REPORT ON

ASEAN

FUNGAL AND MYCOTOXIN

RESEARCH ACTIVITIES

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Executive summary:

From the period January 26 to March 26, 1993 institutions in Malaysia, Singapore, Indonesia, the Philippines and Thailand where fungal and mycotoxin research is conducted were visited. Meetings were arranged with research groups in order to determine past activities and research strengths. Scientists were asked to identify areas of fungal and mycotoxin research that would be of interest to pursue in the future. Wherever possible laboratory facilities were viewed.

ASEAN researchers were familiar with mycotoxin problems in agricultural commodities associated with aflatoxigenic fungi. They have acquired significant human resource and equipment capabilities in aflatoxin research over the years. Internationally-funded fungal and mycotoxin projects in ASEAN have done much to increase technical capabilities for research on aflatoxin and some other mycotoxins. Short-term training programs conducted through these projects as well as mycotoxin training programs offered by several international agencies have strengthened human resource capabilities and stimulated independent mycotoxin research. Indeed, at least one research institution within each of Malaysia, Indonesia, the Philippines and Thailand has acquired proficiency in the identification of many mycotoxigenic fungi and/or chemical analysis of mycotoxins in addition to aflatoxins. Generous equipment donations from several international agencies have contributed considerably towards the technical capabilities of ASEAN fungal and mycotoxin laboratories and research institutions in general.

Research efforts in Malaysia have dealt mainly with monitoring of aflatoxins in agricultural commodities. Some chemical and biological detoxification investigations have also been conducted. Significant potential exists for research in human epidemiological studies of mycotoxins in Malaysia. Laboratory equipment for the conduct of fungal and mycotoxin research is generally good.

Singapore, being almost entirely an importing nation, has devoted most of its fungal and mycotoxin activities towards aflatoxin monitoring to ensure that imported commodities meet its stringent regulations. A culture collection of plant pathogenic fungi is maintained in Singapore. Some research efforts have been directed towards human health aspects of aflatoxins. In most Singaporean laboratories, the standard of equipment is excellent.

Indonesian scientists have acquired mycological and analytical chemistry expertise in the determination of aflatoxins and other mycotoxins in agricultural products. Several small culture collections of fungal pathogens are maintained. Some research efforts have also been aimed at the development of postharvest storage and handling technologies. The effect of mycotoxins on animal health and production has been investigated. Technical and institutional potential exists for the establishment of a network of clinical and research institutions to determine the significance of animal mycotoxicoses in Indonesia. Breeding for resistance to toxigenic fungi has commenced for maize and peanuts. Most laboratories are adequately equipped. Several institutions in Indonesia have been provided with excellent laboratory facilities.

Fungal and mycotoxin research has been in place for many years in the Philippines. Laboratory activities have recently been hindered by severe power shortages. Capabilities have been developed in mycology, chemical and biological control methods, postharvest storage and handling technologies, human health epidemiology and the effects of aflatoxins on animals. Research institutions in the Philippines have conducted extensive surveys for aflatoxins in a variety of agricultural commodities. Many institutions within the Philippines have endeavoured to develop rapid aflatoxin screening kits for use by farmers and merchants. Some research has also been aimed at resistance breeding in maize and peanuts. Most research facilities are furnished with adequate equipment while several others have been provided with excellent equipment, some specifically for mycotoxin analysis.

Scientists in Thailand have experience in almost every aspect of fungal and mycotoxin research. Proficiency in mycology and the analysis of many mycotoxins has been achieved. Chemical and biological controls have been investigated extensively. In addition, significant research has been conducted on the effects of some mycotoxins on animals. Epidemiological studies for suspected aflatoxin related diseases in humans have been carried out. The monitoring of various mycotoxins in foods and feeds has been conducted for some years. Through a network of Field Crops Research Institutes, opportunities for research in resistance breeding in maize and peanuts exist in Thailand and some research to this effect has already been conducted. One of the network of the UNESCO/UNEP funded Microbial Resources Centre (MIRCEN) culture collections is maintained in Thailand along with several other smaller fungal culture collections. Laboratory facilities in Thailand are generally well equipped. In addition to these, an excellent facility has been specifically established for the conduct of many aspects of fungal and mycotoxin research.

In general, ASEAN scientists feel that despite the continuous efforts that have been devoted towards addressing aflatoxin contamination, adequate solutions have not been achieved. Aflatoxin remains a priority in mycotoxin research in ASEAN, particularly in light of the recent EEC discussions to decrease permitted levels for aflatoxin in imported commodities from 10-20 parts per billion (ppb) to two ppb. The development of sampling methodologies, inexpensive and rapid test kits for aflatoxin detection as well as postharvest storage and handling technologies are regarded as essential if aflatoxin contamination is to be controlled. Financial incentives for farmers to produce high quality commodities for domestic use are lacking.

Small pockets of research expertise on mycotoxins other than aflatoxin exist throughout the region. However, the significance of such mycotoxins in ASEAN remains unknown. Their occurrence has never been systematically studied in the region. There is a need to determine the importance of mycotoxins other than aflatoxins in agricultural commodities. It would be useful to determine their effects on animal production and human health in the ASEAN region. An initial step towards achieving this lies in increasing the level of fungal identification and analytical chemistry capabilities throughout the region. Development of analytical methods appropriate to the climate and financial constraints of the ASEAN region is needed. The compilation of a manual for fungal identification, analytical methods and sampling techniques would be useful for standardization and to facilitate the interpretation of analytical data by researchers throughout the ASEAN region.

An investigation as to the frequency and nature of field outbreaks of animal mycotoxicoses provides a system for the determination of the respective significance of mycotoxins in feeds. A network of animal disease investigation centres exists in both Indonesia and Thailand for the collection of clinical information. Collaborative agreements could be established between clinical investigation centres and appropriate research institutions possessing both the mandate and equipment required to conduct analyses in feeds and tissues. If this approach were to be taken it would be necessary to train field veterinarians and pathologists in the recognition of symptoms and histopathological changes associated with suspected mycotoxicoses. In addition, training would have to be undertaken if the analysis of mycotoxin residues in animal tissues were to be an integral part of these studies. Some training in mycology and chemical analysis in feeds may also be needed.

Human resource potential and equipment for fungal and mycotoxin research is existent in the ASEAN region but remains scattered. The need is apparent for a network of fungal and mycotoxin researchers to facilitate scientific information dissemination and to develop and coordinate research activities within the region. Many ASEAN researchers have expressed the need for a fungal and mycotoxin database to manage various kinds of information. Cost and accessibility to mycotoxin standards were also commonly identified constraints to progress in research. Some scientists have suggested that a network of regional scientists may facilitate accessibility to mycotoxin standards.

Canada has considerable expertise in research in mycotoxins other than aflatoxin. This expertise lies within the government (Agriculture Canada, Health and Welfare Canada), in the universities (Laval, Carleton, Guelph, Saskatchewan) and in the private sector. There exist many opportunities to strengthen the human resource base in ASEAN countries within existing Canadian research programs.

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1. INTRODUCTION

The occurrence of mycotoxins in foods and feeds is associated with a number of diseases in humans and animals. Some of these toxic fungal metabolites, such as aflatoxins, are known carcinogens while others have been shown to exert a variety of toxic effects.

Human health and animal production implications have stimulated worldwide regulatory activities to limit allowable levels of certain mycotoxins in imported commodities. This has serious trade implications for exporting countries. In some cases, these regulations are very stringent. Particular concern has been expressed over the recent EEC consideration to decrease tolerable levels for aflatoxin in imported commodities to two parts per billion (ppb). Furthermore, the use of mycotoxins as non-tariff trade barriers could have serious trade implications in both developed and developing countries.

Reports have been published on the occurrence of toxigenic fungi and mycotoxins in many countries throughout the world. Global concern has arisen from the recognition of mycotoxins, in general, as a problem not limited to any particular country or border. Environmental and climactic conditions, however, may favour the growth and toxin production by certain fungi. As a result, national and international research efforts have been devoted to the identification of the specific fungi and mycotoxins that constitute a problem in a given country or region and to strategies for the control of such fungi and mycotoxins.

This report was commissioned by the Canadian International Development Research Centre to investigate fungi and mycotoxin research strengths in the respective ASEAN institutions for the purpose of identifying possible areas of fungi and mycotoxin research. These efforts and funding should be directed in the future.

Presented in the report are general observations respecting fungi and mycotoxin research facilities, human resource capabilities, information dissemination, constraints to research, and institutional strengths of ASEAN laboratories visited as well as research activities in some ASEAN institutions not visited. Recommendations are made for the improvement of the conduct of future fungi and mycotoxin research activities. Existing fungal culture collections are described.

During the period from January 26, 1993 to March 26, 1993 laboratories conducting fungi and mycotoxin research in Malaysia, Singapore, Indonesia, the Philippines and Thailand were visited. These included government, university and private sector laboratories as well as ASEAN regional laboratories.

Scientists were requested to identify:

- current activities in fungi and mycotoxin research;
- institutional mandate and capacity for interacting with other sectors in research;
- aspects of fungi and mycotoxins in which they feel their is a need to pursue research;
- scientific indications for the justification of additional research and development in fungi and mycotoxins;
- human resource capabilities in various aspects of fungi and mycotoxin research;
- facilities and equipment available for fungi and mycotoxin research;
- areas of study in fungi and mycotoxin research in which they feel they would most benefit from training;
- availability of reference material,

and to provide:

- hard copies of completed research reports.

Laboratory facilities were visited whenever possible and equipment available for fungi and mycotoxin research noted.

2. GENERAL OBSERVATIONS AND CHALLENGES

2.1 FACILITIES, EQUIPMENT AND METHODS

Generous equipment donations from various international agencies (World Bank, USAID, JICA, NRI) have contributed enormously towards the research capabilities in several institutions. Entire facilities fully equipped with almost every imaginable piece of new, high tech laboratory equipment have been established at the Food and Nutrition Development Research Centre located at Gadjah Mada University in Indonesia; the Bureau of Food and Drug in Manila; and the Biotechnology Laboratory in Bogor, Indonesia. In addition, the Thai Maize Quality Improvement Centre was specifically established for the conduct of mycotoxin and phytotoxin research, again complete with outstanding laboratory equipment. Mycotoxin research laboratories with HPTLC equipment including densitometers and integrators have been set up at the Philippine Coconut Authority and the Bureau of Animal Industry, also in the Philippines.

When one excludes the exceptional facilities mentioned above, the general quality and abundance of basic laboratory equipment varies from country to country within ASEAN. In general, Singaporean laboratories are world class, with those in Thailand and Malaysia following. Laboratories in Indonesia and the Philippines are generally not as well equipped. Equipment in other research institutions can sometimes be accessed.

Due to a lack of safety equipment and information on occupational safety for laboratory technicians, a limited number of laboratories have not embarked upon mycotoxin activities even though the technical know-how may be available. A manual for occupational health and safety in mycotoxin research is needed.

A general lack of standardization of sampling and analytical methods exists throughout the ASEAN region. The concern that analytical data cannot be compared within the region due to this lack of standardization was frequently expressed by ASEAN researchers. The participation by some laboratories in international sample crosscheck programs through AOAC and IARC alleviates this problem to a certain degree. Crosscheck programs are extremely beneficial in that mycotoxin standards are provided that may not normally be obtainable due to cost and/or accessibility. A third crosscheck program for aflatoxin is currently being initiated through the National Institute of Nutrition in India with FAO assistance and will soon be commenced in several ASEAN institutions.

