causal association between ATA in man and ingestion of specific trichothecenes can be formulated based on present knowledge.

7. CITRININ

7.1 Citrinin is produced by several *Penicillium* species including *P. citrinum*, *P. viridicatum*, *P. expansum* and *P. spinulosum*. Citrinin-producing isolates of *P. citrinum* have been obtained from yellow-coloured rice of the type associated with yellow rice disease in Japan. *P. viridicatum* appears to be most frequently associated with citrinin production in barley, wheat and oats, whereas citrinin-producing isolates of all species except *P. viridicatum* have been obtained from fermented sausages. Citrinin, however, was not reported in the sausages. Moisture contents of 16.5 to 19.5% have been stated as minimum levels for growth of *Penicillium* strains in cereal grains. In maize having a moisture content of 22%, *P. viridicatum* grew well in the temperature range 4° to 30°C. (87, 88, 88, 65, 66)

7.2 Citrinin has been found in barley in Denmark as a co-contaminant with ochratoxin. In a 1971 survey of 33 samples of barley associated with swine nephropathy, 3 of the 19 samples in which ochratoxin had been detected also contained citrinin at levels of 0.16, 1.0 and 2 mg/kg. In Canada, citrinin was detected in 13 of 18 samples of wheat, barley, oats and rye that had heated in storage. Levels ranged from 0.07 to 80 mg/kg. Ochratoxin A was present in all but one of these grain samples. Citrinin appears to be lost in fermentation. Experimental beers made from malted barley with citrinin added at 1 mg/kg malt had no detectable citrinin. (89, 90, 91)

7.3 The kidneys appear to be the major target organ in citrinin toxicosis. When citrinin was administered to rats, they developed enlarged turgid kidneys that were grey-white to pale yellow-brown in colour. Some kidneys contained sub-capsular small cysts. Other viscera were normal. (92)

8. PATULIN

8.1 Patulin is produced by several species of *Penicillium* and *Aspergillus*, including *P. urticae*, *P. claviforme*, *P. expansum*, *A. clavatus*, *A. giganteus* and *A. terreus*. *P. urticae* was isolated from a malt feed believed to be responsible for the deaths of over 100 dairy cattle in Japan. In France death occurred in cattle fed wheat contaminated with *A. clavatus* and the same mould species was found in malt that had intoxicated cattle in Germany. However, patulin was not isolated from the feedstuff in any of these cases. (93, 94)

8.2 *P. expansum* is a common decay organism in fruits and patulin has been found in West Germany in apples, pears, peaches, apricots, bananas, pineapples and grapes. Patulin was found in about 50% of 120 samples of different varieties of apples and pears suffering from brown rot. Patulin levels as high as 1 g/kg of decayed material were found 2 to 3 days after the fruit was removed from cold storage. A significant diffusion of patulin into healthy tissue was not observed in apples and pears but was in peaches and tomatoes. (95)

8.3 Patulin was found in 1 of 12 samples of apple juice at 1 mg/l in a limited Canadian survey of the commercial products. In a 1973 survey of apple juice on the U.S. market, patulin was detected in 37% of 136 samples at an average contamination level of

69 $\mu\text{g}/\text{l}$ (range 40 to 440 $\mu\text{g}/\text{l}$). The presence of patulin was shown earlier to be related to the inclusion of decayed apples with the fruit going to the juice press. (96, 97)

8.4 Although patulin-producing strains of P. expansum have been used for ripening some types of fermented sausage, patulin is not detectable in the finished product, nor is there detectable biological activity that could be related to patulin. Patulin is stable in apple juice and grape juice and dry corn, but not in orange, orange juice, flour, baked bread, cheese, wet corn or apple juice fermented with Saccharomyces spp. The disappearance of patulin has been attributed to its ready reaction with sulphhydryl-containing amino acids and proteins. (98, 99, 94)

8.5 Subcutaneous injection of patulin into rats twice a week for 61 to 64 weeks resulted in sarcomas at the site of injection. (100)

9. PENICILLIC ACID

9.1 Penicillic acid is synthesized by a large number of Penicillium and Aspergillus species, including P. puberulum, P. martensii, P. cyclopium, P. palitan, A. ochraceus and A. sulphureus. This mycotoxin was first isolated in 1913 from P. puberulum grown on maize and was shown to be toxic to laboratory animals. More recently, several strains of P. martensii were isolated from maize described as "blue-eye" diseased. The maize was high in moisture and had been stored at 5°C. Feeding this mouldy grain to mice resulted in death. Penicillic acid was identified as the toxic factor. In laboratory cultures of P. martensii, penicillic acid was produced between 5°C and 32°C with maximum production between 15°C and 20°C. There was a sharp decrease at 25°C and above, with no mould growth above 32°C. (93, 101,)

9.2 In a survey of the 1972 U.S. maize crop, penicillic acid was detected in 7 of 20 random samples at levels of 5 to 231 $\mu\text{g}/\text{kg}$. Analysis of 48 samples, all having the "blue-eye" discolouration, demonstrated penicillic acid in all samples at concentrations in the range 5 to 184 $\mu\text{g}/\text{kg}$ (average 46 $\mu\text{g}/\text{kg}$). Penicillic acid also has been detected in dried beans. Of 20 samples selected for analysis because a high proportion contained viable Penicillium cyclopium, 5 contained penicillic acid in the range 11 to 179 $\mu\text{g}/\text{kg}$ (average 82 $\mu\text{g}/\text{kg}$). (97)

9.3 P. cyclopium and other penicillic acid-producing Penicillium species have been isolated from fermented sausages and country cured ham. However, penicillic acid has not been detected in these products. Only 5% of penicillic acid intentionally added to sausage meat could be recovered after 3 days. Loss was attributed to the formation of adducts with cysteine or glutathione in the meat. (98)

9.4 Subcutaneous injection of 1 mg/dose of penicillic acid twice weekly into experimental animals produced transplantable tumours after 64 weeks. (100, 102)

9.5 There appear to be no reports of disease outbreaks in farm animals attributed to penicillic acid in mouldy feed. However, the information on its occurrence in maize, although limited, suggests that research attention should be given to its possible adverse effects.

10. ERGOT ALKALOIDS

10.1 The ergot alkaloids are produced by the fungus Claviceps purpurea, a parasite which infects rye, wheat and other small grains. The ergot disease is characterized by large purplish-black bodies or sclerotia (ergot) that replace the kernel of the infected grain. Spores of the fungus infect the plant at the time of flowering. Ten so-called peptide type alkaloids have been isolated from rye ergot: ergotamine, ergosine, ergocristine, ergocryptine, ergocornine and isomers of them. (103)

10.2 Ergotism, the disease associated with consumption of ergot infected grains, was widespread in Europe and the Far East from the Middle Ages to the early 20th century. Although large-scale episodes are now uncommon in man, small outbreaks still occur, and ergot toxicosis is frequently seen in domestic animals. (104, 105, 106)

10.3 Ergot is a serious disease in rye, a crop produced largely in Europe and the U.S.S.R. Ergot also has widespread sporadic occurrence in wheat. In the United States, it is most prevalent in the spring and the durum wheat region in the North Central States but seldom attacks other wheats. In India, the incidence of ergot in wheat is less than in rye and pearl millet (Bajra). Ergot in millet has been a serious problem in the States of Gujarat, Rajasthan and Karnataka, but outbreaks of ergotism have not been reported before 1956. The infecting agent in millet has been identified as Claviceps fusiformis (f). Cool humid weather favours the infection. The alkaloids produced are of the clavine group consisting mainly of ergoclavine and traces of elymoclavine, chanoclavine, penniclavine and setoclavine, and differ from the major alkaloids formed by Claviceps purpurea. (107, 108)

10.4 Ingestion of rye ergot has a contractile effect on the circulatory system and the uterus leading to abortion in cattle and gangrene in the extremities and at the end of the tail. Side effects include digestive derangement, hyper-sensitivity, muscular incoordination, increased glandular secretion and accelerated pulse rate. (109, 110, 111)

10.5 Ergot can be removed from grain by air classification or by flotation in salt solutions. Grade standards for small grains in many countries limit the content of ergoty kernels to safe levels, and it is only in countries where these standards are not observed that risk of ergot poisoning exists.

11. OTHER MYCOTOXINS OF POSSIBLE IMPORTANCE

Several other mycotoxins deserve mention as possible contaminants of foods or feeds. All have been obtained from cultures of moulds isolated from plant materials. In some cases, the infected plant material has been associated with a disease outbreak in farm animals or man, but the mycotoxin has not been isolated from the food or feedstuff and has not been established as the causal agent. Included in these mycotoxins are: citreoviridin, cyclochlorotine, cyclopiazonic acid, luteoskyrin, rubratoxin, sterigmatocystin, Penicillium roqueforti (PR) toxin, sporidesmin and the tremorgens.

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II. REVIEW BY COMMODITIES

12. INTRODUCTION

12.1 Since the recognition of the hazards of mycotoxin contamination in food and feed commodities that came with the discovery of aflatoxin in the early 1969's, a number of countries have conducted surveys on the incidence of mycotoxins in their agricultural produce. Most of these surveys have concerned the occurrence of aflatoxin but information on other mycotoxins, particularly zearalenone, ochratoxin A and the trichothecenes, also has been developed. This information should be useful to countries which have not yet conducted such surveys.

12.2 In this report, information on mycotoxin incidence available from the literature as well as that generously contributed directly by certain countries is collected and presented on a commodity basis. Because the incidence of mycotoxin contamination is closely associated with climatic conditions, both as it affects the particular mycotoxin likely to occur and as it creates problems in the growing, harvesting and storage of various crops, considerable emphasis is given to rainfall, temperature, and geographic regions. These data for the regions where mycotoxin contamination has been found should provide some guidance to countries which have not yet conducted surveys as to the likelihood of contamination in their domestic produce.

12.3 For each commodity attention is given to the sites at which mould infection and mycotoxin contamination is likely to occur in the growing, harvesting and storage operations so far as information is available. Data are also presented on adverse effects of contaminated grains and oilseed feeds on livestock and poultry production as observed in various countries in outbreaks of mycotoxicoses. In relation to human health, attention is given to the importance of the various commodities as foods, e.g. daily per capita consumption figures for countries and regions.

12.4 Although mycotoxin incidence, (aflatoxin incidence in particular) has been reported in countries in all regions of the world, yet data are available for only a few countries. This cannot be taken as an indication that the mycotoxin problem is limited to these countries or that the incidence and levels of contamination is necessarily higher than in other countries. Rather, it suggests that these countries are aware that a problem may exist, have been willing to discuss and share their problem with the remainder of the world and have taken steps necessary in developing a control programme.

12.5 Special thanks are expressed to the countries that have made unpublished information available which has greatly aided the preparation of this report.

13. CEREAL GRAINS: MAIZE

13.1 Maize Producing Regions, World Production and Trade

Maize is produced from about latitude 50°N which marks the northern limit of the growing areas in Europe and Asia to about 40°S in South America. It is unique among the cereals in the enormous diversity of strains that have developed to fit the widely different conditions of temperature, moisture, length of frost-free season, and other environmental conditions. Maize is a major world crop, production in 1975 totalling about 320 million metric tons. Although about one-half of the world crop is produced in North America, large quantities are produced in Central and South America, Europe, Asia and Africa. Maize is an important commodity in international trade, about 50 million tons being traded annually.

(1, 2)

13.2 Use of Maize as a Food and Feed Grain

Maize serves both as a human food and as a feed for livestock. A substantial part of the crop is used for the latter purpose, particularly in the developed market economy nations. Although maize food products are consumed to some extent in all producing nations, it is a major dietary staple in most countries of Central America, northern South America including regions in Brazil, East Africa, Southern Africa and parts of West Africa as well as Indonesia, the Philippines and sections of India. In several Central American and African countries, maize may provide 40-50% of the calories and protein in the average diet and also a significant part of the fat. The possible public health hazard from the ingestion of mycotoxin contaminated maize deserves special consideration in these areas. (3)

13.3 Mycotoxins Found in Maize

Five mycotoxins have been reported in samples of maize collected from farms or market channels. These are aflatoxin, zearalenone, ochratoxin and two trichothecenes: T-2 toxin and deoxynivalenol. Of these, aflatoxin has been investigated most intensively and most information is available concerning its occurrence and biological properties. (4,5)

13.4 AFLATOXINS

13.4.1 Aflatoxins: geographic distribution in maize in the producing regions

Distribution of the occurrence of aflatoxin in maize appears to be world-wide. Aflatoxin has been reported in samples collected in countries of North America, South America, Africa and Asia. Levels as high as 12,500 µg/kg have been found; however, most levels reported are below 1,000 µg/kg. Although analyses for aflatoxin in maize in many producing countries appear not to have been conducted, the information from other countries on conditions for its occurrence in the field and during harvesting, storage and marketing makes it probable that the aflatoxin problem exists in all maize producing countries. (6,11)

13.4.2 Aflatoxins: sites of contamination in the growing, harvesting and storage of maize

13.4.2.1 Aspergillus flavus infection and aflatoxin production in the field prior to harvest: traditionally, A. flavus development has been considered a problem that affects cereal grains after harvest. Over the past five years, however, it has been firmly established that both A. flavus infection and aflatoxin contamination may occur in maize prior to harvest. Aflatoxin in freshly harvested corn has been reported in the United States and the Philippines. Indeed, field infection may be the principal source of aflatoxin contamination in U.S. maize. In the Philippines field contamination with aflatoxin is reported to be a sporadic occurrence and when present is found below 30 µg/kg. Studies on toxin occurrence in ears of field corn inoculated with A. flavus 20 days after silking demonstrated that corn grown in areas in southern United States had significantly higher levels of toxin than did similar samples from the Midwest. Surveys conducted in Missouri, a south central state, and South Carolina, a southeastern state, showed the presence of aflatoxin in freshly harvested corn in both states. In the Missouri survey, 60 ears were collected from each of 60 fields and 600 ears in a more intensive survey of two other fields. Aflatoxin tests showed that 120/3,600 ears in the general survey and 6/600 ears in the more intensive survey contained aflatoxin B₁ at levels exceeding 20 µg/kg. No aflatoxin was detected in 750 ears collected in five fields in the adjoining state of Illinois. European corn borer and corn ear worm were the predominant types of insect damage observed on sample ears. By statistical analysis, the incidence of aflatoxin positive corn was significantly higher on ears with earworm damage than on those without. Of 195 insects collected from sample ears, 29 carried A. flavus. (12,13,14,15,16,17)

13.4.2.2 In the South Carolina survey, samples of freshly harvested corn were collected from 92 fields in a 5 000 square kilometre area. An additional 113 samples were collected at elevator delivery points during harvest. Samples from half of the fields were aflatoxin positive; those from one-third of these fields contained aflatoxin above 20 µg/kg and levels above 80 µg/kg were found in samples from 10% of these fields. The same incidence of aflatoxin positive samples was found in the elevator samples. Out of 375 insects collected, 274 contained A. flavus. Because 78 of 85 rice weevils exhibited A. flavus, it was concluded that this insect might be involved in the infection of developing corn ears with the toxin-producing fungus. There was, however, no statistically significant association between insect damage and aflatoxin contamination, and factors in addition to insect attack appear to be involved in A. flavus infection of maize in the field.

13.4.2.3 In a comparison of normal and opaque-2 endosperm counterparts of two maize hybrids that were inoculated by injection of A. flavus spores into the growing ear, fewer toxin-contaminated ears were found in one of the hybrids but there was no significant difference between normal and opaque-2 endosperm types in aflatoxin incidence. Earlier laboratory studies in India had demonstrated that kernels of an opaque-2 variety did not support as much toxin production as that from normal corn lines when tested in in vitro culture. This resistance appears not to extend to the growing ear. Further examination of strain difference showed that there was less toxin development in hybrids adapted to the growing region than in a non-adapted hybrid. Insecticide treatment reduced but did not eliminate aflatoxin in the test varieties. In this study, developing ears were inoculated with A. flavus spores by introducing the inoculum into the silk bundle. (18,19,19)

13.4.2.4 Although aflatoxin contamination in the field appears to be most likely to occur in regions of higher temperature and humidity, it can occur under other climatic conditions. In 1976, pre-harvest contamination was found in fields in some countries of western Iowa (U.S.). About 17% of 214 samples collected contained detectable levels of aflatoxin. However, only a few contained toxin at levels exceeding 20 µg/kg. The highest level observed was 56 µg/kg. Infection of ears and stalks with A. flavus was strongly associated with damage by the second generation corn borer. Drought in the affected counties was considered to be a stress factor contributing to the insect damage and A. flavus infection. (20)

13.4.2.5 Much more work is needed to explore the possibilities of developing varietal resistance to pre-harvest infection with A. flavus. Attention should be given in other regions that have warm, humid climates to the possible incidence of aflatoxin contamination of maize in the field.

13.4.2.6 Aflatoxin contamination in harvesting and storage: The moisture level in harvested maize is the most critical factor to be controlled to prevent mould growth and mycotoxin formation in the grain. This factor becomes more critical for aflatoxin contamination in regions of high prevailing temperatures since growth of A. flavus is accelerated with increasing temperature up to about 32°C. Even though growth rate may decline at higher temperatures, A. flavus continues to be competitive with other fungi up to 40°C or more when the relative humidity of the air in equilibrium with the grain is 84 to 88%.

13.4.2.7 Various conditions and faulty practices can result in mould growth and mycotoxin formation during harvesting and storage of maize. Some commonly encountered are:

- - Delay before initiation of drying of grain harvested at high moisture levels. Shelled maize is particularly at risk since there is little circulation of air through the corn to remove the heat in bulk lots whereas air movement can occur in bulk lots of ear corn. Kernel damage from shelling also facilitates mould attack.

- - Prolonged drying periods due to high humidity conditions in regions where sun-drying is practiced. This problem is important in regions where the maize crop is harvested in the rainy season. As a reference point, it should be noted that in laboratory tests aflatoxin contamination in maize has been detected 72 hours after inoculation with A. flavus and high levels were present at 96 hours.
- - Allowing the grain to be rewetted during the drying process. This may result from inadequate protection from rain during the sun drying process. When plastic sheets are used to cover the piled grain at night, condensation of water vapour from the maize on the cooler plastic sheet may wet the grain in contact with the sheet. The drying period for the grain portions that have been rewetted is prolonged and opportunity for mould growth and toxin formation is increased.
- - Failure to dry the grain to safe moisture levels before placing it in storage. The level that is safe depends on the prevailing temperature and the time that the grain will be stored. Higher moisture levels can be tolerated in cool climates. In the United States a moisture content of 13% or less is recommended for prolonged storage. However, local experts should be consulted for their recommendations. (21)
- - Storage structures which leak rain or permit seepage or migration of ground water into the grain. The latter condition presents a relatively high risk in underground storage.
- - Moisture migration and condensation that results from thermal gradients within the stored grain mass. Moisture migrates from warm to cool areas and can readily attain levels at which mould growth takes place. Lowering of external temperatures with change of season results in cooling of the grain at the walls of the storage bin and moisture migration to this cooler area. Thermal gradients set up also cause air currents to rise from the warm centre of the bulk of grain and fall down along the cooler walls of the bin. As the convected air passes through the cooler grain at the surface of the bulk, it gives up moisture and increases the moisture content of the surface layers. The moist regions that form become foci for mould growth and grain deterioration. Provision for forced air circulation or mixing the grain by moving it from one bin to another destroys the temperature gradients and reduces risk of moulding.
- - Absorption of moisture by stored grain from the atmosphere in high humidity climates. Grain drying facilities may be installed to meet this condition or the dry grain may be hermetically sealed to prevent contact with external air. In warehouse storage of bagged grain, dry grain can be protected against moisture absorption by enclosure of stacks of bags in plastic sheeting using the "ballooning" technique. (22)
- - Insect infestation of the grain. Insect damage presents a hazard to stored grain in all climates but the risk is particularly great in hot and tropical regions. Grain-infesting insects usually are inactive when the moisture content of the grain is below 9% or if the temperature is 4°C or less. At moisture contents about 13% and temperatures above 21°C insects are very active. Dry grain reduces the risk of insect infestation. Low temperatures also reduce insect activity. Insect activity can raise the moisture content of grain, particularly that which is at or above marginally safe moisture levels, to levels at which moulds will grow and produce mycotoxins. Insects also promote mould damage by serving as carriers of various mould species including A. flavus. Stored grain should be treated with fumigants and/or other insecticides to destroy the insect population, eggs and larvae. Certain fumigants such as phosphine-ammonia not only destroy insects, but also are lethal to fungi. (23)

13.4.3 Aflatoxins: incidence in maize in North America

13.4.3.1 The United States and Mexico are the principal maize-producing countries in North America. Some maize is grown in Canada but production is relatively small. Maize is the largest grain crop in the U.S. and extensive surveys for aflatoxin have been conducted, beginning about 1965. No surveys of maize for aflatoxin have been reported from Mexico, but on basis of similarities of climate to that in areas of southern United States, occurrence seems likely.

United States

13.4.3.2 The major part of the 150 million tons of maize produced annually in the United States comes from the Corn Belt States that lie between latitudes 45°N and 38°N and range from Ohio on the East to Nebraska on the west. Substantial but lesser quantities of maize are produced in the southern and southeastern states that lie between 38°N and 30°N. Average rainfall in the Corn Belt States in the growing season (April to September) varies from 50 to 60 cm and average July temperatures from 21° to 27°C. In southeastern U.S., where surveys have indicated a significant incidence of aflatoxin in maize, mean July temperatures and rainfall in the growing season range from about 25° to 28°C and 60 to 70 cm, respectively. (24)

13.4.3.3 Most of the U.S. maize crop is harvested as shelled maize with picker-shellers or combine harvesters; some is picked mechanically and stored in slatted cribs on the farm before shelling. The majority of the corn that is marketed meets the requirements for U.S. grade No. 2 which allows no more than 15.5% moisture. If this corn is to be stored for prolonged periods and into the spring and summer months, it is dried to 13% moisture or below, depending on the prevailing temperature at the storage location.

13.4.3.4 About three quarters of the U.S. maize crop is used as livestock feed and most of this in the farms where it is grown. In recent years over one-fifth of the crop has been exported and about 6-7% used in the dry- and wet-milling industries for the production of grits, meal and starch. Per capita consumption of maize, mainly in the form of grits, meal, prepared breakfast foods and snack items, is estimated to be about 18 g per day. (3)

13.4.3.5 Several surveys have been made of the incidence of aflatoxin in maize grown in different regions of the U.S. (Table II/1). Samples have been collected from fields at harvest, from farm storage and from principal markets. In sampling lots received at market centres, the lower grades represented a greater percentage of total samples collected than were received at the markets since it was expected that aflatoxin, if present, would most likely be found in these poorer grades. As Table II/1 shows, the incidence and level of aflatoxin were low in maize received in the principal Corn Belt markets in 1964, 1965 and 1967. Of 1,594 samples analyzed, 41 were positive for aflatoxin. Maize samples from export cargoes collected at 9 U.S. ports and at Montreal in 1968 and 1969 also showed a relatively low incidence of aflatoxin contamination. Only 8 of 293 cargoes were positive and of these, 3 exceeded 20 µg/kg aflatoxin. The origin of the maize was not determined but that shipped from Baltimore and Norfolk, Virginia may have been produced in Southeastern U.S.

13.4.3.6 The survey of maize received in markets in Southeastern states in 1969 and 1970 showed that 30% of the samples were positive as compared to 2.5% for Midwest maize. Twenty percent had more than 20 µg/kg aflatoxin, and the average level in all contaminated samples was 66 µg/kg. Analysis of 297 samples collected in the field at harvest in Southeastern U.S. in 1973 showed both a high incidence of positive samples and a relatively high level of contamination, supporting the result of the 1969-1970 survey of market samples. It also demonstrated that infection of maize with *A. flavus* in the field was an important source of aflatoxin contamination in the southeast. (Ref. foot-note "c" to Table II/1)

Table II/1. INCIDENCE OF AFLATOXIN IN MAIZE IN THE UNITED STATES

Collection period or crop year	Region	Total no. samples	Number posi- tive	Number >20 µg/kg	Total aflatoxins µg/kg		Description	Ref.
					Ave.*	High		
Collected 1964/1965	Midwest	1 311	35	0	10	19	All grades received at principal Mid- west markets	(a)
Collected 1967	Midwest	283	6	2	17	37	" " "	(b)
Collected 1968/1969	-	293	8	3	20	43	All grades received at 10 ports for export	(c)
Collected 1969/1970	Southeast	60	21	12-B1	66	348	All grades received at southeastern markets	(d)
Crop year 1969/1970	Southcentral	1 283	394	113	31	306	On-farm storage	(e)
Crop year 1972	Southcentral	4 200	-	126**	-	-	Field at harvest	(f)
Crop year 1973	Southeast	297	152	94	60	>640	Field at harvest	(g)

* Average of all positive samples

** Samples were analyzed by a screening method which had a sensitivity of 20 µg/kg.

References - Table II/1

- (a) Shotwell, O.L., Hesseltine, C.W., Burmeister, H.R., Kwolek, W.F., Shanmore, G.N. and Hall, H.H. 1969. Survey of cereal grains and soybeans for the presence of aflatoxin II. Corn and Soybeans. Cereal Chem. 46: 454-456.
- (b) Shotwell, O.L., Hesseltine, C.W., Goulden, M.L. and Vandegraft, E.E.. 1970. Survey of corn for aflatoxin, ochratoxin and zearalenone. Cereal Chem. 47: 700-707.
- (c) Shotwell, O.L., Hesseltine, C.W., Vandegraft, E.E. and Goulden, M.L. 1971. Survey of corn from different regions for aflatoxin, ochratoxin and zearalenone. Cereal Sci. Today. 16: 266-273.

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References - Table II/1 Cont'.

- (d) Shotwell, O.L., Hesseltine, C.W. and Goulden, M.L. 1973. Incidence of aflatoxin in Southern corn, 1969-70. *Cereal Sci. Today*. 18: 192-196.
- (e) Shotwell, O.L., Kwolek, W.F., Goulden, M.L., Jackson, L.K. and Hesseltine, C.W. 1975. Aflatoxin occurrence in white corn under loan. I. Incidence and levels. *Cereal Chem.* 52 : 373-380.
- (f) Lillehoj, E.B., Kwolek, W.F., Fennel, D.I. and Milburn, M.S. 1975. Aflatoxin incidence and association with bright greenish-yellow fluorescence and insect damage in a limited survey of freshly harvested high-moisture corn. *Cereal Chem.* 52: 403-412.
- (g) Lillehoj, E.J., Kwolek, W.F., Shannon, G.M., Shotwell, O.L. and Hesseltine, C.W. 1975. Aflatoxin occurrence in 1973 corn at harvest. I. A limited survey in southeastern U.S.A. *Cereal Chem.* 52: 603-611.

13.4.3.7 Seizure by the Food and Drug Administration of a lot of corn meal milled from maize grown in southeastern Missouri led the U.S. Department of Agriculture to analyse all lots of corn delivered by farmers in that area to the Department under the conditions of a commodity loan contract. Of 1 283 truck loads delivered, 113 had aflatoxin levels greater than 20 $\mu\text{g/kg}$ (Table II/1). Average level in all positive samples was 31 $\mu\text{g/kg}$. Moisture level in the grain and examination of farm storages and storage practices gave no evidence of improper storage and suggested that aflatoxin contamination may have occurred in the field prior to storage. In a field survey undertaken in this area in 1972, 4,200 ears were collected from 66 fields. Three percent of the ears contained more than 20 $\mu\text{g/kg}$ aflatoxin, the sensitivity of the rapid screening method used. This incidence was considerably less than that found in the earlier monitoring of farm storage, but presence of pre-harvest aflatoxin contamination was demonstrated. (Ref. foot note "e" to Table II/1) (16)

13.4.4 Aflatoxins: incidence in maize in Central America

13.4.4.1 The countries of Central America, (Guatemala, Honduras, Nicaragua, Costa Rica, Dominican Republic, El Salvador and Panama) all produce maize for domestic consumption as a food grain. Some are also importers of maize. All lie within the tropics and the seasons are more marked by differences in precipitation than in temperature. Lowlands tend to be hot, but at higher altitudes along the mountain ranges, the climate is more temperate. Precipitation is divided in most regions into a rainy and a less rainy or dry season. Annual rainfall may range up to 200 cm or more. (25)

13.4.4.2 Aflatoxin contamination in maize has been reported in two of the countries of Central America (Costa Rica and Dominican Republic). The similarities in climatic conditions in regions of the other Central American countries suggests that aflatoxin may also occur in maize grown in all countries of this region. (26,27)

13.4.4.3 In a preliminary survey conducted in one mainland country in 1975, mouldy samples of grain were selected as the most likely to contain aflatoxin. Most of the maize samples analyzed contained from 150 to 800 $\mu\text{g/kg}$ aflatoxin and some samples contained 1,200 $\mu\text{g/kg}$. Subsequently a routine surveillance of grains was initiated. Results obtained from this surveillance and from samples submitted by other government agencies and private organizations are given in Table II/2. Factors contributing to aflatoxin contamination were considered to be slow drying of maize after harvest and inadequate storage facilities near the fields. Possibility of pre-harvest contamination was recognized. (27)

13.4.4.4 In a survey of food and feedstuffs conducted in a Caribbean country (Table II/2), twenty of 24 samples of maize analyzed were positive for aflatoxin. Concentrations ranged from 45 to 900 $\mu\text{g/kg}$ with an average of 229 $\mu\text{g/kg}$. Four samples of maize flour were analyzed; all were negative. (26)

13.4.4.5 A disease in poultry, suspected to be a mycotoxicosis, has caused a high mortality in chicks in some flocks in one mainland country. Although signs are similar to those of aflatoxicosis, aflatoxin has not been detected in the livers of sick birds. (27)

Table II/2. INCIDENCE OF AFLATOXIN IN MAIZE SAMPLES IN CENTRAL AMERICA

Type of sample	No. of samples analyzed	No. positive	Aflatoxin concentration $\mu\text{g/kg}$	
			Range	Highest
<u>Mainland country</u>				
<u>Maize:</u>	120	77	30 - 70 100 - 500	3,787
flour, starch	79	50	-	-
prepared foods	12	1	-	-
<u>Feedstuffs:</u>				
dairy and pigs	25	19	30 - 70	320
poultry	50	13	30 - 60	194
<u>Caribbean country</u>				
Maize	24	20	45 - 900	900
Maize flour	4	0	-	-

13.4.5 Aflatoxins: incidence in maize in South America

13.4.5.1 Maize is an important cereal crop in nearly all of the countries of South America where it is grown under climatic conditions ranging from the tropical climate of the northernmost countries to the temperate climates of the south. It is a dietary staple in most countries. Limited information is available on the incidence of aflatoxin in maize grown in South America, reports on its occurrence coming only from Colombia in the tropical region and Brazil, where maize is grown in the southern temperate region of that country.

Colombia

13.4.5.2 Colombia is in the northernmost part of South America, lying between 12°N and 2°S latitude. It has a generally tropical climate without any real change in season. Maize is a major cereal grain and dietary staple in Colombia; total production was 723,000 tons in 1975 and per capita consumption is about 45 g daily. Maize is grown in every state west of the Andes. Rainfall ranges from 25 - 150 cm in some states, to 200 - 350 cm in others. (23, 7)

13.4.5.3 Several cases of aflatoxicosis in fowl have been reported in Colombia during the past two years, 14 cases in 1975 and 41 in 1976. Diagnoses were based on necropsies of the birds. Poultry feeds are formulated primarily from maize, sorghum, soybean meal and cottonseed meal. (7)

13.4.5.4 A limited exploratory survey of cereals and legumes in an area in one state revealed aflatoxin contamination in half of the white maize samples analyzed and in all of the samples of yellow maize. (7)

Brazil

13.4.5.5 Maize is Brazil's largest cereal crop. Production in 1975 was 16.5 million tons of which 1.2 million tons were exported. The remainder was used as food and as livestock feed. In food, maize is used as a meal to make special cakes; another product is fermented macerated corn, wet milled and roasted. Per capita daily consumption was 53 g in 1964-66, the latest date for which figures are available. (2,3,6)

13.4.5.6 The largest production of maize in Brazil is in the southeastern states in regions lying between about 15° and 30°S. Most of the southeast receives abundant rainfall but in the interior the rains are concentrated in a rainy season from October to April. (25)

13.4.5.7 No surveys of maize for aflatoxin appear to have been conducted in Brazil. However, toxicoses in chicks attributed to aflatoxin in maize have been reported. Aflatoxin was found in the range of 1,000-2,000 µg/kg. (6)

13.4.6 Aflatoxins: incidence in maize in South and Southeast Asia

13.4.6.1 Aflatoxin in domestically produced maize has been reported in India, Thailand and the Philippines. Indonesia, the other Asian country which has reported analyses of maize, found no aflatoxin in the samples examined.

India

13.4.6.2 Maize is grown in 16 of the 21 states of India, 14 in central and northern India, and 2 in southern India. Thus it is grown in tropical as well as temperate regions. Rainfall in the maize producing regions ranges from about 50 to 125 cm or more, coming in the monsoon season which extends from June-July up to the end of September. Droughts and floods occur alternately in various states due to the vagaries of the monsoons. Maximum daily temperatures in most of the states range from 23-26°C but in some corn-growing states, it reaches 42°C in the summer months. In the cold season beginning in December, temperatures fall 5° to 11°C below normal and frost occurs in Punjab and more northern areas. (10)

13.4.6.3 Maize is harvested by cutting the ears from the stalk in the field, spreading them in the sun to dry and then beating them with sticks to separate the grain. (10)

13.4.6.4 Maize production in India is about 6.1 million tons, a large part of which is consumed as human food. Per capita consumption on a national basis is about 20 g daily but is higher in regions where maize is a dietary staple. (3, 10)

13.4.6.5 No systematic survey of maize in India for aflatoxin contamination has been reported but aflatoxin contaminated maize was identified as the causative agent in a toxicosis which occurred in two western states in 1974. About 400 people, predominantly males in the age groups of 5-14 and above 30, were affected and about 25 percent of them died of the disease. Maize, the staple food in the area, was found to be contaminated with Aspergillus flavus and aflatoxins levels ranged from 25 to 12,500 $\mu\text{g}/\text{kg}$. The disease was characterized by jaundice, rapidly developing ascites and portal hypertension. In an autopsied human liver, bile duct proliferation and multinucleate giant cells were found on histopathological examination. Heavy rains had occurred in October, drenching the maize in the field and continuing during harvest. Poor storage conditions contributed further to mould damage (10,28,29,30)

13.4.6.6 The outbreak of aflatoxicosis in humans was accompanied by aflatoxicosis in dogs. In the households where humans were affected, dogs were also affected and most of them died. The dogs were fed leftovers from the diets of humans. Signs were anorexia in the early stages and rapidly developing ascites later, resulting in high mortality. (10)

Thailand

13.4.6.7 Maize is second to rice in cereal grain production in Thailand; about 3 million tons were produced in 1975. However, about 80% of the crop is exported and little is used for human food. Per capita daily consumption is about 0.8 g. (2,3)

13.4.6.8 Thailand has a rainy season from July to October, a dry season from November through February, followed by a hot season from March through June. Monthly rainfall in the rainy season exceeds 20 cm, while in the dry season it averages 1 to 2 cm per month. Seasonal variation in temperature is relatively small. The average temperature in the dry season is 24-26°C; that in the hot season is 28° to 30°C. (31)

13.4.6.9 A survey was made of market samples of foodstuffs collected from more than 100 towns and villages in Thailand during 1967-1969. Among the foods collected were 62 samples of maize. On analysis for aflatoxin, 35% were positive. Average level in the contaminated samples was 400 $\mu\text{g}/\text{kg}$ total aflatoxins; highest level observed was 2,730 $\mu\text{g}/\text{kg}$. Since these samples were collected from local food markets, the results may not apply to maize that is exported. (11)

13.4.6.10 For all Thai foods examined, the mean concentration of aflatoxins was highest in the rainy season and lowest in the hot season. There was, however, a poor correlation between aflatoxin contamination and rainfall in different regions. There was a better association between areas in which frequent short-term floods occurred and high levels of aflatoxins in foods. Stored foods were damaged by the flood waters and also, as the flood waters receded many crops were harvested and sun-dried, a process which was slow because of the high humidity and intermittent rains. (11)

The Philippines

13.4.6.11 Maize is the second major food crop, next to rice, produced in the Philippines. Average production, 1971-1975, was 2.1 million metric tons. Maize is grown in each of the eleven administrative regions but, on the average, more than half of the corn produced is grown in the Mindanao area; over one quarter is produced in Luzon, while less than one fifth is produced in the Visayas. About 80% of the maize crop is used as human food and most of this is in the form of corn grits; on a per capita basis this amounts to 65.9 g per day. Animal feed takes about 14% of the crop and industrial uses about 3%. (32)

13.4.6.12 The Philippines has a climate that is generally tropical. Mean temperatures vary little from 27°C either monthly or regionally. Maximum temperatures average 31.1°C while minimum temperatures average 22.8°C. Rainfall in the Philippines varies considerably as a result of the diverse topography. Average annual rainfall is 253 cm with an average of 176 rainy days per year. Annual rainfall in the principal corn-growing regions is 233 cm in Mindanao, 272 cm in Luzon and 239 cm in the Visayas. January through April are months of relatively lower rainfall, particularly in the Luzon and Visayas regions.