In general, equipment necessary to conduct advanced research exists within the region but is limited and scattered in different laboratories throughout the respective countries. Due to a lack of coordination of research activities, this equipment is not optimally utilized. Often, high tech equipment such as HPLC, laminar flow hoods, ultracentrifuge etc. is out of order due to lack of maintenance contracts and the inability to purchase supplementary parts. The cost of standards, solvents and reagents required for the use of some high tech equipment is sometimes constraining. Indeed, high tech equipment is not appropriate in some laboratories. Thus, there is a need to develop quantitative analytical methods appropriate for climactic and environmental conditions in ASEAN that do not require high tech equipment. Every attempt should be made to standardize these methods throughout the region.

Microscopes are sometimes rendered useless owing to fungal growth within the barrel.

Customs restrictions at borders can cause delays in receiving standards, reagents and equipment as well

as decrease purchasing potential. Irregular release of research funds from administrating offices sometimes causes delays in the purchase of supplies and equipment which, in turn, caused delays in operations. Daily power outages lasting between four and eight hours a day have been scheduled in the Philippines for the past year and are still ongoing. This has severely limited research activities requiring even the most basic equipment (eg. freezer). In addition, it has burdened researchers with extra costs that are taken from research budgets for the purchase of generators, fuel and uninteruptible power sources etc. In some laboratories that cannot afford generators, even lighting and telephones remain inoperable for the period of the power outage. Furthermore, these power outages and surges have destroyed expensive laboratory equipment in the past.

2.2 HUMAN RESOURCES AND TRAINING

Although ASEAN researchers tend to be familiar with mycological and analytical research methods involving aflatoxins and aflatoxigenic fungi, the majority of expertise related to other toxigenic fungi is generally restricted to those researchers who have a) completed graduate studies abroad; b) participated in international research collaborations in which a training component was included; or c) participated in international short-term training programs. Note that collaborative research associations, in which acquired knowledge is actually used during the course of the collaboration, tend to stimulate continued independent research in fungi and mycotoxins at a higher frequency than short-term training programs. There is a need to increase the general level of proficiency in mycological and analytical ability. As is the case with equipment, improved coordination of fungi and mycotoxin research activities would optimize the use of the human resource capability that does exist in the region.

Almost without exception, researchers at institutions visited in which screening activities are currently conducted indicated a keen interest in learning techniques to screen for mycotoxins other than aflatoxin.

Veterinary capabilities within private industry appears to far exceed those in the public sector. This deficiency may be due to limited mechanisms within institutional frameworks to correlate low levels of mycotoxins in feeds with symptoms in animals. Increased veterinary capabilities in the recognition of subacute symptoms associated with animal mycotoxicoses could provide direction for other fungi and mycotoxin researchers as to which mycotoxins are predominant and problematic in the region. Interest in pathology training was indicated.

One month training courses have been provided to approximately a dozen ASEAN scientists participating in collaborative research with the Commonwealth Scientific and Industrial Research Organization (CSIRO). These courses have included training in food mycology methods and/or identification of a wide range of food-borne fungi to species level or analysis of mycotoxins by TLC and HPLC techniques.

Training has also been provided to ASEAN researchers through collaborations with Japanese institutions.

See also Section 6.4.1 for information on the regular short-term mycotoxin training program offered by the National Resources Institute of the United Kingdom.

It is very common for scientists in some ASEAN countries to undertake research in more than one major area of study. The amount of time that a scientist is able to devote to fungi and mycotoxin research may, therefore, be limited. This is largely due to a shortage of highly educated research personnel. It is necessary not only to increase the level of capabilities but also the number of researchers with mycotoxin expertise.

2.3 INFORMATION DISSEMINATION AND COMMUNICATIONS

Scientists in Indonesia, the Philippines and, to a certain extent, Malaysia indicated difficulty in obtaining reference material. Most research institutes in these countries have small libraries which subscribe to nationally or regionally published journals specific to the institute's area of study. Accessibility to international journals and searchable databases is often restricted to central library facilities which may not be optimally utilized.

Many scientists are not aware of, or do not have access to, international conference proceedings, relevant newsletters, and information resources available to them. The cost of photocopying reference material that is available in libraries is a major barrier to information dissemination in Indonesia and the Philippines.

A study on publication and referencing patterns in a research institute in Indonesia showed that scientists frequently failed to cite relevant local literature and literature from international journals even when it was accessible. Indonesian researchers tend to publish in house journals rather than international journals because a) a smaller piece of work may be publishable, b) critical review and manuscript rejection are avoided, c) equal credit points are obtained for locally published papers or papers in international journals and d) it is difficult for non-native speakers to write in English. The study suggests that the problem can be circumvented if small units of research are published locally in the Indonesian language to obtain maximum credit points and maximum information transfer locally. Larger units of the same work in English could be submitted to an international journal for international visibility and more credit points.

The ASEAN Food Handling Bureau (AFHB) provides a Selective Dissemination of Information Service through the ASEAN Food Post-Production Information Exchange Project (APEX) Databases. These databases include bibliographic information on published and unpublished mycotoxin reports as well as contacts lists and mailing lists in given areas of interest. A modest annual subscription fee of US $25 entitles users to literature searches from any of the AFHB databases as well as hard copies of a limited number of reports. Many ASEAN researchers were not aware of this APEX Dissemination of Information Service although the need for such a database was universally recognized as imperative. Some scientists in Indonesia and the Philippines that were aware of the service felt the subscription fee to be burdensome and the number of hard copies that could be obtained free with the annual subscription to be insufficient.

A database is currently being developed at the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Australia from scientific data collected during the conduct of collaborative studies with institutions in Thailand, Indonesia and the Philippines. It will provide an authoritative picture of the occurrence of specific fungi in Asian foods and feeds. This will be an invaluable source of information for grain handlers, government bodies and public health authorities. Bibliographic information on fungi and mycotoxins, including abstracts, that is relevant to the ASEAN region has also been compiled in a database at the CSIRO.

Regional and national communication between fungi and mycotoxin researchers is inadequate in ASEAN. As a result, scientists remain unaware of national or regional expertise that may be available to enhance the pace and success of mycotoxin research. Research activities are often duplicated and standardization of research results is lacking. Given that funding for research is in very short supply, every effort should be made to avoid such inefficiencies. The compilation of a regional directory of mycotoxin scientists with a listing of their respective research activities may alleviate some of these problems. The need for national and regional bodies for coordination of fungi and mycotoxin research and dissemination of information is urgent.

International telecommunications with Indonesia and the Philippines are, to be conservative, tedious, and sometimes virtually impossible. In addition, because of foreign currency constraints, even in countries where telecommunications systems are functional, authorization to transmit an international facsimile must often be sought from high up officials. Thus, repeated international communication by facsimile is inappropriate in countries other than Singapore. This further accentuates the need for the establishment of national fungi and mycotoxin coordinating committees and a directory of researchers.

3. INSTITUTIONAL STRENGTHS

3.1 MALAYSIA

Crop production:
Principal crops in Malaysia are rice, pepper and cocoa. Two rice crops are harvested per year. The moisture content of rice may be as high as 32% if harvested during rainy season. Malaysia also produces copra. Although they have tried to grow maize and wheat, the per hectare yield was so low that efforts to produce these crops have been abandoned.

Animal production:
Malaysia is a net exporter of poultry products and has a limited livestock industry. Most meat is imported from Thailand. Raw maize is imported, mainly from Argentina and Thailand, for use as a feedstuff. Tapioca and soybean are also imported for feed. Groundnut is imported from Thailand, India and Indonesia.

Committees:
There is an informal Malaysian Mycotoxin Committee. A Working Group on Sampling Procedures for Mycotoxins was recently formed and is coordinated by the Standards and Industrial Research Institute of Malaysia (SIRIM).
The National Codex Committee proposed the formation of a National Committee on Research and Control of Mycotoxins in Malaysia in 1992.

Regulations:
The regulation for aflatoxin in 35 ppb. Codex Malaysia has been approached to reduce the aflatoxin regulation to 10 ppb, but it has indicated that more studies are necessary if this were to be implemented. Regulations for mycotoxins other than aflatoxin do not exist.

3.1.1 NATIONAL RICE AND PADDY BOARD

The National Rice and Paddy Board (Lembaga Padi dan Beras Negara) (LPN) conducts no research and has no analytical laboratories. Rather, it is responsible for the marketing, pricing, administration and milling of paddy and rice. Rice and paddy samples are submitted to the Chemistry Department of Malaysia or the Standards and Industrial Research Institute of Malaysia for analyses. Malaysia is represented on the AGPP Grains Working Group by a member from the Research and Planning Division of the National Rice and Paddy Board.

3.1.2 MINISTRY OF SCIENCE, TECHNOLOGY AND ENVIRONMENT CHEMISTRY DEPARTMENT OF MALAYSIA •HEALTH DIVISION •TOXICOLOGY DIVISION

Although it falls under the Ministry of Science, Technology and Environment, the Chemistry Department of Malaysia provides a food quality service to the Ministry of Health for the purpose of monitoring and enforcement. Samples are also submitted for analysis by the National Rice and Paddy Board.

HEALTH DIVISION:
A survey of aflatoxin by TLC and HPLC in groundnut and groundnut products, edible oils, mushrooms and canned meat was conducted at the Chemistry Department in the early 1980's. The Health Division continues to conduct aflatoxin analyses by TLC and HPLC on foods and processed foods such as peanut butter, maize flour, cakes, spices etc. and plans to learn methods for the analysis of other toxins in foods depending on directives from the Ministry of Health.

The Health Division participates in an AOAC crosscheck program for aflatoxin and occasionally trains university students in analytical methodologies.

TOXICOLOGY DIVISION:
The Toxicology Division is responsible for the conduct of aflatoxin analyses in feeds and human and animal tissues for toxicological purposes.

3.1.3 DEPARTMENT OF VETERINARY SERVICES •VETERINARY PUBLIC HEALTH LABORATORY

VETERINARY PUBLIC HEALTH LABORATORY:
The Veterinary Public Health Laboratory conducts routine screening of animal feeds and feed ingredients for aflatoxin as well as feed analyses in response to suspected aflatoxicoses.

Feed samples are screened for aflatoxins by TLC, positive samples are subsequently confirmed for aflatoxins B_1 and G_1 by HPLC with fluorescent detection. The laboratory typically analyzes 500-800 feed samples per year. The staff chemist is currently developing a method for determination of aflatoxins in animal fluids and tissues by HPLC.

This laboratory has never analyzed for mycotoxins other than aflatoxin but feels that this may be an important activity. Although the Veterinary Public Health Laboratory is not mandated to conduct research, it is responsible for substances that affect animal performance.

Neither equipment nor the cost of purchasing standard was identified as a constraint to the conduct of mycotoxin analyses. A lack of manpower, however, would be a barrier if analyses for other mycotoxins were undertaken.

3.1.4 STANDARDS AND INDUSTRIAL RESEARCH INSTITUTE OF MALAYSIA

The Standards and Industrial Research Institute of Malaysia (SIRIM) conducts aflatoxin analyses in rice as requested by the National Rice and Paddy Board. Other fungi and mycotoxin activities are unknown as this institute was not visited.

3.1.5 MALAYSIAN AGRICULTURAL RESEARCH AND DEVELOPMENT INSTITUTE •FOOD TECHNOLOGY DIVISION

The Malaysian Agricultural Research and Development Institute (MARDI) is responsible for research and development in 202 agricultural crops.

FOOD TECHNOLOGY DIVISION:
Researchers at the Food Technology Division have published several reviews and research reports on subjects including aflatoxin contamination in agricultural commodities, the status of mycotoxin research in Malaysia, the chemistry and biochemistry of aflatoxins, the dangers of mycotoxins in foods, the biosynthesis of aflatoxin and the biodegradation of aflatoxin.

Mycotoxin research activities at MARDI which relate to the Sixth Malaysia Plan (1991-1995) for development are at various stages of completion. They include the following projects:

- Evaluation of raw groundnut quality during storage - status: initial lab studies have been conducted on proper storage parameters for groundnut;
- Laboratory and pilot scale studies on maize storage parameters with regard to aflatoxin control - status: a storage system incorporating in-bin drying and aflatoxin detoxification facilities has been designed; tender for fabrication is in progress;
- Evaluation of mycotoxins in foods and agricultural products to provide information and guidelines for the purpose of setting mycotoxin standards in local food regulations;
- Screening of aflatoxin in foods and agricultural products - status: 42 samples of groundnut and maize have been evaluated for aflatoxin contamination;
- Study on analytical methods for other mycotoxins (patulin, ochratoxin, zearalenone) - status: not active.

The Food Technology Division conducted a survey of aflatoxin in grains and grain products, feedstuffs, groundnut and groundnut products, cocoa, spices, copra and rice from 1981-1984.

Researchers at the Food Safety Laboratory of the Food Technology Division continue to conduct routine screening of foods and agricultural products (groundnut cake, copra cake, maize, paddy, spices, pepper, cocoa beans) for aflatoxin by minicolumn or TLC. ELISA screening kits are sometimes available for this purpose. Rice samples periodically submitted by the National Rice and Paddy Board are tested for aflatoxin contamination. Other mycotoxins including patulin, ochratoxin and zearalenone have been tested for in the past by TLC but analyses are not conducted on a routine basis.

In addition to routine screening for aflatoxin, the Food Technology Division has undertaken a biological detoxification/control project for reducing/controlling aflatoxin contamination in copra and groundnut cake using Rhizopus oligosporus and R. oryzae.

Scientists in the Division identified the lack of manpower and an HPLC as significant constraints to mycotoxin analysis.

Apart from facilities for mycotoxin analyses, the Division also has comprehensive facilities to conduct microbiological studies but none are currently conducted.

The Food Technology Division indicated that any mycotoxin research undertaken at MARDI must comply with the priorities set forth in the Sixth Malaysia Plan and must be of practical use. Studies on storage parameters with the aim of reducing mycotoxin contamination in grain is perceived as imperative.

3.1.6 INSTITUTE OF MEDICAL RESEARCH
•DIVISION OF NUTRITION
•DIVISION OF EPIDEMIOLOGY
•DIVISION OF MICROBIOLOGY

Studies related to the occurrence of aflatoxin in local foodstuffs have been conducted intermittently at the Institute of Medical Research (IMR) since the 1960's. These studies were initially carried out by TLC in groundnut and groundnut products, soybean and soybean products, rice, maize, feed and other miscellaneous items. More recently, a mycotoxin laboratory, equipped with an HPLC, was established in the Division of Nutrition for the analysis of aflatoxin in foods and feedstuffs.

The Institute of Medical Research is mandated to conduct research and to provide training to various categories of staff involved in food, nutrition and health activities, as well as university students locally and internationally.

DIVISION OF NUTRITION:
In 1990, the Division of Nutrition and the Division of Microbiology at IMR undertook a two year study aimed at determining the frequency and levels of contamination by aflatoxin and aflatoxigenic fungi of a variety of commonly consumed foods (grains and flours, nuts, root crops, pulses and spices) from retail outlets in selected geographical locations in Peninsular Malaysia. In an associated study, food consumption patterns and exposure to aflatoxins and toxigenic molds in selected population groups was investigated. It is envisaged that findings of the project will facilitate a meaningful evaluation of the significance of aflatoxins as environmental carcinogens in Malaysia and provide input for the formulation of suitable control measures by the authorities. The project has been delayed due to a manpower shortage but will be resumed when a technician is recruited.

DIVISION OF EPIDEMIOLOGY:
The Division of Epidemiology is coordinating a study with the Drug Research Centre at the University Sains Malaysia and Ipoh General Hospital (see also Section 3.1.9). The objectives of the study are to determine baseline levels of aflatoxin in normal children as well as to correlate levels of aflatoxin in the tissues of sufferers of hepatic encephalopathy. Blood and urine analyses are conducted at the Drug Research Centre and clinical data is provided by Ipoh General Hospital.

DIVISION OF MICROBIOLOGY:
The Division of Microbiology is responsible for mycological isolations associated with the studies at the Division of Nutrition. A small culture collection is maintained which acts as a reference centre for microorganisms, including fungi, of medical importance.

3.1.7 •UNIVERSITY PERTANIAN MALAYSIA
FACULTY OF FOOD SCIENCES AND BIOTECHNOLOGY
•DEPARTMENT OF FOOD SCIENCE
FACULTY OF SCIENCE AND ENVIRONMENTAL STUDIES
•DEPARTMENT OF BIOLOGY
•DEPARTMENT OF CHEMISTRY
FACULTY OF AGRICULTURE
•DEPARTMENT OF PLANT PROTECTION
•FACULTY OF VETERINARY MEDICINE AND ANIMAL SCIENCES

UNIVERSITY PERTANIAN MALAYSIA (UPM):
The Deputy Vice-Chancellor of Academic Affairs indicated a keen interest in undertaking mycotoxin research coordinated between several UPM Departments.

DEPARTMENT OF FOOD SCIENCE:
A considerable amount of research has been conducted by a Malaysian food microbiologist from the Department of Food Science while doing graduate studies at the University of Wisconsin-Madison. These studies showed the effects of organic acids, salts and pH on the growth and aflatoxigenicity of fungi in culture.

Mycotoxin work has not been pursued at the Department of Food Science due to a lack of laboratory equipment, safety equipment and trained personnel. An interest in surveying for other mycotoxins and fungi in foods was indicated.

DEPARTMENT OF BIOLOGY:
A mycologist/plant pathologist in the Department of Biology formerly conducted studies on Fusarium oxysporum as a plant pathogen causing vascular wilt in oil palm. This work was discontinued in 1988.

Another scientist with the Department of Biology is currently conducting studies on antifungal metabolites produced by Trichoderma for the control of pathogenic fungi. A small culture collection consisting mostly of Penicillium, Aspergillus and Trichoderma is maintained.

DEPARTMENT OF CHEMISTRY:
Although no mycotoxin work is currently conducted at the Department of Chemistry, related activities include antifungal assays as routine checks for biological activity of plant extracts and natural products as potential biological insecticides. The Department is well equipped with modern facilities and instrumentation including NMR, UV/VIS, IR and fluorescence spectrophotometers as well as chromatographic equipment including GC, HPLC and GCMS. In addition, the Department has access to specialized facilities through cooperative arrangements with other research institutions and UPM Departments.

UPM has been earmarked as a possible centre of excellence for a regional centre for natural products chemistry, environmental studies and materials science.

DEPARTMENT OF PLANT PROTECTION:
Research interests at the Department of Plant Protection include studies on the seed-borne infection of Colleotrichum truncatum in soybean and its control, and seed-borne diseases of rice and Penicillium chrysogenum on yams.

DEPARTMENT OF VETERINARY MEDICINE AND ANIMAL SCIENCE:
A researcher at the Department of Animal Sciences who is a member of the ASEAN Food Handling Project Grains Working Group, has undertaken studies on the toxicity of signal grass in sheep.

3.1.8 UNIVERSITY OF MALAYA
•DEPARTMENT OF GENETICS AND CELLULAR BIOLOGY

DEPARTMENT OF GENETICS AND CELLULAR BIOLOGY:
Very little work on mycotoxins has been undertaken at the University of Malaya since 1980; however, a researcher at the Department of Genetics and Cellular Biology contributed a paper entitled 'Overview of Mycotoxins in the Asian Region' at the First Asian Conference on Food Safety, 1990, Kuala Lumpur.

3.1.9 UNIVERSITY SAINS MALAYSIA
•DRUG RESEARCH CENTRE
•SCHOOL OF BIOLOGICAL SCIENCES
•SCHOOL OF CHEMICAL SCIENCES

DRUG RESEARCH CENTRE:
The Drug Research Centre has developed an HPLC procedure for identification and quantitation of aflatoxins in human and animal tissues and fluids. A mass spectrometer is also available. Blood and urine specimens have been collected to determine types and levels of aflatoxins found in tissues and to correlate clinical, pathological and biochemical and histopathological parameters with types and levels of aflatoxins found. It will provide useful baseline levels of aflatoxin and will help to determine the extent to which the population is exposed to aflatoxin. This research is ongoing and funded by the government of Malaysia. Scientists at the Drug Research Centre work collaboratively with the Institute of Medical Research in Kuala Lumpur (see also Section 3.1.6).

SCHOOL OF BIOLOGICAL SCIENCES AND SCHOOL OF CHEMICAL SCIENCES:
A considerable amount of expertise in Fusaria exists at the School of Biological Sciences and the School of Chemical Sciences. Past mycotoxin activities have included studies of Fusaria associated with soil and naturally diseased plants, toxigenicity of animal and plant pathogenic Fusaria, surveys of feeds for toxigenic fungi and their related mycotoxins and toxicity of crude extracts of Fusaria rice cultures to chicks.

Facilities at the School of Biological Sciences include glasshouses, experimental plots and all necessary equipment for the analysis of mycotoxins (ie. TLC, GC, HPLC). GCMS and NMR are available at the School of Chemical Sciences.

A mycologist/plant pathologist at the School of Biological Sciences is familiar with Fusarium taxonomy and maintains a significant culture collection of 2,500 strains, mostly Fusarium.

3.1.10 ASEAN FOOD HANDLING BUREAU

The ASEAN Food Handling Bureau (AFHB) was established to provide administrative and technical support to the ASEAN Food Handling Projet initiated by the ASEAN-Australian Economic Cooperation Program. This is done through various consultancy and project monitoring services, and a Technical Information Service, namely, the ASEAN Food Post-Production Information Exchange Project (APEX) Selective Dissemination of Information service (see Section 2.3).

3.2 SINGAPORE

Crop production:
Singapore is a trading nation that imports all grain commodities for local consumption as well as for re-export. The major marketable grains in Singapore include rice, maize, soybeans, wheat and barley. 95% of rice imports originate from ASEAN.

Singapore currently exercises a stockpile policy in which grain is stored in warehouses for approximately three months for the purposes of food security. In certain high risk commodities, aflatoxin in systematically tested for at the port. Because the turnover of stored commodities is relatively rapid, aflatoxin contamination during storage is not expected to be a problem.

Animal production:
Singapore has a small poultry production industry and a small aquaculture industry. Beef and lamb are imported from Australia, New Zealand and the United States. Pork is imported from a small Indonesian island located close to Singapore where animals are raised specifically for this purpose.

Committees:
The ASEAN Grains Working Group Member is from the Ministry of Trade and Industry. There is no other mycotoxin coordinating committee.

Regulations:
There is a zero tolerance for mycotoxins in Singaporean foods.