13.4.6.13 In Luzon, two crops of maize are generally planted each year; a third crop is usually planted in Mindanao. Harvesting in Luzon and Visayas is done as much as possible on a day without rain. In Mindanao, due to the year-round rainfall and the scheduled day of pick-up of the grain by the buyers, harvesting is done irrespective of the weather. Most Mindanao farmers sell their maize as soon as possible after maturity. (32)

13.4.6.14 Corn is harvested by picking and husking by hand. Shelling may be done by the buyer who does the shelling at the farm site upon purchase. Drying is then undertaken after the shelled corn has been taken to the buyers assembly place. Several days may sometimes elapse before drying can be started. Drying is accomplished by spreading the corn in the sun on mats or cemented drying areas. The lot is piled at night to protect it from the high humidity air, or when it rains during the day, by covering it with plastic sheeting. Problems encountered are rain seepage under the plastic sheeting and sweating on the inside surface of the plastic cover from moisture condensation. Some large farm owners and grain buyers have mechanical driers and use these for commercial corn and rice. Generally driers are used only for corn that cannot be accommodated in the sun during space, or on rainy days. Corn retained for home consumption is dried on the cob, stored under the house or in a storage hut, shelled, dried again and again stored until milled and used. (32)

13.4.6.15 Common containers for stored shelled corn are sacks, wooden boxes and cans. Larger wholesalers and millers use metal tanks, concrete storage silos, warehouses and other sophisticated storage facilities. The large millers move their stored corn from one bin to another if storage is to be longer than two months.

13.4.6.16 A large number of samples of maize and maize products have been analyzed for aflatoxin by the Food and Nutrition Institute of the Philippines government. Their findings are presented in Table II/3. Highest incidence and level appears to occur in dried shelled maize and grits milled from shelled maize. Nearly all samples were positive for aflatoxin; average level in those positive was 77 µg/kg. (32)

13.4.6.17 Results of a survey of maize and maize milling products collected in different areas of the Philippines are given in Table II/4. Aflatoxin was found in products collected in all areas. The number of samples collected was insufficient for conclusions regarding the relative incidence and levels of aflatoxin contamination. Important to note, however, is the lower content of maize grits, the most important food product from maize in the Philippines, as compared to the maize from which this product is milled. Comparison of samples D-1 and D-2 shows a six-fold lower aflatoxin content in grits than in the whole kernel. (32)

13.4.6.18 Incidence of aflatoxin contamination of maize in the field is reported to be sporadic and, when present, is found only below tolerance levels. The major contamination is considered to occur in drying and handling after harvesting and in subsequent storage of the grain. (32)

Table II/3. AFLATOXIN LEVELS IN PHILIPPINE MAIZE FOOD PRODUCTS

	No. of samples	Percent negative	Percent > 20 $\mu\text{g/kg}$	Average of positive samples $\mu\text{g/kg}$	Range $\mu\text{g/kg}$
<u>Maize</u>					
dried, shelled & grits	675	6	66	77	neg-1,331
prepared with salt	51	61	10	19	neg- 78
prepared with sugar & coconut	45	38	22	27	neg- 160
prepared with sugar	29	59	3	8	neg- 36
boiled	24	33	0	9	neg- 20
other prepared with corn	12	50	33	25	neg- 62
raw, freshly harvested	9	100	0		
canned	4	25	0	6	neg- 9
starch	6	50	17	8	neg- 25
bran	2	0	100	54	37-71

Table II/4. AFLATOXIN CONTENT OF SAMPLES OF MAIZE AND MAIZE PRODUCTS COLLECTED IN DIFFERENT AREAS OF THE PHILIPPINES

Sample description	Area collected	Aflatoxin B ₁ $\mu\text{g/kg}$
B-1 Whole corn on cob, drying yard, rural area	Bacolod	343
B-2 Whole corn, shelled, drying yard, related to B-1	"	107
B-3 Corn grits for human consumption, mill in rural area	"	29
C-1 Whole corn, shelled, used in milling	Cebu	343
C-2 Corn starch derived from C-1	"	14
C-3 Corn gluten meal derived from C-1	"	333
C-14 Whole corn, shelled, representative of animal feed; grade available on Cebu market	"	817
D-1 Whole corn, shelled, dry, ready for milling into human food	Davao City	503
D-2 Corn grits from D-1	"	86
D-3 Corn germ from D-1	"	233
D-8 Whole corn, shelled, for milling	"	6
GS-1 Corn grits, freshly milled	General Santos	86
GS-4 Corn germ cake	"	83
GS-5 Corn oil filter residue from oil refining	"	600
GS-6 Whole corn, shelled, drying on slab	"	traces
I-7 Corn grits, human food, made from local corn obtained at mill	Iloilo	43
I-8 Whole corn on cob, dry, from farmers stock, presumably for feed but probably for food, too	"	378

13.4.7 Aflatoxins: incidence in maize in Africa

13.4.7.1 Maize is an important cereal crop in most countries of East, West and Southern Africa. It is used both as food and as feed for farm animals. Per capita daily consumption as human food ranges from about 40 g to over 300 g. Aflatoxin incidence in maize has been reported in 3 countries in East Africa - Mozambique, Kenya and Uganda; Swaziland in Southern Africa; and Ghana and Nigeria in West Africa.

13.4.7.2 Data on aflatoxin incidence in foods in Mozambique, Kenya and Swaziland were obtained in surveys designed to investigate the possible relationship of aflatoxin intake to incidence of primary liver cancer. The areas selected yielded data on aflatoxin incidence in relation to the varied climatic conditions that exist within a country. The information gained in these surveys together with that in reports from the other countries indicates that aflatoxin incidence in maize is a widespread problem in Africa.

Kenya

13.4.7.3 Maize is the dietary staple in Kenya. Per capita daily consumption is about 285 g, providing about 45% of the average calorie intake. The Murang'a region of Kenya in which samples were collected for aflatoxin analysis has an annual rainfall ranging from 87 cm in the low altitude area (1,200 m) to 200 cm in the area of high altitude (2,400 m). Rainfall in the middle altitude areas is 100-125 cm annually. (33)

13.4.7.4 Information on aflatoxin contamination of foods in Murang'a was obtained in an epidemiological study of primary liver cancer. Although this restricted region provides a diversity of climatic conditions, it cannot be taken as representative of the temperatures and rainfall conditions met in all of Kenya. The eastern plateau region, for example, receives only 50 to 75 cm rainfall annually, while the arid regions receive less than 50 cm. The coastal region, however, has a mean annual temperature of more than 27°C and rainfall between 100 and 150 cm.

13.4.7.5 In this epidemiological study, samples of prepared meals were collected from houses at several locations within each altitude area in each of four seasons for one year. Frequency and level of aflatoxin contamination increased in going from high to low altitude areas. Although maize was not analyzed separately for aflatoxin, it was the principal suspect dietary staple and comprised about 40% of the dry weight of average diets. No groundnuts are grown or eaten in the Murang'a district. Based on a contamination level of 1 to 21 µg/kg in the positive diets, these levels would be equivalent to contamination levels of 7 to 160 µg/kg in the maize. (33)

Swaziland

13.4.7.6 Maize is the staple diet in Swaziland; per capita daily consumption is about 315 g providing about 40% of the dietary calorie intake. Production in 1975 was 120,000 tons of which about half was grown in the middleveld which averages about 650-700 m in altitude. Maize is also grown in the highveld (1,000-1,350 m altitude) and lowveld (150-300 m altitude). The highveld has a near temperate climate with warm summers while the lowveld is subtropical and semi-humid. Mean maximum temperature in the lowveld is 29°C, about 6°C higher than that for the highveld. Frosts are common in the winter in the highveld and occur, to a lesser extent, in the lowveld. Rainfall is greatest in the highveld and averages about 127 cm while that in the lowveld is about 66 cm. (34)

13.4.7.7 Under the conditions of high temperatures and low humidity in the lower altitude areas, maize loses moisture rapidly at maturity and is harvested in fairly dry conditions. Maize is stored in the ear on open storage platforms in the lowveld but cribs inside storage huts with open-structured walls are used in the middle- and highvelds. After storage for 3-4 months, the maize is shelled and the grain is stored in underground pits, clay pots and woven grass baskets. Pit storage presents more hazard from water damage than

the other two containers which are kept under cover. Other types of storage which are gradually replacing the traditional pits, clay pots and baskets, include small tins, 200-l drums, sacks and metal silos. (34)

13.4.7.8 Samples of composite prepared meals were collected at three principal locations in each of the altitude areas every two months for one year. In addition to the meals, a sample of beer, sour porridge, sour drink and groundnuts, were collected. The main meal consisted of maize porridge with one or more of the following: vegetables, meat, spinach, groundnuts, sourmilk, pumpkin, sweet potato and various kinds of beans and pulses. Greatest frequency and highest level of aflatoxin was found in meals collected from the lowveld region. Unfortunately, maize samples were not analyzed separately and since practically all meals contained maize, its contribution could not be estimated. It is noteworthy, however, that some samples of the sour porridges and beer, fermented maize and/or sorghum products, were positive for aflatoxin. In another study, 4.3% of 418 samples of maize collected in Swaziland were found to be positive for aflatoxin and contained 10 µg/kg or more. The samples were described as mostly good quality grain and were from maize stored above and below ground. (34, 35)

Uganda

13.4.7.9 Maize ranked first among the cereal grains in production in 1975, 870 thousand tons being produced in that year. Utilization is mainly as human food. Per capita daily consumption of maize meal was 40 g in 1964-66. (2,3)

13.4.7.10 Uganda lies in equatorial East Africa. The climate is characteristically tropical; in the north the climate is roughly divided into a wet season from April to October and a dry season that lasts from November to March. In the south there are two rainy seasons, April to May and October to November. Annual rainfall ranges from less than 37 cm in the northeast to 200 cm in the southeast. (25)

13.4.7.11 A survey conducted in 1966-1967 has provided information on the incidence of aflatoxin in Ugandan cereals, legumes and other foodstuffs. A total of 480 samples were collected from mud and thatch family graneries where food was stored for use between harvests, or from foodstuffs for sale in local village markets. All districts in Uganda were sampled. Of 49 maize samples analyzed, 45% were aflatoxin positive, 26% had aflatoxin levels between 1 and 100 µg/kg and 9% had levels between 100 and 1,000 µg/kg. (8)

13.4.7.12 No difference was observed between toxin levels in foods grown and consumed at home and those purchased locally. Incidence of toxin contamination in foods did not appear to correlate with mean temperature or humidity of the area in which the samples were collected. Indeed, the highest incidence of aflatoxin contamination was found in the district which has the lowest average annual rainfall. Despite its low average annual rainfall, however, rainfall is concentrated into a short rainy season once or twice a year, providing conditions for mould growth on freshly harvested or stored grains. A similar condition of heavy rains and flooding was held responsible for fungal infection and aflatoxin contamination of grains in Thailand.

Mozambique

13.4.7.13 Maize is the major cereal crop and the principal dietary grain staple in Mozambique. Per capita daily consumption is about 145 g. The Inhambane area, where the samples of maize for aflatoxin analysis were collected, lies in the coastal region of the tropical savanna country of Mozambique which extends south of the Tropic of Capricorn. Average annual temperature is about 23°C. There is a distinct rainy season in southern Mozambique which extends from October or November until March. The dry season usually lasts from April into September and sometimes longer. Southern Mozambique suffers from drought and low rainfall except in the coastal region and against the Rhodesian Plateau slopes. In the Inhambane area, typical tropical heat and humidity prevail during summer with only slight abatement during winter. (3,25,9)

13.4.7.14 Samples of stored dry foods, including maize, were collected from locations within each of six subdistricts in the district of Inhambane. Collections were made during each season from each subdistrict over a period of 3 years. In this period, 168 samples of maize were collected. Average aflatoxin level of all samples was 2.4 $\mu\text{g}/\text{kg}$. No values were reported for the percentage of samples that were aflatoxin positive nor for the average level in the contaminated samples. (9)

Ghana and Nigeria

13.4.7.15 Ghana and Nigeria lie in equatorial West Africa. Both have tropical climates with wet and dry seasons. Maize is a dietary staple in both countries. No systematic surveys of the incidence of aflatoxin contamination in maize appear to have been made. In Ghana, analyses of maize samples declared unfit for food use were found to contain 180-250 $\mu\text{g}/\text{kg}$ aflatoxin B₁. (25, 36)

13.4.8 Aflatoxins: incidence in maize in Europe

13.4.8.1 The maize producing countries in Europe lie in a belt bounded on the north by 48°N latitude and extending eastwards from France through central Europe. Reports on analyses of maize are available from only three of these countries: France, Yugoslavia and Hungary. All report a low incidence of aflatoxin.

France

13.4.8.2 France produces about 8 million tons of maize annually, largely in the southwestern part of the country. A 1970 report on the analysis of samples taken from 34 commercial lots of maize showed that 5 contained aflatoxin B₁ concentrations greater than 10 $\mu\text{g}/\text{kg}$. The maximum level observed was 187 $\mu\text{g}/\text{kg}$. Analyses of 75 samples of visibly mouldy maize from the 1974 crop that had been stored in cribs until March detected aflatoxin in only 2 at levels than than 10 $\mu\text{g}/\text{kg}$. (37, 38)

Yugoslavia

13.4.8.3 Maize production in Yugoslavia is about 9 million tons annually, most of which is used domestically as food and livestock feed. Per capita daily consumption as human food was 74 g in 1964-1966, the latest dates for which figures are available. (2,3)

13.4.8.4 Yugoslavia has a temperate climate with cold winters and hot summers in much of the interior area. Annual rainfall is 60 to 90 cm in coastal areas, increasing to 250 cm in some inland regions. (25)

13.4.8.5 Unsuitable climatic conditions during the ripening and harvesting periods often result in mould invasion of maize. In 1972, more than half the maize production was infected by fungi. of 50 samples of maize collected that year, nine contained aflatoxin at levels of 4 to 20 $\mu\text{g}/\text{kg}$ in 7 of the samples, and 130 to 150 $\mu\text{g}/\text{kg}$ in 2 of the samples. (39)

13.4.8.6 A survey of the 1975 maize crop revealed no aflatoxin contamination. Samples were collected in the autumn following harvest and in the spring from terminal elevators, feed processing plants and farms. (39)

13.4.8.7 A survey of foodstuffs reported in 1976 showed that 20% of 141 samples contained detectable levels of aflatoxin B₁. (39)

Hungary

13.4.8.8 Maize production in Hungary was 7.1 million tons in 1975, most of which was used for livestock feed. Hungary has a temperate climate with cold winters and warm summers. Annual rainfall ranges from 50 to 60 cm in lowland and 60 to 70 cm in upland areas; most falling in the growing season. (25)

13.4.8.9 It is reported that although Hungarian strains of Aspergillus flavus often produce aflatoxin in laboratory cultures, this toxin has never been encountered in domestic products. (40)

13.5 ZEARALENONE

13.5.1 Zearalenone: geographic distribution in maize in the producing regions. Zearalenone has been reported in maize grown in North America, Europe and Africa. Highest incidence is in regions having low temperatures following the harvest season. These regions include northern areas of the Corn Belt in the United States, a belt in Europe extending through France and the middle European corn-growing countries, and regions in southern Africa. An exception appears to be Zambia which is located in the tropical zone of Africa. (2,3)

13.5.2 Zearalenone: sites of contamination in the growing, harvesting and storage of maize. Present evidence indicates that production of zearalenone in maize prior to harvest is not a major source of contamination. Zearalenone has been found in freshly harvested maize in Indiana but at a low incidence and level. This finding in the field is supported by experiments in which growing ears of maize were inoculated with spores of F. roseum. Zearalenone was produced in some ears but levels never exceeded 5 mg/kg.

13.5.2.1 Although zearalenone formation in the field appears not to be the major site of contamination, the producing Fusarium species are common field moulds and also may invade the ear prior to harvest. Fusarium infection in the field is favoured by wet weather and late harvest. It would be expected that zearalenone formation would occur in mould infected mature maize that remains in the field for a prolonged period during cold weather.

13.5.2.2 Storage of high-moisture maize with an established infection presents a high risk of toxin production particularly if the grain is stored at low ambient temperature. The higher incidence of hyperestrogenism in swine from midwinter to early spring in northern United States indicates that accumulation of zearalenone occurs in stored maize.

13.5.3 Zearalenone: incidence in maize in North America

13.5.3.1 Zearalenone has been reported in maize in two North American countries: the United States and Mexico. Surveys for zearalenone in the United States have been more extensive and the situation in that country will be discussed first.

United States

13.5.3.2 Climatic conditions have been described in Section 13.4.3. Results of surveys of maize for zearalenone will be presented in this section.

13.5.3.3 Surveys have been reported by the Northern Regional Research Centre, U.S. Department of Agriculture and the U.S. Food and Drug Administration (FDA). Results are given in Table II/5. In the first survey listed, maize samples were collected by the Grain Inspection service of the U.S. Department of Agriculture from principal corn markets in the Corn Belt and, in the second survey, from export cargo at 9 U.S. and 1 Canadian port. In the first survey, zearalenone was detected in 1 of 283 samples at a level between 0.4 and 0.9 mg/kg; in the second survey, 2 samples out of 293 analyzed had zearalenone concentrations in the same range. Higher incidence and levels were found in a survey of the 1972 crop corn

conducted by FDA. In this survey samples were collected in areas where Fusarium damage had been reported or where potential for Fusarium contamination was considered to be high. Weather conditions in 1972 were favourable for Fusarium contamination in certain northern areas of the Corn Belt. Thirteen of the 223 samples collected contained zearalenone and four had zearalenone levels exceeding 1 mg/kg. (Ref. foot note "c" to Table II/5)

13.5.3.4 In two other surveys conducted by FDA, 160 samples of 1973 crop year maize were collected from farms and country elevators in the Corn Belt and 146 samples from similar locations in southern states of the U.S. Levels in positive samples were below 0.4 mg/kg in both surveys but a higher incidence of positives, 10/169, was found in the Corn Belt samples than in the samples, 1/146, from the southern states. This is consistent with laboratory findings that low temperatures increase zearalenone production by F. roseum. (41)

13.5.3.5 Levels of zearalenone in maize associated with disease outbreaks in farm animals in the U.S. are much higher than those found in the surveys just discussed. It would be expected that disease outbreaks would identify lots of heavily contaminated grain. Unfortunately, the cultural, harvesting and/or storage conditions contributing to zearalenone contamination in these lots was not reported. Although the incidence of zearalenone toxicosis reported in the United States is greater than that reported from other countries, this might be expected in view of the large animal population fed maize in the U.S. and the experience and facilities that have been developed for identification of the causative agent involved.

Mexico

13.5.3.6 Maize is a dietary staple in Mexico; production in 1975 was 9 million tons and per capita daily consumption about 310 g. Only one report on the incidence of mycotoxins in maize grown in Mexico has been available. This was a study of the incidence of zearalenone conducted by the University of Mexico in cooperation with the University of Minnesota. Of 139 samples analyzed, 6 were positive for zearalenone. However, levels of zearalenone in the samples was not stated. (e) (Ref. foot-note "e" to Table II/5)

Table II/5. SURVEYS OF CORN FOR ZEARALENONE IN THE UNITED STATES

(From: Shotwell, O.L., 1976. Assay methods for zearalenone and its natural occurrence. U.S. - Japan Conference on Mycotoxins in Human and Animal Health. University of Maryland, U.S.A.)

Year	Agency surveying	Origin samples	Type sample and source	No. of samples assayed	Per cent samples with indicated level of zearalenone (mg/kg)				Reference
					ND ^a	<0.4	0.4-0.9	1.0-5.0	
1967	NRRC	Corn belt	Grain inspection AMS	283	99		1		(a)
1968-1969	NRRC	Export cargo	Grain inspection AMS	293	98		2		(b)
1972	FDA	Corn belt ^b	Elevator & food processing	223	83	9	4	4	(c)
1973	FDA	Corn belt	Farm & country elevator	169	90	10			(d)
1973	FDA	South ^c	Farm & country elevator	146	99	1			(d)
	University of Minnesota	Mexico	For human consumption	139 ^d	96				(e)

^a ND - Not detected.

^b Area where potential for Fusaria contamination was considered to be high or where Fusaria damage had been reported.

^c Includes Southeast, Appalachia,

^d Levels in six positive samples were not reported.

References - Table II/5

- (a) Shotwell, O.L., Hesseltine, C.W., Goulden, M.L. and Vandegraft, E.E. 1970. Survey of corn for aflatoxin, zearalenone and ochratoxin. Cereal Chem. 47: 700-707.
- (b) Shotwell, O.L., Hesseltine, C.W., Vandegraft, E.E. and Goulden, M.L. 1971. Survey of corn from different regions for aflatoxin, ochratoxin and zearalenone. Cereal Sci. Today. 16: 266-273.

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- (c) Eppley, R.M., Stoloff, L., Truckress, M.W. and Chung, C.W. 1974. Survey of corn for Fusarium toxins. J. Assoc. Off. Anal. Chem. 57: 632-635.
- (d) Stoloff, L., Henry, S. and Francis, O.J. 1976. Survey for aflatoxins and zearalenone in 1973 corn crop stored on farms and in country elevators. J. Assoc. Off. Anal. Chem. 59: 118-121.
- (e) Mirocha, C.J., Christensen, C.M. and Nelson, G.H. 1972. Analysis of zearalenone (F-2) and its naturally occurring derivatives. 2nd I.U.P.A.C. Symposium "Control of Mycotoxins". Kungälv, Sweden, page 30.

13.5.4 Zearalenone: incidence in maize in Africa

13.5.4.1 Although most countries of East, West and Southern Africa produce maize, zearalenone has been reported in only three: Swaziland, Lesotho and Zambia. This may be ascribed to the lack of exploratory surveys, but it would also be expected on basis of climate, experience in Europe and N. America indicating that highest incidence of zearalenone in maize and other cereals occurs in temperate zones. In this respect, occurrence of zearalenone in maize in Zambia seems to be exceptional. However, a strain of Fusarium roseum "Gibbsum" was isolated from sorghum grain in Texas which, in laboratory culture, produced higher yields of zearalenone at 25° than at 10°C and such strains may represent an adaptation of a zearalenone-producing organism to a warmer region. (45)

Zambia

13.5.4.2 Zearalenone contamination of maize in Zambia is associated with ear rot disease which has increased incidence in seasons when late rains occur and ears are prevented from drying at harvest. The problem in market maize appears to have been accentuated by the introduction of mechanical harvesting, possibly by reducing the amount of hand-cleaning normally practiced on the farm prior to marketing. In 1974, a season of relatively high incidence of zearalenone contamination, up to 25% of survey samples in southern and central provinces contained between 6 and 12% mouldy grain, with Fusarium spp. being the main fungi present. In contrast, in 1975 mouldy grain levels did not exceed 5% in any sample and Diplodia were the main mould species present. The occurrence of zearalenone in relation to extent of mould damage found in the 1974 crop is given in Table II/6. Zearalenone content paralleled the percentage of diseased grain; the maximum level found was 1.8 mg/kg. In 1975 the maximum level found was 0.15 mg/kg. Results for 1976 are presented in Table II/7. It is interesting to note that no aflatoxin was found in the grain. (45)

Table II/6. ZEARALENONE CONTENT AS RELATED TO MOULD DAMAGE IN THE 1974 MAIZE CROP IN ZAMBIA

Diseased grain category	Percent of samples in each diseased grain category		Zearalenone content mg/kg	
	Southern Province	Central Province	Southern Province	Central Province
0 - 2%	20	10	0.2	0.2
2 - 4%	30	30	0.2	0.2
4 - 6%	25	18	0.8	-
6 - 8%	0	18	-	0.3
8 - 10%	5	6	1.5	-
10 - 12%	5	0	-	-
> 12%	15	12	1.8	-

Table II/7. ZEARALENONE CONTENT OF MAIZE RECEIVED AT LUSAKA DEPOT, ZAMBIA, JULY 1976

Diseased grain category	Zearalenone content mg/kg
Reject	4.0
Cleaned	None detected
Uncleaned	None detected
Incoming samples:	
7.0% Fungal infestation	0.06
5.0% Fungal infestation	* < 0.05
4.5% Fungal infestation	* < 0.05

* 0.05 is the limit of detection

13.5.4.3 Zearalenone was found in commercial and home-made beer brewed from maize. Levels found in home-made beers prepared from the 1974 crop harvested in different areas are given in Table II/8. The zearalenone content of the maize (in a quantity equivalent to a litre of beer) from which the beer was brewed also is given in Table II/8. Little, if any, zearalenone appears to be lost in the brewing process. The high level, 2.47 mg/l, in Mumbwa beer was attributed to the local practice of using mouldy maize for brewing. Intake of zearalenone among beer drinkers in such a high level area was estimated to be about 0.45 mg per day. Zearalenone was present in samples of commercial beer from one of the two sources surveyed at a level of 0.34 mg/litre. (46)

13.5.4.4 Although no cases of overt disease had been definitely attributed to feeding mouldy grain to livestock, a common practice in Zambia, there is concern that instances of poor growth rate, infertility, leg-weakness and scouring in pigs could be related to the feeding of mouldy maize.

13.5.4.5 In 1975, the National Agricultural Marketing Board (NAMB) of Zambia introduced two additional market grades for maize, D and E, which permitted <2 to 5%, and <5 to 10% mouldy grain content, respectively. Upon introduction of the new grades, NAMB installed grain cleaners in order to recover grain suitable for human consumption from the mouldy grain purchased from farmers. Cleaning is accomplished by removal of the lighter mouldy kernels by air separation. Average yield of cleaned maize in 1975 was 75% of the contaminated stock. (46)

13.5.4.6 Prices paid to the farmers for grades D and E averaged about 35% of the prices for acceptable grades. However, even at this discounted price, it was considered doubtful whether the return realized from the cleaned grain recovered cleaning, handling, storage, and other costs involved.

Table II/8. ZEARALENONE CONCENTRATION OF BEER IN ZAMBIA AS RELATED TO DISTRICT OF MAIZE GROWTH

District	Beer		Maize	
	mg/litre*	No. of samples	mg/litre**	No. of samples
Lusaka Urban	0.21	3	0.30	3
Mumbwa	2.47	8	0.48	6
Mozabuka	0	8	0	7
Lusaka Rural East	0.25	4	0.28	4

* Ng/litre = ppm

** Ng of zearalenone in the maize used to make one litre of beer

Swaziland

13.5.4.7 Climatic conditions are described in section 13.4.7 together with grain storage practices in Swaziland. Mixed foodstuffs from the survey of home-prepared meals for aflatoxin, also described in section 13.4.7 were analyzed for zearalenone, as were the 55 samples of sour drinks, sour porridges and local beers that were collected concurrently with the prepared meals. These products are prepared by fermentation of maize and sorghum meals or flours for various lengths of time. Zearalenone was not detected in the meal samples. Of the 55 samples of fermented products, six contained zearalenone at concentrations of 800 to 5,300 $\mu\text{g/kg}$. Five of the samples were collected from higher altitude regions where the climate is cooler and apparently more favourable for zearalenone formation. Two samples of mouldy maize collected from the field, one contaminated with Fusarium moniliforme and the other with Diplodia zeae, contained zearalenone. Aflatoxin, ochratoxin, sterigmatocystin and patulin were not detected in these two maize samples. (47)

Lesotho

13.5.4.8 Lesotho is located in southern Africa between 29° and 31°S latitudes. The westward side, comprising about one quarter of the total area of Lesotho, is flat or rolling country at an altitude of 1,460 to 1,830 m. The greater part of the country to the east consists of high mountain chains and deeply incised valleys. Because of the high altitudes, temperatures are generally lower than in neighbouring Swaziland.

13.5.4.9 Products surveyed for zearalenone content in Lesotho were samples of a common local alcoholic beer. Ingredients are malted maize or sorghum, flour and hops, and occasionally various fruits such as grapes and pineapple. In making the beer, the village brewer spreads the germinating grain over the floor of his hut to dry. After fermentation is completed, the beer is boiled but not filtered and it contains considerable solids. Of 140 samples collected in 1974, 12% (17 samples) contained zearalenone at concentrations ranging between 300 and 2,000 $\mu\text{g/kg}$. (47)

13.5.5 Zearalenone: incidence in maize in Europe

13.5.5.1 Three countries in Europe - Yugoslavia, Hungary and France - have reported detection of zearalenone in maize.

Yugoslavia

13.5.5.2 The climatic conditions in Yugoslavia, described in section 13.4.8, appear to be conducive to Fusarium infections in maize and to a relatively high incidence of zearalenone contamination.

13.5.5.3 Outbreaks of estrogenism in swine attributed to zearalenone in maize feeds occurred in 1963, 1968, 1969, 1972 and 1974. The disease was observed in most parts of Yugoslavia in 1968 which was a very rainy year and the maize, harvested at high moisture levels, was strongly contaminated with moulds. In 1972, more than half of the maize crop was attacked by moulds, especially Fusarium graminearum, as a result of unfavourable weather during the ripening and harvesting periods. Hyperestrogenism in swine was widespread and infertility in dairy cows also occurred. Twenty-three (42%) of 54 samples of maize analyzed contained zearalenone at concentrations ranging from 0.7 to 37.5 mg/kg. (42)

13.5.5.4 A survey reported in 1976 found 2.6% of 191 samples of maize to be contaminated with zearalenone at an average level of 5.1 mg/kg. In another investigation, 2 of 15 samples of maize collected at farms contained zearalenone at a level of 0.65 mg/kg. (39, 43)

13.5.5.5 The site at which zearalenone formation occurs in maize subsequent to the field infection with Fusarium was not clearly identified. It was stated, however, that "it seems that intense activity of Fusarium takes place during storage causing a further contamination with mycotoxins", indicating that formation occurs both in the field and during storage.(42)

13.5.5.6 One noteworthy report concluded that some samples of maize, that appeared to be of good quality macroscopically, were found to contain as much as 200 mg/kg of zearalenone.(39)

13.5.5.7 In another report it was estimated that about 14% (210,000 tons) of stored grains in the Vojvodina region of Yugoslavia was lost because of mould damage on mycotoxin contamination. Swine frequently refused to eat the contaminated grain, and weight gains were low.(39)

Hungary

13.5.5.8 As in neighbouring Yugoslavia, the climatic conditions in Hungary are favourable for Fusarium infections of maize and zearalenone contamination.

13.5.5.9 Zearalenone contents of 70-80 mg/kg were found in maize in a study reported in 1968. In the same study, 200 zearalenone positive samples were found in a survey of "many" hundred samples of maize, wheat and mixed feed. In another investigation, 3 samples of maize grits intended for feed use were found to contain 50-75 mg/kg zearalenone. (40)

13.5.5.10 Cases of hyperestrogenism in swine associated with feeding Fusarium-infected maize have been reported since 1960, a massive outbreak occurring in 1969. In a recent outbreak, 106 gilts fed a Fusarium-contaminated diet showed a marked prolongation of the breeding period and only 38% of the gilts became pregnant. Among the pregnant sows, 2 aborted and 2 produced mixed litters of normal piglets and deformed fetuses. The infertile sows showed enlargement of the uterus, also indicating the presence of zearalenone. (40)

13.5.5.11 Fusarium toxicosis was also observed in a herd of 138 dairy cows fed a diet in which certain components contained 5-75 mg/kg zearalenone. Signs of the toxicosis were refusal of the toxic feed, edema of the vulva and marked decrease in milk yield. (40)

France

13.5.5.12 Zearalenone was the principal mycotoxin found in a survey of the 1974 maize crop in France. Samples of maize grown in southwest France were collected in March after overwinter storage in cribs. The samples selected showed visible mould growth. Of 75 samples analyzed, 82.5% contained zearalenone at detectable levels up to 170 mg/kg. (44)

13.6 TRICOTHECENE TOXINS

13.6.1 Trichothecene toxins: geographic distribution in maize in the producing regions. Trichothecene toxins have been reported in maize in two maize-growing countries - the United States and Yugoslavia. However, members of this group of toxins have been found in other cereal grains, straw or hay in Russia and other countries of eastern Europe, Finland and Japan. It seems likely that the trichothecenes also occur in such agricultural products in other countries which have similar climates.

13.6.2 Trichothecene toxins: sites of contamination in the growing, harvesting and storage of maize. No surveys have been reported on the occurrence of trichothecenes contamination in maize at the time of maturation in the field. The producing Fusarium species are field fungi and can invade the ear but the extent of toxin production prior to maturation is uncertain. It is known, however, that millet and wheat allowed to overwinter in the field were infected with F. sporotrichoides and F. poae and caused illness and deaths

in humans consuming these grains. It can be expected that toxin production would also occur in Fusarium infected corn stored under similar temperature and humidity conditions. Delayed harvest of late maturing maize, high in moisture at the time of the first killing frost, has been associated with Fusarium damage in the stored grain in the U.S. (48)

13.6.3 Trichothecene toxin: incidence in maize in the producing regions

13.6.3.1 Trichothecenes have been reported in maize in only two countries which produce this grain. These are the United States and Yugoslavia. Hungary has reported finding trichothecenes in surveys of feeds which may have included maize. No surveys have been reported elsewhere. This may be attributed in part, at least, to the lack of analytical methods suitable for routine application.

United States

13.6.3.2 The U.S. Food and Drug Administration assayed 173 samples of maize from the 1972 crop collected in the spring of 1973 from elevators in areas of the Midwest where Fusarium damage had been reported. Late planting and rainy weather had delayed harvest in these areas. The harvest lagged 4 to 6 weeks behind normal in the North Central States. About one quarter of the corn was still in the fields in December, when normally all the crop has been harvested. The moisture of the late harvested corn was higher than normal and because of a fuel shortage, some corn could not be dried immediately after harvest.