3.2.1 MINISTRY OF THE ENVIRONMENT
•FOOD CONTROL DEPARTMENT

FOOD CONTROL DEPARTMENT:
The Food Control Department of the Ministry of Environment is the body responsible for regulating mycotoxin contaminants in foods while laboratory analyses are conducted at the Institute of Science and Forensic Medicine under the Ministry of Health. 100% of the consignments of certain prescribed high risk commodities are sampled at the port and tested for aflatoxin. These include groundnut, maize, sunflower and wheat. Other commodities and processed foods are randomly sampled at the consumer end.

The Ministry of Environment intends to subscribe to several on-line information services including the US FDA database, Dialog and US News Bulletin in order to obtain up-to-date postharvest information. It is hoped that this information system will contribute to a forecasting system that canbe used by government and traders to estimate the availability and quality of commodities and may include information on mycotoxins. Access to postharvest information was identified as a constraint to an efficient food and feed grain industry at the ASEAN Grains Postharvest Program Workshop in Singapore in February, 1993.

3.2.2 MINISTRY OF HEALTH
INSTITUTE OF SCIENCE AND FORENSIC MEDICINE
•FOOD LABORATORY

FOOD LABORATORY:
The Food Laboratory is responsible for laboratory monitoring of aflatoxin levels in foods for the purposes of enforcement under the Food Control Department of the Ministry of Environment. In addition, the Food Laboratory provides technical advice to the Food Control Department and provides certification of food for export.

Before 1989, the number of food items analyzed for aflatoxin was limited to approximately five aflatoxin analyses per month. However, since then, the Food Laboratory has adopted immunoaffinity columns for aflatoxin analysis and has been able to increase its capacity to 40 food samples per month. From November 1989 to November 1992, 1434 food samples have been analyzed for aflatoxin B_1,including peanuts and peanut products, cereals and cereal products, coffee, tea, seeds and legumes. In positive samples, aflatoxin levels are subsequently quantitatively determined by HPLC with fluorescence detection. Their presence is confirmed by pre-column derivitization with trifluoroacetic acid. Milk and milk products are also screened for aflatoxin M_1. Results from the last several years indicate that aflatoxin contamination in foodstuffs is not a serious problem in Singapore.

One of the current research projects at the Food Laboratory is the role of aflatoxins in the aetiology of hepatocellular carcinoma in Singapore. Studies to date have shown that aflatoxin intakes in the Singaporean population are very low.

Equipment and facilities at Institute of Science and Forensic Medicine (ISFM) are superior and include excellent general laboratory equipment, HPLCs, GC/MS, scanning electron microscope, GC, atomic absorption spectrometry and FTIR. The ISFM analyzes many different food contaminants (colour, preservatives, heavy metals, composition analysis etc.) and additives and thus has a requirement for sophisticated equipment and has acquired a large repertoire of analytical expertise.

The Food Laboratory is a designated WHO Collaborative Centre for Food Contaminants Monitoring and mycotoxins are included under the monitoring program as a joint project with UNEP/FAO/WHO on Analytical Quality Assurance.

Technicians from Macau, Brunei and Thailand have been trained in aflatoxin detection at this laboratory as part of the WHO program.

Under the direction of The Food Control Department, the Food Laboratory will soon be undertaking analyses for ochratoxin A. An interest in initiating screening activities for other mycotoxins was indicated by the Food Laboratory. Due to a shortage of manpower, it would be preferable if simple screening kits were available for routine monitoring. However, for the purposes of prosecution, AOAC methods would have to be employed and training for such obtained by Food Laboratory technicians.

3.2.3 MINISTRY OF NATIONAL DEVELOPMENT
•DEPARTMENT OF PRIMARY PRODUCTION

DEPARTMENT OF PRIMARY PRODUCTION:
The Primary Production Department is responsible for testing mycotoxins in feeds, however, for practical purposes, most testing is done at the Food Laboratory of the Ministry of Health (see Section 3.2.2).

3.2.4 NATIONAL UNIVERSITY OF SINGAPORE
FACULTY OF SCIENCE
•BOTANY DEPARTMENT
•ZOOLOGY DEPARTMENT
FACULTY OF MEDICINE
•DEPARTMENT OF MICROBIOLOGY

BOTANY DEPARTMENT:
The recently retired former Head of the Botany Department conducted surveys on the mycoflora of rice and groundnut for many years. 2500 pure cultures of these fungi have been isolated and are maintained on slants in the Departments culture collection. No information on their toxigenicity is available.

The current Head of the Botany Department is a mycologist. Although his interests focus on aquatic and degradative fungi, he has undergone training for mycotoxin analysis in Japan. No mycotoxin research or work with mycotoxigenic fungi is currently being conducted in the Department. The capability to conduct mycological screening, isolation and maintenance exists, however interests lie in the chemical aspects of mycotoxin research (ie. isolation and identification of toxins). Mechanisms are in place to conduct collaborative inter-, intra-departmental and international research.

Facilities in the Botany Department include excellent general laboratory equipment, HPLC, GC, access to an NMR (through the Chemistry Department), a fluorescent microscope and a freeze drier with ampoule attachment. Significant expertise exists in the Department of Botany in the areas of biochemistry and molecular biology. Equipment needs were not identified as a constraint to mycotoxin research at the National University of Singapore, however, manpower capability would have to be increased.

ZOOLOGY DEPARTMENT:
A few years ago, the Zoology Department, jointly with the Singapore Storage and Warehouse Pte Ltd., undertook a research project to study problems associated with grain storage and handling in Singapore. From these studies several problems were identified including mycotoxin contamination. Preliminary results show that at a particular time of the year imported maize has a high moisture content. This corresponds to the rainy season in exporting countries.

DEPARTMENT OF MICROBIOLOGY:
Knowledge of aflatoxin screening exists at the Department of Microbiology through a researcher who was formerly involved in routine screening for aflatoxin in groundnut and dried shrimp at the Department of Primary Production. She is no longer involved in mycotoxin research.

A collaborative study with the Institute of Molecular and Cellular Biology and the Institute for Community and Occupational Family Medicine was initiated last year by the Department of Microbiology on aflatoxin and liver cancer development in rats and humans. The hypothesis is that aflatoxin induces a point mutation in the RAS oncogene which leads to primary liver cancer. Studies are being conducted to induce liver cancer in rats. Publications are not yet available.

Laboratory facilities at the Department are now shared with other Departments. Researchers indicated that if efforts in mycotoxin research were to be increased, equipment would have to be procured and up-to-date laboratory techniques would have to be learned and adopted.

Scientists at the Department of Microbiology saw a need to conduct base line studies on mycotoxin exposure in humans. This would involve mycological and toxin screening in foods in order to determine on which mycotoxins it would be most important to focus. This information could then be used to investigate links between mycotoxin levels in foods, exposure to humans and potential health implications.

Fragmentation in fungi and mycotoxin research was recognized. A need for a coordinating committee was expressed.

3.2.5 SIN HENG CHAN (SINGAPORE) PTE. LTD

This company has four feedmills in Malaysia with the main office and laboratory in Singapore.

Each of the four feedmills in Malaysia conduct on-site routine screening for aflatoxin in raw maize, groundnut and soybean using commercially available immunocolumn kits. The laboratory in Singapore has an HPLC for quantitative aflatoxin determinations in positive samples. The company does not screen for mycotoxins other than aflatoxin.

3.3 INDONESIA

Crop production:
The government of Indonesia, through its five-year development plan policy (REPELITA I-IV, 1969-1988), has placed the highest priority on achieving self sufficiency in staple food crops. Agriculture (ie. self sufficiency, widened product diversification and accelerated growth of exports) will continue to be the foundation in Indonesia's second 25-year development plan. In the case of rice, self-sufficiency was attained in 1985, however, in recent years, the rate of increase in rice production has been less than the population growth. With a limited arable land and a slow rate of increase in rice production, it is essential and crucial to reduce postharvest losses. Several government officials indicated that the political climate is now suitable to initiate research focusing on increasing grain quality.

Secondary crops in Indonesia, often referred to as 'palawija', include maize, groundnut, soybean and cassava. Maize is imported in some years and exported in others depending on annual yield. Wheat is imported from Canada, Brazil, Australia and Saudi Arabia.

In the past, raw feed materials were imported, but in recent years Indonesia has been able to produce most of its domestic grain requirements for feed. Copra, manioc (pelletized, dried cassava) and sometimes groundnut are exported. Although soybean is produced in Indonesia, some is still imported.

Animal production:
Indonesia has substantial poultry and beef cattle industries, however, both are still imported. The pork production industry is limited except on islands where it is produced specifically for export or on non-Moslem islands for local consumption.

Committees:
The Working Group on Food Crops Post Harvest is chaired by the Head of the Directorate of Food Crop Economics. This Working Group acts as a clearing house for results of postharvest research, and is involved in monitoring and evaluating postharvest activities conducted at the various institutions.

Regulations:
No regulations exist for aflatoxin or other mycotoxins in Indonesia.

3.3.1 BADAN URUSAN LOGISTIC (NATIONAL LOGISTICS AGENCY)

Badan Urusan Logistic (BULOG) is the agency responsible for stabilizing food prices and maintaining national stocks of rice, soybean, soybean meal, wheat, sugar and raw feed materials. In the past BULOG was also responsible for maize but this has since been privatized. The Centre for Research and Development within BULOG acts as the executing agency for donor funded research.

In 1984, BULOG, in collaboration with the National Resources Institute (NRI) of the United Kingdom, conducted surveys in North Sulawesi, South Sulawesi, East Java and Jakarta to determine the degree of aflatoxin contamination in cereal maize, and at what stage of postharvest processing it occurs. The survey showed that aflatoxin levels increased after harvest.

High moisture content, high incidence of mould growth and heating of grain were factors involved in the postharvest yellowing of rice as identified in a collaborative study between NRI and BULOG. A further study investigated aflatoxin contamination in rice.

For the last three years, research has been conducted with the Commonwealth Scientific and Industrial Research Organization in Australia to study aflatoxin contamination in maize and soybean and to investigate fumigation during storage.

BULOG is planning a three year research collaboration with the Southeast Asian Regional Centre for Tropical Biology (BIOTROP) to study the impact of packaging on quality of grain and grain products. This study, funded by the EEC, will have a small mycotoxin component.

The BULOG laboratory uses the Bright Green Yellow Fluorescence test (BGYF) as a presumptive test for aflatoxin since no HPLC or GC are available. Scientists recognize the need to commence systematic screening of agricultural products for aflatoxin in response to the EEC consideration to decrease tolerance levels for aflatoxin to two ppb. The need for laboratory equipment was indicated.

The Indonesian mycologist involved in past mycotoxin research is no longer working at BULOG, however, an entomologist is active in some aspects of grain storage as related to mycotoxins. Scientists

felt it crucial to increase both the number of mycotoxin researchers and the technical knowledge of existing researchers through graduate studies, programs abroad, and short-term training courses, respectively.

Because BULOG does not share the same administrative system as the rest of the Government of Indonesia, the executing and partial funding of collaborative research by BULOG is facilitated. This avoids delays in release of research funds that is sometimes a problem in other government departments.