13.6.3.3 The rabbit skin irritation test was used as an indicator of trichothecene toxins. Quantitation was based on T-2 toxin as the reference compound. Of the 173 samples assayed, 54% were positive by the skin irritation test and 9% had levels of 0.2 to 1 $\mu\text{g}/\text{kg}$ T-2 toxin equivalent. (49)

13.6.3.4 Deoxynivalenol was isolated from maize of the 1972 crop and was identified as the swine emesis and swine refusal factor. In another study, deoxynivalenol (40-1,800 $\mu\text{g}/\text{kg}$) and zearalenone (175-3,600 $\mu\text{g}/\text{kg}$) were found in five samples of feedstuffs which swine refused to eat and deoxynivalenol (100 $\mu\text{g}/\text{kg}$) only was found in one sample which caused vomiting in dogs. Diacetoxyscirpenol at levels of 380 and 500 $\mu\text{g}/\text{kg}$ was found in two samples that were associated with hemorrhagic bowel syndrome in swine. One sample of feedstuff associated with bloody stools in bovines contained 70 $\mu\text{g}/\text{kg}$ T-2 toxin and 700 $\mu\text{g}/\text{kg}$ zearalenone (5,50,49)

13.6.3.5 In an earlier study, T-2 toxin was isolated from mouldy maize associated with mycotoxicosis in dairy cattle that resulted in the death of 7 of the 35 animals affected. Signs (oral lesions) in broiler flocks have been observed which closely resemble those found in chicks fed T-2 toxin, strongly suggesting the presence of trichothecenes in the feed. (51,52)

13.6.3.6 Studies in the U.S. have shown that trichothecene (deoxynivalenol) contaminated maize causes emesis in swine at relatively low levels of contamination resulting in their refusal to eat the grain. Reduced growth rate results from inadequate feed intake. Poultry and cattle are less susceptible to emesis and will accept contaminated maize. However, broiler and turkey poults show reduced growth rates and feed efficiencies if fed contaminated feeds. Trichothecene-contaminated maize fed to laying hens resulted in decreased egg production. This effect was breed dependant. A herd of dairy cows that had received rations containing maize with T-2 toxin for a prolonged period had a high incidence of abortion and also suffered a 20% mortality rate. (5,50,52,53,54,55,51)

Yugoslavia

13.6.3.7 In a survey of maize for trichothecenes conducted by the Poljoprivredni Fakultet, Zagreb, reported in 1976, 8.3% of the samples analyzed contained T-2 toxin at an average level of 2.2 mg/kg . No information is available concerning the crop year or weather conditions during the growing, harvesting and storage of the maize. (39)

13.6.3.8 It has been reported that about 14% (210,000 tons) of stored maize of crop year 1974/1975 was lost because of mould contamination in the Vojvodina region of Yugoslavia. Principal fungi present were Fusarium graminearum, F. moniliforme and other Fusarium spp. The contaminated maize contained the refusal and emesis factor and zearalenone. Weight gains in swine fed the maize were very low; very often pigs refused to eat the contaminated grain. (39)

Hungary

13.6.3.9 The Central Veterinary Diagnostic Institute of Hungary has conducted extensive surveys for T-2 toxin in various domestic feeds. Analysis of 629 samples (the plant material was not specified) showed 2.4% to be contaminated with T-2 toxin. Of 16 samples analyzed for diacetoxyscirpenol, 6.3% were positive, and 1 of 10 samples analyzed for stachybotrys toxin contained the toxin. (40)

13.7 OCHRATOXIN

13.7.1 Ochratoxin: geographic distribution in maize in the producing regions.

Ochratoxin has been reported in only a few of the maize-growing countries. In Europe, ochratoxin has been found in maize in France and Yugoslavia. Hungary has reported occurrence of this mycotoxin in "feeds" which may include maize. In North America, ochratoxin has been detected in maize only in the United States. Absence of reports from other countries, particularly those having temperate climates, may only signify that analyses for ochratoxin have not been conducted.

13.7.2 Ochratoxin: sites of contamination in the growing, harvesting and storage of maize. Little information is available concerning the development of ochratoxin in cereal grains in the field or after harvest. The growth characteristics of Penicillium viridicatum, as observed in laboratory experiments, and its frequent incidence in stored maize, however, indicates that this fungus could proliferate and produce ochratoxin in maize stored at moisture levels of about 22%. Development in storage is indicated by the isolation of ochratoxin from wheat that had entered storage at high moisture and had subsequently heated. (56)

13.7.3 Ochratoxin: incidence in maize in North America

United States

13.7.3.1 Two surveys of maize for ochratoxin have been conducted by the U.S. Department of Agriculture (USDA). In 1967-1968, 283 samples of maize were collected mainly from principal markets in the Corn Belt but including some samples from markets in southern states. All grades of maize were represented. Ochratoxin A was detected in one sample at a level of 110-150 µg/kg. This sample was USDA Sample Grade, the lowest official grade, and showed over-mould damage.

13.7.3.2 In a second survey, 293 samples of export maize were collected at 9 U.S. ports and one Canadian port. In this, as in other surveys, a greater percentage of Sample Grade maize was collected than was representative of that marketed in the expectation that this grade would be most likely to contain mycotoxins. The presence of ochratoxin A was confirmed in one U.S. No. 3 grade sample and two U.S. No. 4 grade samples representing an overall incidence of one percent. These samples had more than 5% total damaged kernels. Aspergillus ochraceus was not observed in any of the samples containing ochratoxin, but Penicillia were isolated from all of them.

13.7.3.3 No reports of toxicosis of farm animals resulting from the ingestion of ochratoxin contaminated maize have been found. However, outbreaks of porcine nephropathy in Denmark have been traced to ochratoxin contaminated barley as the causal agent.

13.7.4 Ochratoxin: incidence in maize in Europe

Yugoslavia

13.7.4.1 Climatic conditions, rainfall and maize production in Yugoslavia were described in section 13.4.8.4

13.7.4.2 In a survey of maize conducted by the Poljoprivredni Fakultet, Zagreb, 172 samples were analyzed for ochratoxin A. Of these, 43 samples (25%) were positive and contained levels in the range 0.04-5.1 mg/kg. In another study, a survey of 900 samples of various cereals and of pork collected from a village in Croatia indicated 8 to 10% of the samples were contaminated with ochratoxin A. (39)

13.7.4.3 It has been proposed that ochratoxin in the food supply may be a factor in Balkan nephropathy, a disease endemic in some areas of the Balkan countries. Maize may possibly be involved since it is a part of the diet in several of these countries and, in at least one, has been found to contain ochratoxin. (57)

Hungary

13.7.4.4 Climatic conditions, rainfall and maize production in Hungary are described in section 13.4.8.9

13.7.4.5 Surveys of feeds, composition not stated, in which samples were collected from various areas in Hungary have been conducted by the Central Veterinary Diagnostic Institute. Of 29 samples analyzed for ochratoxin A, four samples (14%) were found to contain the toxin. (40)

13.7.4.6 No disease outbreaks in farm animals attributed to ochratoxin in feedstuffs were described in a recent report on mycotoxicoses in Hungary. (40)

13.8 PENICILLIC ACID

13.8.1 Penicillic acid: geographic distribution in maize in the producing regions. Penicillic acid has been reported only in maize grown in the United States.

13.8.2 Penicillic acid: sites of contamination in the growing, harvesting and storage of maize. There is little information in the literature of the sites of contamination of the penicillic acid-producing fungi on maize. However, maize which is stored under high moisture conditions and at a low temperature (about 5°C) is susceptible to infection by Penicillium species which are responsible for the production of penicillic acid. In the laboratory, it has been shown that penicillic acid was produced by cultures of P. martensii at temperatures between 5 and 32°C. There is a sharp decrease in production at 25°C and above and no mould growth above 32°C. (58)

13.8.3 Penicillic acid: incidence in maize in the producing regions.

13.8.3.1 The mycotoxin, first isolated in 1913 from P. puberulum grown on maize, was shown to be toxic to laboratory animals. In 1972, an FDA survey of U.S. maize detected penicillic acid in 7 of 20 random samples at levels of 5 to 231 ug/kg. Subsequent analysis of 48 samples of maize, selected because they were infected with "blue-eye" disease, demonstrated penicillic acid in all samples at concentrations ranging from 5 to 184 ug/kg (average 46 ug/kg). (59, 48, 13)

14. CEREAL GRAINS: WHEAT

14.1 Wheat Producing Regions, World Production and Trade

The major wheat regions of the world are found in the temperate zones between 30° to 60°N and 25° to 40°S latitudes, but it is also grown in most of the subtropical countries as well as at high elevations in some tropical countries. In 1975, world production of wheat was over 355 million metric tons, exceeding that of any other cereal crop. The principal regions of wheat production are in North America, Western and Eastern Europe, China, India, Argentina and Australia. Countries of North Africa and the Near East produce important quantities and significant amounts are produced in many other countries. About four-fifths of the wheat crop is used in the countries where it is grown, and the remaining one-fifth, about 70 million tons in 1975, enters into international trade. (2)

14.2 Use of Wheat as a Food and Feed Grain

Wheat is used mainly as a food grain and is a dietary staple in wheat-producing countries. Per capita daily consumption may exceed 300 g in some North African and central European countries, supplying 40 to 60% of the calorie and protein intake. Some wheat is fed to farm animals, particularly in years of surplus production in the large producing countries. Low quality wheat rejected as human food also goes into livestock feed in most countries. (3)

14.3 Mycotoxins Found in Wheat

Four mycotoxins - aflatoxin, ochratoxin, citrinin and zearalenone - have been reported in wheat. Ochratoxin and citrinin are produced by the same fungal species, Penicillium viridicatum, and frequently occur together in contaminated grain.

14.4 AFLATOXINS

14.4.1 Aflatoxins: geographic distribution in wheat in the producing regions

Aflatoxin has been found in wheat in two countries in south central Europe, one in Asia, one in Central America and one in North America. Surveys of wheat in market channels have been reported in only one country. The few analyses that have been reported give no indication of greater incidence of aflatoxin contamination in any geographic region. No cases of aflatoxicoses have been reported. This might be expected in view of the low incidence and levels of aflatoxin that have been found in wheat.

14.4.2 Aflatoxins: sites of contamination in the growing, harvesting and storage of wheat

The information given on past history of the grain in those cases where aflatoxin has been found is insufficient to indicate the site of contamination. The very low incidence of the aflatoxin-producing fungus, Aspergillus flavus, in wheat as collected from the field in the United States reduces the probability of pre-harvest contamination. Low incidence has been found even in samples collected from fields that, because of heavy rains, remained unharvested for several weeks after the grain had ripened. In contrast, the relatively high inocula of Aspergillus flavus to which wheat and other grains are exposed after harvest in handling and storage facilities provide a favourable condition for fungal growth and toxin production if the grain has a moisture content above 18%. Until contrary evidence is presented, it may be assumed that the major precaution to be taken in preventing aflatoxin contamination in wheat is to ensure that it be handled and stored at a safe moisture level. For long-term storage this is 13% or less, depending mainly on the temperature, but also on the amount of broken kernels, rubbish and other foreign material present, and the extent of fungal contamination. (60)

14.4.3 Aflatoxins: incidence in wheat in North America

United States

14.4.3.1 In two surveys of wheat conducted by the U.S. Department of Agriculture, samples representing all market grades and all classes of wheat from the different growing regions were collected from principle markets and analysed for aflatoxin. In a survey of the 1964 crop, 531 samples of mainly hard red winter wheat were collected from three mid-western markets. Aflatoxin was detected in two samples by thin-layer chromatographic analysis at levels of 9 $\mu\text{g/kg}$ total aflatoxins. Both were U.S. Sample Grade grain, the lowest grade in quality. Administration of extraots of these samples to ducklings gave an inconclusive, but probably negative test for aflatoxin. (61)

14.4.3.2 In a second survey, samples were collected during crop years 1970-1973; 291 hard red winter, 286 hard red spring and 271 soft red winter wheat samples were analysed for aflatoxin. Aflatoxin was not detected in any sample including those in Sample Grade which were collected in disproportionate numbers relative to their occurrence in the market. (62)

Canada

14.4.3.3 Analyses of 32 samples of heated grains, including at least 19 wheat samples, collected from farm storage bins in Saskatchewan gave no positive tests for aflatoxin although other mycotoxins were found. The grain was from the 1968 crop that was harvested under unfavourable weather conditions. An estimated 70% or more of the crop was stored at high moisture levels, resulting in heating and spoilage. (60)

14.4.4 Aflatoxins: incidence in wheat in Central America

14.4.4.1 One mainland Central American country has reported analyses of two wheat products, semolina and "german". One sample of each was analysed and contained 10 $\mu\text{g/kg}$ aflatoxin B₁. (27)

14.4.5 Aflatoxins: incidence in wheat in Asia

14.4.5.1 Flood conditions in Pakistan in 1976 resulted in aflatoxin contamination in wheat and other grains. Concentrations in wheat were low, ranging up to 5 $\mu\text{g/kg}$. (64)

14.4.5.2 In 1976, ten other samples of mould damaged wheat collected in various areas of Pakistan (not necessarily local produce) were tested at the Tropical Products Institute for contamination with aflatoxin. Four of the samples had been stored in covered stores but the other six had remained uncovered. The aflatoxin level in all the covered samples was 5 $\mu\text{g/kg}$ but ranged, in the uncovered samples, from 5 to 240 $\mu\text{g/kg}$ with an average contamination level of 100 $\mu\text{g/kg}$. (65)

14.4.6 Aflatoxins: incidence in wheat in Europe

14.4.6.1 Reports are available from only two countries - Yugoslavia and Greece. In Yugoslavia, 8 of 15 samples, or 60%, of wheat analysed were found to contain aflatoxin at an average concentration of 6.4 $\mu\text{g/kg}$. No further information on the sample was given. (39)

14.4.6.2 In Greece, 2 of 17 samples (12%) of wheat analysed contained 10 to 50 $\mu\text{g/kg}$ aflatoxin. Harvesting and storage conditions were not described. A higher incidence was found in kolyva, a boiled wheat. Five of 17 samples (about 29%) contained 5 to 48 $\mu\text{g/kg}$ aflatoxin. (63)

14.5 ZEARALENONE

14.5.1 Occurrence of zearalenone in wheat has been reported in only one country, Hungary. Three samples of wheat contained from 5-10 mg/kg zearalenone. In the Canadian investigation of mycotoxins in heated wheat described in section 14.4.3.3, analyses for zearalenone were made but all samples were negative. (40)

14.5.2 The conditions under which zearalenone developed in wheat in Hungary were not described. It is known, however, that Fusarium roseum, a fungus that produces zearalenone, is one of the Fusarium spp. associated with scab, a disease of wheat and other small grains. Scab occurs in wheat when subjected to extended periods of cool, rainy weather prior to harvest. Serious epidemics of the disease occurred in wheat in the United States in 1919, 1928 and 1935. Scab is well known as a disease in wheat and other small grains in Japan where it is described as "Akakabibyō" or red mould disease. However, hyperestrogenism, the characteristic sign of zearalenone intoxication, has not been reported in Japan in animals fed scabby grains and it appears that other Fusarium toxins were present. (1, 66)

14.5.3 No association of zearalenone-contaminated wheat with mycotoxicosis in farm animals or humans has been established.

14.6 OCHRATOXIN AND CITRININ

14.6.1 Ochratoxin and Citrinin: geographic distribution in wheat in the producing regions

Analyses for ochratoxin in wheat or a wheat product have been reported from Canada, the United States and Yugoslavia. Citrinin is frequently found to accompany ochratoxin since both are produced by Penicillium viridicatum, one of the Penicillia found in stored grains contaminated with ochratoxin. However, analyses for citrinin were conducted only in Canadian studies of samples of grain that had heated in storage.

14.6.2 Ochratoxin and Citrinin: incidence in wheat in North America

Canada

14.6.2.1 In a Canadian study, 32 samples of heated grains collected from farm storages were analysed for ochratoxin, citrinin, aflatoxin and zearalenone. Ochratoxin A was found in 14 of 19 samples of wheats ranging in concentration from 0.03 to 27 mg/kg. Citrinin was detected in 11 of 19 samples, at levels ranging from 0.07 to 20 mg/kg. The wheat was stored damp as a result of unfavourable weather at harvest and mould growth and heating resulted. Levels of ochratoxin A found in some samples of heated wheat in a Canadian study appear to be high enough to cause adverse effects if fed as a major feed component to swine or chicken. (56)

14.6.2.2 In a U.S. Department of Agriculture survey of 848 samples of wheat collected in the 1970-1973 crop years at principal markets, 11 samples were found to contain ochratoxin A at levels ranging from a trace to 35 µg/kg, generally much lower than found in the heated grain in Canada. Eight of the contaminated samples were in the poorest market grades, U.S. Grade No. 5 and Sample Grade, and two were in Grade No. 4. The trace level of ochratoxin occurred in one Grade No. 3 sample. It is noteworthy that ochratoxin A was not detected in any of the 271 soft red winter wheat samples included in the survey although rainfall is generally higher in the region growing this class of wheat than in the regions growing the hard red winter and hard red spring wheats, the other two classes of wheat analysed. (62)

14.6.3 Ochratoxin and Citrinin: incidence in wheat in Europe

Yugoslavia

14.6.3.1 In Yugoslavia, survey of a large number of samples of foodstuffs, including cereals and bread, indicated that 8-10% were contaminated with ochratoxin A. The survey was conducted in a village in Croatia where Balkan endemic nephropathy is prevalent. Conditions of the wheat at harvest and in storage were not described. However, it is evident from the Canadian studies that high levels of ochratoxin A and citrinin can develop in wheat if it is stored above safe moisture levels even in a cool climate. (39)

14.6.3.2 It has been suggested that ochratoxin A may be a dietary factor contributing to Balkan endemic nephropathy, a disease in humans that occurs in certain areas of Yugoslavia, Bulgaria and Rumania. Ochratoxin A has been found in wheat and bread in an affected area of Yugoslavia and possibly may be involved in this disease. However, the role of ochratoxin A remains to be established. (57)

15. CEREAL GRAINS: RICE

15.1 Rice Producing Regions, World Production and Trade

15.1.1 The principal rice-producing countries of the world lie between 30°N and 30°S latitudes. Notable exceptions, however, are Japan and the Koreas in Asia, Italy in Europe, and California in the United States, each of which produces more than 1 million tons annually. About 90% of the world crop of 344 million tons in 1975 was produced in Monsoon Asia. This area extends across southeastern Asia from India to Japan, and includes practically all of the adjacent tropical and subtropical islands. The other 10% of world production was reported in forty countries in Africa, 14 in North and Central America, 13 in South America, 10 in Europe and Australia.

15.1.2 Of the 344 million tons of rice produced in 1975, only 12 million tons, or 3.5%, entered into international trade. (67)

15.2 Use of Rice as a Food Grain

Rice is used almost entirely as a food. Only low quality grain rejected as food because of mould or other damage is used as feed for livestock. Per capita consumption in south and southeast Asia ranges from 170 to over 440 g daily, supplying up to 70% of the calorie intake and over 50% of the dietary protein. (3)

15.3 Mycotoxins Found in Rice

Aflatoxin is the only mycotoxin reported in naturally moulded rice. Other mycotoxins have been found in rice inoculated with spores of Penicillium species associated with yellow rice disease. Rice imported into Japan at the end of the second world war was affected with this disease and caused illness and death in humans. Incidence of yellow rice disease since that time appears not to have been significant. (68, 69)

15.4 AFLATOXINS

15.4.1 Aflatoxins: geographic distribution in rice in the producing regions

Aflatoxin has been detected in rice in six countries in south and southeast Asia, one country in Africa, one in South America and two in Central America. Despite its culture in tropical and subtropical regions of high rainfall and humidity, the reported incidence of aflatoxin contamination in raw rice is relatively low. However, because of the high consumption in many areas, even a low level of contamination may have adverse effects on human health.

15.4.2 Aflatoxins: sites of contamination in harvesting, storage and processing of rice

15.4.2.1 There appears to be little specific information in the literature on the stage in harvesting or subsequent handling at which aflatoxin contamination of rice most frequently occurs. There are, however, several stages where moisture conditions could provide opportunity for growth of the aflatoxin producing fungus, Aspergillus flavus, and toxin production. Conceivably, fungal infection might occur in the field prior to harvest. There is no evidence for this but scientists at the University of Agricultural Sciences, Bangalore, India have initiated a study to find whether A. flavus infection occurs during the milky stage of the rice grain.

15.4.2.2 At harvest, the moist grain provides opportunity for mould growth unless properly dried. Rice harvested with combines usually contains 18-25% moisture and is dried gradually down to 12 or 13% moisture in artificial driers. Drying must start within 24 hours after combining to avoid mould growth and heating of the grain. In most Asiatic countries, the rice is harvested by cutting straw bearing the panicles; the straw may be left on the ground to dry or tied in bundles and suspended from poles. After drying to a moisture level suitable for threshing, the grain is beaten out, cleaned, and dried for 3-4 days on straw mats until the moisture level in the grain is reduced to 13 or 14%. If the drying operation is prolonged by rainy weather, opportunity for mould growth is presented. (1, 10)

15.4.2.3 Parboiling of rice is a process which provides particular opportunity for mould growth if drying to a safe moisture level of 12 or 13% is not accomplished in a short time.

15.4.2.4 Storage under improper conditions also provides opportunity for mould growth and mycotoxin formation. Among frequent causes are moisture migration and condensation arising from inadequate ventilation of the stored grain that frequently provide conditions for mould growth. Generation of high moisture levels by insect activity is another condition that favours mould development.

15.4.2.5 Mould growth may also result from blending lots of high and low moisture of grain to reach a safe average level. Unless mixing is thorough and equilibrium is reached quickly, the high moisture component provides high risk of moulding.

15.4.3 Aflatoxins: incidence in rice in Central America

15.4.3.1 Analyses of rice for aflatoxin have been conducted in two Central American countries. (26, 27)

15.4.3.2 Analyses conducted in one mainland Central American country showed that 4 of 13 samples of rice contained aflatoxin; the highest level detected was 146 $\mu\text{g}/\text{kg}$, while in a Caribbean country, no aflatoxin was detected in an analysis of 6 samples of polished rice. Two samples of rice bran were positive and contained 15 and 50 $\mu\text{g}/\text{kg}$ aflatoxin.

15.4.4 Aflatoxins: incidence in rice in South America

Colombia

15.4.4.1 Colombia is the only country in South America that has reported analyses of rice for aflatoxin. In an exploratory survey of food and feed grains conducted in the vicinity of Medellin by the National University of Colombia, 83% of the rice samples collected contained over 20 $\mu\text{g}/\text{kg}$ aflatoxin. Possible causes of contamination mentioned were prolonged drying periods after harvesting and deficiencies in storage conditions. (7)

15.4.5 Aflatoxins: incidence in rice in South and Southeast Asia

India

15.4.5.1 India is the world's second largest producer of rice, growing this crop in 19 of its 21 states. However, limited information is available on the incidence of aflatoxin contamination. Only one study has been reported. Six percent of the samples collected in an epidemiological survey in a coastal area of Mangalore in Karnataka State contained aflatoxin. Aflatoxin levels and number of samples analysed were not given. (10)

Thailand

15.4.5.2 Rice is the major dietary staple in Thailand. Annual production is 15 million tons and per capita daily consumption is about 440 g. Incidence of aflatoxin contamination in market rice was determined in a survey of all Thai foods for aflatoxin conducted in 1967-1969. Of 364 samples of raw rice collected, 2% contained aflatoxin at an average level of 20 $\mu\text{g/kg}$; the maximum level found was 98 $\mu\text{g/kg}$. In another part of the same study, aflatoxin levels in prepared foods as sampled in individual homes were investigated in three areas of Thailand. In Singburi, 3% of the samples of prepared rice contained aflatoxin at levels up to 600 $\mu\text{g/kg}$; in Ratbiri, 10% were contaminated with levels up to 180 $\mu\text{g/kg}$; and in Songkhla, 1% contained aflatoxin at concentrations up to 71 $\mu\text{g/kg}$. Aflatoxin contamination in foods collected from markets, farms, distributors, warehouses and other sources was greatest in the second half of the rainy season and lowest during the hot season. In the Ratburi area where incidence in prepared foods was highest, aflatoxin contamination was attributed to heavy rains that occurred in the year the dietary survey was made. Villages were flooded and unmilled rice stored under the stilted houses may have been wetted. (11)

15.4.5.3 In Thailand, rice is a major dietary staple and contributed to the intake of aflatoxin. The incidence of primary liver cancer was found to be correlated with the incidence of aflatoxin-contaminated prepared foods as well as the incidence and level of aflatoxin in rice as sampled from individual homes. (70)

15.4.5.4 One case of acute toxicosis resulting in death of a child has been reported in which aflatoxin in contaminated rice was the suspect agent. A 10 kg child consumed steamed rice contaminated with aflatoxin for two days prior to the fatal illness. It has been estimated that the child received 6 mg of aflatoxin daily.

The Philippines

15.4.5.5 Rice is a major crop in the Philippines and is produced in all administrative regions. Annual production in 1975 was 6.5 million tons and per capita daily consumption is about 240 g.

15.4.5.6 Surveys have been made of many foods, including rice, for aflatoxin content in the Philippines. Findings on rice are presented in Table II/9. Levels in rice were relatively low and generally under 20 $\mu\text{g/kg}$.

Table II/9.

AFLATOXIN LEVELS IN RICE IN THE PHILIPPINES

Rice product	No. Samples	Percent Positive	Percent > 20 $\mu\text{g/kg}$	Ave. of Positives, $\mu\text{g/kg}$	Highest level, $\mu\text{g/kg}$
Raw, polished	72	21	1	10	22
Rough rice	10	30	0	15	18
Factory processed	20	0	-	-	-
Poprice	6	17	0	3	3
Rice bran	15	80	33	16	38

Taiwan

15.4.5.7 Rice is the most important staple food in Taiwan. A study published in 1968 reported that no aflatoxin was detected in rice collected from markets in Taiwan. However, aflatoxin was found in one of the samples of mouldy rice that was collected from families in which 25 people became ill and three children died of unknown cause. One of five samples analysed contained about 200 $\mu\text{g}/\text{kg}$ aflatoxin B₁. Necropsies of the children were not performed. The evidence is insufficient to implicate aflatoxin as any more than a possible causative agent. (71)

Indonesia

15.4.5.8 Rice is the main staple food in almost every region of Indonesia. Production in 1975 was about 15.3 million tons and per capita daily consumption about 300 g. Traditional cultivation practices are largely employed but a corps of 6,300 extension workers have introduced new high yielding varieties, fertilizers, pesticides and other improved practices. Greatest possibility of mould contamination is considered to be in rice stored by the collector who purchases rice from the farmers. However, analyses of samples taken from storage facilities have given negative tests for aflatoxin. Aflatoxin has been detected in rice handled by distributors. Analysis of four samples suspected to be contaminated showed the presence of aflatoxin G₁ in one at a concentration of 15 $\mu\text{g}/\text{kg}$. A more extensive survey of rice for aflatoxin is planned because of its importance in the Indonesian diet. (72)

Vietnam

15.4.5.9 Rice is the principal cereal crop in Vietnam and is the major dietary staple in most regions. Results of a survey of rice for aflatoxin in the Saigon region were reported in 1971. Samples were collected from storage facilities and from the market. A minicolumn screening method sensitive to 20 $\mu\text{g}/\text{kg}$ was used for analysis. Thirty-one percent of 139 samples collected were positive for aflatoxin. Incidence was the same in market and storage samples, but was higher in the inexpensive grades than in the medium and expensive grades. Price range reflected different kinds of rice, the degrees to which the rice was milled, as well as evidence of mould contamination. (73)

15.4.6 Aflatoxins: incidence in rice in Africa

Mozambique and Uganda

15.4.6.1 Analysis of rice for aflatoxin has been reported in surveys conducted in two countries in Eastern Africa - Mozambique and Uganda. Per capita daily consumption of rice in the former country is about 20 g; in the latter, about 5 g. Analyses of 34 samples of rice collected in Mozambique from home storage over a period of three years showed an average level of 3.4 $\mu\text{g}/\text{kg}$ aflatoxin. In a similar survey of foodstuffs conducted in Uganda in 1966-1967, samples of rice, among other foods, were collected from mud and thatch family graneries where food was stored for use between harvests, or from material on sale in local village markets. Aflatoxin was not detected in the 11 samples of rice analysed. On the basis of the findings in these two countries, aflatoxin contamination in rice appears not to be a serious problem. Both levels of consumption and levels of contamination are relatively low compared to other dietary items. (9, 8)

15.4.6.2 No reports on analyses of rice are available from West African countries where rice is a major dietary staple and per capita daily consumption may exceed 200 g. The possible effect of long-term ingestion of low levels of aflatoxin on public health has been explored in epidemiological studies conducted in 4 countries in Africa. In these studies, the relationship between dietary aflatoxin and the incidence of primary liver cancer was investigated.

16. CEREAL GRAINS: SORGHUM

16.1 Sorghum Producing Regions, World Production and Trade

Sorghum is cultivated from the tropics to latitudes as high as 45° North and South. It is grown largely in regions of Africa, Asia, North America and Australia that are too hot or too dry for successful maize production. World production in 1975 was about 50 million tons of which Africa, Latin America and the Far East each produced about one-fifth, North America about one-third and the Near East the remainder.

16.2 Use of Sorghum as a Food and Feed Grain

Sorghum meal is a dietary staple in Central India and in countries of Central America and Africa. Per capita daily consumption ranges from 20 to 140 g are reported for countries in Africa and from 1 to 50 g daily for Central American countries. In other producing countries, sorghum grain is mainly used as a livestock feed. (3)

16.3 Mycotoxins Found in Sorghum

Two mycotoxins have been found in sorghum, aflatoxin and zearalenone. Zearalenone has been reported in only one sample of sorghum grain and that one had been damaged by head blight. It has, however, been found in fermented sorghum products.

16.4 AFLATOXINS

Analyses for aflatoxin in sorghum grain have been reported in only five countries - India, Uganda, two in Latin America, and the United States. Findings in these countries are given in Table II/10. Systematic surveys of market sorghum have been carried out only in the United States and Uganda. Both incidence and level of contamination were low in the United States indicating that sorghum grain does not present a problem in the United States. The levels found in Uganda and Central America indicate that a problem may exist and that this commodity merits further attention.

Table II/10.

AFLATOXIN INCIDENCE IN SORGHUM GRAIN

Country	No. Samples	No. Positive	Aflatoxin Range µg/kg	Ref.
India	Found in mouldy heads		?	(a)
Uganda	69	26	<100 - >1 000	(b)
Colombia	?	All	>20	(c)
Central American country	15	2	100 - 200	(d)
United States	599	2	13 - 50	(e,f)

References - TABLE II/10

- (a) India, unpublished data submitted to FAO, 1977.
- (b) Alpert, M.E., Hutt, M.G.R., Wogan, G.N. and Davidson, C.S. 1971. Association between aflatoxin content of food and hepatoma frequency in Uganda. *Cancer*. 28:253-260.
- (c) Colombia, unpublished data submitted to FAO, 1977.
- (d) Central America, unpublished data submitted to FAO, 1977.

- (e) Shotwell, O.L., Hesselstine, C.W., Burmeister, H.R., Kwolek, W.L., Shannon, G.M. and Hall, H.H. 1969. Survey of cereal grains for the presence of aflatoxins. I, wheat, grain, sorghum and oats. Cereal Chem. 46:446-454
- (f) FDA Surveillance Survey, unpublished communication from L. Stoloff.

16.5 ZEARALENONE

16.5.1 Zearalenone has been reported in only one sample of sorghum grain that was damaged by head blight. This was sorghum grown in southwestern United States. Zearalenone has, however, been found in sorghum malt and fermented sorghum products in Lesotho and Swaziland in southern Africa. The fermented products include sour drinks, sour porridges and beers. (61, 47)

16.5.2 Sorghum malt is prepared by germinating, drying and milling raw sorghum grain. The grain may be damp for five days or more during the germination and drying stages of the process. All samples of sorghum malt examined, both factory and village-produced, contained zearalenone. Sorghum malt is used in preparing the local beers. Sour porridges are prepared by a yeast fermentation of sorghum or maize meals, whereas sour drinks apparently depend on fermentation by adventitious microorganisms. (47)

16.5.3 Of 140 Lesotho beers analysed, 17 contained zearalenone in the range 300 to 2,000 µg/kg; about the same percentage of Swaziland beers were positive. A total of 110 samples of beer, sour drink and sour porridges were collected and analysed in Swaziland. Zearalenone was present in 10% of these at levels ranging from 8,000 to 53,000 µg/kg. (47)

16.5.4 It has been suggested that regular ingestion of zearalenone in fermented products in Swaziland may be related to the high incidence of cervical cancer in certain population groups. (74)

17. CEREAL GRAINS: BARLEY

17.1 Barley Producing Regions, World Production and Trade

Barley is grown in nearly all areas of the temperate zones, in many subtropical areas, and in high altitude sections of the torrid zones of both hemispheres. It is an important crop in Europe, North America, North Africa, regions in Asia, Australia and Argentina. World production in 1975 was about 155 million tons, of which 12.6 million tons entered international trade. (2)

17.2 Use of Barley as a Food and Feed Grain

Barley is used mainly as livestock feed although small quantities in most producing countries are used as human food.

17.3 Mycotoxins Found in Barley

Four mycotoxins - aflatoxin, ochratoxin A, citrinin and zearalenone - have been detected in barley.

17.4 AFLATOXINS

Aflatoxin has been reported in barley in only two countries. In a Central American country, one sample of 6 analysed contained 146 µg/kg aflatoxin. In Greece, aflatoxin was present in 2 of 27 samples analysed at levels between 10 and 50 µg/kg. Aflatoxin has not been

reported in the principal barley producing countries which have cool climates. It appears that the incidence of aflatoxin may be limited to the warmer growing areas but the limited data make this a tentative conclusion. (27, 63)

17.5 OCHRATOXIN

Ochratoxin A has been detected in barley in the United States, Denmark, Sweden and Canada. It appears to be a problem in barley principally in Northern Europe where high levels have been found.

17.5.1 Ochratoxin: incidence in barley in North America

United States

17.5.1.1 In a survey of market barley in the United States conducted by the Food and Drug Administration, ochratoxin A was detected in 14% of 159 samples analysed. Half of the positive samples contained trace amounts ($<10 \mu\text{g/kg}$); the range in the measurable detections was 10 to $29 \mu\text{g/kg}$. In a follow-up survey of malt barley and beer samples collected at 138 breweries, no ochratoxin was found in either the barley or the beer. (13)

Canada

17.5.1.2 Ochratoxin A was detected at a level of $22,000 \mu\text{g/kg}$ in one sample of mixed oats and barley that had heated in farm storage. (56)

17.5.2 Ochratoxin: incidence in barley in Europe

Sweden

17.5.2.1 In Sweden, 7 of 84 samples of barley and oats analysed contained ochratoxin at concentrations of 16 to $410 \mu\text{g/kg}$. These samples were collected in the spring of 1972 from farm storages of the 1971 crop. Grain was harvested at moisture levels ranging from 16 to 57%. Drying was carried out on some farms whereas other farms stored the cereals without any treatment. There was a low correlation between percentage germination of the stored barley and ochratoxin content. (75)

17.5.2.2 Ochratoxin A was detected at levels $>2 \mu\text{g/kg}$ in 32 of 129 kidneys from normally slaughtered swine collected at four slaughter houses in Sweden. The kidneys analysed deviated in some respects from normal kidneys on macroscopical inspection. Ochratoxin A levels of 5 to $104 \mu\text{g/kg}$ were demonstrated in 7 kidneys that showed histological changes expected to occur in ochratoxicosis in swine. (75)

Denmark

17.5.2.3 Ochratoxin A-contaminated barley in swine rations has been associated with porcine nephropathy in Denmark. A survey of barley samples collected in an area where swine were affected revealed that 58% of 33 lots contained ochratoxin A. The highest concentration was 27.5 mg/kg . In a survey of barley intended for beer production, 6% of 50 lots contained ochratoxin A. Highest concentration was $189 \mu\text{g/kg}$. (76)

17.5.2.4 Experimental beers made from ochratoxin A-contaminated barley were found to retain about 4% of their original ochratoxin. At this rate of ochratoxin loss, barleys with sufficient ochratoxin A to result in detectable ochratoxin in the beer would likely be rejected for malting because of lack of germination. (13)

17.5.2.5 Ochratoxin A residues appeared in the tissues of swine fed a ration containing 1 mg/kg of added crystalline ochratoxin A. At slaughter, after 3 months on this ration, 28 µg/kg ochratoxin A was found in kidney tissues, 11 µg/kg in liver, 9 µg/kg in muscle and 3 µg/kg in adipose tissue. (78)

17.5.2.6 A Danish survey of pig kidneys from cases of nephropathy of which most of the carcasses had passed meat inspection, showed residues of ochratoxin A in 36% of those examined; the highest concentration was 68.2 µg/kg. In a subsequent survey, 34% of the nephropathy kidneys contained ochratoxin A. (79)

17.5.3 Ochratoxin: sites of contamination in the growing, harvesting and storage of barley

The site at which ochratoxin contamination occurred in the barley samples collected in Denmark and in the United States has not been identified. Neither has it been identified in the Canadian or Swedish samples since samples of grain entering storage were not analysed for ochratoxin. However, the heating that occurred in storage and isolation of the producing mould, *P. viridicatum*, in the Canadian samples, is strong evidence that the ochratoxin A was formed in storage. The high moisture levels at which some of the lots of grain in Sweden entered storage were in the range for *P. viridicatum* growth and possibly toxin formation occurred at this site. It is evident that research is needed to identify the site and conditions under which ochratoxin contamination occurs.

17.6 CITRININ

17.6.1 Citrinin has been reported in barley in Denmark and in Canada. In Denmark citrinin occurred with ochratoxin A in some of the samples of barley that were associated with porcine nephropathy. Ochratoxin A was detected in 58% of 33 samples of barley; citrinin was found in 9% of the samples at concentrations up to 2 mg/kg. (76)

17.6.2 In Canada, one sample of barley and one of mixed barley and oats were analysed for citrinin. Both had heated in farm storage. Citrinin was detected in the mixed grain sample at a level of 60 mg/kg. (56)

17.6.3 It has been suggested that citrinin has a role in porcine nephropathy that results from feeding mould-damaged barley.

17.7 ZEARALENONE

17.7.1 Zearalenone in barley has been reported only in the United Kingdom. However, barley may have been a component of feeds in other countries in which zearalenone was detected.

17.7.2 Decreases in egg production of large poultry flocks in northwest England in the winter of 1967-1968 led to the examination and finding of zearalenone in barley being fed as a component of the rations. Zearalenone also was detected in other samples collected in a random sampling of barley offered for poultry feed at that time. (80)

17.7.3 Zearalenone was found in barley feed in an incident in which reduction of litter size of pigs from the normal 10 to 12 down to 4 to 5 occurred in 1967-1968; contaminated barley was found again in a similar incident in 1968-1969. Both incidents occurred in Scotland. In 1971, also in Scotland, zearalenone was demonstrated at levels of 500 to 750 µg/kg in barley fed to sows that had produced litters containing stillborn pigs or pigs with a "splayleg" condition. The barley had been stored without drying after harvest but was treated with propionic acid as a preservative. (80, 81)

17.7.4 A survey of the 1974 barley crop in the East Midlands revealed no incidence of zearalenone. (82)

18. CEREAL GRAINS: OATS

18.1 More than 80 % of the world acreage of oats lies in moist temperate areas, particularly North America, northern Europe and Soviet Russia. The major producing regions are bounded on the north in Finland by latitude 65°N and on the south in the United States by latitude 30°N. Total world production in 1975 was 49 million tons of which about 1 million tons entered international trade. (2,67)

18.2 Only a small percentage of the world oats crop is used as human food, the bulk of the crop being used for livestock feed. Per capita daily consumption as human food ranging from about 1 to 10 g is reported for the producing countries. (3)

18.3 Two mycotoxins have been detected in oats - aflatoxin and ochratoxin A. Few analyses have been reported and incidence of mycotoxins appears to be low.

18.4 Aflatoxin was detected in 3 of 304 samples of oats collected at principal markets in a survey of the 1964 U.S. oats crop. Trace levels (6 µg/kg) were found in 3 samples all in the lower U.S. market grades. (61)

18.5 In Greece, analysis of 14 oat samples revealed one that contained between 10 and 50 µg/kg aflatoxin B₂. (63)

18.6 Ochratoxin A in oats has been reported in two countries - Canada and Sweden. In both cases, ochratoxin was detected in grain that had entered storage after harvest at high moisture levels and was analysed in the following spring. The two Canadian samples (one a mixture of oats and barley) contained 3 and 22 mg/kg ochratoxin A respectively. The latter sample also contained 60 mg/kg citrinin. In Sweden, 2 oat samples of 84 samples of oats and barley analysed contained 28.5 and 76.5 µg/kg ochratoxin A respectively. (56, 83)

19. CEREAL GRAINS: MILLET

19.1 About one half of the world millet crop is grown in China, one-fifth in India, one-fifth in West and Central Africa and the remainder largely in other countries in Africa and Asia. World production in 1975 was about 47 million tons, most of which was used in the countries where it was grown. Millet is used both as a food and feed grain with per capita consumption as food ranging from about 10 to 300 g daily in Africa and India. (2,3)

19.2 Aflatoxin has been found in millet in two recent surveys of home-stored and market foods in Uganda and Thailand, both tropical countries. In the Uganda survey 9 of 55 samples (about 16%) analysed contained from 1 to 100 µg/kg aflatoxin. In Thailand, 10% of 44 samples were positive. Average level of aflatoxin in the positive samples was 64 µg/kg; highest level detected was 248 µg/kg. (11)

19.3 Outbreaks of alimentary toxic aleukia which occurred in Russia between 1942 and 1947 have been attributed to Fusarium toxins in millet that overwintered in the field. F. sporotrichoides and F. poae were principal fungi in the infected grain. Recently, T-2 toxin and other trichothecenes have been identified in cultures of F. sporotrichoides isolated from grains associated with clinical cases of alimentary toxic aleukia. (84)

19.4 From the available data, it appears that aflatoxin is likely to be the mycotoxin that occurs most frequently in the millet-producing regions of Africa and India.

20. OILSEEDS: GROUNDNUTS

20.1 Groundnut Producing Regions, World Production and Trade

Groundnuts are grown in the warmer regions of all six continents. In 1975, 38 countries were reported to have each produced 50 thousand tons or more of in-shell groundnuts: 21 countries in Africa, 12 in Asia, 2 each in South and North America and 1 in Central America. Producing areas lie in temperate, sub-tropical and tropical regions. World production in that year was over 19 million tons. Over 815 thousand tons of shelled nuts and about 1.1 million tons of groundnut cake entered international trade. (2, 67)

20.2 Use of Groundnuts as Food and Feed

Groundnuts are an important source of food protein in countries of Africa and Asia. They also are a major source of food oil in these countries. The cake, a by-product of oil production, is fed to domestic livestock or is exported. A small quantity of food grade cake is used as a food ingredient.

20.3 Mycotoxins Found in Groundnuts

Two mycotoxins have been detected in groundnuts - aflatoxin and citrinin. There has been, however, only one report of the occurrence of citrinin.

20.4 AFLATOXINS

20.4.1 Aflatoxins: geographic distribution in groundnuts in the producing regions

Groundnuts are highly susceptible to aflatoxin contamination and aflatoxin has been reported in this crop in the producing regions of all continents.

20.4.2 Aflatoxins: sites of contamination in the growing, harvesting and storage of groundnuts

20.4.2.1 Aflatoxin contamination of groundnuts can occur in the field prior to lifting, during harvesting, farm and commercial storage and transport. Aspergillus flavus infection of the kernel and formation of aflatoxin has been found to occur under drought conditions when plant growth is retarded and resistance to mould invasion is reduced. In the 1972 crop in Southeastern USA, aflatoxin contamination was present in groundnuts lifted from drought-stricken fields whereas those lifted from adjacent irrigated fields, or from irrigated parts of the same field, were free from aflatoxin. Drought conditions may encourage termite damage to the pod, providing a route for entry of A. flavus into the kernel. Such damaged kernels from plants grown under adequate rainfall conditions may be aflatoxin contaminated at lifting, as frequently are also kernels from plants that have died prematurely. Pods left in the ground after maturity have less resistance to mould invasion, and may be contaminated at lifting.

20.4.2.2 Aflatoxin contamination often occurs during the period that groundnuts are drying after lifting. If the drying period is prolonged beyond 5 to 7 days, there is high risk of A. flavus growth and aflatoxin formation. Damage to the pod in harvesting increases the risk of mould invasion of the kernel. Various practices have been introduced to hasten drying. These include inverting the windrow to expose the pods to the sun and air, picking the pods from the vine and sun-drying in a thin layer, and drying the picked pods with artificial heat.

20.4.2.3 Storage is a third site where aflatoxin contamination frequently develops. Mould growth and toxin formation are likely to occur if the groundnuts have not been dried to a safe moisture level of 9% or less, the level depending on the ambient temperature, extent of existing mould infection and intended period of storage. Accumulation of moisture in local regions of bulk stored groundnuts can raise the moisture content above safe levels and initiate mould growth and toxin formation. Moisture accumulation can result from temperature differences between the interior and exterior of the bulk, causing moisture migration to the cooler areas. Forced ventilation of the storage reduces the temperature gradients and redistributes the moisture, thereby preventing mould growth. Leaky storage facilities that allow the groundnuts to be wetted by rain are a more obvious cause of mould damage. Wetting frequently occurs in open storage that relies on tarpaulins and dunnage to protect bagged groundnuts from the rain. Leaky tarpaulins that cover the pile, or faulty dunnage beneath the pile, can allow serious wetting.

20.4.2.4 Wetting during transport of groundnuts in open vehicles also has been reported as a cause for subsequent mould growth and mycotoxin contamination.

20.4.3 Aflatoxins: incidence in groundnuts in North America

20.4.3.1 United States and Mexico are the principal producers of groundnuts in North America. Reports on aflatoxin incidence are available only from the former country.

United States

20.4.3.2 Groundnuts are grown in three principal regions of the United States - the Southeast, Southwest and the Virginia-Carolina region. Different varieties are grown in each region. Total production was 1.75 million tons (in shell) in 1975. About 230 thousand tons of shelled nuts were exported that year. Major domestic use is as human food. (2)

20.4.3.3 Soon after aflatoxin contamination was discovered in the domestic crop, a programme was initiated to control the aflatoxin content in marketed groundnuts. A system of analyses and certification was developed by the responsible units of government (U.S. Department of Agriculture and the Food and Drug Administration) and industry which provides comprehensive data on the crop as farmers stock groundnuts (in shell) or as shelled nuts.

Groundnuts are rejected for food use at the first buying point if they show visible Aspergillus flavus mold growth. The variation in rejections by year and by growing region are shown in Table II/11. A. flavus incidence is greatest in the Southeastern region. The rejected nuts are used for oil extraction; limitations are placed on the use of the groundnut cake. (13, 90, 91)

Table II/11. FARMERS' STOCK GROUNDNUTS REJECTED FOR VISIBLE ASPERGILLUS FLAVUS BY YEAR AND BY AREA, AS PER CENT OF TOTAL CROP.

Area	Year					
	1968	1969	1970	1971	1972	1973
Southeast	6.3	1.4	3.4	2.6	13.5	2.6
Southwest	1.2	2.4	1.4	2.5	1.9	0.8
Virginia-Carolina	0.5	0.07	0.4	0.3	0.2	0.1
All Areas (average)	3.7	1.4	2.1	2.1	8.1	1.6

20.4.3.4 A second control point is after shelling. Maximum level permitted in shelled nuts that will be subjected to further processing before becoming available as a consumer produce is 25 $\mu\text{g}/\text{kg}$. The percentage rejected on this basis is given in Table II/12. Rejections were highest in the Southeastern region and generally paralleled rejection of farmers' stock groundnuts. (91)

Table II/12. SHELLED GROUNDNUT LOTS CONTAINING TOTAL AFLATOXINS GREATER THAN 25 $\mu\text{g}/\text{kg}$ - PERCENT OF ALL LOTS FROM EACH PRODUCING AREA FOR CROP YEAR 1971-1975.

Crop Year	Area			
	Southeast	Southwest	Virginia-Carolina	All areas
1971	6.1	1.8	1.4	4.1
1972	12.7	1.4	0.5	7.7
1973	6.9	4.0	1.5	5.1
1974	2.0	2.4	0.6	1.8
1975	3.4	4.2	0.7	3.1
Average	6.2	2.8	0.9	4.4

20.4.3.5 The manufacturers of groundnut consumer products such as groundnut butter reduced the aflatoxin level in the shelled product to concentrations below 20 $\mu\text{g}/\text{kg}$, the level permitted by FDA. This is accomplished by screening to remove broken and shrivelled nuts and by electronic sorting to remove discolored kernels. Roasting also causes some reduction in aflatoxin content. The effectiveness of these procedures is indicated by FDA data on consumer products given in Table II/13. (92)

Table II/13. CONSUMER GROUNDNUT PRODUCTS. PERCENT OF SAMPLES EXAMINED BY FDA WITH AFLATOXIN LEVELS ABOVE THE GIVEN VALUES FOR FISCAL YEARS 1973-1975.

Total Aflatoxin $\mu\text{g}/\text{kg}$	Fiscal Year		
	1973	1974	1975
50	0	0,5	0
20	3	3	2
15	5	6	3
10	7	8	3,5
5	9	13	5
No. of samples examined	526	169	317

20.4.3.6 Samples of finished products with levels that exceed raw material certification guidelines can be attributed to the great variance inherent in sampling and analyzing groundnuts for aflatoxin contamination. (13, 93)

20.4.4 Aflatoxins: incidence in groundnuts in Central America

Groundnut production is relatively small in Central America; about 100 thousand tons were grown in 1975. Analyses for aflatoxin have been reported from one Central American country. Of 7 samples analyzed, aflatoxin was detected in one at a level of 32 $\mu\text{g/kg}$. (27)

20.4.5 Aflatoxins: incidence in groundnuts in South America

20.4.5.1 One country, Brazil, has reported analyses of groundnuts for aflatoxin. That country grows 300 to 500 thousand tons of groundnuts annually, largely in the state of São Paulo. In years of continuous rain during harvest of the rainy season crops, 60-80 percent of the crop may be contaminated with aflatoxin as a result of insufficient drying prior to picking and storage. Less contamination occurs in the dry season crop but poor drying and storage practices sometimes result in mould growth and toxin formation. (6)

20.4.5.2 Levels of contamination vary from year to year. In crop year 1966-1967, aflatoxin levels were in the range 2,600 to 3,400 $\mu\text{g/kg}$ for 268 samples of groundnut flour analyzed with some samples containing more than 10,000 $\mu\text{g/kg}$. Samples collected from the rainy season crop averaged about 5,000 $\mu\text{g/kg}$, those from the dry season crop about 1,000 $\mu\text{g/kg}$. In 1967-1968, 240 samples collected from 4 oil mills in 6 different periods had an average aflatoxin content of 1,000 $\mu\text{g/kg}$; maximum level observed was 15,000 $\mu\text{g/kg}$. Periods of rain during and after harvest are the major factors that determine the aflatoxin content of the groundnuts. (6)

20.4.5.3 Groundnut meals intended for prepared feeds are now commonly analyzed for aflatoxin. Export meals also are usually analyzed. However, small food processors are not generally aware of the aflatoxin problem and may use contaminated material. (6)

20.4.6 Aflatoxins: incidence in groundnuts in Asia

20.4.6.1 Five countries in Asia have reported detection of aflatoxin in groundnuts: India, Thailand, the Philippines, Indonesia and Taiwan. Not all have conducted extensive surveys but the reports indicate a high incidence of aflatoxin contamination in the crop produced in these countries.

India

20.4.6.2 India produced nearly 7 million tons of groundnuts in 1975. They are grown in 17 of the 21 states, ranging from Punjab in the north to Kerala in the south of India. Rainfall in the growing areas varies from 60 cm to 125 cm or more annually; some groundnuts are grown under irrigation. (10)

20.4.6.3 Several studies have been conducted on aflatoxin contamination in groundnuts grown and harvested under different conditions of rainfall and humidity. In a study of groundnuts contamination in Andhra Pradesh, reported in 1965, samples were collected from crops of both the dry and the rainy seasons grown in coastal districts where there is generally high humidity. A total of 743 in-shell samples and 141 samples of groundnut cake were analyzed. Aflatoxin levels between 1,000 and 5,000 $\mu\text{g/kg}$ were found in 12% of the samples. Levels were highest in samples of the rainy season crop. (10)

20.4.6.4 A 1965-1967 survey of groundnuts and groundnut cake samples collected from districts of Gujarat, Andhra Pradesh and Madras states showed that 10% to 40% of the groundnuts and 82% of the cakes contained fairly high levels of aflatoxin. (10)

20.4.6.5 Nearly 50% of 500 samples of groundnuts collected in a 1967-1968 survey of the West Coast crop contained aflatoxin B₁ at levels between 100 to 250 µg/kg. (10)

20.4.6.6 A study reported in 1976 of groundnut cake samples collected mostly from Madhya Pradesh region revealed that more than 50% of the samples were positive for aflatoxin. (10)

20.4.6.7 A process has been developed for detoxification of groundnut protein by treatment with hydrogen peroxide in an aqueous solution. (10)

20.4.6.8 Aflatoxin contaminated groundnut meal has been implicated in several disease outbreaks in farm animals in India. Groundnut cake was the major protein component of the ration of 126 crossbred cattle involved in a toxicosis in which 58 died. Level of aflatoxin in the ration ranged from 1100 to 25000 µg/kg. Aflatoxin contaminated groundnut cake was held responsible for aflatoxicosis in pigs in an outbreak in Kerala state. Levels in samples causing disease in this and other cases were estimated to be generally above 20,000 µg/kg. (10)

Taiwan

20.4.6.9 Groundnuts are among the important daily foods in Taiwan and were included in a survey of foods for aflatoxin in 1966. Samples of groundnuts from storages of 3 of the 8 oil mills surveyed contained aflatoxin B₁ levels in the range of 40 to 430 µg/kg. Nine of 13 samples of peanut butter from 8 manufacturers had no detectable aflatoxin. Groundnut cake samples collected from 4 of 12 oil mills were positive for aflatoxin and contained from 80 to 290 µg/kg of B₁. (71)

Thailand

20.4.6.10 Thailand produces about 260,000 tons of groundnuts annually. Some of the crop is crushed for oil and part of the groundnut cake is exported. Most groundnuts are eaten as snack items; data were not available on the quantities consumed as food. (2, 11)

20.4.6.11 Aflatoxin levels in groundnuts were determined in a survey of all Thai foods carried out over a 2-year period in 1967-1969. Food samples were collected from over 100 towns and villages during both the rainy and dry seasons. Of 219 samples of groundnuts analyzed, aflatoxin was detected in 49% at an average level of 1,530 µg/kg (total aflatoxins) in the contaminated samples. Highest level observed was 12,300 µg/kg. Among all foods analyzed, incidence and levels were highest in groundnuts. Contamination was most frequent in samples collected in the rainy season, when average concentrations of total aflatoxins in all groundnut samples reached almost twice the values observed in the hot season. (11)

Indonesia

20.4.6.12 Average annual production of groundnuts in Indonesia is about 291,000 tons. About 10% of the crop is exported. The major production is in 8 of the 27 provinces. Indonesia is located in the tropics with annual rainfall ranging from 80 to 144 inches. Groundnuts mature and are harvested in the dry season which usually extends from June to October.

20.4.6.13 A comparison of aflatoxin contamination of groundnuts samples collected from 3 points in the marketing system in 1970 and 1976, is given in Table II/14. (72)

Table II/14. AFLATOXIN CONTENT OF GROUNDNUT SAMPLES TAKEN FROM COLLECTORS, SUB-DISTRIBUTORS AND RETAILERS IN INDONESIA, 1970 and 1976

Date	Source	No. Samples	Aflatoxin level, ug/kg	
			B ₁	G ₁
1970	Collector	2	ND*	ND
	Sub-distributor	4	trace	trace
	Retailers**	10	ND-4100	ND-460
1976	Collector	2	ND	ND
	Sub-distributor	4	ND	ND
	Retailers***	12	ND-67	ND-8
* ND = not detectable ** Rainy season *** Dry season				

20.4.6.14 The higher level of aflatoxin in retail groundnuts, particularly in 1970, is attributed to inadequate protection from wetting during rainy weather. (72)

20.4.6.15 In West Java, groundnut press cake is fermented to make a food called "oncom". The press cake is the by-product of oil extraction in manually operated presses. Aflatoxin levels found in a study of the process are tabulated in Table II/15. Aflatoxin level was reduced in both the fermentation of the press cake to make "oncom" and the subsequent frying operation. The higher level of aflatoxin in groundnuts used to make groundnut oil, Table II/15, as compared to those destined for the retail market, Table II/14, was attributed to the use of the lower grades of groundnuts for oil extraction. Manufacturers of groundnut food products tend to use the lower grades of groundnuts for products in which the appearance of the kernel is not important. This is reflected in the aflatoxin levels of the products as shown in Table II/16. (72)

Table II/15. AFLATOXIN CONTENT OF GROUNDNUTS, GROUNDNUT PRESS-CAKE, GROUNDNUT OIL AND FRIED "ONCOM" TAKEN FROM BANDUNG, WEST JAVA

Food/Commodities	No. Samples	Aflatoxin content (average value) ug/kg	
		B ₁	G ₁
Peanuts	20	180	353
Peanut presscake	20	126	174
Peanut oil	20	61	82
Oncom	39	67	120
Fried oncom	16	41	83

Table II/16. AFLATOXIN CONTENT OF GROUNDNUT PRODUCTS MARKETING IN INDONESIA

Groundnut Product	No. samples	Aflatoxin content (average) $\mu\text{g/kg}$	
		B ₁	G ₁
Fried groundnuts	4	ND	ND
Flour coated fried groundnuts	3	ND	3
Sweetened groundnut cake	2	170	83
Groundnut sauce	5	83	49
Groundnut butter	3	13	ND

20.4.6.16 Most Indonesian groundnuts that are exported go to the Netherlands. Those containing more than 5 $\mu\text{g/kg}$ of aflatoxin B₁ are considered to be positive. Analysis of groundnuts exported in 1975/1976 showed 75%¹ to be free of aflatoxin. (72)

20.4.6.17 Special attention is being given by the Indonesian government to educational programmes on preventative measures that should be taken by the farmers and collectors to avoid aflatoxin contamination.

Philippines

20.4.6.18 Groundnut production in the Philippines was 36,000 tons in 1975, about double that in 1971-1973. Over half of the crop is grown in the Cagayan Valley. Much of the crop is shipped to the peanut processing industry in Manila. Over 90% of the production is used as human food, mainly in the form of snack items. None is fed to livestock. (32)

20.4.6.19 A survey of Philippine foods carried out in 1967-1968 by the Food and Nutrition Research Institute showed that 66% of 173 samples of raw groundnuts analyzed contained aflatoxin. Twenty percent had aflatoxin levels in the range 10 to 885 $\mu\text{g/kg}$ (average of all positives: 56 $\mu\text{g/kg}$). Higher incidence and levels were found in groundnut butter. Aflatoxin was detected in 99% of 485 samples analyzed; 91% had levels in the range 20 to 8600 $\mu\text{g/kg}$. Average level of all positives was 196 $\mu\text{g/kg}$. Mean level of positives in 173 samples of sidewalk-vended roasted peanuts was 49 $\mu\text{g/kg}$. Aflatoxin was detected in 70% of the samples with 25% containing between 10 and 791 $\mu\text{g/kg}$. (32)

20.4.6.20 Aflatoxin concentration in groundnut butter and other groundnut products has been monitored each year, since 1968. The results are tabulated in Table II/17. (32)

20.4.6.21 The level of aflatoxin appears to have decreased since 1973. The improvement in quality of groundnut butter and other processed products is attributed to joint efforts of the Philippine Food and Drug Administration and the groundnut processing industry. The Food and Drug Administration initiated an educational programme and surveillance of processed products. The groundnut products manufacturer whose product was rejected in the United States undertook a massive educational programme involving farmers and middlemen. In the years that followed, rejection of groundnuts by the company decreased from 8% to less than 2% in 1976, mainly due to improvements in harvesting, drying and storage procedures. For instance, farmers invert their groundnut plants immediately upon harvest, middlemen now dry their product immediately whereas in the past they never undertook drying of their products. The company established an aflatoxin laboratory at the factory and now monitor their product. (32)

Table II/17. AFLATOXIN LEVELS IN PHILIPPINE PROCESSED GROUNDNUTS, 1968-1975

(32)

Item	Number Analyzed	% Negative	% > 30 µg/kg	Range µg/kg	Mean of Positives µg/kg
a. Groundnut butter					
1968	21	0	100.0	85 - 428	166
1969	88	0	97.7	tr - 742	152
1970	65	0	100.0	41 - 1064	185
1971	34	0	100.0	43 - 575	176
1972	29	0	96.6	18 - 1705	296
1973	61	0	90.2	tr - 265	97
1974	111	0	82.9	9 - 332	90
1975	18	11.11	33.3	neg - 155	38
b. Other Groundnut Products					
1968	30	6	3.3	neg - 112	34
1969	55	25.4	23.6	neg - 885	73
1970	37	10.8	35.1	neg - 975	90
1971	15	13.3	33.3	neg - 220	67
1972	27	18.5	18.5	neg - 673	63
1973	35	37.1	17.1	neg - 223	36
1974	14	0	35.7	tr - 110	32
1975	27	14.8	11.1	neg - 204	34

20.4.7 Aflatoxins: incidence in groundnuts in Africa

20.4.7.1 Aflatoxin in groundnuts has been reported in three countries in East Africa - the Sudan, Uganda and Mozambique; two countries in West Africa - Senegal and Nigeria; and one in Southern Africa - Swaziland. It may be concluded from this distribution of incidence, that aflatoxin contamination is likely to be found in groundnuts in all producing countries of Africa.

Nigeria

20.4.7.2 Average annual groundnut production in Nigeria for 1973 was about 340,000 tons from which 60 thousand tons of derived groundnut cake were marketed abroad each year. Groundnuts are the major cash crop of Northern Nigeria and are also valued as a food crop by the farmer. (85)

20.4.7.3 The crop is grown in two main zones: (1) the northern zone with a drier climate, an annual rainfall of 75 to 90 cm, and a rainy season from June to September; and (2) the riverain zone with a humid climate, an annual rainfall of 100 to 125 cm, and a rainy season from April to November.

20.4.7.4 Samples of groundnuts for aflatoxin analyses were collected in 1973 from major storage sites, lots delivered to oil mills and from vendors in the Kano city market. Aflatoxin content of 35 samples taken from storage sites ranged from 50 to 200 $\mu\text{g/kg}$ (mean, 230 $\mu\text{g/kg}$). The range in concentrations in 51 samples from groundnuts delivered to oil mills was 50 to 1000 $\mu\text{g/kg}$ (mean, 150 $\mu\text{g/kg}$). Seven samples purchased from vendors had levels ranging from 50 to 60 $\mu\text{g/kg}$. Groundnut cakes from 5 oil mills in Kano also were sampled and analyzed. Aflatoxin levels were between 270 and 400 $\mu\text{g/kg}$ and averaged 380 $\mu\text{g/kg}$, excluding one sample that had a level greater than 1000 $\mu\text{g/kg}$. The high level was in a sample from a mill which drew groundnuts from the riverain zone. For comparison, analyses of groundnut cakes from these oil mills in 1966 showed an average aflatoxin content of 250 $\mu\text{g/kg}$ and a range from 10 to 1000 $\mu\text{g/kg}$. (86)

20.4.7.5 Handpicked selected groundnuts (HPS) from Kano State usually contain less than 5 $\mu\text{g/kg}$ aflatoxin and rarely more than 50 $\mu\text{g/kg}$. In the riverain area, however, HPS groundnuts in 1973 contained more than 70 $\mu\text{g/kg}$ aflatoxin. Rejected nuts contained about 400 $\mu\text{g/kg}$ toxin and were sold in the local market. (86)

20.4.7.6 Experimental studies carried out in Northern Nigeria showed that the incidence of aflatoxin can be greatly reduced by simple methods that can be understood and used by the farmers. These methods, listed below, are part of an extension programme farmers in the major groundnut areas, operated by the Field Service Division, Northern Nigeria Ministry of Agriculture: (85)

- plants should be lifted as soon as the crop is ripe;
- care should be taken to avoid damaging the pods during harvesting;
- damaged pods and pods and kernels that have been attacked by moulds should be destroyed;
- in the riverain zone, when the crop is harvested under wet conditions, the pods should be picked from the vine and sun-dried in a layer one pod deep;
- once dried, pods and kernels should be stored under dry conditions pending sale to the Marketing Board.

Senegal

20.4.7.7 Groundnuts are a major crop in Senegal, over 1 million tons being produced annually. They are an important domestic food but most of the crop is crushed for oil and practically all the groundnut cake is exported for animal feed. Senegal has a hot, generally dry climate. Rainfall occurs between July and October and dry weather prevails the remainder of the year. Groundnuts are harvested during the first week in October. (87)

20.4.7.8 Aflatoxin contamination is the principal problem in the production of groundnuts in Senegal. Aflatoxin content of groundnut cake from Ziguinchor ranged from 70 to 350 $\mu\text{g/kg}$ in 1970; from 140 to 700 $\mu\text{g/kg}$ in 1971; from 70 to 140 $\mu\text{g/kg}$ in 1972 and from 140 to 750 $\mu\text{g/kg}$ in 1973. (88)

20.4.7.9 Studies on chemical methods of detoxification by ammonia treatment have been conducted by the government and industry and research is being supported on agronomic, biological and chemical aspects of the aflatoxin problem. (87)

The Sudan

20.4.7.10 Production of groundnuts has increased almost four-fold in the last 15 years and was over 1 million tons of in-shell nuts in 1975. In that year, about 203,000 tons of shelled groundnuts and 32,000 tons of groundnut cake were exported. In 1970, about two-thirds of the crop was produced in rainfed areas and the remainder under irrigation. Expansion of the crop was expected in irrigated areas. (2, 89)

20.4.7.11 In a 1969-1970 survey of the crop for aflatoxin contamination, samples from the rainland 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 aflatoxin. Levels in five samples were below 50 µg/kg, one contained 50 to 250 µg/kg and concentrations in three were greater than 1000 µg/kg. In contrast, aflatoxin was detected in 62 of the 63 samples collected from the rainfed areas; levels in 23 were less than 50 µg/kg, 27 had concentrations between 50 and 1000 µg/kg, and 11 had levels above 1000 µg/kg. (89)

20.4.7.12 The high incidence of contamination in the rainland samples was attributed to improper drying during the six or seven days immediately following harvest. Post-harvest washing to remove soil from nuts grown under irrigation in heavy clay soils also was identified as a cause for aflatoxin contamination. (89)

20.4.7.13 It was estimated in 1967 that losses of around three-quarters of a million pounds per annum were being incurred because no systematic aflatoxin analysis of groundnut kernels, or cake, for export were being carried out. (89)

Uganda

20.4.7.14 Groundnuts are an important food crop in Uganda. Per capita daily consumption was about 26 g in 1964-1966 and was among the highest reported in the FAO Food Balance Sheets. Production was 215,000 tons in 1975, most of which was used domestically. (3,2,67)

20.4.7.15 In a survey of foodstuffs conducted in 1966-1967, samples of foods were collected from randomly selected native homes and markets from every part of Uganda. Of 152 samples of groundnuts assayed, 18% contained aflatoxin, 5% had levels in the range of 100 to 1000 µg/kg and 5% had concentrations greater than 1000 µg/kg. The report did not relate the incidence of aflatoxin in groundnuts to cultural practices or climatic conditions but did note that incidence of aflatoxin contamination in foods generally was highest in two provinces, one of which had a dry climate. (8)

Mozambique

20.4.7.16 Groundnuts are produced in Mozambique both for use as a domestic food and as a cash crop for export. Production in 1975 was about 120,000 tons. (2, 67)

20.4.7.17 In a survey of foods conducted in the Inhambane district, groundnuts were found to be the main source of dietary protein and were also identified as the main source of aflatoxin contamination. Analysis of 153 samples of groundnuts collected from dry stored foods available to households showed the mean aflatoxin concentration to be 1036 µg/kg. In-shell nuts contained less aflatoxin than shelled nuts. Average level in the in-shell nuts was 233 µg/kg whereas the exposed shelled nuts averaged 1838 µg/kg. However, aflatoxin levels in prepared foods were relatively low when compared with those found in stored groundnuts, suggesting a considerable degree of selection by the housewife prior to the preparation of food. (9)

Swaziland

20.4.7.18 The groundnut is an important source of dietary protein in Swaziland. The crop is dried in a variety of ways after harvest including windrows, platforms and small stacks. After hand picking, the pods are given a further period of sun-drying prior to storage. (34)

20.4.7.19 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. Of 93 samples of groundnuts, 11 contained aflatoxin. Average concentration of all samples, including negatives, was 10.6 µg/kg. Groundnuts that had been stored five or six months contained about five times as much aflatoxin as those sampled shortly after entering storage (34)

21. OILSEEDS: COTTONSEED

21.1 Cottonseed Producing Regions, World Production and Trade

Cottonseed is a by-product of the production of cotton. Cotton is grown in tropical, subtropical and warmer areas of temperate regions of all continents. Major producing countries are China, India, Pakistan and Iran in Asia; Brazil, Argentina, Colombia and Peru in South America; the United States and Mexico in North America; Nicaragua, Guatemala and El Salvador in Central America; Egypt, the Sudan, Nigeria, Rhodesia, Uganda and South Africa in Africa; and Greece in Europe. World production of cottonseed in 1975 was about 22.5 million tons. About 10% of it entered international trade either as cottonseed or as cottonseed cake. (2)

21.2 Use of Cottonseed as Food and Feed

Some cottonseed is fed to livestock but most of the cottonseed produced is crushed or extracted for recovery of cottonseed oil that is used as a food oil. The residual cake or meal is almost entirely used as livestock feed. Small quantities are used as a food ingredient in some countries.

21.3 Mycotoxins Found in Cottonseed

Only one mycotoxin - aflatoxin - has been reported in cottonseed or in cottonseed cake.

21.4 AFLATOXINS

21.4.1 Aflatoxins: geographic distribution in cottonseed in the producing regions

Analyses of cottonseed for aflatoxin have been reported in two countries in Asia - India and Iran; one in Europe - Greece; one in North America - the United States; and three in Central America - Guatemala, El Salvador and Nicaragua. This represents a small sampling of the cottonseed producing countries. However, the information obtained indicates the incidence of aflatoxin and levels that may be expected under various cultural conditions.

21.4.2 Aflatoxins: sites of contamination in the growing, harvesting and storage of cottonseed

21.4.2.1 Aflatoxin contamination of cottonseed has been detected in the field prior to harvest and is a serious problem in some areas; it also has been demonstrated in storage of high-moisture seed. Field contamination is associated with a boll rot caused by Aspergillus flavus. A characteristic sign of A. flavus infection is a bright, greenish yellow fluorescence of the fibre or seed. A. flavus boll rot is most prevalent in hot, dry regions where cotton is grown under irrigation. Average daily maximum temperatures of 35 to 42°C during the boll opening period have been found to be associated with a high incidence of boll rot and aflatoxin contamination. (94)

21.4.2.2 Storage of cottonseed at 15%, 18% and 22% moisture at 27° or 29°C resulted in A. flavus growth and aflatoxin contamination which reached a maximum within 30 days. Other studies have indicated that cottonseed could be stored for several months at 9 to 10% moisture without increase in aflatoxin content. (95, 96)

21.4.3 Aflatoxins: incidence in cottonseed in North America

21.4.3.1 The United States and Mexico are both producers of cotton and cottonseed but analyses for aflatoxin have been reported only in the United States.

21.4.3.2 In the United States, cotton is grown in most of the southern states, ranging from Georgia on the Atlantic coast to California in the Far West. Cultural and climatic conditions vary widely in the cotton-growing areas and are differentiated by the very hot temperatures and low rainfall in the Far West and Southwest areas where cotton is grown under irrigation as contrasted to the higher rainfall and humidity, but lower temperatures of the Southcentral and Southeastern states. These differences in climatic conditions are associated with the incidence and level of aflatoxin detected in cottonseed as shown in Table II/18. Cottonseed grown in the Far West and, to a lesser extent, in that grown in the Southwest, had higher incidence and levels of contamination than the other two regions in the 1964-1966 crops. For all regions there was a marked variation in incidence by year. From 4% to 16.6% of the cottonseed in the Far West had aflatoxin levels above 30 µg/kg in 1964-1966; whereas only 0 to 2.2% exceeded this limit in the Southeastern and Southcentral states. (94,)

21.4.3.3 Detoxification of cottonseed and cottonseed meal by ammonia treatment is practiced in oil mills in the Far West and Southwest. (98, 99)

21.4.3.4 Aflatoxin contamination has been shown to be associated with Aspergillus flavus boll rot which is prevalent in hot, dry cotton-growing areas where exceptionally high temperatures (35° to 42°C average maximum daily temperatures) prevail at the time of boll opening. Surveys of the cotton crop over several years showed that boll rot was rare in most U.S. cotton-growing areas but occurred regularly in the Imperial Valley of California and frequently in Arizona. Although pink boll worm damage increases the incidence of boll rot and aflatoxin contamination in the hot and dry regions, the pink boll worm has been present in the crop for many years in other regions where the occurrence of A. flavus infection is rare. In these more humid regions it appears that other mould species are competitive with A. flavus and dominate growth in wounds caused by the boll worm. (94)

21.4.4 Aflatoxins: incidence in cottonseed in Central America

Cotton and cottonseed are major exports of Central America. Cottonseed production in 1975 was over 500,000 tons from which about 150,000 tons of derived cottonseed cake were exported.