3.3.2 MINISTRY OF HEALTH
•NUTRITION RESEARCH AND DEVELOPMENT CENTRE

NUTRITION RESEARCH AND DEVELOPMENT CENTRE:
In 1969, a study in collaboration with the Nutrition Research and Development Centre (NRDC) was initiated by medical physician who observed a high incidence of liver cancer in his patient load. An investigation of levels of aflatoxin B_1 in food, urine, liver showed a strong indication for a positive correlation between aflatoxin levels in food and the incidence of liver cancer in humans. Aflatoxin B_1, G_1 and M_1 determinations were done by TLC and UV/visible spectrophotometry, thus, aflatoxins in tissues were reported as 'detectable'.

Other research conducted at NRDC include studies of aflatoxins in groundnut and groundnut products at different times between harvest and consumption; surveys of aflatoxins in candle nut, peanut, maize, soybean and soybean products (tempe and fermented peanut presscake), rice and green gram; the effect of length of storage on aflatoxin contamination of cassava; and the effect of drying, storage procedures and cultural practices on aflatoxin levels in peanuts in rural and urban communities. Equipment for these studies was provided by UNICEF.

No research on mycotoxins has been conducted at NRDC for the last five years.

The laboratory at NRDC is furnished with TLC equipment, UV/visible spectrophotometer, microscopes and HPLC.

3.3.3 INDONESIAN INSTITUTE OF SCIENCES
•NATIONAL INSTITUTE FOR CHEMISTRY

NATIONAL INSTITUTE FOR CHEMISTRY:
Research activities at the National Institute for Chemistry in the mid 1980's included studies on the reduction of aflatoxin B_1 at various stages in the fermentation of peanut presscake, and a survey of aflatoxin contamination in market samples of tempe. The method used for analysis of tempe was TLC with confirmation by HPLC.

3.3.4 MINISTRY OF TRADE
•CENTRE FOR TESTING AND QUALITY CONTROL

CENTRE FOR TESTING AND QUALITY CONTROL:
Commodities for export are tested for aflatoxin to ensure compliance with importing country regulations at the Centre for Testing and Quality Control in Caracas, just outside of Jakarta. Commodities analyzed include raw maize, coconut and rice. TLC is used as an initial screening with subsequent confirmation by HPLC. The development and harmonization of sampling methods, and analytical techniques were identified as areas requiring further research.

3.3.5 MINISTRY OF AGRICULTURE
AGENCY FOR AGRICULTURAL RESEARCH AND DEVELOPMENT
CENTRAL RESEARCH INSTITUTE FOR FOOD CROPS
•BOGOR RESEARCH INSTITUTE FOR FOOD CROPS
CENTRAL RESEARCH INSTITUTE FOR ANIMAL SCIENCES
•BOGOR RESEARCH INSTITUTE FOR VETERINARY SCIENCES

BOGOR RESEARCH INSTITUTE FOR FOOD CROPS:
The Bogor Research Institute for Food Crops (BORIF) is one of six research institutes for foods crops in Indonesia under the umbrella of the Central Research Institute for Food Crops (CRIFC) the latter institute, in turn falls under the direction of the Agency for Agricultural Research and Development (AARD).

BORIF has the responsibility of pioneering research and commodity analysis on rice, cereals, grain legumes and root crops. This is in contrast to the other research institutes under CRIFC which are mandated to conduct research only on commodities grown in specific agroecological zones (eg. highland wet crop production, dryland crop production etc.).

BORIF is involved in the study of ecological factors and field resistance to mycotoxigenic fungi. Relevant research conducted at the Plant Breeding Division in collaboration with the Plant Diseases Division of BORIF includes:

- breeding for resistance of peanut to Aspergillus parasiticus using germplasm obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT);
- the effectiveness of fungicides in controlling Fusarium head blight in wheat;
- natural resistance of maize cultivars to Fusarium ear rot;
- natural resistance of maize cultivars to infection by Aspergillus flavus;
- field screening for natural resistance to Fusarium in maize;
- influence of planting dates and meteorological factors on leaf blight and purple ear rot incidence and on the yield of maize;
- breeding for ear rot resistance in maize was initiated two years ago.

The staff mycologist at BORIF indicated that significant field infestation by Fusarium and Aspergillus occurs in maize during rainy season. Fusarium graminearum occurs in highland maize, whereas Fusarium moniliforme and Aspergillus flavus are prevalent in lowland maize.

Given the high incidence of Fusarium infestation in sorghum during rainy season, scientists at BORIF would like to initiate resistance breeding in sorghum.

A new JICA, USAID funded biotechnology facility with modern equipment, including growth rooms, tissue culture rooms, greenhouses, molecular biology laboratory with polymerase chain reaction equipment as well as common laboratory equipment (eg. HPLC, GC, laminar flow hoods), is associated with BORIF. Experimental plots are available in BORIF and in the other food crops research institutes under CRIFC throughout Indonesia.

In a paper presented at an ICRISAT Workshop in 1987, BORIF researchers identified the following as important prospects for future research on mycotoxins in groundnut:

- surveys on the distribution and incidence in groundnut seed of the fungi involved in preharvest aflatoxin contamination;
- studies on the relationship among the fungi, the groundnut hosts, and environmental factors in

relations to aflatoxin production;
- further studies on the effect of aflatoxin on man and animals;
- studies on practical methods that could be adopted to reduce aflatoxin production in groundnuts in the field and during storage.

BORIF currently has international research links with IDRC through the Soybean Project, IRRI, and ACIAR on field rhizobia. An agreement through the Ministry of Agriculture allows BORIF to conduct collaborative research with the universities.

BORIF was identified to act as one of the agronomic teams to participate in the 1992 Draft Proposal - Investigation and alleviation of the effect of aflatoxins on food safety, quality and resultant health economics in Indonesia (see Section 6.5.2).

BOGOR RESEARCH INSTITUTE FOR VETERINARY SCIENCES (BALITVET):
A Canadian veterinarian was responsible for the initial development of the Toxicology Department at BALITVET, and was very active in introducing and encouraging mycotoxin research, including mycotoxins other than aflatoxin. BALITVET has continued commitment to mycotoxin research and still has one of the broadest technical capabilities for mycotoxin analysis and collection of standards in the ASEAN region.

Mycotoxin research activities at BALITVET are directed towards examining the significance of mycotoxins on animal health and production. To this end, determinations of mycotoxins in feeds, especially chicken feeds, and feeding trials on domestic animals fed mycotoxin contaminated grain, are carried out.

In the past, BALITVET scientists have conducted determinations on the following mycotoxins in feeds, sometimes in response to reports of suspected mycotoxicoses: aflatoxin, zearalenone, ochratoxin A, cyclopiazonic acid, deoxynivalenol, nivalenol T-2 and moniliformin. With the exception of T-2 and moniliformin, BALITVET chemists have the capability to determine most of the above mycotoxin both by TLC and by HPLC. In addition, liver and serum samples have been tested for aflatoxin at the BALITVET laboratory.

BALITVET is one of three institutions in Indonesia which participates in a collaborative project with the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Australia to investigate the mycological status of Indonesian grains with respect to both the fungal flora and mycotoxins (see also Sections 3.3.8 and 3.3.10). In this regard, BALITVET contributes towards the mycotoxin analysis component of the project.

BALITVET participates in sample crosscheck for ochratoxin A and aflatoxins B_1, B_2, G_1, G_2 and M_1.

Comparative studies of levels of mycotoxins in grains and feeds in different seasons and altitudes have been conducted. Chemists at BALITVET indicated that zearalenone and deoxynivalenol were relatively common in maize grown in high altitude areas, whereas zearalenone, moniliformin and T-2 toxin are relatively common in maize grown in low altitudes. This year, the Mycology Department will collaborate with the Toxicology Department to investigate the presence of Aspergillus and Penicillium species in agricultural wastes (eg. rice straw, bran, maize stems, etc.) used as animal feeds. Mycotoxins will also be determined.

Feeding trials conducted at BALITVET include acute experimentally induced aflatoxicosis in ducks and chickens, and deoxynivalenol feeding trials in goats. Cultures extracts are used in these experiments since pure toxins are too expensive and difficult to obtain.

The use of activated charcoal and aluminosilicate have been studied for their efficacy in preventing aflatoxicosis in ducks.

Scientists at BALITVET are able to examine pathological and histopathological effects of mycotoxins on animals. An experimental pathologist is interested in starting work on pharmacology and kinetics of mycotoxins in animals, however, it was suggested that some training in histopathology would be useful if these studies were undertaken.

Other areas identified in which it would be useful to obtain training are sampling techniques, toxin isolation, and familiarization with ELISA kits.

BALITVET is one of the institutions identified in a proposal for the improvement of post harvest handling of palawija crops to reduce aflatoxin content (see Section 6.5.6).

The Director of BALITVET has himself, been involved in mycotoxin studies. The Head of the Toxicology Division has an M.Sc. in mycology from an Australian university, but is now working on toxicology. Two chemists in the division have graduate degrees (one M.Sc. and one Ph.D.), one of whom has undergone training in Australia for mycotoxin analysis by HPLC. The chemist at BALITVET responsible for mycotoxin analyses is particularly diligent.

Laboratory equipment at BALITVET is adequate to conduct mycotoxin analyses and includes HPLC, GC, TLC, spectrophotometer and rotary evaporator. Some analytical equipment was in disrepair due to the lack of maintenance contracts. Animal housing facilities for large domestic animals are available at BALITVET.

It is within the mandate of BALITVET to train university and other public sector employees in laboratory techniques.

The Mycology Division houses a small culture collection of approximately 60 isolates of fungi, mostly freeze dried. Expertise in the identification of mycotoxigenic fungi is limited.

3.3.6 MINISTRY OF AGRICULTURE
DIRECTORATE GENERAL OF LIVESTOCK SERVICES
•ANIMAL DISEASE INVESTIGATION CENTRE

ANIMAL DISEASE INVESTIGATION CENTRE:
Under the Directorate General of Livestock Services seven Animal Disease Investigation Centres (DIC) have been established in different provinces in Indonesia. The most advanced of these is strategically located in Java (the island having the highest animal population) near Gadjah Mada University. This Centre is a service laboratory providing:

- diagnostic services and disease control advice to farmers;
- disease investigation services;
- applied research;
- animal health examinations;
- training for animal health laboratory staff and workers;
- disease mapping;
- active field service;
- vaccine monitoring;
- disease surveillance;
- veterinary drug potency tests;
- veterinary public health assistance.

The Centre has close professional relations with faculties of veterinary medicine and animal science at universities in Yogyakarta, and other cities in Java, and maintains frequent contact with other institutes in the Directorate General of Livestock Services network, including the Animal Disease Research Institute and the Livestock Research Institute.

Over the period 1984-1989 the facility, equipment and library were significantly upgraded with funding provided by the Canadian Government. Canadian contributions also included veterinary consultancies and in-country and overseas training at the University of Guelph and the University of Saskatchewan. In 1989, funding was extended to the Centre for a further three years to allow for a more comprehensive program of human resource development and institutional strengthening. Canadian consultancies, including two veterinarians and one administrator, terminated at the end of March, 1993.

Veterinarians at the DIC reported frequent suspected toxicoses, particularly in ducks and rabbits. Although feed samples were submitted, the Centre has no capacity to analyze for mycotoxins. The technician in the Bacteriology/Mycology Section reported frequent isolation of Aspergillus flavus from feed and tissue samples submitted by farmers. This technician has undergone some training in Canada. Herbal medicines administered to sick animals are an additional suspected source of mycotoxin exposure in animals.