21.4.4.1 In a survey of the 1971 cottonseed crop, 99 samples of freshly ginned cottonseed were collected during the harvest season (November to April, 1971), at various gins throughout the cotton-producing areas of Guatemala, Nicaragua and El Salvador. Aflatoxin was detected in 4 of the samples - one from Guatemala (60 µg/kg), one from Nicaragua (30 µg/kg) and two from El Salvador at levels of 30 and 43 µg/kg respectively. The 4% incidence is about the same as that in the United States for rainfall-grown cotton. (100)

Table II/18. PERCENT OF SAMPLES OF COTTONSEED ANALYZED BY THE U.S.D.A. WITH LEVELS OF AFLATOXIN B₁ ABOVE THE GIVEN LEVELS FOR CROP YEARS 1964-1966, BY AREA.

(from: Marsh, P.B. and Simpson, M.E. 1976. Influence of some field conditions on the occurrence of aflatoxin in cottonseed at harvest. Pro. Conf. of Collaborators from Southern Agricultural Experiment Stations., New Orleans, U.S.A. Dept. Agr. Bull. ARS-S-7)

Region	Crop year	Number of samples	Aflatoxin B ₁ µg/kg			
			>10	>30	>70	>150
Southeast	1964	123	2.4	1.6	0.8	0.8
	1965	172	3.0	2.0	0.5	0
	1966	140	0	0	0	0
South Central	1964	339	0.9	0.6	0.3	0
	1965	474	3.9	2.2	1.7	0
	1966	386	0	0	0	0
Southwest	1964	246	0	0	0	0
	1965	344	4.2	2.5	1.0	0.5
	1966	280	1.1	0.7	0.3	0.3
Far West	1964	227	18.1	15.2	9.8	5.9
	1965	317	6.8	4.0	1.6	0.8
	1966	259	23.4	16.6	13.0	9.4

21.4.5 Aflatoxins: incidence in cottonseed in Asia

21.4.5.1 Two countries in Asia - India and Iran - have reported analyses and detection of aflatoxin in cottonseed and cottonseed cake.

Iran

21.4.5.2 In a survey conducted by the Regional Mycotoxin Training and Research Institute at the University of Isfahan, 147 samples of cottonseed and 143 samples of cottonseed cake were collected from widely distributed Iranian cities. Aflatoxin was detected in 6 of the cottonseed samples whereas 75 of the cottonseed cake samples contained levels up to 46 µg/kg. Aflatoxin in cottonseed cake fed to dairy cows was considered to be a contributing source of aflatoxin contamination found in milk in Iran. (97, 131)

India

21.4.5.3 About 2.1 million tons of cottonseed are produced annually in India. Most of it is fed as such, or after oil extraction, to domestic livestock. However, about 200,000 tons of cottonseed cake are exported each year. (2, 10)

21.4.5.4 Cotton is grown in areas of India having annual rainfall ranging from 75 cm in Central India to over 250 cm in Assam and Kerala states. It is also grown under irrigation in drier states. (10)

21.4.5.5 Surveys for the incidence of aflatoxin in cottonseed have shown that more samples from humid areas were infected with Aspergillus flavus than those from dry areas; there was a positive correlation with aflatoxin incidence. About 48% of humid area samples showed aflatoxin levels higher than 500 µg/kg whereas 28% of the dry area samples did not exceed 50 µg/kg. (10)

21.4.5.6 It was considered that harvesting of well dried bolls, proper management practices after harvest and storage of cottonseed at a moisture level of 7% would prevent aflatoxin incidence in cottonseed. (10)

21.4.6 Aflatoxins: incidence in cottonseed in Europe

21.4.6.1 Analyses of cottonseed for aflatoxin have been reported from Greece, one of the ten producing countries in Europe. Analyses of imported cottonseed cake have been reported by Germany, Sweden and Denmark.

Greece

21.4.6.2 In a survey of farm products grown in various regions of Greece, three samples of cottonseed and 17 of cottonseed cake were collected. Aflatoxin B₂ was detected in one sample of cottonseed at a level between 10 and 50 µg/kg and aflatoxin B₁ was found in one sample of cottonseed cake at a concentration between 50 and 100 µg/kg. (63)

Federal Republic of Germany

21.4.6.3 A survey of imported oilseed meals conducted in farm year 1972-1973 showed that 13 of 14 samples of cottonseed products contained aflatoxin at concentrations in the range of 35 to 3,220 µg/kg. Average concentration was 786 µg/kg. In 1974, analyses of 7 samples of cottonseed meal for aflatoxin B₁ gave an average value of 39 µg/kg; highest concentration was 93 µg/kg. (101, 102)

Denmark

21.4.6.4 A survey, reported in 1970, of imported cottonseed products from eight major exporting countries (Brazil, Colombia, Guatemala, Nicaragua, El Salvador, Syria, Turkey and the U.S.S.R.), found aflatoxins in one-third of 120 samples tested. Average level of total aflatoxins was 30 µg/kg; range, 5 to 120 µg/kg. The incidence by country ranged from 21 to 100% of the samples tested. (103)

Sweden

21.4.6.5 Five samples of cottonseed cake were included in a survey of imported oilseed meals reported in 1970. Aflatoxin was detected in one sample at a level above 50 µg/kg. (104)

22. OILSEEDS: SOYBEANS

22.1 Soybeans are a major world oilseed crop; production in 1975 exceeded 68 million tons. Major producing countries are the United States, Brazil, Argentina, China, N. and S. Korea, Indonesia and Romania. Soybeans are an important food crop especially in Eastern Asia where the domestic production is largely processed for food. In other countries the oil is extracted for food purposes and the soybean cake is mainly used for livestock production. Over the past few years, however, an increasing quantity of the meal has been processed into flour, protein concentrates and protein isolates for edible uses. Soybeans and soybean cake are important commodities in world trade; over 16 million tons of soybeans and 8.7 million tons of soybean cake were exported in 1975. (2)

22.2 Survey of the soybean crop in the United States indicates a very low incidence of aflatoxin. Of 852 samples collected at principal markets in 1964 and 1965, two contained aflatoxin at levels of 7 and 10 $\mu\text{g}/\text{kg}$, respectively. Both were USDA Sample Grade, the lowest of the 5 quality grades. (105)

22.3 Aflatoxin was not detected in 4 samples collected in Sweden; 4 in Indonesia or 2 in a Central American country. (104, 72, 26)

22.4 Analysis of 4 samples of soybean meal imported into West Germany were negative for aflatoxin. (101)

22.5 However, Hungary reported that 13.8 percent of 582 samples of soybean meal imported in 1975 contained aflatoxin at levels between 100 and 400 $\mu\text{g}/\text{kg}$; 5 percent of 1018 samples taken in 1976 contained aflatoxin concentrations in the same range. Origin of the meal was not stated. (40)

22.6 One of a number of samples of soybeans tested in Italy was found to contain aflatoxin B₁ at a concentration of 180 $\mu\text{g}/\text{kg}$. The origin of the beans was not stated.

22.7 An analysis of two fermented soybean products was reported from Indonesia. Aflatoxin was not detected in 10 samples of tempe but aflatoxin G₁ was found in 2 samples of soybean ketchup at a concentration of 345 $\mu\text{g}/\text{kg}$. (72)

23. OILSEEDS: COPRA

23.1 Copra is the dried meat of the coconut from which coconut oil is extracted. It should not be confused with desiccated coconut which is prepared from fresh coconuts for food use. World production in 1975 was 4.1 million tons. Over three-quarters of this was produced in South and Southeast Asia. Almost half of the world output of copra is found in a single country, the Philippines, which supplies more than 53 percent of copra and 23 percent of world coconut oil exports. (2, 32)

23.2 Copra is prepared by drying the husked and split nuts either by sun-drying or by drying with artificial heat in suitable kilns. Copra cake is the residual from the extraction of oil from copra. It is eaten by low-income population groups in some countries but is principally used as livestock feed. (10)

23.3 Aflatoxins: incidence in copra in Asia

Philippines

23.3.1 Most information concerning incidence of aflatoxin in coconut products comes from the Philippines. Results of a survey of coconut products conducted by the Food and Nutrition Research Institute are given in Table II/19. (32)

Table II/19. AFLATOXIN LEVELS IN PHILIPPINE COCONUT PRODUCTS

Product	No. of samples	Percent negative	Percent > 20 µg/kg	Mean of positives µg/kg	Highest level µg/kg
Coconut, fresh, grated	4	100	--	--	--
Coconut milk, fresh	1	100	--	--	--
Coconut with sugar	47	79	17	18	110
Copra	182	29	40	39	513
Copra cake	26	65	31	11	26

India

23.3.2 India has reported that various types of moulds have been found in wet copra commonly used for culinary purposes in households and other eating establishments. However, no systematic survey has been conducted for aflatoxin in copra. Significant levels of aflatoxin have been detected in copra cake which is mostly used as cattle feed. No adverse effects in cattle have been reported. (10)

23.4 Aflatoxins: incidence in other producing regions

Ghana

23.4.1 Samples of copra were collected from Axim in the Western region of Ghana. They had a greenish-yellow mould contamination. Analysis of an unstated number of samples showed aflatoxin (B₁ and G₁) concentration to be 88 µg/kg. (106)

United States

23.4.2 Imports of copra into the United States have been discontinued since FDA surveillance activity found aflatoxin in 88 percent of 72 samples of copra and copra meal tested. Total aflatoxin average was 46 µg/kg; range, trace to 200 µg/kg. (13)

Finland

23.4.3 Analyses of 16 samples of imported copra showed 63 percent contained aflatoxin in concentrations between 10 and 100 µg/kg (average 37 µg/kg). (107)

24. OILSEEDS: SUNFLOWER SEED

24.1 Largest production of sunflowers as an oilseed crop is in Central and Eastern Europe where U.S.S.R. is the largest single producer. Other countries producing substantial quantities include Argentina in South America, United States in North America, and Turkey in Asia. World production in 1975 was 9.6 million tons. (2)

24.2 No reports are available from the producing countries on the incidence of aflatoxin in domestic sunflower seed or cake. However, three countries have reported results of surveillance of imported sunflower cake. In Hungary, 9.6 percent of 73 samples analyzed in 1975 were contaminated with aflatoxin; in 1976, no aflatoxin was detected in 52 samples analyzed. In Germany, 4 samples of sunflower cake were analyzed in farm year 1972/1973. One sample contained 17 $\mu\text{g}/\text{kg}$ aflatoxin. No information is available concerning the conditions or site of contamination. (40, 101)

24.3 In Italy two samples of sunflower seeds analysed were found to contain 50 and 90 $\mu\text{g}/\text{kg}$ of aflatoxin B₁.

25. VEGETABLE OILS

Aflatoxin has been detected in crude oils extracted from aflatoxin-contaminated oil-seeds, including groundnuts, copra and olives. In some countries the crude oil is preferred for food use; in others, refined oil is the product of choice. The customary method of refining involves an alkali treatment which removes essentially all aflatoxin. In many countries the refined oils are bleached by absorption of dissolved plant pigments on special clays. This treatment also removes aflatoxin. (108)

25.1 GROUNDNUT OIL

25.1.1 In India aflatoxin concentrations between 20 and 200 $\mu\text{g/kg}$ (average 100 $\mu\text{g/kg}$) have been found in samples of groundnut oil collected from local markets. Investigations conducted at the Central Food Technological Research Institute, Mysore, have shown that 65% of the aflatoxin is in particulate matter in the oil, most of which can be removed by filtration. By adding an additional frame containing an absorbent to remove dissolved aflatoxin, 95% removal could be accomplished in a conventional plate and frame filter press. In other experimental studies it was found that exposure of groundnut oil contained in glass bottles to bright sunlight for one hour destroyed the aflatoxin. (109, 10)

25.1.2 In Taiwan analyses of groundnut oil samples collected from 17 mills in 1966 showed aflatoxin B_1 concentrations in the range 10-70 $\mu\text{g/kg}$ in oil from eight mills. Alkali refining is not practised in Taiwan. (71)

25.1.3 In Indonesia, 20 samples of groundnut oil were collected in Bandung, West Java for aflatoxin analysis. Average concentration of total aflatoxins ($B_1 + G_1$) was 143 $\mu\text{g/kg}$. (72)

25.1.4 Assays of samples of Nigerian groundnut oil taken in 1972 showed values between <5 and 40 $\mu\text{g/kg}$. Alkali refining is not generally practised in Nigeria, although some mills use a Fuller's earth bleaching treatment. (86)

25.2 COCONUT OIL

25.2.1 Analyses of 25 samples of coconut cooking oil in the Philippines showed that 60% contained aflatoxin at an average concentration of 3 $\mu\text{g/kg}$. Highest concentration was 7 $\mu\text{g/kg}$. No aflatoxin was detected in two samples of refined coconut oil. (32)

25.2.2 Coconut oil in India was found to contain aflatoxin at concentrations in the range 5-100 $\mu\text{g/kg}$. (10)

25.3 OLIVE OIL

25.3.1 Analysis of 12 samples of crude farm olive oil collected from different parts of Greece revealed total aflatoxins in 11 samples at levels ranging from 2 to 20 $\mu\text{g/kg}$. Four samples of commercial olive oil contained concentrations between 2 and 7 $\mu\text{g/kg}$. (63)

26. PULSES (Beans and peas)

26.1 Pulse Producing Regions, World Production and Trade

26.1.1 Pulses are produced throughout the world in both tropical and temperate regions. Approximately 64% of the world produce is grown in Asia, where the main producing countries are China, India and Pakistan. Africa and the U.S.S.R. each grow about 10% of the total world crop of pulses. In Africa the main producing nation is Nigeria, followed by Ethiopia and Morocco. A smaller quantity is grown in North and Central America, South America and Europe. (2)

26.1.2 A very large variety of pulses are produced, the variety being dependent on the area of production. However, the major crops can be broadly classified as follows:

About 30% of the total world product of pulses are dry beans (haricots)

"	23%	"	"	"	"	dry peas
"	12%	"	"	"	"	dry broad beans
"	10%	"	"	"	"	chick peas
"	2%	"	"	"	"	dry cow peas
"	5%	"	"	"	"	pigeon peas

26.2 Use of Pulses as Food and Feed

26.2.1 Legumes are very important in the dietary requirements particularly of vegetarians, as they are normally the only protein rich ingredient of their diet. These crops are of importance not only in providing the necessary proteins needed to maintain the health and growth of the population; they also fix atmospheric nitrogen in the soil and in this way fertilize the soil and thus enhance crop production.

26.2.2 The following are the pulses widely produced in countries where aflatoxin contamination is a problem:

India	:	Tur dal or pigeon peas (<u>Cajanus cajan</u>) Bengal gram (<u>Cicer arietinum</u>) Mung beans (<u>Phaseolus radiatus</u>) Black gram (<u>Phaseolus mungo</u>) White peas (<u>Pisum sativum</u>) Green gram (<u>Phaseolus aureus</u>)
Thailand and Hong Kong	:	Mung beans, soybeans, red, black, brown, white, horse, yellow, Burmese, kidney beans; chickpeas
Philippines	:	Mung beans, kidney beans, peas, chickpeas, pigeon peas, rice beans, lima beans, string beans
Costa Rica and Brazil	:	Black beans (<u>Phaseolus vulgaris</u>)
North America	:	Black eye, black turtle, garbanzo, great northern, lima, navy, pink, pinto and red beans

26.3 Mycotoxins found in Pulses

Aflatoxin has been shown to be the main mycotoxin contaminant of pulses. However, ochratoxin A was shown to be present in various samples of Canadian white beans and penicillic acid in certain bean samples from the U.S.A.

26.4 AFLATOXINS

26.4.1 Aflatoxins: geographic distribution in pulses in the producing regions

Aflatoxin has been detected and described in four countries in Asia, two in Africa, one in Central America, one in South America, and one in North America. The majority of the reports indicate the presence of a low concentration of aflatoxin in the samples of pulses examined. However, certain samples, particularly those from Asian and African countries, showed a fairly high level of aflatoxin contamination.

26.4.2 Aflatoxins: sites of contamination in the growing, harvesting and storage of pulses

There is little information in the literature on the stages of preharvest, harvesting, processing and storage at which aflatoxin contamination of pulses most frequently occurs. There is the possibility of preharvest contamination in the field particularly if there has been insect damage to the crop. At this stage the moisture content of the seeds will be high and therefore conditions will be conducive to the growth of invading moulds. At harvest the moist seeds, unless they are properly and rapidly dried, will present a good substrate for mould growth. Poor conditions of storage provide opportunities for mould growth and aflatoxin formation. Most leguminous seeds are subject to infestation by weevils. Such infestation may serve as a carrier for fungal spores but fungal growth will only occur when the moisture content of the pulses is at a suitably high level.

26.4.3 Aflatoxins: incidence in pulses in Central America

26.4.3.1 Pulses are grown in most of the countries of Central America. The annual production is approximately 2 million tons. Aflatoxin analyses of pulses have been reported only from one Central American country. (2, 3)

26.4.3.2 Beans are normally grown as a mixed crop on small Central American farms; they are mainly harvested during the dry period from December to January, thus minimizing the possibility of moulding. The beans have been studied because the presence of aflatoxin was suspected. When lots are found to be slightly contaminated with aflatoxin they are used as animal feed. When the concentration is higher, the lot is rejected. (27)

26.4.3.3 In a preliminary survey in 1975, 100 food samples, most of them mouldy, were analysed for aflatoxin B₁. The bean samples (mainly black beans) were contaminated at levels of 80-400 µg/kg. Subsequently, monitoring of food products, especially grains, was begun. The results obtained for beans showed that 7 of 32 samples contained aflatoxin. The maximum concentration found was 194 µg/kg with a range of 100-194 µg/kg. (27)

26.4.4 Aflatoxins: incidence in pulses in South America

26.4.4.1 South America produces 3 million tons of pulses annually. This represents approximately 15% of the world total production. Aflatoxin has been reported only in samples of pulses tested in Colombia. (2)

Colombia

26.4.4.2 Colombia produces 157,000 tons of pulses annually and the daily per capita consumption is approximately 12 g. There is little information on the aflatoxin contamination of pulses in Colombia. However, in 1975 a limited survey was carried out in and around the city of Medellin. Fifty percent of the bean samples tested were contaminated with aflatoxins B₁ and B₂. (2,3,7)

26.4.5 Aflatoxins: incidence in pulses in Asia

26.4.5.1 Asia produces 26.5 million tons of pulses annually, the majority being dried beans, dried broad beans, dried peas and dried chickpeas. Aflatoxin has been reported in pulses in four Asian countries - Iran, Thailand, Hong Kong and the Philippines. Work in India has indicated the presence of aflatoxin-producing moulds in pulses. (2)

India

26.4.5.2 India is one of the world's largest pulse-producing nations and pulses are the main protein-rich ingredient of the Indian diet. The annual production is 10.5 million tons and the per capita daily consumption is about 20 g. There have been no reports of aflatoxin contamination in India. Aflatoxin-producing strains of A. flavus have been isolated

from several varieties of beans but no analyses for aflatoxin have been reported. Mould damage to dry beans has not been reported to be a problem in India. (2,3,10)

Thailand and Hong Kong

26.4.5.3 Thailand produces 350 thousand tons of pulses annually and the per capita daily consumption is about 10 g. There is a similar rate of consumption in Hong Kong. Dry beans were among the foods included in a survey for aflatoxin conducted in Thailand and Hong Kong in 1971. In Thailand 140 samples of mung beans (*Phaseolus aureus*) were analysed and showed a contamination rate of 5%. The mean concentration for all the samples of total aflatoxins was 1 µg/kg, whereas for contaminated samples the maximum concentration of total aflatoxins was 112 µg/kg. The results for all other types of beans (e.g. soya, red, black, brown, white, horse, yellow, Burmese, peas and chickpeas) were pooled. The 322 samples assayed showed a contamination rate of 3%. The mean concentration of total aflatoxins in all samples was 3 µg/kg; in contaminated samples it was 213 µg/kg. The maximum concentration of total aflatoxins found in a contaminated sample was 1,620 µg/kg. In Thailand the foods most frequently contaminated were corn, millet, wheat and barley. (2,3)

26.4.5.4 In Hong Kong 12 samples of mung beans were assayed and 8% of these were shown to be contaminated; of the 68 samples of other beans assayed (e.g. soya, red, black, brown, white, horse and kidney) 13% were contaminated. The concentrations of the aflatoxins in Hong Kong beans were not determined; however, it was concluded that beans of various strains were the chief source of aflatoxin contamination of the diet in Hong Kong. (9)

Philippines

26.4.5.5 The Philippines produce annually 29 thousand tons of pulses but only 4 g are consumed daily per capita. A large variety of beans and peas have been assayed for aflatoxin content. The results are shown in Table II/20. (2,3, 32)

Table II/20.

AFLATOXIN LEVELS IN PHILIPPINE PULSES

(32)

Pulse	No. samples	Percent negative	Percent >20 µg/kg	Mean of positives µg/kg	Range µg/kg
Mung beans	33	12	18	13	0-46
Soya beans	22	59	9	18	0-48
Kidney beans	20	10	45	62	0-222
Peas	8	63	12	13	0-46
Chickpeas	6	0	0	6	tr-18
Cow peas	16	37	19	16	0-86
Pigeon peas	9	44	12	7	0-23
Rice beans	4	25	50	17	0-27
Lima beans	6	33	50	58	0-118
String beans	3	0	0	10	tr-16

26.4.5.6 The results indicate a fairly high percentage of contaminated samples but the mean concentration of aflatoxin in the contaminated samples, with two exceptions, is low. (32)

Iran

16.4.5.7 The annual pulse production of Iran is 179 thousand tons and the daily per capita consumption is 10 g. One survey has been carried out for the detection of aflatoxin in

local legumes. Samples (220) of peas and of four varieties of beans were collected at different seasons. Only one of the samples of beans was found to contain a small concentration of aflatoxin B₁. (97)

26.4.6 Aflatoxins: incidence in pulses in Africa

26.4.6.1 Five million tons of pulses are produced annually in Africa, the majority being dried beans, broad beans and cow peas. Aflatoxin has been reported in pulses in only two African countries, Mozambique and Uganda. The daily per capita consumption in Uganda is about 45 g; in Mozambique it is about 20 g. (2,3)

Mozambique

26.4.6.2 Pulses form only a minimal part of the diet in Mozambique; most of the people obtain the majority of their protein requirements from groundnuts.

A survey was conducted in 1974 to determine the correlation between aflatoxin intake in contaminated foods and incidence of primary liver cancer. Aflatoxin was shown to be present in 6.6% of the bean samples tested. The mean aflatoxin level in 65 samples of beans was 12.6 µg/kg. (9)

Uganda

26.4.6.3 Pulses form part of the staple diet in Uganda. In 1966-67 aflatoxin levels were determined in 480 food samples stored for consumption between harvests and collected from different parts of Uganda. Among the samples investigated were beans and peas. The aflatoxin levels found in these samples are given below (Table II/21). Also included for comparison are the figures for groundnuts. (8,)

Table II/21.

AFLATOXIN LEVELS IN PULSES FROM UGANDA

(8)

	Number of Samples	% Positive	Total Aflatoxin Concentration, µg/kg		
			1-100	100-1000	>1000 µg/kg
Beans	64	71.9	30	11	5
Peas	19	15.8	3	0	0
Groundnuts	152	8.0	11	8	8

26.4.6.4 All the samples contained aflatoxins B₁, B₂ and G₁, but there were only traces of aflatoxin G₂. These results indicate that in Uganda beans were very susceptible to contamination with aflatoxin. Groundnuts, in this instance, appear to be less susceptible to contamination. (8)

26.5 OCHRATOXIN

16.5.1 Ochratoxin A, at a concentration of 0.02-2.1 mg/kg, was found in three of four samples of mouldy white beans, which were obtained from elevators in southwest Ontario, Canada. The toxin was produced by strains of Penicillium viridicatum or Penicillium patulans. As the toxin was concentrated in those beans with a darker surface, it was possible to select the reject material by hand. (56)

26.6 PENICILLIC ACID

26.6.1 Dried bean samples were selected for analysis in the United States because of evidence of contamination with the mould Penicillium cyclopium. Five of the 20 samples tested contained penicillic acid at a concentration of between 11 and 179 µg/kg. (13)

27. ROOT CROPS

Aflatoxin has been detected in seven root crops used as human food: cassava, yams, sweet potatoes, taro, carrots, radish and onions.

27.1 CASSAVA

27.1.1 Cassava is a staple food in countries of Africa, South and Southeast Asia and South America. World production of the fresh root was estimated to be 105 million tons in 1975. Cassava is exported from some producing countries in the forms of flour or pellets and as dried chips. (2)

27.1.2 Some cassava root is sold fresh for immediate consumption in the growing areas. The remainder is peeled and usually sun-dried for 1-2 weeks. If the drying period is prolonged there is high risk of mould contamination. The dried cassava is processed into cassava chips, cassava pellets or cassava flour. (72)

27.1.3 Analyses of cassava and cassava products in Indonesia gave the following results: no aflatoxin was detected in two samples of fresh cassava or in two samples of "good looking dried cassava"; levels of 210 $\mu\text{g/kg}$ total aflatoxins were found in samples of rural dried cassava; 733 $\mu\text{g/kg}$ was found in mouldy dried cassava which is used in certain areas in making a special snack called "gatot". (72)

27.1.3 Analyses of 26 samples of cassava collected in a food survey in the Philippines showed aflatoxin in all samples at levels up to 1114 $\mu\text{g/kg}$. Average level was 158 $\mu\text{g/kg}$; 74% of the samples had concentrations greater than 20 $\mu\text{g/kg}$. (32)

27.1.4 Aflatoxin in dried cassava was found in food surveys conducted in three countries in Africa. An overall average level of 0.1 $\mu\text{g/kg}$ was reported for 89 samples of cassava meal collected from homes and local markets in Mozambique; four of 34 samples collected in a similar survey in Uganda contained concentrations in the range <100 to >1000 $\mu\text{g/kg}$ and chips and flour selected from local markets in Ghana contained concentrations between 20 and 70 $\mu\text{g/kg}$. (9,8,36)

27.1.5 Aflatoxin was detected in two of three samples of cassava flour analysed in a Central America mainland country; highest level found was 2915 $\mu\text{g/kg}$. (27)

27.1.6 Although only a few samples of cassava have been analysed in each of the reporting countries, the high percentage of positive samples and the relatively high levels found indicate that attention should be given to improved methods for drying this food.

27.2 SWEET POTATOES

27.2.1 Two countries have reported analyses of sweet potatoes for aflatoxin - Taiwan and the Philippines. (32, 71)

27.2.2 In Taiwan, sweet potatoes are sliced in small pieces and sun-dried on the ground preparatory to storage as a staple food by farmers in certain areas. It was observed that the dried sweet potatoes were highly contaminated with mould and contained between 10 and 180 $\mu\text{g/kg}$ of aflatoxin. Of 37 samples of sweet potatoes (presumably dried) analysed in the Philippines, 78% were positive and contained an average concentration of 39 $\mu\text{g/kg}$; 24% contained levels between 20 and 780 $\mu\text{g/kg}$. (71, 32)

27.3 OTHER ROOT CROPS

27.3.1 The following incidence and levels of aflatoxin in selected root crops were found in a survey of Philippine foods: yams - 61% of 46 samples contained an average concentration of 175 $\mu\text{g/kg}$ (highest - 1107 $\mu\text{g/kg}$); 30% of 10 samples of carrots were positive (highest - 14 $\mu\text{g/kg}$); traces of aflatoxin were detected in 30% of 10 samples of radish; of 14% of onions

assayed, one contained trace levels of aflatoxin; 18% of 38 samples of taro were positive; two samples contained levels between 20 and 86 µg/kg. Presumably, the samples analysed were dried products. (32,)

28. TREE NUTS

Mycotoxins have been reported in the following tree nuts: pistachio nuts, almonds, walnuts, brazil nuts, pecans, hazel nuts and filberts.

Aflatoxin is the major contaminant of tree nuts. Zearalenone and sterigmatocystin have been found in pecan nuts in the U.S.A.

Aflatoxin has been reported in all the tree nuts mentioned above. The incidence and extent of contamination is dependent on the degree of protection (e.g. spraying with fungicides and insecticides), time of harvest, and the methods used for the storage of the nuts.

Although most of the work on the determination of the presence of aflatoxin in tree nuts has been performed in the U.S.A., contamination normally occurs in the country of origin. The countries are as follows: pistachio nuts are mainly obtained from Iran and Turkey; almonds come from the Near East and also the U.S.A.; walnuts and hazelnuts come from Turkey, Cyprus and the U.S.A.; brazil nuts are obtained from Brazil; and the country producing pecans is the U.S.A.

Contamination may occur at three different times. It may first occur while the nuts are on the tree prior to harvesting. At this stage, particularly when nuts have ripened and the hulls have opened, they are often attacked by airborne and insect borne spores of Aspergillus flavus. After harvesting, the tree nuts are de-hulled, washed and sorted. The washing water can be a source of contamination and if the nuts are allowed to remain wet they will be very susceptible to mould growth and aflatoxin contamination. The third stage is storage; once again, the nuts are very susceptible to contamination if they are stored under adverse conditions of temperature and humidity.

28.1 PISTACHIO NUTS

28.1.1 Two types of Iranian pistachio nuts are marketed - the round (Owhadi) type, which is the more esteemed and ripens earlier on the trees, and the almond shaped (Badani) nut, which ripens later. There is no contamination of the nut on the tree prior to ripening and opening of the shells unless there has been insect damage to the nuts. However, the fungal spores which enter the nuts at the damaged areas are airborne and insects appear not to be directly responsible for the contamination. After the nuts have ripened, the extent of contamination is proportional to the time delay in harvesting. This contamination, which has been designated as first stage contamination, is present in up to 100% of nuts obtained from unsprayed trees. Early control (i.e. spraying with a fungicidal agent two weeks prior to ripening and then at fortnightly intervals) reduces the contamination to below 30%. (110, 111)

28.1.2 Contamination can also result from the washing water used during the dehulling and cleaning operation. However, this second stage contamination is of little industrial importance if the nuts are rapidly patio dried after processing. (111)

28.1.3 The nuts are normally stored at 4° to 10°C after harvesting at a relative humidity of 25 to 40%, the moisture content being maintained at 5.3%. Under these conditions there is no spread of the mould but there may be an increase in the aflatoxin content. (112)

28.1.4 Incidence of aflatoxin in Turkish pistachio nuts has resulted in the following recommendations: (113)

- (a) the nuts should be rapidly dried to a moisture content of 7.5% or less;
- (b) de-hulled nuts should be sorted to remove nuts with split shells;
- (c) de-hulled nuts should be sorted using fumigation or other methods to prevent insect damage;
- (d) pistachio nuts with split shells should be marketed first;
- (e) storage facilities should be improved and split nuts should be separated from unsplit nuts.

28.1.5 Canada has reported that of seven samples of imported pistachio nuts tested, one was found to contain 120 $\mu\text{g}/\text{kg}$ aflatoxin. (114)

28.1.6 Most of the pistachio nuts imported into the United States are produced in Turkey and Iran. In 1972, samples of pistachio nuts entering the United States were analysed for aflatoxin content. Positive findings were made of levels of aflatoxin which exceeded permissible limits (20 $\mu\text{g}/\text{kg}$) and more than 80% of the entering lots were detained. As a result of this, a team of aflatoxin experts visited the two countries to help determine how aflatoxin contamination could be prevented. Subsequently, the detentions of pistachio nuts dropped substantially. (13)

28.2 ALMONDS

28.2.1 Most of the work on the incidence and extent of aflatoxin contamination in almonds has been performed in the U.S.A. and Canada.

28.2.2 A. flavus has been found in both sound and insect-damaged kernels. One in 2000 of sound and one in 200 of damaged kernels were infected. Fruits were susceptible to A. flavus contamination from the time of commencement of rapid drying to the time when the moisture content dropped to below 5% (based on the fresh weight of the kernel). Major aflatoxin contamination has been traced to damage by the navel orange worm (Paramyelois transitella). Sorting of shelled almonds for specific kinds of damage is effective in segregating nuts contaminated with aflatoxin. In the U.S.A., survey of in-shell and shelled almonds before sorting showed aflatoxin at concentrations below 20 $\mu\text{g}/\text{kg}$ in many samples. In Canada, no aflatoxin was found at a concentration greater than 15 $\mu\text{g}/\text{kg}$ in any of eight samples tested. (115, 13, 116, 114)

28.3 WALNUTS

28.3.1 Major contamination has been traced to those varieties which are susceptible to sunburn. Of the in-shell and processed Californian (U.S.A.) nuts examined, many were found to contain aflatoxin, usually at a concentration below 20 $\mu\text{g}/\text{kg}$. Sorting procedures were used to concentrate the aflatoxin into the reject material. (13, 116)

28.3.2 Samples of walnuts have also been analysed for aflatoxin content in Canada, Yugoslavia and Germany. In eight Canadian samples tested no aflatoxin was found in concentrations greater than 15 $\mu\text{g}/\text{kg}$. There was no detectable aflatoxin in the seven German samples tested, while three of 14 Yugoslavian samples were shown to contain aflatoxin at a concentration of 3.25 $\mu\text{g}/\text{kg}$. (114, 101, 39)

28.4 PECAN NUTS

28.4.1 Aflatoxins: incidence in pecan nuts

28.4.1.1 Although intact shells provide a barrier against the penetration of A. flavus into some varieties of pecan, the shell structures of other varieties permit the invasion and growth of A. flavus. (117)

28.4.2 Zearalenone: incidence in pecan nuts

28.4.2.1 Zearalenone has been shown to be present in samples of pecan nuts in the U.S.A. It has been extracted from kernels with sound shells which indicates that, as with A. flavus, the Fusarium spp. which produce zearalenone can pass through the intact shells of certain varieties of pecan. There are no reports of the presence of zearalenone in other tree nuts. (117)

28.4.3 Sterigmatocystin: incidence in pecan nuts

28.4.3.1 Sterigmatocystin has been shown to be present in the kernels of certain varieties of pecan nuts. (117)

28.5 BRAZIL NUTS

28.5.1 The nuts grow on small trees in the equatorial forests of Brazil. The fruits fall on the ground during the harvest season from January to March. They are collected daily. After harvest the hard shells of the nuts are broken and the shells and nuts are put into a perforated basket and immersed in running water. The good kernels sink to the bottom while the remainder float. The wet kernels are put into a storage room without further drying and are shipped after a few days to assembly points for sale in either domestic or export markets. The wet kernels are susceptible to invasion by A. flavus.

28.5.2 Transportation of the nuts from the producing areas to the processing points is carried out in bulk by boat. The trip can last as long as two months under conditions of high moisture and relative humidity. It is at this stage that fungal development and toxin production are most likely to occur. (6)

28.5.3 Of 23 samples of imported Brazil nuts tested in Scandinavia, all were shown to contain aflatoxin. Those nuts classified as edible had 520 $\mu\text{g/kg}$ of aflatoxin, while those kernels which were classified as being "possibly edible" or certainly unfit for consumption had concentrations of aflatoxin of up to 10,000 $\mu\text{g/kg}$. (119)

28.5.4 In 1968, an importer control programme was instituted in the U.S.A. Since that time, the incidence of aflatoxins in in-shell Brazil nuts has been reduced markedly. In 1967, 20% of the lots entering the country were embargoed because of aflatoxin contamination, while in 1975 less than 3% of the lots were detained. (120)

28.6 FILBERTS

28.6.1 A major proportion of shelled filberts consumed in the U.S.A. are imported from Turkey. No aflatoxin was detected in any of the domestic filberts sampled, but about 8% of 142 samples of imported filberts tested were positive for aflatoxins (total aflatoxin averaged 33 $\mu\text{g/kg}$; range 2-100 $\mu\text{g/kg}$). (13)

28.6.2 Low concentrations (<10 $\mu\text{g/kg}$) of aflatoxin have been detected in filberts tested in Scandinavia. (119)

28.6.3 Of the filberts grown in Turkey about 10% are consumed in the country and the remainder are exported. They play a very important part in the economy of the country, constituting approximately 1% of the total annual export.

28.6.4 In 1967, about tentons of Turkish filberts were rejected by the Canadian authorities. It was stated that they were contaminated with aflatoxin. Subsequent analysis of the nuts did not confirm this claim. (136)

29. MISCELLANEOUS VEGETABLE PRODUCTS: Coffee Beans, Cocoa Beans, Fruits and Fruit Juices

Aflatoxin has been shown to be present in samples of green coffee beans, cocoa beans, figs and wine. Ochratoxin A and sterigmatocystin have also been reported in green coffee beans while patulin has been shown to be a contaminant of apple juice and other fruits such as peaches, pears, apricots and cherries.

29.1 GREEN COFFEE BEANS

29.1.1 Almost 100% of the annual world green coffee crop of 4.5 million tons is produced in the developing countries. Nearly 3 million tons are produced annually in Latin America, the main producers being Brazil, Colombia and Mexico. Africa produces 1.1 million tons annually in Ethiopia, Ivory Coast, Uganda and Kenya. Aflatoxin, ochratoxin A and sterigmatocystin have been found in samples of green coffee beans. For a number of years green coffee beans have been recognized as one of the commercial products more susceptible to mould contamination. In 1968, numerous samples of mouldy beans obtained from the majority of the producing countries were tested for the presence of aflatoxin. There was no indication of the presence of aflatoxin in any of those samples. (2, 7, 121)

29.1.2 Greece has reported that a survey was conducted on samples of green coffee beans which were organoleptically changed. One of the samples, imported from Brazil, was shown to contain aflatoxin at a level of 48 $\mu\text{g}/\text{kg}$. (63)

29.1.3 Samples (118) of imported coffee beans were analysed in Yugoslavia; 70% were contaminated with aflatoxins B₁ and G₁, the mean level of contamination being 3.79 $\mu\text{g}/\text{kg}$. (39)

29.1.4 A sample of green coffee beans which had become wet and mouldy due to improper handling and abnormally long storage in 1971, was tested for possible mycotoxin contamination. Various Aspergillus spp. were found and ochratoxin A was present in four of the five samples tested at levels ranging from <20 to 400 $\mu\text{g}/\text{kg}$. Concurrently, 19 samples from 267 bags of beans originating in six countries were shown to contain ochratoxin A. In the samples from which obviously spoiled beans had been removed, ochratoxin A was present at an average concentration of 47 $\mu\text{g}/\text{kg}$ (range 20 to 360 $\mu\text{g}/\text{kg}$). The study was continued on another 68 samples, half of which had been directly flown in from the country of origin, to avoid the factor of sea transport, the other half being laboratory-cleaned samples of green coffee beans. Ochratoxin A was found in two of the samples at about 20 $\mu\text{g}/\text{kg}$ and in one sample at 80 $\mu\text{g}/\text{kg}$. The moulds present in the coffee beans were A. ochraceus and a Penicillium species. Roasting of the coffee destroyed most of the ochratoxin contamination of the beans. (122)

29.1.5 Sterigmatocystin at a concentration of 1143 $\mu\text{g}/\text{kg}$ was present in one of two samples of green coffee beans from Southwestern Africa; these had been condemned as unfit for human consumption. Ochratoxin A and sterigmatocystin have been shown to be present in fairly high concentration in mouldy coffee beans. However, samples of commercial and of hand-selected beans were contaminated to a much lower degree with aflatoxin and ochratoxin. (135)

29.2 COCOA BEANS

29.2.1 The annual world production of cocoa beans is about 1.5 million tons, of which approximately two-thirds are grown in Africa (where the main producing countries are Ghana, Nigeria and the Ivory Coast). The other one-third is produced in Latin America, mainly in Brazil and Ecuador. (2,3)

29.2.2 Aflatoxin is the only mycotoxin which has been found as a contaminant of cocoa beans.

29.2.3 In Yugoslavia, 21 samples of imported beans were analysed and of those, 70% were contaminated. Aflatoxins B₁ and G₁ were found at a mean total concentration of 4.87 $\mu\text{g}/\text{kg}$. (39)

29.2.4 During 1976, various chocolate products were tested in Canada for aflatoxin content. A total of 15 samples were analysed but none was found to contain aflatoxin at a concentration greater than 15 $\mu\text{g}/\text{kg}$. (114)

29.2.5 Three samples of sweet chocolate were tested in a Caribbean Central American country and of these one had a concentration of aflatoxin of 10 $\mu\text{g}/\text{kg}$. (26)

29.3 FRUITS (fresh and dried)

29.3.1 FIGS

29.3.1.1 Figs are susceptible to aflatoxin contamination either fresh or as the dried product. The ripe fruit on the trees was very susceptible to attack by A. flavus and subsequently to aflatoxin production when inoculated experimentally with A. flavus spores. Firm-ripe fruit developed high levels of aflatoxin (5 to 6200 $\mu\text{g}/\text{kg}$) while shrivelled ripe fruits had the highest aflatoxin content (1950 to 7200 $\mu\text{g}/\text{kg}$) after injection of spores. Figs that were allowed to sun dry on the trees were particularly susceptible to fungal infection when artificially inoculated. However, an FDA survey conducted in the United States found aflatoxin in only six of 165 samples of dried figs, at an average level of 13 $\mu\text{g}/\text{kg}$, the range being 2 to 29 $\mu\text{g}/\text{kg}$. (123, 13)

29.3.1.2 Dried figs play an important part in the economy of Turkey. They constitute 1.3% of the total annual exports. (136)

29.3.1.3 There are two instances of contamination of dried figs with aflatoxin. In 1972 samples of Turkish dried figs, collected from Danish markets, were found to be contaminated with aflatoxin. The Danish authorities warned the exporters that they would discontinue their imports if subsequent lots were found to be contaminated. There have been no further reports of incidence in Denmark. (136)

29.3.1.4 In 1973-74, three of 38 shipments of dried figs to the U.S.A. were detained because they were contaminated with aflatoxin. Studies conducted in the Department of Agricultural Microbiology on the presence of aflatoxin in dried figs have shown that there was no aflatoxin in 60 samples of various kinds of dried figs and fig pastes tested. Studies have indicated that figs can only be contaminated with detectable levels of aflatoxin during the drying stage. Once they are dry, the high sugar content decreases the available water and thus the relative humidity to levels which will not allow the proliferation of A. flavus. (136)

29.3.2 OTHER FRUITS

29.3.2.1 Patulin was found in West Germany in about 50% of 240 samples of apples and pears suffering from brown rot disease. Levels ranged up to 1 g/kg in the decayed material. There was no significant diffusion of the patulin into the healthy tissue of apples and pears but diffusion did occur in peaches and tomatoes. Patulin was also found in apricots, bananas, pineapples and grapes. (124)

29.3.2.2 Many different types of fruit were tested for aflatoxin in Canada but none of the samples analysed contained more than 15 $\mu\text{g}/\text{kg}$. (114)

29.3.2.3 Samples of dried fruits such as dates and raisins have also been surveyed in the U.S.A. However, no aflatoxins were found in 108 samples of raisins and 62 samples of dates analysed. (13)

29.4 FRUIT JUICES

29.4.1 Patulin has been reported in apple juice in Canada, the United States and Sweden. A level of 1 mg/l was found in one of 12 samples analysed in Canada in 1972. In a 1973 survey of apple juice on the U.S. market, patulin was detected in 37% of 136 samples at an average level of 69 $\mu\text{g}/\text{l}$ (range 40 to 440 $\mu\text{g}/\text{l}$). Of 66 samples of commercial apple juice analysed in Sweden, 24 contained patulin levels greater than 10 $\mu\text{g}/\text{l}$; highest level found was 54 $\mu\text{g}/\text{l}$. (125, 126)

29.5 WINE

29.5.1 There is little evidence of aflatoxin contamination of wines even though A. flavus often attacks grapes. German wines have been tested for aflatoxin content and no aflatoxins were found even in wines produced in those years when grape rot was a problem. A study of 33 German wines from the South Baden vineyard area revealed the presence of aflatoxin at a concentration of less than 1 $\mu\text{g/L}$ in one sample only. (13)

30. ANIMAL PRODUCTS

Mycotoxin contamination in animal products may occur as tissue residue from mycotoxins ingested by the animal in contaminated feeds. Animal metabolic products of the ingested mycotoxin may also be present. Thus, when dairy animals are fed aflatoxin B_1 , the metabolite aflatoxin M_1 appears in the milk. M_1 is also found in the liver of animals; B_1 itself has also been detected in this organ.

Another way that mycotoxin contamination can take place is through mould growth on the commodity. This may be an adventitious infection that occurs in the storage of either raw or prepared foods (especially the latter), or, in the case of fermented foods, mycotoxins may possibly be formed by the fermentation (e.g. cheese and fermented sausages) microorganisms. Risk of this happening is greatest when, in the production of a fermented food, natural starter cultures are employed rather than a pure culture of a single mould species. (127)

Three mycotoxins have been reported in commercial animal products - aflatoxin M_1 in milk, aflatoxin M_1 and roquefortine in cheese, and ochratoxin A in pork and poultry. Aflatoxin B_1 has been detected in organ and muscle tissues of livestock and poultry and in eggs of chickens fed aflatoxin B_1 experimentally but has not been reported in market products.

30.1 MILK

30.1.1 Milk is the animal product most commonly found to be contaminated with a mycotoxin. Indeed, the content of aflatoxin M_1 in milk is a good indicator of the level of aflatoxin B_1 in the feed. The first report of aflatoxin M_1 in market milk was in 1968 in South Africa. Three surveys in Germany have detected aflatoxin M_1 in fluid and dried milk. A survey reported in 1972 found that 5% of 166 samples of dried milk contained 0.07 to 0.2 $\mu\text{g/L}$, expressed on a fluid milk basis. In a second study reported in 1974, aflatoxin M_1 was found at concentrations between 0.02 and 0.4 $\mu\text{g/L}$ (fluid milk basis) in 62% of 120 samples collected at monthly intervals over a period of $1\frac{1}{2}$ years. In a 1976 survey of fluid milk, samples were collected at farms during the months February to April when the cows were fed stored feeds. Nineteen percent of 419 samples contained aflatoxin M_1 at levels between 0.05 and 0.54 $\mu\text{g/L}$. (128, 129, 130, 101)

30.1.2 Aflatoxin M_1 has been found in milk in Iran. Samples collected from village producers near Isfahan in 1973 were positive for M_1 in 50% of 30 samples analysed (range was from 50 to 500 $\mu\text{g/kg}$); 57% of 37 samples analysed in 1974 were positive (range was 50 to 250 $\mu\text{g/kg}$). Incidence in milk from large producers was 10% in 20 samples assayed in 1973 (range was 8 to 10 $\mu\text{g/kg}$); none was detected in 1974. Source of contamination was attributed to mouldy hay that had been improperly dried. (13)

30.1.3 A 1973 survey in the U.S.A. of milk products (cottage cheese, nonfat dried milk and evaporated milk) produced in areas where consumption of aflatoxin-contaminated feeds was suspected, showed aflatoxin M_1 in all of 16 samples taken in one area but only in 8% of 320 samples taken in two other areas. (13)

30.2 CHEESE

30.2.1 In an examination of samples of cheese imported into the U.S.A., aflatoxin M_1 was found in two of eight samples from Germany, two of four samples from Switzerland, and in one of 11 samples from France. Levels ranged from 0.1 to 0.6 $\mu\text{g/kg}$. No aflatoxin was found in

12 samples of cheese from Italy and Greece. However, a recent examination of cheese in Greece showed aflatoxin in all of six samples analysed at concentrations of between 14 and 30 $\mu\text{g/kg}$. Identity of the aflatoxins was not confirmed. Concentrations up to 2 $\mu\text{g/kg}$ were detected in Tunisia. (13, 63, 132)

30.2.2 Roquefortine, an alkaloid produced by Penicillium roqueforti, was detected in 16 samples of blue cheese imported into Canada from France (roquefort), England (stilton), Denmark, West Germany, Canada, and Finland and Italy (gorgonzola). The significance of these findings is not clear in view of the very limited toxicological data on roquefortine. More recently it was reported that long-term feeding tests of P. roqueforti cultures, as well as cheese made with this starter, showed no acute, chronic or carcinogenic effects in rats. Neither was any deleterious effect found in rainbow trout fed the mould for 100 days. (133, 101)

30.3 ANIMAL TISSUES

30.3.1 Ochratoxin A has been detected in market swine or poultry in three countries - in swine and poultry kidneys in Denmark, in swine kidneys in Sweden, and in pork meat in Yugoslavia. Maximum level reported was 67 $\mu\text{g/kg}$ in nephropathic kidneys of swine in Denmark. The tissue residues were associated with ochratoxin A-contaminated feed. (57, 75,)

30.3.2 Tissue residues found when young swine were fed, for three months, rations containing 1 mg/kg ochratoxin A, were 28 $\mu\text{g/kg}$ in kidneys, 11 $\mu\text{g/kg}$ in liver, 9 $\mu\text{g/kg}$ in muscle and 3 $\mu\text{g/kg}$ in adipose tissue. The level fed is within the range of concentrations found in naturally-contaminated feeds in some countries. Ochratoxin A disappears from bacon pig tissues when the swine are placed on ochratoxin-free feeds. Residual life (RL50) of the toxin in the tissues is about 3.3 to 4.5 days. Maintenance on a toxin-free diet for four weeks would result in essentially residue-free meat. (78, 57)

30.3.3 Aflatoxin has been detected in organ and muscle tissues of beef and swine and in meat and eggs of chickens fed aflatoxin-contaminated feeds in experimental studies but no cases of natural occurrence in market products have been reported. Ratios of aflatoxin B₁ concentration in the feed to that in liver of several animal species and in the eggs of chickens has been determined in a number of studies. Average of the range of values reported for these ratios are: beef - 14000:1; swine - 800:1; broiler - 1200:1; and eggs - 2200:1. (134)

30.3.4 Toxin-producing strains of Penicillium expansum (citrinin), P. verrucosum (ochratoxin A), P. cyclopium (penicillic acid and patulin) and other toxigenic Penicillia have been isolated from fermented sausages but no mycotoxins have been detected in the products. (127)

30.4 FISH AND FISH PRODUCTS

30.4.1 Reports from Southeast Asia indicate the occurrence of aflatoxin contamination in some fish products. A Philippine survey revealed low levels of aflatoxin in several products: 83% of 24 samples of dried fish were positive, average concentration being 3 $\mu\text{g/kg}$; 93% of 15 samples of smoked fish contained aflatoxin, average level being 5 $\mu\text{g/kg}$; 42% of salted and fermented small shrimps contained an average concentration of 2 $\mu\text{g/kg}$; and 68% of the 25 samples of fish sauce analysed had an average concentration of 2 $\mu\text{g/kg}$. (32)

30.4.2 Of 139 samples of dried fish and shrimp analysed in a survey of Thailand foods, aflatoxin was detected in 5% of the samples at an average concentration of 166 $\mu\text{g/kg}$ (total aflatoxins). (11)

30.4.3 In Indonesia, two samples of salty fish of the kind commonly eaten by patients suffering from liver cancer were analysed for aflatoxin. The average level of aflatoxin B₁ was 5 $\mu\text{g/kg}$. (72)

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FOOD AND AGRICULTURE ORGANIZATION
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UNITED NATIONS ENVIRONMENT
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Agenda Item 4

MYC - 4 b

JOINT FAO/WHO/UNEP CONFERENCE ON MYCOTOXINS
Nairobi, Kenya, 19-27 September 1977

HEALTH AND TOXICOLOGICAL ASPECTS OF MYCOTOXINS

SUMMARY

This paper assesses the potential public health hazards arising from ingestion of food contaminated by mycotoxins. It deals specifically with the more important mycotoxins, namely aflatoxins, ochratoxins, trichothecenes and zearalenone. The document is based on the deliberations of the WHO Task Group on Environmental Health Criteria for Mycotoxins held in Geneva from 1-7 March 1977.

1. INTRODUCTION

1.1 The material contained in this document is based principally on a health criteria document elaborated by the WHO Task Group for Mycotoxins which met in Geneva from 1-7 March 1977. The criteria document summarizes the relevant data on which the task group based their evaluation. The group also made recommendations which are reproduced in the present document.

1.2 The general remarks on mycotoxins, the evaluations and recommendations as discussed by the Task Group have been further elaborated and interpreted in the present document to the better understanding of the health risks involved, with a view to providing an overview of the potential problem areas. In addition, and for better appreciation of the problems by primarily non-scientific oriented readership, particularly those concerned with policy matters related to production, distribution, safety and control of foods at national level, a final section on the possible risks has been included which in fact generally translates the scientific facts into an over-all summary form.

1.3 The above facts have been revealed from research data and represent the current knowledge for which we can be reasonably certain with regard to human health effects.

1.4 The primary objectives of this paper are to highlight the state of knowledge of the principal mycotoxins (aflatoxins, ochratoxins, trichothecenes and zearalenone) in the human environment with particular focus on their occurrence, stability, toxicological properties, potential populations at risk and potential toxicological consequence, both acute and chronic.

1.5 The effects of mycotoxins on animal health and productivity are known to a greater extent. This document touches on these aspects only in so far as toxicological evidence mentioned is largely derived from experimental or epidemiological studies. Other Conference documents, in particular MYC-4a "Global Perspective on Mycotoxins" and MYC-4c "Economic and Trade Aspects of Mycotoxins" will include selected information referring to effects in domesticated animals. For considerations of national control measures to reduce contamination of foods and feeds by mycotoxin reference is invited to document MYC-7 and the Mycotoxins Surveillance Guidelines available (in English) to delegates at the Conference.

2. GENERAL REMARKS

2.1 Fungal species are major sources of organic compounds of considerable structural diversity and complexity. While some fungal metabolites such as penicillin and ergot alkaloids have been widely employed therapeutically, others have been shown to be toxic.

2.2 Many toxin-producing fungi are ubiquitous and can thrive in a wide variety of environmental situations. The extent of the growth of the fungal flora is dependent upon temperature, relative humidity, moisture content of the substrate, maturity of the seed or legume and accessibility of the fungal spores through the seed or legume protective covering.

2.3 Mycotoxicooses, a broad spectrum of diseases of both acute and chronic nature resulting from the consumption of mycotoxins, have been encountered in humans and livestock in many parts of the world including both developed and developing countries.

2.4 While the toxicologic diseases of man and animals associated with moulds growing on foods have long been known, the recognition of the broad occurrence of both the toxigenic fungi and the mycotoxin(s) is of considerably more recent vintage.¹⁻³ The discovery of the association between ingestion of rye infected by Claviceps purpurea, the best known genus of fungi capable of forming ergot alkaloids and the clinical features of ergotism was recognized in the mid-19th century.⁴ This was followed in 1911 by reports of an "inebriated bread syndrome" associated with breads infected by Fusarium graminearum. Later studies recognized human stachybotryotoxicosis⁵ as well as the association between alimentary toxic aleukia (ATA) and infestation of over-wintered wheat with Fusarium poae and Fusarium sporotrichioides,^{1,2,6} and health problems with "yellow rice" apparently associated with Penicillium toxins have also been identified.⁷⁻⁹

2.5 More recent studies have linked aflatoxin consumption with liver disease.¹⁰⁻¹²

2.6 Despite the increased interest in the aetiology of mycotoxicoses in man, the majority of data available on mycotoxins and mycotoxicoses have been derived from the field of veterinary medicine or through the accumulation of evidence on mould toxicity in a wide spectrum of experimental species. Such studies indicate that the potential of mycotoxins as toxic compounds is considerable and hence future more definitive investigations may well establish a causal role of mycotoxins in other human diseases.

2.7 A wide variety of plant products are subject to contamination by fungi and their metabolites during harvest, storage, transportation and processing, hence providing the potential for contamination of human food. An additional source for human exposure to mycotoxins from food may occur as a consequence of ingestion of milk and meat from animals which had consumed feed contaminated with mycotoxins. Occupational exposure may also occur.

2.8 The diagnosis of mycotoxicoses in man or animals is an exceedingly difficult matter. However, advances in chemical methodology for a large number of mycotoxins has enabled quantitative measurement. Hence, it has been possible to characterize the exposure of humans to specific mycotoxins, notably aflatoxins, thereby facilitating epidemiological investigations.

2.9 Other mycotoxins which are present in the human environment and may present a health hazard are listed in Table 1 with their fungal sources. Relatively less is known about the nature and effects of these compounds than of those considered in this document.

3. SPECIFIC MYCOTOXINS

3.1 Aflatoxins

3.1.1 Aflatoxins generally refer to a group of toxic crystalline, highly fluorescent bis-furanocoumarin metabolites (B₁, B₂, G₁ and G₂) produced by Aspergillus flavus and A. parasiticus. These fungi are ubiquitous and the potential for contamination of food and feed is widespread. The occurrence and magnitude of aflatoxin contamination varies with geographical, seasonal, crop harvesting and storage factors.

TABLE 1. SOME OTHER MYCOTOXINS (AND THEIR FUNGAL SOURCES)
PRESENT IN THE HUMAN ENVIRONMENT WITH POTENTIAL AS HUMAN HEALTH HAZARDS

<u>Toxin</u>	<u>Producing fungi</u>
Citreoviridin	<u>P. citreoviride</u>
Citrinin	<u>P. viridicatum</u> , <u>P. citrinum</u>
Cyclochlorotine	<u>P. islandicum</u>
Luteoskyrin	<u>P. islandicum</u>
Maltoryzine	<u>A. oryzae</u>
Patulin	<u>P. expansum</u> , <u>P. uriticae</u> , <u>P. claviforme</u> , <u>P. patulum</u>
PR Toxin.	<u>P. roqueforti</u>
Rubratoin	<u>P. rubrum</u>
Rugulosin	<u>P. islandicum</u>
Sterigmatocystin	<u>A. flavus</u> , <u>A. versicolor</u> , <u>A. nidulans</u> , <u>A. parasiticus</u>
Tremorgens	<u>Penicillium</u> and <u>Aspergillus</u> species
Vomitoxin (Deoxynivalenol)	<u>F. graminearum</u>

3.1.2 Of the four major aflatoxins, usually B₁ is found in the greatest concentration. The four toxins may occur together although they need not, and their concentration relative to each other and occurrence may vary depending on the fungus strain and substrate.

3.1.3 Although many studies have confirmed the presence of aflatoxins in virtually every area of the world and in nearly every major dietary staple at one time or another, it is not known definitively in all areas to what extent agricultural commodities are contaminated with aflatoxin or to what extent they are used for food.

3.1.4 Of the crops widely grown for food and feed throughout the world, groundnuts (peanuts) and maize (corn) constitute the highest potential aflatoxin hazard.¹³⁻¹⁸ In some field samples of groundnuts and maize, the concentration of aflatoxin B₁ has ranged up to several thousand µg/kg or more.

3.1.5 Aflatoxins are also found in coconut (copra), manioc, common beans, wheat, barley, rice, oats, rye, millet, sorghum, cocoa and green coffee beans, cotton seed meals and meal, tree nuts such as Brazil nuts, almonds, walnuts, pistachios, pecans and filberts.

3.1.6 The heterogeneity of aflatoxin distribution in a contaminated unprocessed commodity stresses the importance of adequate sampling technique, clean-up procedures, sample preparations and analytical variance.

3.1.7 Toxic aflatoxin derivatives such as M₁ have been detected in the milk and/or urine of animals and humans ingesting contaminated feeds and food. Although aflatoxin has been found in tissues of animals consuming rations of toxin-contaminated feed, the toxin has not been identified in commercial meat products.

3.1.8 Optimal conditions for toxin accumulation are prevalent in areas combining high humidity and temperatures. Toxin-producing fungi can infect developing crops and produce toxins prior to harvest as a consequence of insect or other damage.

3.1.9 Reduction of insect infestation by the use of resistant crop lines and insecticides can reduce contamination by aflatoxins. The toxin is not eliminated from food or feed by ordinary cooking or processing practices and since pre- and post-harvest procedures do not ensure total protection from aflatoxin contamination, techniques for decontamination have been developed. Segregation of discoloured seed by sorting can distinctly reduce the aflatoxin level in a commodity.

3.1.10 Aflatoxin B₁ causes chromosomal aberrations and DNA breakage in plant and animal cells. It has also been demonstrated to cause gene mutations in several bacterial test systems when "activated" by microsomal preparations from rat and human liver.

3.1.11 The liver is the predominant target of aflatoxin. Comparative *in vitro* studies in liver metabolism would suggest that man is relatively refractory to aflatoxin toxicity.

3.1.12 Available epidemiological data (primarily in regions of sub-Saharan areas of Africa and South-east Asia)^{10-12,16,19-24} support the positive association of aflatoxin ingestion and liver cancer in man in studies of populations in which estimates of aflatoxin intake and the incidence of primary liver cancer have been made concurrently. For example, studies in Uganda,¹⁸ Swaziland²² and Thailand¹⁶ reveal positive indications between the frequency of aflatoxin contamination of foods offered for sale in markets and present in

home stores, and the frequency of liver cancer in the study areas. Studies in Thailand,^{23,24} Kenya,¹² Mozambique¹¹ and Swaziland¹⁹ have related the actual concentrations of aflatoxin in meals about to be eaten, to the incidence of primary liver cancer in the areas from which the meal samples were taken. These studies are summarized in Table 2.

3.1.13 Daily dietary aflatoxin exposures estimated as varying from 3.5 to 222.4 ng/kg body weight/day have been positively correlated with crude incidence rates of primary liver cell cancer ranging from 1.2 to 13.0 cases/100,000 people/year in regions of Kenya, Mozambique, Swaziland and Thailand.

3.1.14 The highest liver cancer incidences in Kenya and Swaziland were noted in the low altitude regions in contrast to the middle and high altitude regions concomitant with the aflatoxin daily intake for these regions, e.g., in the case of the respective regions of Kenya (10.0, 5.9 and 3.5 ng/kg body weight respectively) and for those of Swaziland (43.1, 8.9 and 5.1 ng/kg body weight respectively).

3.1.15 There is also the possibility of a viral involvement (hepatitis B) in the aetiology of aflatoxin-associated liver cancer in these areas. Hepatitis B infection is common in countries with a high incidence of primary liver cancer and hepatitis B anti-genaemia is more common in individuals with liver cancer in these countries, than in normal subjects. However, the association, if any, between hepatitis infection and aflatoxin ingestion has yet to be studied adequately.

3.1.16 Chronic ingestion levels are more related to human liver cancer than sporadic incidences of high concentrations of aflatoxin. Dose-response correlations have also been established experimentally for aflatoxin B₁ carcinogenesis in rats and rainbow trout. Aflatoxins have been demonstrated to be carcinogenic in at least 8 species including non-human primates.

3.1.17 The acute, subacute and chronic toxicity of the aflatoxins varies greatly depending upon the species, age, sex and nutritional status of the species, the dose of the particular aflatoxin, and the length and repetitiveness of the exposure. Individuals of a group may exhibit marked variations in susceptibility.

3.1.18 Lethality occurs in animals and animal cells in culture upon acute exposure to a variant range in doses, while subacute doses of the toxin may result in certain pathological lesions.

3.1.19 Young laboratory animals are more susceptible than adults to the acute and chronic effects of these toxins and males have an enhanced susceptibility compared to females. Individuals of a group may exhibit marked variations in susceptibility. Hormonal involvement in toxicity syndromes can be demonstrated in laboratory animals, and this may be reflected in the male preponderance of human liver cell cancer. Dietary status is important in the expression of acute and chronic toxicity in animals. In animals protein-deficient diets are generally protective; diets low in vitamin A gave rise to colon tumours in addition to liver tumours.

3.1.20 While the main target organ of aflatoxin is the liver, aflatoxin B₁ and its metabolites have been found in heart, kidney, brain tissues, and urine or faeces of non-human primates as well as humans.

TABLE 2. SUMMARY OF AVAILABLE DATA ON AFLATOXIN INGESTION LEVELS AND
PRIMARY LIVER CANCER INCIDENCE IN ADULTS (MODIFIED
FROM PEERS, GILMAN AND LINSELL, 1976)¹⁹

<u>Country</u>	<u>Area</u>	<u>Aflatoxin</u>	<u>Liver Cancer</u>	
		Estimated average daily intake in adults: ng/kg body weight/day ^b	No. of cases	Incidence per 10 ⁵ of total population/ year ^a
Kenya	High altitude	3.5	4	1.2
Thailand	Songkhla	5.0	2	2.0
Swaziland	High veld	5.1	11	2.2
Kenya	Middle altitude	5.9	33	2.5
Swaziland	Mid-veld	8.9	29	3.8
Kenya	Low altitude	10.0	49	4.0
Swaziland	Lebombo	15.4	4	4.3
Thailand	Ratburi	45.0	6	6.0
Swaziland	Low veld	43.1	42	9.2
Mozambique	Inhambane	222.4	- ^c	13.0

^aRevised incidence estimate taken from Van Rensburg²¹

^bExcludes any aflatoxin present in native beers

^cNumber of cases not available, probably >100

3.1.21 The differences in responses to aflatoxins in different animals has been attributed to their differential metabolism. The rates of metabolism are an important factor in determining the type of toxic action of aflatoxin B₁. Animals that actively metabolize aflatoxin to the hemiacetal (B_{2a}) form appear to be vulnerable to acute liver damage. However, once aflatoxin is in the liver and it is metabolized slowly, the unchanged molecule is available for conversion to an epoxide, and the animal's susceptibility to the chronic effects of aflatoxin becomes more manifest.

3.1.22 Immunosuppressive effects have been demonstrated with aflatoxins in animal studies but the extent to which chronic ingestion of these substances may impair immunogenic mechanisms in man is not known.

3.1.23 Data on the teratogenic effects of the aflatoxins are limited, and in the hamster, although not conclusively demonstrated, indicate that these toxins can cross the placental barrier. Aflatoxin has been rarely found in mother's milk, but there have been very few studies in this respect. Hazards to infants consuming food from this source are presently unknown. However, nursing mothers should avoid consumption of mouldy foods.

3.1.24 While acute toxicity data are relatively scarce in relation to human exposure, there are reported epizootics of apparent aflatoxicoses, the primary manifestation of which is an acutely diseased liver.²⁵ This latter sign parallels the observed effects in experimental animals.²⁶ Although idopathic syndromes in man such as Reye's syndrome²⁵⁻²⁷ and Indian infantile cirrhosis²⁸ have been linked to aflatoxin ingestion, the current available evidence is inconclusive. In terms of human risk, as small as the dose may be, it is not possible to say that there is no risk, although it may be minimal.

3.2 Ochratoxins

3.2.1 Ochratoxins are compounds elaborated by several species of the genera Aspergillus and Penicillium. These moulds are widely distributed and can be found in agricultural products where they can produce ochratoxins if conditions become suitable for their development. This toxin, mainly ochratoxin A, is shown to occur naturally in maize (corn), barley, wheat, oats, rye, dried white beans, green coffee beans and animal tissues (as residue), and has been found in 10 countries thus far.^{29,30} Concentrations ranging between 0.02 mg/kg - 28 mg/kg have been found in feedstuffs. Although ochratoxin A has been detected in barley intended for beer production, its presence in beer has not been established.

3.2.2 Since the toxin is relatively heat stable it will not be destroyed during normal cooking and processing procedures. However, it is largely destroyed under coffee roasting conditions.

3.2.3 Ochratoxin A possesses nephrotoxic properties, as demonstrated experimentally in many species of animals.^{31,32} The lowest dose tested so far (200 ppb) has proven nephrotoxic in two species of animals; as yet no attempt has been made to determine a no-effect level. The compound has been found as a major disease determinant of nephropathy in swine and poultry in several countries.³⁰⁻³³

3.2.4 Although in general the main target organ of ochratoxin A is the kidney, the liver can also be affected in several animal species.

3.2.5 Ochratoxin A has been found in foodstuffs in an area of the Balkans, where endemic nephropathy is prevalent in the human population. This disease entity is comparable, in terms of changes of renal structure and function, to the animal nephropathy induced by ochratoxin A, thus raising the possibility that it may also be due to ochratoxin. Therefore, further investigation of a possible association between ochratoxin A ingestion and human nephropathy is warranted.

3.2.6 Information is scant concerning the teratogenic and mutagenic effects of the ochratoxins. While ochratoxin A is teratogenic in rats and mice, ochratoxins A and B are non-teratogenic in swine, and no toxin was found to cross the placenta to the foetus.

3.3 Trichothecenes

3.3.1 Trichothecene toxins belong to a group of closely-related chemical compounds produced by several species of Fusarium cephalosporium, Myrothecium, Trichoderma and Stachybotrys.^{34,35}

3.3.2 As far as food and feed products are concerned, the major producers of toxic trichothecenes are members of the F. tricinctum group which are widely distributed in the world. The fungus has been found on a number of cultivated plants including: wheat, maize, oats, barley, soybean, millet, sorghum, forage grasses, sugar beets, stone fruits, citrus fruits, peas and tomatoes.

3.3.3 F. tricinctum (syn. F. sporotrichioides) is consistently the most toxic fungus isolated from mouldy corn in low temperature storage. The occurrence of mycotoxicosis in cattle ingesting trichothecene-containing corn has been reported.

3.3.4 Available information suggests that toxin is stable under ordinary food and feed processing.

3.3.5 The biological activities of the naturally occurring trichothecenes are in many respects quite similar, causing a non-specific dermal toxicity in all animals tested. Of the trichothecenes, T-2 toxin, a diacetoxyscirpenol, fusarenon-X and deoxynivalenol are most commonly encountered and are among the most acutely toxic to test animals at levels of less than 10 µg/kg. They are associated with some degree of hemorrhaging although there are differences in target organ affected.

3.3.6 Sub-lethal oral doses in chronic feeding studies in rodents result in necrotic oral lesions and severe gastroenteritis. In addition to generalized toxicoses, emesis is induced by several of the more toxic trichothecenes in some animals.

3.3.7 The character of the trichothecene-induced lesions in actively dividing cells resembles to some degree those induced by radiation.

3.3.8 T-2 toxin appears to be the only trichothecene tested for teratogenicity and has been shown to cause malformations in mice. It is not possible to predict with a reasonable degree of certainty the potential hazard of pre-natal exposure in other species to T-2 toxin or other trichothecenes.

3.3.9 Alimentary Toxic Aleukia (ATA) is a disease of man, acute in appearance characterized by inflammation of the skin and mucous membrane and vomiting and followed by a sub-acute phase of which bone marrow depression is the predominant clinical feature.

3.3.10 The disease was associated with ingestion of overwintered grains invaded by moulds, particularly Fusarium poae and F. sporotrichioides. The disease occurred epidemically in the Soviet Union during the period 1931-1943. Similar symptoms as in ATA have been reproduced in cats by feeding cultures of the two Fusarium species. However, the fungal metabolites causally associated with the changes in the cat were not isolated. In one sample supposed to contain the toxic principle the following trichothecenes were detected: T-2 toxin, neosolaniol, and T-2 tetraol.

3.3.11 No relevant reproduction of the ATA symptoms in test animals by trichothecenes has been reported. Virtually no data on human exposure to trichothecenes relating to ATA are available. Thus no association between ATA in man and ingestion of trichothecenes can be formulated based on present knowledge.

3.4 Zearalenone

3.4.1 Zearalenone is the parent member and an oestrogenic metabolite, of a group of 5 derivatives produced by various species of Fusarium, and is commonly found occurring with trichothecene toxins.^{36,37} The major producers of zearalenone are members of the Fusarium roseum complex. Zearalenone is a contaminant of cereal grains such as maize, wheat, barley, sorghum and oats. It is most commonly associated with maize in concentrations ranging from a few $\mu\text{g/kg}$ to 3000 mg/kg . Zearalenone has been found in almost all parts of the world including Europe, Africa, North and Central America and Australia.

3.4.2 Zearalenone is stable to the conditions normally encountered during processing of feedstuff.

3.4.3 Zearalenone has been demonstrated to have oestrogenic and other effects in animals. Since it has been found in foodstuff destined for human consumption, then there is the possibility of it being involved in oestrogen-related diseases.

3.4.4 There are no data on the carcinogenicity and mutagenicity of zearalenone. In a limited study in rats fed high doses of zearalenone, fertility was impaired and a teratogenic response was noted. While zearalenone is often found together with the trichothecenes in nature, there are no data on their possible synergistic effects.

4. CURRENT CONCEPTS OF MYCOTOXINS AND PUBLIC HEALTH

Based on the scientific evidence contained in the above document, the following could be summarized:

4.1 There are many mycotoxins in the environment with the existence of a number of them recognized for centuries, such as large scale epidemics of ergotism in middle and western Europe caused by the consumption of rye infected by ergot.

4.2 Because of the wide-spread nature of the fungi producing aflatoxins both in terms of the geographical distribution and range of crops which can be infected, the aflatoxins undoubtedly have been in the environment for a long period of time.