Research on field outbreaks in animals could be correlated to levels of mycotoxins in feeds in countries that are self sufficient in major feed components. This is a system for "fast-tracking" the identification of prevalent fungi and mycotoxins that cause actual field problems in the region. Such a project would involve the identification of field outbreaks or chronic exposure symptoms by an institution with a service function (eg. DIC), and coordinated with the analysis of feed and tissue samples by an institution with facilities and the human resource capability to do so (eg. BALITVET). A mechanism for coordination of veterinary research activities by BALITVET (under the Agency for Agricultural Research and Development) and the DIC (under the Directorate General of Livestock Services) was established through the National Research Coordinating Committee for Veterinary Sciences (NRCC). The coordinator of the NRCC is the director of BALITVET.

3.3.7 BOGOR AGRICULTURAL UNIVERSITY
FACULTY OF AGRICULTURAL TECHNOLOGY

- **DEPARTMENT OF FOOD TECHNOLOGY AND HUMAN NUTRITION**
  LABORATORIUM TERPADU (INTEGRATED LABORATORY)
- **MICROBIOLOGY DIVISION**
- **FOOD TECHNOLOGY DEVELOPMENT CENTRE**

DEPARTMENT OF FOOD TECHNOLOGY AND HUMAN NUTRITION:
Mycotoxin research at the Department of Food Technology and Human Nutrition (FTHN) is confined to aflatoxins. The effect of processing on aflatoxin production in peanuts and peanut products has been studied. The Department of Agricultural Engineering is also involved in this research sometimes. In addition, the FTHN is presently studying the degradation of aflatoxin by Rhizopus oligosporus in fermented products. Some work has been conducted on aflatoxin in 'jamu', a popular Indonesian herbal medicine tonic.

A food chemist at the FTHN indicated that most mycotoxins can be analyzed, however, experience is limited to aflatoxin. Research on mycotoxins other than aflatoxin is limited by lack of funding.
In addition to good general laboratory and analytical equipment (safety hood, freeze drier, HPLC with autosampler, GC, UV/visible spectrophotometer, densitometer, laminar flow hood), FTHN has a laboratory animal testing facility. All faculties at Bogor Agricultural University have the use of the Inter University Centre (IUC) for Food and Nutrition which includes a pilot plant for food processing.

Obtaining of standard was identified as an obstacle to mycotoxin research since it is expensive and difficulties are often presented in clearing customs.

Concern was expressed that if training in fungi and mycotoxins were provided to graduate students that expertise would be lost to the Department when the student graduated. Therefore, training of a staff member was regarded as a preferable alternative.

MICROBIOLOGY DIVISION:
The Microbiology Division of the Integrated Laboratory does not currently participate in fungal and mycotoxin research but maintains a small culture collection of bacteria and fungi important in agricultural biotechnology. The Division is involved in a proposal for the development of a central facility for an Indonesian culture collection of microorganisms for agricultural biotechnology. This would include a training component in the isolation and identification of mycotoxigenic fungi (see Sections and 4.3 and 6.5.3).

FOOD TECHNOLOGY DEVELOPMENT CENTRE:
The Food Technology Development Centre (FTDC) of Bogor Agricultural University is set apart from the faculties in that it has a research mandate rather than a teaching mandate. The research and development functions of the FTDC cover, among other things, grain storage and infestation control at the village level, and quality control and assurances.

The FTDC, financed by and in collaboration with the Dutch, undertook the Street Foods Project (1988-1992) which with the aim to improve quality sought to investigate the safety of street foods in West Java. Among the components studied was the microbial content and aflatoxin levels in street foods by TLC. No other mycotoxins were investigated.

One of the researchers at FTDC has conducted aflatoxin surveys in peanuts, soybean, green gram, gado gado, sate sauce, and maize, and was trained in the Netherlands at TNO to do analyses for zearalenone, ochratoxin and islanditoxin. Analyses for these toxins are not currently conducted at FTDC. The most suitable method for the extraction and detection of zearalenone in maize with subsequent determination by GC or HPLC, was investigated by an FTDC researcher.

An entomologist/microbiologist is currently conducting studies on aflatoxin to determine if there is a difference between aflatoxin levels in groundnut harvested after the rainy season and those harvested after the dry season. Variations in aflatoxin levels in peanuts from small traders as compared to major traders is also being investigated.

The Director of the FTDC did his Ph.D. on the storage of rice. One of the modules of his thesis was a study of the microorganisms and aflatoxin in rice. Research on the effect of hydrothermic parameters on the production of aflatoxin B_1 in stored rice was also conducted. Aflatoxin was determined by HPLC. A general textbook entitled "Mycotoxins in Foodstuffs" has been written in Bahasa Indonesia (Indonesian language) by the FTDC Director. Chapters include:

- General Introduction;
- Fungi in Foodstuffs (biological aspects, ecology, control);
- General Review of Mycotoxins (source of contamination, control, regulations etc.);
- Aflatoxin (fungus, structure and characteristics of toxins, detoxification, occurrence, analytical methods);
- 19 other chapters on fungi, characteristics, toxicity, analysis and detoxification of the following mycotoxins: sterigmatocystin, ochratoxin, ergot, trichothecenes, zearalenone, rubratoxin, islanditoxin, citrinin, sporidesmin, cyclopiazonic acid, Phoma toxins, tremorgenic toxins, slafranin, penicillic acid, patulin, Stachybotrys toxins, sweet potato toxins, Alternaria toxins, Penicillium roqueforti toxins.

Because the book would be for limited distribution (approximately 250 copies), the author has been unable to find a publisher for the manuscript. Because of the language similarity, it was indicated that even if published in Bahasa Indonesia, the book would be useful to researchers in Malaysia and Brunei.

Researchers at FTDC felt that studies on mycological infestation, and contamination by mycotoxins other than aflatoxin in maize and rice, would be useful. A description of FTDC research interests is presented in Section 6.5.4.

Equipment available for fungi and mycotoxin research includes adequate general laboratory equipment, HPLC, GC/MS, UV spectrophotometer, NMR, GC, laminar flow hood, safety hood, UV chamber and incubators.

3.3.8 GADJAH MADA UNIVERSITY
FACULTY OF AGRICULTURE
•DEPARTMENT OF PLANT PESTS AND DISEASES
•FOOD AND NUTRITION DEVELOPMENT AND RESEARCH CENTRE
FACULTY OF AGRICULTURAL TECHNOLOGY
•LABORATORY OF FOOD AND NUTRITION

DEPARTMENT OF PLANT PESTS AND DISEASES:
In the early 1970's this department was involved in research on the mycotoxic effects of yellow rice in pigeons and poultry. Feeding studies on mycotoxicoses in mice and pigeons fed yellow rice were conducted by a graduate student at the Department two years ago.

A monoclonal antibody system as a rapid screening method for aflatoxins in grain is currently being developed.

In the future the Department will conduct research on mycotoxins in herbal medicines.

Researchers indicated a difficulty in obtaining chemicals with limited shelf lives due to the prolonged shipping periods. Stringent regulations at customs present a problem in obtaining mycotoxin standards.

Researchers felt that short term workshops in analytical methodologies for mycotoxins would be a more useful training avenue than bilateral training agreements that would involve only one graduate student.

A French entomologist from the Institut Francais Scientifique pour le Developpement en Cooperation (ORSTOM) is on a four year assignment with the Department. He previously spent a term in Ivory Coast where he was involved in a study to determine the relationship between aflatoxin contamination in food and the incidence of primary liver cancer. He was the initiator of the 1992 draft proposal entitled 'Investigation and alleviation of the effect of aflatoxins on food safety, quality and resultant health economics in Indonesia' (see Section 6.5.2).

FOOD AND NUTRITION DEVELOPMENT AND RESEARCH CENTRE:
The Food and Nutrition Development and Research Centre (FANDARC), established with funding provided by the World Bank in 1990, is an inter-university centre located at Gadjah Mada University (UGM) in Yogyakarta. The Graduate Studies Program at UGM is responsible for the administration of the Centre. The main objective of the centre is to provide facilities for research and training and to facilitate the promotion of science and technology in the area of food and nutrition. FANDARC is mandated to promote research cooperations with other research institutions in Indonesia, as well as overseas, and to promote industry-university interactions for the advancement of science and its application for the improvement of food supply and quality.

The Laboratory of Food Microbiology is one of the four main laboratories within the Centre. It undertakes the following activities related to fungi and mycotoxins:

- collection and identification of food microbes;
- food biotechnology;
- food fermentation;
- microbial toxins and enzymes.

Equipment already installed and functional include HPLC and autosampler, low-field NMR, AAS, GLC, 2X80L fermentors, 16L fermentor, ultracentrifuge, incubators, safety hoods, -80 °C freezer, fluorescent microscope, UV-vis spectrophotometer, TLC scanner, freeze drier with ampoule attachment as well as a full complement of other common laboratory equipment. Laboratory facilities and equipment are outstanding at FANDARC. FANDARC also has new animal housing and greenhouse facilities and a very well equipped food processing laboratory.

Researchers from the Laboratory of Food and Nutrition of the Faculty of Agricultural Technology use the FANDARC facilities in the conduct of fungi and mycotoxin research (see below).

The Rector, from whom approval to develop bilateral agreements must be sought, is a faculty member of the Faculty of Agricultural Technology.

LABORATORY OF FOOD AND NUTRITION:
In 1983, the Faculty of Agricultural Technology undertook a study on maize losses after harvest in which it was determined that the percentage of samples contaminated with aflatoxin increased from the farmer level, middleman level through to the processor level.

More recently, two mycologists and one chemist at the Laboratory of Food and Nutrition have participated in a collaborative project with the Commonwealth Scientific and Industrial Research Organization (CSIRO). An extensive survey of the mycological status of peanuts, maize, rice, mung beans, soybean, red kidney beans, sorghum, coriander seeds, black peppercorns, nuts and soy flour with respect to both the fungal flora and mycotoxins was commenced in 1990 under this project. The most significant mycotoxigenic species isolated to date are Aspergillus flavus and Fusarium moniliforme. Mycotoxins analyzed for include aflatoxins B_1, B_2, G_1 and G_2; sterigmatocystin, cyclopiazonic acid, ochratoxin A, penicillic acid, veruculogen, citrinin, zearalenone, T-2 toxin, diacetoxyscirpenol, deoxynivalenol, nivalenol, verrucarol, alternariol, alternariol monomethyl ether and tenuazonic acid. Some of these analyses may have been done in Australia. Chemists at UGM are able to analyze for aflatoxin, cyclopiazonic acid and kojic acid.

In addition to the results of aflatoxin and aflatoxigenic fungi, researchers reported the occurrence of Fusarium moniliforme in black soybeans and other Fusarium species in rice and paddy.

An investigation was carried out in 1991 to determine the effect of different postharvest handling techniques on mould growth patterns (number and identity of fungi) and the critical hydrothermic parameters for aflatoxin production in maize stored in village unit cooperatives.

Several researchers at the Laboratory of Food and Nutrition have been involved in studies of mycotoxigenic contaminants and stability of mycotoxins in the traditional fermentation of soy sauce, kecap and koji. Although most of these studies involve aflatoxin, some work has been conducted on cyclopiazonic acid and kojic acid in processed soysauce. In one study, Aspergillus oryzae was found to be effective in deactivating aflatoxin produced by Aspergillus flavus. Aflatoxin was quantified by TLC and subsequent optical spectrophotometry determination.

A graduate student is conducting studies on the stability of aflatoxin during food processing in copra.

The effect of zinc and phytic acid on the growth and aflatoxin production by A. flavus has been studied.

Diligent scientists at the Laboratory of Food and Nutrition have substantially added to the pool of human resource capabilities in fungi and mycotoxins at UGM. Three researchers at the Faculty of Agricultural Technology have undergone one month training courses in Australia, one in the analysis of mycotoxins by TLC and two in food mycology methods and identification of common fungal genera to species.

Mycotoxin research at the Laboratory of Food and Nutrition is not limited by lack of equipment since scientists are able to make use of the FANDARC facilities (see above). Scientists, however, indicated that manuals on fungal taxonomy and isolation techniques are in shortage. In addition, the need for training in Fusarium toxin analysis was identified.

A scientist with the Laboratory of Food and Nutrition is the curator for a fungal culture collection which is housed at FANDARC. It consists of approximately 500 isolates of fungi, including those isolated in the conduct of collaborative research with the CSIRO project. A freeze drier and -80 degree freezer are available at FANDARC.

3.3.9 DR. SARJITO HOSPITAL

Clinicians, biostatisticians and epidemiologists at Dr. Sarjito Hospital have participated in community health and epidemiology studies on the correlation between vitamin A and other health related parameters. Through these studies, experience in processing and analyzing large data sets has been gained, and a framework for the collection and long term follow-up of community based data has been established.

Dr. Sarjito Hospital has never been involved in mycotoxin research but it was approached to participate in the Human Health Component of the 1992 draft proposal entitled 'Investigation and alleviation of the effect of aflatoxins on food safety, quality and resultant health economics in Indonesia' (see Section 6.5.2).

3.3.10 SOUTHEAST ASIAN MINISTERS OF EDUCATION ORGANIZATION SOUTHEAST ASIAN REGIONAL CENTRE FOR TROPICAL BIOLOGY TROPICAL AGRICULTURE PEST PROGRAMME

SOUTHEAST ASIAN REGIONAL CENTRE FOR TROPICAL BIOLOGY:
Since the initiation of fungi and mycotoxin research at Southeast Asian Regional Centre for Tropical Biology (BIOTROP) in 1983, mycological studies on grain have been conducted. These include:

- an inventory of fungi in stored grains (maize, soybean and groundnut);
- an inventory of Fusarium spp. and toxigenicity (deoxynivalenol, moniliformin and zearalenone) associated with some economically important crops in Java;
- surveys of Aspergillus flavus and the level of aflatoxin in maize during drying;
- the effects of moisture content, storage systems and percentage damaged kernels on Aspergillus flavus populations in groundnut;
- the effects of drying and shelling of maize on grain intactness, Aspergillus infestation and aflatoxin production;
- the effect of methyl bromide on fungi of milled rice and soybean;
- fungi on some commodities collected from farmer and retailers in West Java;
- the correlation of insect and fungal infestation in stored maize.

Graduate studies were conducted by students from Bogor Agricultural University to investigate the effects of moisture and types of packaging on fungal infestation of stored mung beans.

During the period 1989-1992, BIOTROP entered into technical cooperation with the National Logistics Agency (BULOG) to conduct research on the toxicity of carbon dioxide and phosphine to insects and fungi in stored products. Research with BULOG has included the following studies:

- the effect of CO_2 on storage fungi and aflatoxin production in maize;
- the effects of different concentrations of CO_2 on mycelial growth, sporulation, germination and aflatoxin production of three isolates of Aspergillus flavus on solid culture medium;
- the effects of phosphine on storage fungi, aflatoxin production, moisture content and seed quality of maize;
- the effects of phosphine and length of storage on fungi, aflatoxin production, protein content and moisture content in soybean meal;
- the effects of phosphine concentration on mycelial growth, sporulation, germination and aflatoxin production of two isolates of Aspergillus flavus grown on solid culture medium;
- inventory of fungi, aflatoxin, protein and moisture content of soybean meal from feedmills and BULOG warehouses;
- the effect of different milling degree of rice to fungi and protein content.

Biotrop will undertake a three year project with BULOG to investigate the effect of type of packaging on rice quality in the near future. This study will include a mycotoxin component.

In 1990, BIOTROP embarked upon the mycological and mycotoxin survey of Indonesian grain in collaboration with the CSIRO in Australia and Indonesian project participants at Gadjah Mada University and the Bogor Research Institute for Veterinary Sciences (see Sections 3.3.5 and 3.3.8).

In the future, BIOTROP will participate in a study to determine the effect of postharvest handling of maize on fungal infection and mycotoxin production. Fungal enumerations of Aspergillus flavus and Fusarium and analyses for aflatoxin, zearalenone, moniliformin and deoxynivalenol will be conducted.

BIOTROP has acquired significant mycological expertise, in part, through cooperative research associations and training programs with the CSIRO in the identification of common storage fungi to the species level. Researchers indicated an interest in investigating the importance toxigenic field fungi and collaborative plant breeding research.

The plant pathologist/mycologist at the Phytopathology Laboratory is a faculty member in the Biology Department of Bogor Agricultural University. This is advantageous since it renders access to analytical equipment, such as an HPLC, which is not available at BIOTROP. Technicians at BIOTROP are able to analyze for aflatoxin, moniliformin, zearalenone and deoxynivalenol by TLC and have some experience with HPLC.

The Phytopathology Laboratory and Chemistry Laboratory are equipped with incubators, a laminar flow hood, UV chamber for isolation, ultracentrifuge, autoclave, GC, microscopes, UV chamber for TLC, moisture meter and rotary evaporator. In addition, an HPLC and freeze drier with ampoule attachment have been ordered but have not yet been received.

BIOTROP has the capacity to cooperate in research, training and information dissemination with university and government sectors as well as with other regional institutions. A program linking BIOTROP to nine universities in nine provinces in Indonesia will be finalized at the end of the year. Funding will be provided for human resource development, equipment and construction of facilities. The director of BIOTROP indicated the possibility of incorporating a mycotoxin component into this program.

A small culture collection of storage fungi and plant pathogens is maintained on agar media.

3.3.11 PRIVATE FEEDMILL COMPANIES

Several of the larger private feedmills screen for some mycotoxins in high risk commodities. Charoen Pokhpand Feedmill conducts routine screening for aflatoxin and ochratoxin in groundnut. Cargill Feedmill screens only for aflatoxin B_1 in maize and groundnut.

3.4 PHILIPPINES

Crop production:
The Philippines is an exporter of copra cake and copra meal. Wheat pollard, maize and soybean are imported as feed ingredients. In the last five years, the Philippines has imported rice from Indonesia, Vietnam or Thailand. Some peanuts are produced but most are imported from China, Vietnam, India and Thailand.

Animal production:
The Philippines is self-sufficient in the production of eggs, poultry, pork and mutton, but it imports beef. Processed pork was exported in past years. There is a strong government initiative to become a net exporter of pork again.

Committees:
In 1992, a number of government institutions led by the National Food Authority put together plans for a mechanism to address the mycotoxin problem in the local food and feed supplies. They submitted a proposal to the Department of Science and Technology for the First National Seminar-Workshop for the Integrated Control Program on Fungi and Mycotoxins in Philippine Food and Feedstuffs. One of the objectives of the Seminar-Workshop was to draw up a strategic plan of action. This was held in Manila in February, 1993. Proceedings will be available from the Food and Nutrition Research Institute.

Regulations:
The *Consumer's Act of the Philippine*s 1991 sets out a guideline of 20 ppb for aflatoxin. This Act is not in effect yet. For imported feed ingredients, a tolerance of 30 ppb for aflatoxin is used. A 20 ppb limit is followed for food, although it is unknown whether there is any legal basis for either of these limits. Exported copra and peanut products are monitored for aflatoxin B_1 at 20 ppb.

3.4.1 DEPARTMENT OF AGRICULTURE
•PHILIPPINE COCONUT AUTHORITY

PHILIPPINE COCONUT AUTHORITY:
The European Community announced that in November 1991 it would lower the limit for aflatoxin in oil-seed cakes for use as straight feeds and for use by unregistered feed mills from 50 ppb to 20 ppb. Complaints of high aflatoxin contamination in copra and loss of the North American market for Filipino copra has prompted intense research into the causes for fungal infestation of copra and possible preventative measures.

A two year, £ 819,000 technical cooperation project between the National Resources Institute (NRI) of the United Kingdom and the Philippine Coconut Authority (PCA) commenced in 1990 (see also Section 3.4.4). The immediate objective was to reduce aflatoxin levels in copra and copra products and to upgrade the quality of copra produced in the Philippines. To do this the following research studies were undertaken:

- survey of copra cake/meal from exporting mills to determine mean current levels of aflatoxin B_1 contamination;

- national aflatoxin surveys to determine regional and seasonal variations and to establish the stage in processing/marketing chain where major aflatoxin contamination occurs;
- the effect of different drying techniques, moisture content and length of storage on aflatoxin and mould contamination in copra and copra meal/cake;
- evaluating and developing rapid methods of aflatoxin analysis and sampling protocols of copra and copra by-products;
- detoxification during processing of copra cake and refining of coconut oil;
- examination of the efficacy of a commercially available mould inhibitor in preventing mould growth and aflatoxin contamination during sun drying;
- the correlation of aflatoxin levels and mould infestation in copra dried by different techniques;
- development of a low-cost moisture meter for copra;
- investigation of social and economic aspects of copra production and marketing systems;
- determination of most efficient drying techniques;
- storage and shipping trials on copra cake to establish whether aflatoxin is formed at these stages and to investigate the causes and possible solutions.

The project also assisted the PCA in developing new grading standards.

Fusarium and Alternaria were found to significantly contaminate copra.

The PCA will continue its responsibility to monitor for aflatoxin in copra and copra by-products for export in all regions of the Philippines. A massive information drive to educate copra producers as to the hazards and possible procedures for preventing aflatoxin contamination in copra is under way.

An aflatoxin laboratory has been fully equipped with excellent laboratory equipment including laboratory scale grinders, extraction and analytical equipment (computerized HPTLC densitometer and integrator, UV-vis spectrophotometer). Staff have been fully trained in aflatoxin analysis by computer controlled HPTLC. A total of thirteen project-related NRI training scholarships were awarded to PCA (7), the Bureau of Animal Industry (4), the Food and Nutrition Research Institute (1) and the National Post-harvest Institute for Research and Extension (1). Seven of these scholarships were in mycotoxin analysis, of which five went to the PCA. In an exercise to provide accreditation to the laboratory, two international inter-laboratory studies were undertaken.

3.4.2 DEPARTMENT OF AGRICULTURE
•NATIONAL FOOD AUTHORITY
TECHNOLOGY RESOURCE DEVELOPMENT DIRECTORATE
TECHNICAL RESEARCH DIVISION

NATIONAL FOOD AUTHORITY:
The primary function of the National Food Authority (NFA) is to stabilize the price of rice and maize. In addition, it is the agency responsible for determining quality in imported commodities.

The NFA samples imported commodities at port and tests for aflatoxin by BGYF at the Technical Research Division. Positive samples are sent to either the Bureau of Plant Industry, the Bureau of Animal Industry or the National Postharvest Institute for Research and Extension for quantitative determination. The NFA also provides analytical services for private importers.

Previously, the NFA conducted aflatoxin analyses by minicolumn with detection up to 50 ppb by fluorotoximeter. However, since the safety hood has been out of order, the chromatoview BGYF test has been employed for the last two years. The NFA does not have the capacity to conduct mycological analyses.