4.3 The recent rapid development of knowledge concerning the aflatoxins has led to an increasing awareness of the potential significance of mycotoxins in general as potential chemical environmental contaminants. For example, the presence of aflatoxins contained in groundnut meal in animal rations was found to be the common factor of an unknown disease (Turkey X disease) which caused the death of many thousands of turkeys, pigs and cattle in the 1960s in England.

4.4 Although aflatoxin has been known for less than two decades, its potential hazard to human health has caused great concern and has resulted in aflatoxin becoming one of the most studied mycotoxins in the human environment. This is so because of its presence in crops widely grown for food and feed throughout the world. While groundnuts and maize constitute the highest potential hazard and are consumed by very large populations, aflatoxins are also found in coconuts (copra), maize, common beans and generally, to a lesser degree, in wheat, barley, rice, oats, rye, millet, sorghum, cassava, cotton seed meats and meals, and a wide range of tree nuts.

4.5 The occurrence and magnitude of aflatoxin contamination varies with geographical, seasonal, crop harvesting and storage factors and is dependent to a large degree on temperature and relative humidity. Aflatoxin-containing mould can be present in overwhelming quantities in a relatively small amount of grain, or even in a single kernel, and thus even visual inspection can greatly reduce the extent of the hazard.

4.6 Aflatoxin is stable to the extent that cooking does not destroy it, although sunlight degrades it to some extent.

4.7 Aflatoxin is particularly noteworthy for its striking toxic features in many animal species. In addition, available epidemiological data indicate the association of aflatoxin daily consumption with an increase of liver cancer incidence in man in specific regions of sub-Saharan areas of Africa and the Far East, namely Kenya, Mozambique, Swaziland and Thailand. Epidemiological studies performed in Kenya and Swaziland involving three distinct altitude levels showed an elevated liver cancer incidence in the lowest altitude region which also had the highest aflatoxin daily intake. High altitudes being less humid, contain less aflatoxin in the foodstuffs. The epidemiological studies described have been carried out in four countries. This is not to say that other countries with significant daily consumption of aflatoxins do not have similar potential risks. The problem is one of dose-relation and hence any reduction in aflatoxin intake will reduce the risk. Although the scientific evidence as a whole questions whether a "no-effect" level exists for all carcinogens, for practical purposes there may be one. It should be noted that in the four epidemiological studies described, daily dietary aflatoxin exposures estimated a 3.5 ng/kg body weight/day were correlated with only 1.2 primary liver cell cancer cases per 100,000 people/year.

4.8 The epidemiology studies were confirmed with animal studies, since cancer was produced in eight species, including monkey. The trout is particularly sensitive, where 0.1 ug/kg of aflatoxin in the diet has caused liver cancer. While it is not possible to extrapolate animal data to man with certainty, animal studies can serve as a potential indicator of risk. From animal studies, it has also been shown that those animals with poor nutrition are more susceptible to the risk of cancer, however, it is not known with certainty whether or not this is true in humans. While in man we cannot be certain what levels in the daily consumption of aflatoxin do not cause liver cancer, with animal studies there can be little doubt. Dose-related studies in man strongly indicate that any reduction in the intake of aflatoxin would help reduce this risk. There are special

potential problems involving women and children and early life. Aflatoxin consumed by pregnant women will have the potential, as indicated by animal studies, to pass the placenta and thus the foetus (especially of very low body weight) may receive a relatively high dose. A similar situation can also exist for nursing children. Thus for the sake of prudence, mothers should be particularly advised to avoid aflatoxin exposure as much as possible. This could be accomplished by reducing (or avoiding as far as possible) their intake of mouldy foods which are a major source of aflatoxin.

4.9 While cancer is a chronic disease which can manifest itself after several years, note should also be made of the potential acute effects in humans of aflatoxins. Acute diseases of the liver affecting children in Thailand and India have been linked to aflatoxin ingestion although the current available evidence is inconclusive. In terms of human risk, as small as the dose of aflatoxin may be, it is not possible to say that there is no risk, although it may be minimal. For the above reasons, the Task Group had as a first recommendation one on control which reads as follows:

"Although a number of questions have to be elucidated by further research, there appears to be little doubt that high exposure of aflatoxin causes a public health problem, thus prudence indicates to initiate preventive measures to reduce aflatoxin intake in populations with high exposure where this is feasible under local conditions."

Very minute amounts of aflatoxin are also present in many foods produced in temperate climates. Most of the developed countries have tolerances for aflatoxin which indicate the concern of these countries regarding the toxins. The national tolerances vary considerably, the lowest being 5 ppb, which is based on the limits of sensitivity of the analytical method employed for checking aflatoxin contamination.

4.10 Although the overwhelming emphasis has been placed on consideration of aflatoxin because of its dominant position amongst the mycotoxins in terms of its potential hazards, the Task Group also considered additional mycotoxins such as the ochratoxins, trichothecenes and zearalenone. Aside from ochratoxin A where there may be a connection between its consumption in foodstuffs in an area of the Balkans and kidney disease in the human population, very much less is known definitively concerning the potential human hazard of the trichothecenes and zearalenone.

4.11 Additionally, other mycotoxins are present in the human environment which may present a health hazard. These have been listed along with their fungal sources. Relatively still less is known about the nature and effects of these compounds.

4.12 It should be restressed that with regard to mycotoxins, emphasis should be given to tropical areas with high humidity. Since hot and moist climates greatly favour fungal growth, the public health problem from aflatoxins produced by them will be greatest in areas where these climates prevail. Thus priority, for preventive measures for the protection of the population, should be given to those at highest risk. Although considerably more is known about the public health hazards of aflatoxin, and much less concerning the other mycotoxins, it must be noted that in the prevention of fungal contamination, other potentially hazardous mycotoxins will be avoided.

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RECOMMENDATIONS*1. General

1.1 The distribution of known toxic fungi and their toxic products in the human environment should be studied in different countries and related to the distribution of human disease. Where positive associations between toxin and disease are observed, they should be submitted to detailed epidemiological study.

1.2 Food and feed should be screened for fungi identified as producing mutagenic, carcinogenic and toxic products.

1.3 Attention should be paid to the possibility of non-alimentary human exposure to mycotoxins as, for example, in workers handling pure mycotoxins or contaminated materials. The identification of such situations may provide a basis for epidemiological studies of the effect of chronic exposure to mycotoxins as well as indicating the need for safety measures.

1.4 Further methods should be developed for the rapid detection, measurement and confirmation of mycotoxins in contaminated materials under field conditions.

1.5 Sampling has proven to be the most difficult step in the control procedure. The development of internationally agreed sampling plans is strongly recommended.

1.6 Further methods should be developed for the measurement and confirmation of human tissues, body fluids and excretion products, of mycotoxins to which significant human exposure has been demonstrated.

1.7 Studies should be undertaken on the possible effects of the use of agricultural chemicals (e.g. pesticides and fertilizer) on toxic fungi and their potential for contamination of the food or feed.

1.8 Where mycotoxins have been identified as presenting a potential human health hazard, monitoring, control measures and decontamination programmes should be introduced. Measures which will prevent initial fungal contamination or toxin production should be encouraged.

1.9 Reference centres for the detection and measurement of the various mycotoxins should be recognized and made available to assist in the elucidation of episodes or outbreaks of human disease thought to be due to mycotoxins.

1.10 FAO/WHO should consider establishing reference centres on mycotoxins with one or more of the following tasks:

* As elaborated by the WHO Task Group on Environmental Health Criteria for Mycotoxins, Geneva, 1-7 March 1977.

educational programme to encompass analytical methods and diagnosis of mycotoxicoses;

confirmation of the identity of toxin by sophisticated physical means and maintaining quality control on methodology;

depository of mycotoxin standards and centre for disbursement of analytical standards;

computerized mass spectral data library encompassing known and unknown toxins as well as those commonly misidentified as mycotoxins;

toxicity studies in animals.

2. Specific

2.1 Aflatoxins

2.1.1 Although a number of questions have to be elucidated by further research, there appears to be little doubt that high exposure of aflatoxin causes a public health problem, thus prudence indicates to initiate preventive measures to reduce aflatoxin intake in populations with high exposure where this is feasible under local conditions.

2.1.2 Reports from developed countries of the presence of aflatoxin in human tissues, body fluids and excretion products should be confirmed using specific assay methods with appropriate limits of detection. Subject to such confirmation, the frequency of this event should be studied in appropriate samples of the general population of various countries and a search made for the sources of aflatoxin exposure.

2.1.3 The implication of aflatoxin as a cause of some cases of Reye's syndrome should be studied further using the case-control approach. For each case and control, similar data should be obtained on the presence of aflatoxins in tissues, body fluids and excretion products and attempts should be made to identify dietary sources of aflatoxins.

2.1.4 The distribution of aflatoxin contamination of food should be studied in relation to the frequency of Indian childhood cirrhosis and primary liver cancer.

2.1.5 The epidemiology of suspected outbreaks of acute aflatoxicosis should be studied in detail. Such studies should include verified measurements of the exposure of aflatoxin through foods and other routes of exposure. This should include aflatoxin and derivatives in and excretion products of individuals both affected and unaffected by the disease. More research is needed to develop and assess the validity of techniques for detecting early effects of the diseases.

2.1.6 The validity of a causal relationship between aflatoxin ingestion and primary liver cancer should be tested further by introduction of control measures to reduce aflatoxin exposure in an area of high liver cancer incidence and high aflatoxin exposure with subsequent monitoring of incidence in this area and in a comparable area where the aflatoxin exposure has been low.

2.1.7 The prevalence of hepatitis B antigen should be determined in areas of varying aflatoxin exposure in countries with a high incidence of primary liver cancer.

2.2 Ochratoxin

The levels of ochratoxin A and possibly citrinin should be measured in food-on-the-plate in areas of the Balkans with different rates of Balkan nephropathy and correlated with the incidence of nephropathy in these areas.

2.3 Zearalenone

Studies should be made of the presence of zearalenone in human food, and, if present, its distribution studied in relation to the incidence of oestrogen-related disease in man.



FOOD AND AGRICULTURE ORGANIZATION
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Agenda Item 4

MYC - 4c

JOINT FAO/WHO/UNEP CONFERENCE ON MYCOTOXINS
Nairobi, Kenya, 19-27 September 1977

TRADE AND ECONOMIC ASPECTS OF MYCOTOXINS

S U M M A R Y

The extent and economic losses due to mycotoxin contamination of food and animal feeds is not known in quantitative terms. The paper attempts to put together some of the broad indications of such losses through a study of the susceptibility of crops to fungal attack and mycotoxin development, the importance of these crops in the national economy and losses in international trade due to the enforcement of regulatory tolerances by importing countries. General indication is also provided on potential risks to animal and human health well-being.

1. INTRODUCTION

1.1 Increased knowledge of and concern over mycotoxins have their modern origin in an economic mishap: the loss in 1960 of at least 100,000 turkey poults in the U.K. due to feeding of aflatoxin contaminated groundnut meal from Brazil. Awareness of the potential losses in animal production due to aflatoxins alone quickly spread in countries of Europe and North America. Governmental barriers to internal and international trade in contaminated feedstuffs were raised, and were followed by even stricter controls when it became known that some foodstuffs were also liable to be contaminated, and that aflatoxin B₁ was the most potent substance known to cause liver cancer in several experimental laboratory animals.

1.2 In the years since 1960, it became clear that aflatoxins were a problem not just in groundnuts but in a wide range of materials used for human food and animal feed, including other oilseeds, the derived vegetable oils, cereals (especially maize), tree nuts and even starchy crops such as cassava. A matter of grave public health concern in some countries was the finding that animal products, especially milk, were contaminated with aflatoxin M₁ and/or B₁ if the feed contained aflatoxin B₁. This widened the range of foods and feeds coming under surveillance for aflatoxin contamination in many countries, with obvious potential and real economic consequences for primary producers and traders.

1.3 The intensive study of aflatoxins also led to the finding of a number of other mycotoxins in human foods and animal feeds. For most of these, the degree of hazard they pose as well as the economic implications are not yet fully known, but a few governments are likely to increase monitoring activities with a view to introducing surveillance and control measures. Zearalenone and ochratoxin A may be candidates for surveillance particularly in feedstuffs for pigs, and trichothecene toxins in feeds as well as in foods. Commercial control is now exercised over the levels of another mycotoxin, ergot which affects mainly rye and millet.

1.4 For a detailed review of the mycotoxins situation, reference should be made to document MYC-4a, Part I offering a general overview and Part II presenting available information on incidence of mycotoxins in the most susceptible commodities in different parts of the world. For an appreciation of the hazard from mycotoxins to human life, health and productivity, reference is invited to document MYC-4b on the health and toxicological aspects. Document MYC-3 outlines the environmental aspects of the mycotoxin problem.

2. ECONOMIC LOSSES AND COSTS

2.1 General

2.1.1 Mycotoxins are produced by moulds. Until recently, when the nature of the toxins became known, losses were associated with consumption of mouldy food or feed. One of the earliest animal feed poisoning on record involved mouldy millet eaten by tigers in India about 300 B.C. In the Middle Ages, ergot in rye and barley led to many human food poisoning outbreaks in Europe, sometimes of epidemic proportions. In the 18th century, mouldy rice consumption claimed many deaths in Japan and Russia, and in the 19th century, certain mouldy breads caused deaths among French troops. During the first half of this century, major human or animal poisoning incidents involved consumption of mouldy maize in southeastern United States and elsewhere, of mouldy overwintered grain in Russia and Eastern Europe, or mouldy U.S. barley fed to pigs in Germany. These and other recorded incidents can be regarded as showing only the tip of the iceberg; numerous deaths of humans and animals and other losses due to mouldy food or feed were not recorded and are therefore not known. Nor can a value be placed now on those losses that were documented.

2.1.2 Epidemiological studies can now usually separate toxic effects due to moulds per se from those due to the mycotoxins produced by the moulds; in most cases examined, the toxins are mainly or solely responsible for observed loss of human or animal health and productivity. The best known and perhaps most widely distributed mycotoxin is aflatoxin. Other mycotoxins that have been characterized and demonstrated to be responsible for adverse effects in animals or man include zearalenone, ochratoxin A, some of the Fusarium toxins collectively known as trichothecenes, citrinin, patulin and penicillic acid.

2.1.3 Reliable published information on the economic and trade aspects of contamination by mycotoxins is extremely limited and is frequently not expressed in quantitative terms. The studies and statistics available are often incomplete and are restricted to one mycotoxin, namely aflatoxin. It is therefore very difficult to express comprehensively the losses due to mycotoxins in economic terms. However there are indications which, if viewed together, may convey an idea of the kind and, less clearly, the extent of these losses. An examination can also be made of the significance of crops, susceptible to contamination by mycotoxins, for the national economy particularly in terms of international trade.

2.1.4 Table 1 attempts to express the kinds of losses and costs in very general terms. Although in some cases it is clear who will bear the loss or cost, in many other cases this is not so. The site or mode of contamination, especially by aflatoxins, may be the farmer's field or his storehouse, or the transport, storage and packing facilities of the purchasing agent, the marketing board or the middleman. The conditions of export and import, handling and storage and other operations carried out by processors, the distribution and marketing to consumers, and finally the preparation of the food or feed for consumption, all determine whether the product will become contaminated or not.

2.1.5 Among the costs must be counted also those incurred in trying to deal with problems due to mycotoxins through efforts of prevention and control. For some appreciation of efforts in these fields, reference is invited to papers MYC-5 and MYC-7 and to the two guideline publications available (in English) to conference delegates.

2.2 Susceptible Commodities and their Geographic Distribution

2.2.1 With increasing survey activity and greater sensitivity of analytical methods employed to study incidence of contamination by aflatoxins or other mycotoxins, the number and kinds of crops and foods or feeds known to be affected has grown rapidly. Beginning with groundnuts, which still remain among the most important of the commodities found to be contaminated, the list has expanded to include groundnut cake and oil, cottonseed and cottonseed cake and oil; other oilseeds and derived products (including sesame seed, soybeans, sunflower seed and copra); tree nuts such as pistachios, Brazil nuts, chestnuts, filberts, almonds, pecans, cashews; beans and other legumes; coffee and cocoa beans; cereal grains such as maize, rice, wheat, barley, oats, rye, sorghum, millet; bread and other cereal products; beer; cassava; figs and other dried fruits, (unsound) apples, apple juice; wine; animal feeds, hay, straw and silage; animal food products including muscle and organ meats, dairy products including milk and cheeses, poultry and eggs. The frequency and degree of contamination observed so far varies greatly, and so does the economic aspect depending inter alia on whether or not official or commercial mycotoxin limits exist, the damage or loss caused by the particular contaminant and the probability of its detection.

2.2.2 Fifteen years ago contamination by mycotoxins was thought to be a problem confined to those tropical or subtropical regions, which provided the temperature and humidity conditions favourable for the growth of Aspergillus flayus. This assumption proved to be incorrect, as several mycotoxins have been found in countries in more temperate regions, e.g. in France, Germany, Yugoslavia, and even in those of colder regions such as Canada, Finland, Denmark, Norway, USSR.

Table 1. TYPES OF LOSSES AND COSTS ASSOCIATED WITH CONTAMINATION OF FOODS OR FEEDS BY MYCOTOXINS - EXPRESSED IN GENERAL TERMS.

BEARER OF LOSSES AND COSTS	LOSSES AND COSTS
<u>1. National Level</u>	
Primary producer	<ul style="list-style-type: none"> - Outright food/feed loss. - Contaminated crops provide less income and may lead to potential loss of outlet. - Reduced productivity of livestock leads to less income from animal products; smaller litters; reduced work output.
Middleman	<ul style="list-style-type: none"> - Less income from products refused, condemned or sold at a discount. - Storage, transport and packing costs on such products. - Potential loss of outlet, trading reputation and also of raw material source.
National Exchequer	<ul style="list-style-type: none"> - Lower foreign exchange earnings from reduced exports. - Costs involved in shipment or trans-shipment, sampling, analysis etc. of exported goods that are subsequently refused import entry; potential loss of overseas outlets. - Costs of detoxification or reconditioning abroad. - Increased costs for food or feed imports; staple food subsidies. - Increased costs of surveillance and control. - Increased need for expenditures on human and animal health facilities and activities. - Increased costs involved in training and extension programmes.
Consumer (human or animal)	<ul style="list-style-type: none"> - Consumption may lead to impaired health and productive capacity. - Lack of food may lead to under nutrition or higher food prices resulting from outside purchase of foods or feeds. - Possible medical and veterinary costs associated with the above.
<u>2. International Level</u>	<ul style="list-style-type: none"> - Reduction in supplies for meeting needs or demands for different commodities; possibility of price fluctuations. - Potential problems would be the increased difficulty involved in arriving at sound international agricultural production adjustments and commodity and food security agreements.

2.2.3 The following is a list of some countries where studies, sometimes of very limited scope, have indicated or demonstrated the presence of one or another kind of mycotoxin in the food or feed supply available locally: Senegal, Upper Volta, Gabon, Mali, Benin, Niger, Togo, Nigeria, Gambia, Ghana, Angola, Zambia, Madagascar, South Africa, Sudan, Mozambique, Mauritius, Malawi, Uganda, India, Sri Lanka, Bangladesh, Burma, Thailand, Philippines, Indonesia, Australia, Japan, U.S.A., Canada, Mexico, El Salvador, Nicaragua,

Guatemala, Argentina, Brazil, Colombia, Denmark, Finland, France, Norway, Sweden, United Kingdom, Yugoslavia, Czechoslovakia, Ireland, Germany, Poland, Portugal, Spain, Turkey, Iran. Considering the potential health and economic implications of the mycotoxin problem and particularly the role of the commodities affected in international trade, it may be suggested that few if any countries have reason to believe that problems due to mycotoxins are not also their concern.

2.2.4 Over 1,000 million tons of major agricultural crops are produced in regions of the world which are or could be menaced by mycotoxin contamination. At stake in international trade could be over 16,000 million U.S. dollars worth of exports. Table 2 gives some particulars for world production and value of world exports of susceptible commodities in 1974. Some of the commodities have a history of frequent and considerable contamination by one or more mycotoxins; for others mycotoxins there may be an occasional problem involving relatively low levels of contamination.

Table 2. WORLD PRODUCTION AND EXPORTS OF SELECTED AGRICULTURAL COMMODITIES SUSCEPTIBLE TO MYCOTOXIN CONTAMINATION - 1975.

Commodity	World Production - million tons	Value of World Exports - million U.S.\$
Groundnuts	19	622 *
Copra	4	332 *
Cottonseed	22	165 *
Sesame seed	2	124
Tree nuts (pistachios, chestnuts, walnuts, almonds, filberts)	2.4	
Cocoa and products	1.6	2,350
Maize	322	6,998
Rice	344	3,318
Barley	155	1,777
Sorghum	54	1,000
Millet	47	10
Oats	49	141
Rye	24	70
Cassava	105	280
* including the value of cake and meal exports		

2.2.5 The position of groundnuts in the economies of member countries of the African Groundnut Council may serve as a more specific example. Table 3 gives the pertinent data. Note the high proportion of the population dependent on groundnuts for income and work,

reaching a level of 60-70% in the Gambia and Senegal; similarly, groundnuts contribute 60-70% to the national income of the Gambia and Niger. These data may be supplemented by the following information. Some sixteen million people in the member states of the African Groundnut Council cultivate approximately 4 million hectares in the groundnut producing areas. Total production, despite the decline in the recent years due to drought, amounted to 12.5% of the total world production.

Table 3. POSITION OF GROUNDNUTS IN THE ECONOMY OF MEMBER STATES OF THE AFRICAN GROUNDNUT COUNCIL.

	The Gambia	Mali	Niger	Nigeria	Senegal	Sudan
Season	1974/75		range 70/72	74/75	range 71/73	1974/75
Contribution of groundnut to the national income (%)	60	4.75	60-70	4.5-5	25-30	20
Area cultivated (hectares)	91,445	200,000	400,000	1,450,000	1,100,000-1,200,000	525,000
Total population (millions)	0.38	4.14	4.13	70.00	4.02	16.09
Population engaged in groundnut industry (% of total)	>60%	15%	35-40%	14%	70%	3%
Total production (tons)	130,000 130,000	90,000 90,000	135,000 135,000	800,000 800,000	575,000-1,000,000 (in shell)	650,000 650,000
<u>Exports</u> (tons)						
- seed	39,865		103	150,000	3,300-32,540	128,398-202,940
- oil	20,000	7,000			crude 5,424-190,693	
					refined 17,673-39,292	50,000 50,000
- cake	23,000				126,204-313,170	

Member states export some 370,000 tons of shelled nuts which is about 40% of the total world exports. Their share of world exports for unshelled nuts is 10-20%, of oil over 50% and of groundnut cake over 25%.

2.3 Situation in the Temperate Regions

2.3.1 As stated in paragraph 2.2.2 the problem of mycotoxins is not limited to tropical and subtropical countries. In addition, at least some of the industrialised countries of Europe as well as Japan are not able to maintain their production and consumption of animal feeds without resorting to imports both from developed and developing countries. To guard against the hazards of mycotoxin contamination, governmental authorities and the section of industry involved in these commodities, often conduct and sponsor surveys to gauge the depth of the problem, or operate control schemes to ensure absence of aflatoxins and some of the other mycotoxins, above levels deemed harmful, in the food and feed supply. For example, surveys and much scientific work has been carried out in France, England, Denmark, Sweden, Finland, Norway, Yugoslavia, USSR, Poland, Czechoslovakia, Japan, Canada and the USA.

2.3.2 Work in Denmark showed ochratoxin A to be the only one of seven ochratoxins giving rise to concern as a toxic natural contaminant in maize, barley, wheat, oats, rice, green coffee beans, broad beans, green beans, groundnuts and hay, and possibly in bread and beer. Residues of ochratoxin A have been found in ham and poultry in Denmark where the incidence of contamination in a random sampling was higher than 20%, and in samplings limited to regions in which swine nephropathy was endemic, incidence was higher than 50%.

2.3.3 In France research on mycotoxins in cereals is conducted by a group made up of research workers and administrators from the public and private sectors concerned. Studies on maize since 1973 have led to detection of zearalenone in 45% of the samples examined, and indicated the occasional presence of ochratoxin A and trichothecene toxins. The degree of contamination observed for zearalenone may be significant in view of this mycotoxin's ability to cause oestrogenic syndromes in pigs. One lot of 1,000 tons contained ochratoxin A at a level of 200 µg/kg which is high enough to induce swine nephropathy. Industrial groups, particularly the manufacturers of cattle feed and vegetable oils, are also carrying out research inter alia on aflatoxins B₁ and M₁, zearalenone, trichothecenes and patulin in maize, rye-grass silage, barley, oilseed cakes and milk. Several incidents between 1973 and 1976 have been reported in which dozens of sows died from vulvo-vaginitis due to zearalenone in maize-based feeds. Reports also indicate problems with dairy cattle fed on farm-produced grass silage. After a few weeks a partial paralysis of the rear legs developed, which was ascribed to patulin intoxication. In sheep, paralysis was shown to have been caused by the consumption of mouldy chestnuts containing the neurotoxic mycotoxin citreoviridin. This disease has been endemic in one region of France for more than 20 years; ewes paralyzed frequently abort or give birth to lambs with poor equilibrium. Recently, fungi widely used in producing ripe cheeses have been shown to elaborate mycotoxins under some conditions. Penicillium roqueforti and Penicillium camemberti are the fungi studied.

2.3.4 In Japan, ochratoxins, zearalenone, sterigmatocystin and aflatoxins have been detected as natural contaminants particularly in certain lots of cereals stored under poor conditions. Mycotoxins have not, however, been reported in consumer food products. It would therefore appear that contamination by mycotoxins does not present a problem for Japan's national agricultural production. However, a report in 1971 indicated that for some years a large part of Japan's production of cereals had been destroyed by mould (red mould disease).

2.3.5 In the USA, numerous studies and surveys have been undertaken, many of the latter on a national scale, in order to establish what problems are posed by mycotoxins for the economy, for public health and for the livestock-industry. Groundnuts have been kept under particularly close scrutiny in view of the known and sometimes considerable incidence of mycotoxins contamination. Nationwide, this incidence averaged 3.7, 1.4, 2.1, 2.1, 8.1 and 1.6% in the years from 1968 to 1973. The highest regional incidence was observed in 1972 in the southeastern USA: this was 13.5%. Cottonseed and cottonseed cake contamination by aflatoxins was surveyed nationally for three consecutive years, 1964-1967. Incidence

detected as aflatoxin B₁ was 8% in cottonseed and 19% in cottonseed cake and contamination level was substantial, averages being 143 and 99 µg/kg respectively. In one survey in a highly contaminated region the incidence in the cottonseed crop was 100%. Surveys of the maize harvests between 1964 and 1969 indicated an incidence of contamination of about 2.5% at a low average level nationwide. However, contamination was decidedly more frequent in the southeast, e.g. 35% in 1969/70 and the range of total aflatoxins was 6 - 348 µg/kg and in 1973 another survey showed an incidence of 8.2%.

2.4 Situation in tropical and subtropical regions

2.4.1 The situation regarding mycotoxins is undoubtedly more alarming in many of the tropical and subtropical countries where mould growth is favoured by conditions of humidity and temperature, cultivation, harvesting, transport and storage. Insect pests, and sometimes also drought may increase the extent and severity of fungal attack of crops. The measures needed to remedy the situation are often difficult to introduce, because efficient systems of extension, supply and control are still under development.

2.4.2 The repercussions of mycotoxin contamination are equally serious, from the point of view both of the health of the population and of economics. The susceptible crops are either local foods, the contamination of which may lead to intoxication to varying degree, or commodities intended for export in which case the loss of foreign exchange and indeed of earnings can be very critical in view of the needs for development. Where a local food is also an export item, selection of the better part for export may lead to local consumption of the more highly contaminated part with increased risk of toxic effects. Another aspect of the problem in certain countries is lack of enough food for the people and for their domesticated animals; losses due to mycotoxins can often not be made up in many localities.

2.4.3 To illustrate some of the problems associated with mycotoxins, selected information from India and certain other countries of the Far East where surveys and investigational work has been carried out, may be cited. In many developing countries, vegetable oils are consumed unrefined, often by preference and also because of lower cost or unavailability of refined products. In the rural areas and in some cities of India, unrefined groundnut oil is a dietary staple. Such oil has often been shown to carry high levels of aflatoxin, and the toxin has in fact been found in considerable quantities in foods fried in such oil. Indian surveys have also shown contamination by aflatoxins of groundnuts, cottonseed, rice, sorghum, maize, wheat, barley and oats. A recent publicized occurrence was the death of more than 100 people from an acute hepatitis disease which struck one rural area after the population had virtually subsisted for weeks on mouldy maize with a very high content (250-15,600 µg/kg; mean 6,000 µg/kg) of aflatoxins. A number of incidents of fatal animal mycotoxicoses have been recorded in the late 1960's, viz. 58 cows and 2219 chickens in Karnataka state, 4000 rabbits in the Kulu valley, 1400 buffalos in the Punjab, and numerous Red Island hens at a state breeding farm. These reports are probably only a small sampling of the largely unrecorded losses due to mycotoxins. In another report on cottonseed in India aflatoxin was found in 54% of 388 samples examined; 12% of the contaminated samples contained more than 500 µg/kg of aflatoxin B₁.

2.4.4 In Thailand, an interesting survey was carried out in 1967-69 to study the relationship between aflatoxins in food and the incidence of human liver cancer. This survey included more than 3,000 samples, representing about 170 commodities, available on the markets of Thailand (and also Hong Kong) over a period of 23 months. Table 4 is a summary of a part of the findings in this survey. Groundnut products were most frequently and most heavily contaminated, followed by maize. Also often contaminated, but at distinctly lower levels, were dried pimentos, millet and other cereals. Significant findings were the aflatoxin contamination of prepared foods such as cookies, candies and cooking oils based on groundnuts or maize. Mean total aflatoxin concentration of contaminated samples varied from 16 to 1530 µg/kg according to the commodity. Occasionally high maximum levels were found even for such items as dried fish/shrimp and beans.

Table 4.

AFLATOXIN CONTENT OF MARKET FOODS OF THAILAND

Foodstuff	No. samples assayed	% samples contaminated	Mean aflatoxin concentration (contaminated samples) $\mu\text{g/kg}$
Groundnuts	216	49	1530
Maize	62	35	400
Chili peppers	106	11	125
Millet, wheat, barley etc.	44	11	67
Prepared foods	364	6	510
Dried fish/shrimp	139	5	166
Mung beans	140	5	16
Other beans	322	3	213
Sago (cassava starch)	65	3	150
Rice	364	2	20

2.4.5 In Indonesia, rice, cassava and maize are staple foods, and groundnuts are consumed to a more limited extent but with regional variations in intake. Cassava and groundnuts are also export crops. Aflatoxins have not so far been reported in the cereal supplies, nor in fresh or properly dried cassava. However, in cassava dried under poor conditions aflatoxin has been found at levels of several hundred $\mu\text{g/kg}$, and in the special snack food "gatot", made from moulded cassava (consumed by only few people), levels over 700 $\mu\text{g/kg}$ have been observed. As in India, unrefined groundnut oil, as well as farm-stored and locally consumed groundnuts frequently contain above 100 $\mu\text{g/kg}$ of aflatoxins.

2.4.6 In the Philippines, maize and groundnuts are staple items of the diet, and both commodities have a history of substantial aflatoxin contamination in the field and in consumer foods. An appraisal of work done during the last ten years suggests average levels of aflatoxins in contaminated maize and its products of around 100 $\mu\text{g/kg}$, and in contaminated groundnuts and groundnut products of around 200 $\mu\text{g/kg}$. In the past, groundnut butter has been a special problem, high levels of aflatoxin in the mg/kg range have been noted. Cassava and cassava products also appear to have a high incidence of contamination, with average levels above 100 $\mu\text{g/kg}$. Local foods showing a low incidence and low levels of contamination were rice, soybeans and coconut products. Mung beans and other beans, yam and sweet potato were in an intermediate position.

2.4.7 Reports from other regions of the world, particularly those with tropical or subtropical climates, indicate that at least aflatoxins pose problems of similar magnitude there. More detailed information is needed for all developing countries to assess the problems and to gauge the economic consequences of contamination by the different mycotoxins shown to be involved.

3. REGULATIONS IN NATIONAL AND INTERNATIONAL COMMERCE

3.1 Aflatoxin is the only mycotoxin currently controlled under national laws and regulations as well as by EEC directive. In view of its nature as an environmental contaminant, maximum tolerance limits for aflatoxin (whether B_1 or the total of $B_1 + B_2 + G_1 + G_2$) have been established in a number of countries. A summary of national tolerance limits² is given in Table 5. Some countries control all human and animal foods (Japan, Malaysia, Poland, Sweden and the United States); in other countries only foods which are more commonly contaminated, such as oilseeds, are controlled (Canada, Denmark, India, Italy, Malawi, Norway, Rhodesia and the United Kingdom). Some countries (Belgium, France and Israel) control only animal feed; and some countries (Brazil, India and Malawi) have established quality control limits for certain commodities intended for export. More of this latter type of limit may be expected from producer countries to stimulate exports of susceptible commodities.

3.2 A recent directive by the European Economic Community (EEC) has established certain maximum tolerance limits for animal feeds as shown in Table 6. All the EEC members, with the exception of Ireland, have informed the EEC secretariat in Brussels that these tolerance limits are being introduced into the national laws. The limits shown in Table 6 were established pursuant to a Directive of the EEC Council on 17 December 1973, which fixes the maximum content of undesirable substances in animal feed. The date of enforcement was 1 January 1976. However, according to this directive the member states are entitled, under certain conditions, to allow animal feed to have a higher content of aflatoxin B_1 than stipulated. This applies particularly to materials intended exclusively for manufacture of compound animal feeds, as long as the final product meets the requirements of the directive after being mixed. The member States also may lower the maximum levels temporarily, may fix maximum levels for other substance, or to forbid completely the presence of these substances.

3.3 In some cases the tolerance levels established are at the limit of sensitivity of the analytical method, in order to ensure minimal exposure of the population to any aflatoxin present in imported commodities. The introduction by governments and the EEC of such low tolerance levels, especially in animal feed so as to avoid the transfer of aflatoxin to food of animal origin, has caused difficulties in the export of oilseeds for some of the developing countries. Where relatively higher tolerance levels are in force for animal feed, e.g. in Japan, Norway and France, the final preparation of the mixed feed is expected to ensure a product with only a low level of aflatoxin. In certain countries like India, the tolerance level was originally established along the guidelines issued by the Protein Advisory Group of the United Nations System. This group recommended lowest possible levels of aflatoxins in foods rich in protein, suggesting on a risk/benefit evaluation that intake should not exceed 30 $\mu\text{g/kg}$ of food ingested where use of contaminated food cannot be avoided. Other developing countries have also looked to the guidelines of the Group as one basis for arriving at practical control levels.

3.4 A significant trend has emerged since regulations and tolerance levels concerning aflatoxins were first introduced: Since 1973 new regulations or amendments to earlier ones have generally set lower tolerances. Examples are the lower limits by the EEC, and a reduction in the 20-25 $\mu\text{g/kg}$ limit for groundnut and its products to 15 $\mu\text{g/kg}$ by Canada.

3.5 Although to date no mycotoxin other than aflatoxin has been made the subject of regulation, it should be noted that India has fixed a provisional limit of 0.05% for ergot in millet, and Sweden has fixed a provisional limit of 50 $\mu\text{g/kg}$ for patulin in apple juice.

3.6 However, there is a definite tendency to regulate other mycotoxins, in particular ochratoxin A, patulin, zearalenone and the trichothecenes. For aflatoxins, the permissible limits have continued to be lowered ever since their establishment, and the number of countries introducing regulations continues to increase. All this indicates that the negative effect of mycotoxins on national economics and international trade will increase in the coming years.

Table 5.

AFLATOXINS LIMITS IN DIFFERENT COUNTRIES - 1976

Country	Commodity	Aflatoxin* limit (ug/kg)
Belgium	animal feed	40 ***
Brazil	groundnut oilseed cake (export)	50
Canada	nuts and their derived products	15 **
Denmark	groundnuts and Brazil nuts	5-10
France	animal feed	700
India	food	30
	groundnut flour for food use	120
	groundnut cake (export)	60-120
Israel	all foods	20
Italy	groundnuts	50 ***
Japan	all foods	10
	groundnuts cake for animal feed mixes	1000
Malaysia	all foods	0
Malawi	groundnuts	5
Netherlands	foods and feeds	5
Norway	oilseed cake	600
Poland	all foods and feeds	5
Rhodesia	groundnuts	25
	animal feed	50-400
Sweden	all foods, particularly Brazil nuts, groundnuts, groundnut butter,	5
	raw materials for further processing in Sweden	20
	confectionery groundnuts	50
U.K.	groundnut flour for animal feeds	0-500 ***
	confectionery groundnuts	20 *
USA	all foods and animal feeds	20-25 **

* Aflatoxin B₁** (total of aflatoxin B₁, B₂, G₁, G₂)

*** see Table 6 - EEC limits may now apply

Table 6. EEC TOLERANCE LIMITS FOR AFLATOXIN B₁ IN ANIMAL FEED

Commodity	Aflatoxin B ₁ Tolerance - not more than (ug/kg)
Produce for processing into mixed feed	50
Complete feed for cattle, sheep and goats (with the exception of dairy animals, calves and lambs)	50
Complete feed for pigs and poultry (with the exception of infant pigs, chicks, ducklings and turkeys)	20
Animal feed supplements for dairy animals	20
Other complete feeds	10

3.7 Necessary though all such regulations are, they nevertheless constitute, as things stand at present, a handicap to international trade, since they involve:

- for exporting countries, increasing difficulties in finding outlets for their products and a threat to their balance of trade;
- for importing countries, a risk of difficulties in obtaining supplies of certain essential commodities, particularly animal feedstuffs;
- for countries which are large consumers of certain food crops, a risk of a drop in the amount of food available, a risk aggravated where supplies are already at a critical minimum threshold.

4. A REVIEW OF RELEVANT INTERNATIONAL TRADE

4.1 Economic Geography of Mycotoxin Contamination

4.1.1 The problem of supplies largely for livestock feed does not appear to be too difficult for most of the countries of the temperate regions. A large number of tropical or subtropical countries offer a range of raw materials, and market considerations then will determine the trade relationships. However at times, for example after the 1972/73 drought of near global dimensions, extra supplies may not be available from any source, even in response to higher purchase price offers. Even then the result for the countries of the temperate regions, because of several options, will not necessarily be of the greatest consequence. One way of dealing with any shortage of feed is increased slaughter and transfer of meat to cold or canned storage. Another way is a withdrawal from the feed and grain reserves which many of the countries maintain inter alia for just such eventualities. A third solution is increased trade in feed materials among industrialised (and centrally planned) countries, especially if surplus inventories have been carried at considerable cost for many years. There is thus a certain amount of flexibility in the system for such nations.

4.1.2 Some of this same kind of flexibility is available for those tropical or sub-tropical countries which normally depend on their non agricultural resources, e.g. petroleum, minerals, metals, the export income or cash reserves or credit from which can be used to maintain food (and perhaps feed) supplies at normal levels. It should be noted that funds may be used which would otherwise have supported further growth and national development. A part of the greater need for food and feed in such countries can be accounted for by a rapidly rising standard of living for much of the population.

4.1.3 For the largely agriculturally based countries little or no flexibility is available other than aid or credit which is already overextended due to higher import bills. Cash crops may dominate the international trade pattern of these countries and will often be their main source of income to be used for development and for needed improvement in the standard of living. Off-farm reserves of food may be low or non-existent. In such a situation, the possibility of loss of earnings from harvest failures is a grave prospect both for the international trade of the country and loss of foreign exchange income or of markets due to mycotoxin contamination can be serious by itself but will also aggravate the problem. Often, unfortunately, conditions which adversely affect the harvest, such as drought, untimely rains, or attacks by pests, will also increase the level of contamination by mycotoxins.

4.1.4 In Table 7 a number of developing countries are ranked in decreasing order of their dependence on agricultural exports. The proportion (and in parentheses the percentage) of the export earnings derived from a few of the more important commodities susceptible to contamination by mycotoxins is indicated in the main body of the table. The data are for the crop year 1974. This may not have been a typical year for some countries and crops, because of the effects of the drought in the preceding years. An adjustment was however made only for groundnuts and groundnut products from Nigeria, as the 1974 and later figures were only a fraction of those according to which Nigeria had long been classified as a major exporter of these commodities. Some increases in prices may have compensated for lower export volume. In general, the data given must be considered as merely indicative of the magnitude of the value of trade involved. A closer examination of the data may nonetheless be instructive in showing the potential for difficulties that could arise from mycotoxin contamination.

4.1.5 There are countries for which more than half the agricultural exports are accounted for by one or two of the crops susceptible to mycotoxins contamination. Where the crops are rice (as for Pakistan or Surinam) or cacao/cocoa (Nigeria), contamination may not in practice have had a measurable influence on trade income because of the absence of regular checks by importing countries. Where any of the other commodities are involved, contamination may lead directly to loss of income from exports and possibly loss of market for the country or the commodity. Among the more endangered examples would appear to be groundnut products from The Gambia (66% of agricultural exports value), Senegal (21.5%), Sudan (18%) and India (11.4%); maize from Argentina (22.8%) and Thailand (17.5%); cottonseed products from India and Iran; copra from Papua-New Guinea (23.2%) and the Philippines (11%) and sesame seed from Ethiopia (16.2%) and the Sudan (14.3%).

4.1.6 Percentages tell only a part of the story. Value, taken as an indication of volume, should sometimes alter the order or ranking, and if population factors were considered a still different order might result. To take groundnut products as an example, exports from Brazil were valued at US\$ 42.2 million, those from Senegal at US\$ 36.8 million. Although in Brazil, groundnut products make up only 0.9% of the agricultural exports, yet the number of farmers and middlemen affected by trade losses would not appear to be lower than in Senegal where groundnuts make up 21.5% of the agricultural exports. It will be useful to reiterate in this context some of the information previously cited for the six member countries of the African Groundnut Council: about 16 million people cultivate approximately 4 million hectares of groundnuts; these people and others who depend on income from groundnuts by work or trade may reach 60-70% of the population in some countries, i.e. Senegal and The Gambia.

Table 7. "VULNERABILITY" OF CERTAIN COUNTRIES TO PROBLEMS OF CONTAMINATION OF SELECTED COMMODITIES BY MYCOTOXINS.

Million US \$ (% of agricultural exports 1974. See text 4.1.4)											
Country	(A) 1974 Total Exports Value	(B) 1974 Agric. Exports Value	(B) as % of (A)	Rice	Maize	Cacao/ Cocoa	Groundnut products*	Cottonseed products*	Copra	Sesame seed	Pistachio nuts
Sudan	440	426	96.8				76.7(18%)	4.8(1.1%)		60.8(14.3%)	
Gambia	39.5	37	93.7				24.4(66%)	3.1(1%)			
Uganda	326	300	92.0				6.4(6%)	1.9(0.8%)		38(16.2%)	
Malawi	119	107	89.9					10.7(0.48%)		3.3(0.8%)	
Ethiopia	262	234	89.3								
Sri Lanka	520	414	79.6								
Argentina	3,930	2,889	73.5	14(0.5%)	658(22.8%)		6.7(0.2%)				
Cameroons	476	343	72.1			161(47%)	7.2(2.1%)				
Tanzania	418	301	72.0					3.9(1.3%)			
Nicaragua	381	270	70.9					14.3(5.3%)			
Guatemala	572	393	68.7					8.5(2.1%)		5.8(1.5%)	
Thailand	2,466	1,670	67.7	484(29%)	292(17.5%)		4.7(0.3%)			4.6(0.3%)	
El Salvador	462	307	66.5					3.8(1.2%)			
Colombia	1,416	930	65.7					4.7(0.5%)			
Ivory Coast	1,213	791	65.2			314(40%)		3.6(0.5%)			
Mali	63	41	65.1					1.1(2.7%)			

* oil excluded

Table 7. (cont.)

Million US \$				Million US \$ (% of agricultural exports 1974. See text 4.1.4)							
Country	(A) 1974 Total Exports Value	(B) 1974 Agric. Exports Value	(B) as % of (A)	Rice	Maize	Cacao/ Cocoa	Groundnut products*	Cottonseed products*	Copra	Sesame seed	Pistachio
Egypt	1,518	984	64.8	101(10%)			10.9(1.1%)	3.1(0.3%)			15(1%)
Turkey	1,532	971	63.4				4(0.4%)	21.7(2.2%)			
Brazil	7,951	4,881	61.4	18(0.4%)	139(2.8%)	322(6.6%)	42.2(0.9%)	9.1(0.2%)			
Guyana	268	160	59.7	22(13.8)							
Philippines	2,725	1,560	57.2						172(11%)		
Senegal	391	171	43.7				36.8(21.5%)				
India	3,843	1,376	35.8	25(1.8%)			157(11.4%)	19.9(1.4%)		6.3(0.6%)	
Mexico	2,850	1,015	35.6			113(30%)					
Ecuador	1,062	371	34.9								
Pakistan	1,026	340	33.1	211(62%)							
Papua/New Guinea	713	151	21.1								
Indonesia	7,426	993	13.4			34(22.5%)	3.5(0.4%)		35(23.2%)		
Surinam	215	17	7.9	13(76.5%)							
Korea, DPR	4,460	272	6.1	60(22%)							
Nigeria	9,529	544	5.7			293(54%)	[97(37%)]**				
Iran	23,806	241	1.0					11.6(4.7%)			20(0.1%)
Venezuela	10,833	85	0.8	20(23.5%)							

* oil excluded

** 1973 basis (see text)

4.1.7 Income from foreign trade in susceptible commodities can therefore be seen to be a very significant factor in the national economy of a number of developing countries. Pistachio nuts may not be important to Iran's trade balance, and may not cause serious distortion to that of Turkey, but in both countries substantial regions and large populations depend on income from this tree crop for their economic wellbeing. Similarly, what might otherwise often be subsistence farm families are enabled to enter the market economy of their countries because of income from sesame seeds intercropped with the staple foods. The solution of a shift from cultivation of susceptible commodities to others is often not feasible under constraints of soil and climate, lack of resources for seed and energy, or lack of infrastructure for bringing about the required changes in post-harvest handling and domestic and foreign marketing for the new commodities.

4.1.8 Eventually, as part of the process of development, appropriate diversification of agricultural production and relatively reduced reliance on foreign exchange earnings from raw commodities will decrease the real and the potential level of susceptibility of countries who suffer serious trade consequences from mycotoxin contamination. Such a prospect, should not be cause for complacency in any country.

4.2 Trade Agreements and the Role of International Organizations

4.2.1 In the past, mycotoxin contamination appears not to have figured prominently among the international and very often private agreements concerning the purchase/sale of susceptible commodities. This reflects mainly an ignorance of the presence of the toxin and/or an inability to detect the toxin or link to it any adverse effects from use of the commodities. With increasing awareness of adverse effects, ability to detect low levels of toxins and concern by government agencies of importing countries over potential hazards to public health, regulations have been put into effect in a number of the importing countries. They are more and more reflected in current trade agreements, especially those covering susceptible commodities. The need for quality control tests at the point of export which would include reliable checking for compliance with stipulated limits for aflatoxins has thus become apparent, and food control laboratories normally meet this need.

4.2.2 International organizations such as FAO, UNEP and WHO in the UN System, the EEC, OECD and other bodies, are engaged in generating further information on different aspects of prevention and control of problems due to mycotoxins. In matters of international trade such knowledge might include development of internationally accepted:- 1) sampling plans for different commodities leading to international agreement on the complete procedure of sampling and analysis, 2) guidelines for surveillance and food control inspection systems, 3) methods of regional forecasting of climatic and other conditions favoring mycotoxin development, 4) guidelines for innocuous use of contaminated produce in feeding of different animals grown for food, 5) protocols for detoxification and the quality control and evaluation of the resulting products. Initial efforts have already been made in the first two of these areas. This Conference may also assist in the identification of problems at the international level, and define areas in which the sponsoring organizations and others may promote progress toward their solution.

5. INTERNATIONAL TRADE - GROUNDNUTS AND GROUNDNUT PRODUCTS

5.1 World Production of in-shell groundnuts was about 19 million metric tons in 1975, about 21% above the average production during the period 1961-65. Asia, with 11 million tons in 1975, accounted for 58% of world production; Africa, with 5 million tons, for 26%; and North America, with 3 million tons, for 10%. Asia's 1975 production was 32% greater than the average 1961-65 production and that of North America was 100% greater, whereas that of Africa dropped slightly, by about 4%, and therefore can be considered stationary.

Five countries with more than 13 million tons, accounted for about 70% of world production in 1975: India, 35%; China, 15%; USA, 9%; Senegal, 6% and Sudan, 5,75%.

5.2. World Export Trade in groundnuts and groundnut products is divided into four categories. In 1975, trade in in-shell groundnuts involved more than 100 thousand tons valued at US\$ 56 million; shelled nuts 886 thousand tons at US\$ 473 million; groundnut cake 1,158 thousand tons at US\$ 149 million, and oil 405 thousand tons at US\$ 348 million. Table 8 shows the distribution of export trade value for the three categories other than oil. Principal exporting countries are the USA, India, Sudan, Senegal, Brazil, South Africa, The Gambia and China. Nigeria is also normally an important exporter.

Table 8. GROUNDNUT PRODUCTS EXPORT TRADE VALUE IN 1975. (million US\$)

Country	Shelled nuts	Oilseed cake	In-shell nuts	Total
USA	148	-	7,7	155,7
India	38	67	-	105
Sudan	98	2,6	-	100,6
Senegal	9	42	-	51
Brazil	33	4,1	10,2	47,3
South Africa	23	11	-	34
The Gambia	25	4,2	-	29,2
China	15	-	11	26
Egypt	7	-	4,4	11,4
Malawi	7,5	-	-	7,5
Cameroun	7	-	-	7
Guinea Bissau	4	-	1,4	5,5
Argentina	-	5,2	-	5,2
Turkey	-	-	-	2,6
Bénin	-	-	-	2,5
Thailand	-	-	-	2,3

5.3 World Import Trade is directed mainly at Europe, with Japan and Canada also taking substantial percentages. In 1975 Europe imported 77% of the total world export crop of in-shell groundnuts, 71% of the total world export crop of shelled groundnuts and 87% of the total world export crop of oilseed cake. France, U.K. and FRG imported each of the products, while Portugal imported both shelled and unshelled nuts. The Netherlands and Sweden imported only shelled nuts and oilseed cake was imported additionally by Poland.

5.4 Groundnut Cake Import and Use in France - Capsule Case Study

5.4.1 Imported groundnut cake is an item of major importance to France, the largest European importer, and to other EEC countries as well. Table 9 shows both usage and import figures for groundnut cake in European countries. Table 10 contrasts the rapidly increasing consumption of soybean cake in France over the years with the decreasing usage levels of groundnut cake.

Table 9. CONSUMPTION STATISTICS FOR GROUNDNUT CAKE IN EUROPEAN COUNTRIES - 1975.

Country	Consumption tons	Importation tons
France	335,000	254,000
U.K.	213,000	211,000
FRG	86,000	83,000
Italy	32,000	9,000
Belgium	32,000	31,000
Netherlands	8,000	9,000
Denmark	0	0

Table 10. REVIEW OF CONSUMPTION OF GROUNDNUT AND SOYBEAN CAKES IN FRANCE, 1938-1975.

Year	Consumption of all oilseed cakes - tons	Consumption of groundnut cake - tons (% of total)	Consumption of soybean cake - tons (% of total)
1938	678,000	-	-
1963	-	372,000 (26%)	-
1965	1,411,000	-	-
1970	2,203,000	384,000 (19%)	167,000 (58%)
1971	2,118,000	-	-
1972	2,314,000	- (17,7%)	- (62%)
1973	2,487,000	407,000 (16%)	1,528,000 (60%)
1974	2,588,000	229,000 (8,9%)	1,931,000 (80%)
1975	2,473,000	335,000 (13,5%)	1,832,000 (75%)

5.4.2 Table 10 shows that the consumption and importation of groundnut cake in France is turning in favour of soybean cake. One reason may be that buyers are looking for reliable availability of supplies, whereas groundnut cake is offered lot by lot, or subject to availability. Then, the number of cattle, relatively lower consumers of soya, has been falling due to recession and for other reasons, while the numbers of pigs and poultry have not gone into a similar decline; these shorter-term livestock consume relatively more soya.

Finally, and not least important, there is the contamination of groundnut cake by aflatoxin and the introduction of regulations concerning acceptable levels of aflatoxin in cattle feed imported into the EEC countries.

5.4.3 It appears that there exists a threshold level of consumption below which a major feed constituent cannot maintain its importance in a given country. When this consumption level, in France for example, falls below 200,000 tons/year, the product will be considered as marginal on the French market and will find it increasingly difficult to compete. This is the situation presently between groundnut and soybean cakes. If consumption of groundnut cake were to fall to a level below 200,000 tons/years, the commodity would risk complete elimination from the market at a fairly early date. In this same way linseed cake was on a previous occasion displaced by groundnut cake. The displacement of groundnut cakes has proceeded progressively, first from poultry feed, then from pig feed, then, starting in 1970/71 to a greater or lesser extent from nearly every use, so that today it remains virtually only in cattle and sheep feed. Volume of consumption is approaching the critical level of 200,000 tons.

5.4.4 Aflatoxin contamination it appears has played a role in the trend toward replacement of groundnut cake in animal feeds in France. In addition irregularity of supply (made more obvious perhaps due to recent droughts e.g. in the Sahel) has contributed to the strength of the trend and to a weakness in price vis-a-vis soybean cake. The price of groundnut cake may be 15 to 20% less than that of soya bean cake and while some of this difference may be based on nutritive value, most of it will, in effect, be an insurance against the risk of contamination by aflatoxin. In conclusion note may be taken that in certain regions of France, where the rearing of pigs and poultry predominates, e.g. in Brittany, use of groundnut cake is already "marginal".

5.5 Some Trade Losses due to Aflatoxin Contamination

5.5.1 During 1971/75 Japan imported nearly 600,000 tons of groundnut cakes, for a total value of \$100 million. The Japanese authorities estimate that an average of 0.1% of these imports contained more than 100 µg/kg of Aflatoxin B₁, and were consequently refused and burned. This would represent a loss of \$100,000 for the exporter.

5.5.2 In some years certain lots of groundnut cake exported from Brazil to Western Europe were reported to contain as much as 2000 to 3000 µg/kg aflatoxin B₁ and were therefore not allowed to be used as cattle feed component. Instead, these lots were sometimes used as fertilizer at discounts of up to 75%. Since 1975 trade in this produce has been completely stopped between Brazil on the one hand and France, Belgium and the Netherlands on the other.

5.5.3 Information from Sudan indicates that excess moisture in stored groundnuts has in the past led to aflatoxin development and to loss of 6-8% of the crop in storage. Considering Sudan's income from exports of groundnuts (Table 8), this reduced availability may be estimated as loss of foreign exchange earning of 6-8 million dollars.

5.5.4 Several European countries, in particular the Netherlands, FRG, Italy and Denmark, have progressively decreased their imports of groundnut cake from all sources and the two countries are reported to be no longer using groundnut cake for cattle feed. Imports into Denmark have been zero since 1970. Had it not been for the problem of contamination by aflatoxin, however, there might be a demand in Denmark for about 100,000 tons per year, to the value of more than 100 million Danish crowns. This is a loss of a significant market for the exporting countries.

5.5.5 Thirteen percent of the groundnut cake received between 1972-1976, amounting to 20,000 tons, were reported by a French firm to contain more than the 700 µg/kg of aflatoxin B₁ then stipulated as the permissible limit in the French legislation, which was less strict than the new EEC directive. Another French manufacturer of cattle feed found the following results in lots of groundnut cake purchased. (Table 11.)

Table 11. AFLATOXIN CONTENT OF SAMPLES OF CATTLE FEED IN FRANCE, 1971-1975

Year	No. of Samples	Aflatoxin B ₁ mean (ug/kg)	Aflatoxin B ₁ max. (ug/kg)	% higher than 700 ug/kg
1971	96	965	2,460	59%
1972	116	1,120	7,700	52%
1973	215	947	5,700	45%
1974	92	687	1,980	43%
1975	130	698	2,700	47%

Thus perhaps about half of the groundnut cake supply in France was above the legal limit for direct feed use. More recently, however, the number of heavily contaminated lots has decreased. One explanation may be that France was the last EEC country to enforce regulatory limits and, up to 1973, absorbed many of such lots refused entry by other EEC countries.

5.5.6 In view of the high levels of contamination found, European manufacturers of compound feedstuffs for cattle consider sampling and control measures indispensable and insist that suppliers of groundnut cake meet specifications with strict clauses concerning refusal of merchandise or price penalties in case of unacceptable contamination by aflatoxin. Increased difficulties in the marketing of groundnut cake are therefore to be expected in coming years, unless strict measures are taken by the exporting countries to control this contamination.

5.5.7 In the U.K., incomplete information indicates that, over the period 1970-73, at least 3,500 tons of groundnuts imported and intended for human consumption were found to contain aflatoxin B₁ at levels higher than 50 µg/kg, the limit for food. The lots involved could therefore find use only in animal feed at a considerably lower price.

5.5.8 In the USA an agreement has been in force for several years between groundnut producers and the federal authorities in order to prevent the marketing of groundnuts visibly contaminated by *Aspergillus flavus*. Over the period 1968-73, nearly 300 thousand tons of groundnuts were involved. Percentages of the quantities (crop) withdrawn in the various regions over the years 1968-73 are as follows. (Table 12.)

Table 12. PERCENTAGE OF QUANTITIES OF GROUNDNUT CROP WITHDRAWN IN VARIOUS REGIONS OF USA, 1968-73.

Regions	1968	1969	1970	1971	1972	1973
Southeast	6,3	1,4	3,4	2,6	13,5	2,6
Southwest	1,2	2,4	1,4	2,5	1,9	0,8
Virginia-Carolina	0,5	0,007	0,4	0,3	0,2	0,1
Avg, USA	3,7	1,4	2,1	2,1	8,1	1,6

If the second stage of segregation, recently introduced and entailing the withdrawal from market channels of groundnuts contaminated by more than 25 µg/kg of aflatoxins, had been applied since 1968, withdrawals for the period 1968-73 would have reached 1,170 thousand tons,

about 400 thousand tons for the Southeast alone. These figures need no comment; they illustrate the enormous problem of aflatoxins in groundnuts in just one country.

5.5.9 Data are available on the costs due to the control of the aflatoxin content in groundnuts in the United States at the three check points of the system, namely: 1) at the first buying point for farmers' stock (in-shell) groundnuts; 2) at the sheller, and 3) at the manufacturer of the consumer products. Rejection at point 1) resulted in a loss in value of farmers' stock groundnuts, which averaged about half a million dollars for 1974-1975 or approximately 0.15% of the total farm value of the nuts. In crop years 1974 and 1975 about \$1.80 per 1,000 lb of shelled groundnuts was paid for sampling and analysis of raw, shelled groundnuts at check point 2) and indemnification losses for payments to shellers, for lots that were outside the permitted aflatoxin levels, which represented about 0.6% of the estimated value of the shelled nuts. The costs of sampling and analysis carried out internally by manufacturers of consumer products and other inspections costs (borne by the government) are not included.

5.5.10 Detoxification of a commodity contaminated with aflatoxin to make it acceptable for food or feed use is another cost involved. There have been a few cases reported in which a shipment of a commodity has been prohibited from use because of contamination and this has resulted in total loss of the commodity. These incidents are exceptional however. The detoxification procedures for groundnuts involve the physical removal of contaminated kernels. The methods are widely used but there are no estimates available on the costs involved or on the losses resulting from the reduced value of the rejected nuts. Conference Document MYC-6 discusses in detail methods and problems associated with the detoxification of food and animal feed contaminated by mycotoxins, including some of the relevant economic considerations.

6. INTERNATIONAL TRADE - OTHER COMMODITIES

6.1 Maize

6.1.1 World production of maize reached a level of more than 322 million tons in 1975, 49% above the average for the period 1961-65. North America accounts for 46% of the total, Asia for 16.7%, Europe for 14.2%, Latin America for 8.5% and Africa for 7.7%. All regions have markedly increased their production in the past 15 years, the advance being 80% for Europe, 56% for Africa, 55% for Latin America, 53% for North America and 45% for Asia. Leading producer countries are the USA (45%), China (10%), Brazil (5%), and South Africa (3%), Yugoslavia (2.8%), Rumania (2.7%), Mexico (2.7%), France (2.5%), Argentine (2.4%), Hungary (2.2%), India (1.7%), Italy (1.6%), Canada (1.1%), Indonesia (1.0%), Thailand (0.9%), Philippines (0.8%) and Egypt (0.8%).

6.1.2 World trade of maize in 1975 involved only 15.8% of total production. This low percentage indicates that maize is an important staple or major component of animal feed in most of the producing countries. 51 million tons entered world trade and its value for 1975 was about 7 billion US dollars, distributed mainly among 7 exporting countries as follows:

(million US\$)

USA	4,447
Argentina	500
South Africa	431
France	415
Netherlands	391
Thailand	336
Brazil	157

6.1.3 More than four-fifths of world imports of maize were accounted for by countries of Europe (55%), by the USSR (10.9%) and Japan (14.5%). The main use of maize imported by these countries is as animal feed. Therefore, lower availability of maize on the world market will adversely affect the livestock industries in these countries, leading to lower production of animal protein products. In view of the widespread and sometimes heavy contamination of maize by aflatoxins, zearalenone and other Fusarium toxins, availability of maize of acceptable quality may be put in question depending on the ability of the producing countries to prevent or control mycotoxin development. In many producing developing countries maize is a staple food, and contamination by mycotoxins may cause or increase food deficits, leading to hunger and/or greater use of scarce foreign exchange reserves to meet the food requirements of the population often at the expense of investment in development.

6.1.4 Trade losses of maize due to contamination by mycotoxins are not widely reported nor necessarily separated from losses ascribed to moulds. An example was cited of a large shipment (20,000 tons) of aflatoxin in contaminated maize imported into Costa Rica from Thailand prior to 1974. A part of the shipment was destroyed (burned or buried) subsequently; another part (1,100 tons) was sent to Nicaragua where analysis showed levels of 150-300 µg/kg of aflatoxin and the lot was destroyed; still another part was shipped to Venezuela but was not accepted for importation, adding transportation and handling costs to the losses.

6.1.5 Loss of 2% of the harvested crop due to aflatoxin contamination has been reported in Swaziland.

6.1.6 In Zambia, maize payments to producers were discounted because of mould damage associated with zearalenone contamination. An effort was made in 1975 to clean grossly infected maize lots in order to recover sound grain suitable for human consumption. Farmers received only about 35% of the average maize price for these lots, and there were further costs involved in re-conditioning. Additional reduced availability of the staple for local consumption led to increased imports and use of foreign exchange reserves.

6.2 Cottonseed and Cottonseed Cake

6.2.1 World production of cottonseed in 1975 was more than 22 million tons, 11% higher than in 1961-65. Nearly 42% of this production is in Asia (China, India, Pakistan and Turkey are the major producers), nearly 23% in the USSR, 14% in USA, 9.5% in Africa (Egypt, Sudan, South Africa, Ivory Coast) and 8% in Latin America (Brazil, Mexico, Argentina, Colombia, Nicaragua).

6.2.2 World trade in cottonseed had an export value in 1975 of about US\$ 30 million, distributed in part as follows: USA, \$9.5 million, Nicaragua \$5.3 million, Ivory Coast, \$3.6 million, Uganda, \$3.2 million, South Africa, \$3 million, Israel, \$1.5 million, Mali \$1.1 million and Thailand \$1.1 million. Other exporting countries include Swaziland, Afghanistan, Bénin, Ethiopia, Togo and Upper Volta. By value, two-thirds of cottonseed world-wide imports were taken by Japan, followed by Greece (12.5%), Lebanon (8.9%) and Costa Rica (2.8%).

6.2.3 Cottonseed may be used in ruminant feed rations, or may be processed to yield edible oil and cottonseed cake for animal feed. By far the greater part of the cottonseed production is processed into oil and cake. Countries with cottonseed cake exports in 1975 exceeding a value of US\$ 1 million were Turkey (26), India (19), USA (9), Iran (8), Argentina (7), Colombia (6.7), El Salvador (6.4), Guatemala (6.1), Sudan (5.8), Tanzania (4.4), Egypt (3.4), Denmark (2.6), Brazil (2.2), Mozambique (1.8), Uganda (1.5), Paraguay (1.5) and Ethiopia (1.4). Europe nearly monopolized world imports in 1975, five countries accounting for four-fifths of the total: Denmark (46%), FRG (15%), Sweden (7.5%), Poland (6.4%) and GDR (5%).

6.2.4 Trade losses due to mycotoxin contamination of cottonseed cake appear not to have been reported. As this commodity is used principally in feeds for ruminant animals, it may be expected that minor levels of contamination will be accepted for some time. Detoxification by ammoniation is practiced to some extent in the USA, with associated costs being on the order of \$10 per ton.

6.3 Copra and Copra Cake

6.3.1 Coconut production of 29.6 million tons in 1975 was dominated by the Philippines, Indonesia and India; Sri Lanka and Mexico also were important producers. Fresh coconuts enter international trade channels to a limited extent only; the main products traded are copra and copra cake. The Philippines by far export the largest share of copra; export value in 1975 was \$172 million out of the \$263 million world total. Papua-New Guinea was another major exporter (\$40 million). Countries of Western Europe and Japan imported most of the copra in trade. For copra cake, the Philippines and Indonesia accounted for 48% and 36% respectively of the 1975 world export trade value of \$69 million. Countries of Western Europe, and particularly the Federal Republic of Germany and the Netherlands, were the importers.

6.3.2 The loss of a substantial market for copra occurred in the past few years because of contamination by aflatoxin. The USA had regularly imported copra from the Philippines, the values for the years 1971, 1972 and 1973 being \$38, \$22 and \$25 million respectively. By 1975 imports of copra and copra cake into the USA had ceased, largely because of governmental surveillance over importation.

6.4 Sesame Seed

6.4.1 World production in 1975 was close to 2 million tons, more than half of it accounted for by India (21%), China (18.6%) and Sudan (13.6%). Other countries producing more than 20,000 tons per year are Mexico, Burma, Ethiopia, Nigeria, Venezuela, Afghanistan, Turkey, Bangladesh and Colombia.

6.4.2 World trade in 1975 had an export value of \$124 million. Most of this trade originated in Sudan (29%), Ethiopia (28%), Mexico (7.3%), Guatemala (5.6%), Afghanistan (4%), Thailand (3%) and Sri Lanka (2.7%). Asian countries imported about 40% of the trade volume, Japan taking one-half (20%). Italy and Greece were the major importers in Europe, and Egypt in Africa.

6.4.3 Trade losses due to mycotoxin contamination of sesame seed appear not to have been reported, perhaps because searches and findings of contamination have been less frequent than for some other oilseeds.

6.5 Tree Nuts

6.5.1 Incidence of mycotoxins contamination has been reported in almonds, pecans, walnuts and filberts, but problems in trade have so far been recorded only for Brazil nuts and pistachio nuts. Almonds appear to be especially susceptible under certain conditions. In Canada, aflatoxin tolerance limits for nut products have recently been made applicable specifically to cashew nuts, suggesting that a problem may exist. The information on imports into the USA of Brazil nuts and pistachio nuts may serve as illustration for the tree nuts. It may be of interest to recall the problem of sampling to determine contamination of nut commodities, already discussed in connection with groundnuts, and to indicate that the relatively high commercial value of many tree nuts may make adequate sampling for quality or governmental control a rather costly matter.

6.5.2 Brazil nuts are collected over wide areas of Brazil having a humid tropical climate. Unless attention is paid to the conditions of collection, transport and handling, aflatoxin contamination may occur. Following detection of contaminated lots, a surveillance programme introduced in the USA in 1967 led to refusal of one in four lots coming from Brazil, and in 1968 to refusal of one in three lots. The value of the unacceptable lots in these two years was \$4.7 million. A quality control programme introduced in Brazil led to a drastic reduction in the proportion of lots arriving in the USA which still had to be refused because of aflatoxin contamination. For the three years 1971-72-73 the refusal rate averaged only 1%, with a total value of \$0.3 million. Estimates have also been made of costs to importers for control of aflatoxins in Brazil nuts marketed in the USA. These costs included sampling and reconditioning. Annually, the average of these costs over the period 1970-76 was \$5.50 per ton (2000 lb) or 1.4% of the value of imported Brazil nuts.

6.5.3 Pistachio nuts are exported mainly from Iran and Turkey. Surveillance activities showed that four out of five lots arriving in the USA during the first four months of 1972 were contaminated with aflatoxins to the point where they had to be refused entry. The value of the unacceptable lots for this part of the year may be estimated conservatively at \$4-5 million. Two years later, 13% of all lots reaching the USA during the first half of the year still had to be refused, the value being estimated at \$1.5 million. An estimate is also available for costs to US importers for control of aflatoxins in pistachio nuts in 1975. These costs, which include the sample, sampling and analysis, and reconditioning, averaged \$13 per ton, or 0.7% of the value of more than \$23 million for the total import volume.

6.6 Other Commodities

6.6.1 The commodities discussed earlier have experienced trade problems due to mycotoxins (aflatoxins) contamination. Judging from checks mainly on suspect lots and a rather small number of surveys of limited scope, incidence of contamination is not insignificant in several further commodities derived from oilseeds (e.g. soybeans and sunflower seed) and grains other than those already mentioned (e.g. rice, wheat, oats, barley, sorghum and millet). Contamination has also been found in coffee and cocoa beans, figs and other dried and fresh fruit, apple juice, wine, beers (particularly those produced from local grains in warm humid climates), and in animal products, especially milk, cheese and liver.

7. CONCLUSION

The economic and trade aspects of mycotoxins contamination are clearly of concern to the world community of nations in regard to problems of balance of payments and the protection of public health. A common approach to their solution would seem appropriate both in the context of the New International Economic Order and to ensure a fuller, safer, more wholesome food supply for mankind.

PUBLICATIONS AND PAPERS ON
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I. PUBLICATIONS*

<u>Title</u>	<u>Series and No.</u>	<u>p.</u>	<u>year</u>
1. Mycotoxin Surveillance - A guideline	FAO Food Control	75	1976
2. Report of the Joint FAO/WHO/UNEP Conference on Mycotoxins. containing in Annex the working papers MYC-5, MYC-6, MYC-7, MYC-8, and MYC-9 (See section III below)	FAO Food and Nutrition Paper No. 2	120	1977
3. Recommended Practices for the Prevention of Mycotoxins in Food, Feed and their Products	FAO Food and Nutrition Paper No. 10	75	1979
4. Perspective on Mycotoxins: (Assessment, Occurrence, Toxicological and Economic aspects)	FAO Food and Nutrition Paper No. 13	175	1979

II. ARTICLES

<u>Title</u>	<u>Author</u>	<u>Journal Citation</u>	<u>year</u>
5. The Occurrence and Control of Mycotoxins and Mycotoxicoses	J.V. Rodricks	FAO Food and Nutrition Vol.2, No.1,9-14	1976
6. Mycotoxins: The FAO Programme	K.O. Herz	FAO Food and Nutrition Vol.2, No.4, 16-18	1976

III. OTHER DOCUMENTS*

<u>Title</u>	<u>Description</u>	<u>p.</u>	<u>year</u>
7. Report of Interagency Meeting (FAO,WHO,IARC, UNEP) on Action to Control Mycotoxin Contamination of Food and Animal Feeds, 26-27 June 1978	Internal Report (offset brochure)	20	1978
8. Prevention of Contamination of Foods and Feeds by Mycotoxins.	Conference (see 2 above) Working Paper MYC-5	20	1977
9. Detoxification of Food and Animal Feed contaminated by Mycotoxins.	" MYC-6	12	1977
10. Surveillance and Control Measures to Reduce Contamination by Mycotoxins.	" MYC-7	10	1977
11. Extension at National Level to Solve Problems of Contamination by Mycotoxins.	" MYC-8	6	1977
12. Priorities for Research and Training in the Field of Mycotoxins.	" MYC-9	10	1977

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2. Report of the joint FAO/WHO/UNEP conference on mycotoxins, 1977 (E* F* S*)
3. Report of the joint FAO/WHO expert consultation on the role of dietary fats and oils in human nutrition, 1977 (E* F* S*)
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