The Technology Resource Development Directorate is currently developing a presumptive aflatoxin test for maize where aflatoxin levels as determined by TLC will be correlated to BGYF test results.

Occasionally, requests from industry to test for toxicity in rice are received. Because the NFA has no capacity in this regard, the samples are sent to the Bureau of Animal Industry for animal toxicity testing.

Mould inhibitors for quality preservation in stored maize are currently under evaluation.

The NFA has conducted international training courses for ASEAN participants in the analysis of physical properties of grain and has instructed university students in practical laboratory techniques.

The NFA is mandated to conduct research in food handling and postharvest technology through the Technical Research Division of the Technology Resource Development Directorate.

3.4.3 DEPARTMENT OF HEALTH
•BUREAU OF FOOD AND DRUG

The Bureau of Food and Drug (BFAD) is not mandated to conduct research. It is responsible for monitoring aflatoxins in domestic processed foods, which to date have been confined to peanut butter, peanut candy and maize based snack foods. Analysis is done by TLC with densitometer detection. Technicians are also familiar with HPLC aflatoxin analyses.

BFAD obtains aflatoxin B_1 standard through participation in the IARC aflatoxin B_1 crosscheck sample program. Scientists are intending to start monitoring aflatoxin M_1 in milk as well as ochratoxin A and zearalenone in processed foods.

Systematic mycological studies are not conducted, but several fungi have been isolated from processed foods; they are included in the small fungal culture collection maintained at BFAD.

The mycotoxin laboratory at BFAD had a five year technical cooperation project with Japan in which there was a mycotoxin component. (One technician at BFAD has been trained in the TLC and HPLC analysis of mycotoxins other than aflatoxin in Japan.) However, researchers feel they would benefit from more training in analytical techniques. If fungi and mycotoxin capabilities were to be expanded, researchers felt they would also benefit from mycology training.

BFAD is a new facility founded with JICA funding in 1987. It is outfitted with new high tech equipment in every division. Laboratory equipment in the facility includes three GCs, HPLCs with fluorescent detector, safety hoods, mass spectrometer, TLC densitometer and integrator, -70 degree freezer, freeze drier with ampoule attachment, laminar flow hoods, CO_2 incubators, fluorescent microscope, light microscope, and plenty of excellent general laboratory equipment. A small-animal experimental facility is available on site with a fully equipped histopathology laboratory. Specific Pathogen Free rabbits, mice, rats and guinea pigs are bred at BFAD.

BFAD scientists suggested the organization of a laboratory network to facilitate the obtaining of mycotoxin standard. They perceive the lack of inexpensive rapid test kits for aflatoxin as a barrier to industry in testing for mycotoxins. It was felt that analytical methods development would be an important component to a coordinated ASEAN mycotoxin research effort.

3.4.4 DEPARTMENT OF AGRICULTURE
•BUREAU OF ANIMAL INDUSTRY

The Bureau of Animal Industry (BAI) is mandated to monitor aflatoxin in domestic feed components and commercial feeds excluding aquaculture feeds. Imported feed grain is the responsibility of the National

Food Authority until it reaches the warehouse at which point it becomes the responsibility of the BAI. BAI is also responsible for livestock, dairy and poultry production.

A survey conducted in 1990 revealed that 30% of feedstuffs and over 65% of poultry and hog feeds contained levels of aflatoxin greater than 20 ppb.

The BAI was involved in studies with the Aquaculture Department of the Southeast Asian Fisheries Development Centre to:

- survey aflatoxin B_1 in commercial shrimp finisher feeds;
- conduct feeding trials to determine the effects and tolerance levels of aflatoxin B_1 on shrimp;
- determine transmission of aflatoxin B_1 to edible shrimp tissue. Aflatoxin determinations were done using HPTLC.

In the past, the BAI has not, conducted routine screening of feeds and feedstuffs as it is mandated to do, due to lack of funding. However, a service arm of the BAI is responsible for aflatoxin analysis in feed samples submitted by the feedmill industry or livestock raisers reporting suspected mycotoxicoses.

A pathologist with the BAI reports symptoms in animals that may indicate zearalenone and ochratoxin A contamination of feeds. For this reason, the BAI has attempted to adapt aflatoxin detection procedures to zearalenone and ochratoxin A analysis using commercially available minicolumn screening kits; however, this failed. This lead to the BAI's interest in learning TLC techniques for analysis of these two toxins and then adapting the technique for HPTLC analysis.

In June of 1991, a collaborative sub-project of the NRI-Philippine Coconut Authority project (see Section 3.4.1) was approved to commission an aflatoxin laboratory at BAI. Studies involved aflatoxin surveys in feeds and pig feeding trials to assess aflatoxin related financial losses. The recently completed feeding trials were conducted using commercial feed naturally contaminated at a known level and feed artificially contaminated to a known level, with mycotoxin binder. Other producers of commercial mycotoxin binder are interested in participating in such studies to prove the efficacy of their product.

Like the PCA project, funding was provided for the BAI sub-project by the EEC with counterpart funding from the Government of the Philippines. Funding was recently discontinued prior to the planned termination date. Feeding trials originally scheduled to take place in regional stations in the regions of Mindanao, Visayas, and Luzon will continue with funding from the Philippine government but on a much smaller scale.

BAI felt that it would gain from the training of staff to increase their capability in the areas of animal toxicology and pathology. In turn, BAI is able to train other government staff and university students.

The NRI project provided the BAI with computerized HPTLC equipment comparable to that at the PCA. The BAI also has a GC, UV-vis spectrophotometer and microtitre plate reader. One senior staff member was trained through the NRI Mycotoxin Program and another was trained in mycology.

3.4.5 DEPARTMENT OF AGRICULTURE •BUREAU OF PLANT INDUSTRY

BUREAU OF PLANT INDUSTRY:
The Bureau of Plant Industry (BPI) provides certification that commodities for export are free of infestation. In addition, the Chemistry Laboratory of BPI monitors aflatoxin in raw beans, and domestic maize and peanuts. Occasionally, analyses are performed on samples passed on from the National Food Authority.

A study funded by the Government of the Philippines was conducted from 1977 to 1979 with the following objectives:

- to identify the different aflatoxin producing organisms that are naturally associated with peanut, maize, rice, legumes, copra and cassava;
- to determine the possible point of entry of the fungi in relation to postharvest handling and processing;
- to determine the moisture content gradient in various agricultural crops at which invasion of aflatoxin producing organisms is minimized or prevented.

The BPI has been involved in the determination of aflatoxin during soysauce fermentation and monitoring for the toxin in commercial soysauces. In order to determine baseline data to be used in the development of guidelines for aflatoxin tolerance under the Consumer's Act, the BPI undertook a survey of aflatoxin in peanuts and beans. BPI screens approximately 30 samples of green lentils, peanuts, soybeans, beans, cassava, nuts and maize per month for aflatoxin. Aflatoxin is determined using two dimensional TLC with UV densitometer detection.

The BPI is nearing completion of the development of a rapid field test kit for aflatoxin in maize.

A project entitled "Monitoring and forecasting system on aflatoxin contamination in raw agricultural crops" was proposed to the Government of the Philippines in 1992 but was not funded (see Section 6.5.8).

The Chemistry Laboratory is equipped with basic laboratory equipment as well as densitometer, GC and HPLC. Additional analytical equipment is available through the pesticide residue laboratory including eight GCs, HPLCs and extraction facilities.

Scientists at the Chemistry Laboratory would like to obtain analytical training for aflatoxin and mycotoxins found in rice, especially citrinin.

The BPI is able to accept some university students for training.

A small fungal culture collection of approximately 50 isolates of plant pathogens is maintained on fresh agar slants at the BPI.

3.4.6 DEPARTMENT OF AGRICULTURE
•NATIONAL POSTHARVEST INSTITUTE FOR RESEARCH AND EXTENSION

NATIONAL POSTHARVEST INSTITUTE FOR RESEARCH AND EXTENSION:
The National Postharvest Institute for Research and Extension (NAPHIRE) is tasked to cut down losses and maintain food and feed quality through research and development work. NAPHIRE applied research and development efforts are geared towards addressing the Philippines postproduction system which includes aflatoxin contamination in maize and peanuts as a priority. Other crops for which NAPHIRE is responsible include soybean, grain legumes, and rice. The Philippine Rice Research Institute (PhilRice) will soon take over all NAPHIRE responsibility for rice research.

In the early 1980's a two-phased project was conducted to determine the nature, causes and extent of paddy deterioration at the farm level. This was a cooperative effort with the University of the Philippines at Los Banos. The effect of different postharvest practices (field stacking, threshing, drying delays, storage) on discoloration and fungal infestation in paddy was examined in Phase I. In Phase II, field conditions were simulated in the laboratory to determine the effect on discoloration of paddy and milled rice brought about by the various fungi identified (Fusarium, Aspergillus, Rhizopus, and Curvularia) in the field study.

NAPHIRE has been involved in several studies to determine the effects of storage and processing on aflatoxin contamination in maize. Varietal differences in the incidence of aflatoxin contamination have also been examined.

The Canadian International Development Research Centre (IDRC) has provided funding for two projects regarding aflatoxin in Philippine commodities. The first, entitled 'Control of Aflatoxin in Maize' commenced in 1987. The general objective of phase I of the project was to determine the effectiveness of the postproduction practices prescribed for the farm level in controlling aflatoxin contamination. The acceptability and economic implications of such practices to the farmers were also considered. A comprehensive field study defined aflatoxin build-up in different varieties of maize after various drying and shelling methods were employed. A second phase to this project was launched in 1991 in South Cotabato with the objectives of:

- determining the effectiveness of controlling aflatoxin in a real farming and marketing situation;
- establishing the economics of the operations and the receptivity of the farmers to the innovations.

The second IDRC funded project, 1987-1990, was conducted as a collaborative study between NAPHIRE and Isabela State University to examined aflatoxin build-up in peanuts on the farm and in storage. Agronomic factors, postproduction handling and primary processing (windrowing, stripping, modified atmosphere storage, storage duration etc.) requirements to control aflatoxin contamination were established. In addition, the use of groundnut varieties resistant to the invasion of Aspergillus flavus have shown promising results.

NAPHIRE is one of the institutions currently participating in the project entitled 'Fungi and Mycotoxins in Asian Food and Feedstuffs' in collaboration with the Commonwealth Scientific and Industrial Research Organization (CSIRO). The project is funded by the Australian Centre for International Agricultural Research (ACIAR). Institutions in Thailand and Indonesia are also involved in similar studies with the CSIRO. NAPHIRE participation commenced in 1990 with the objectives:

- to determine and enumerate prevalent fungal species growing in rice, maize, peanuts, soybeans and mung beans at various postharvest stages;
- to determine the significance of prevalent species as mycotoxin producers;
- to determine aflatoxin status of maize and peanuts.

Aflatoxin is determined by TLC with quantitation by densitometer. Fungal isolations and some analyses are conducted concurrently in Australia. Prevalent fungi reported to produce mycotoxins in grains will be tested for toxigenicity in pure culture. Research efforts will continue until October, 1993 under this project.

In collaboration with Israel, NAPHIRE is undertaking a four year project from 1991-1994 that is funded by USAID, to study the efficacy of modified atmospheres in the outdoor storage of maize. This will involve aflatoxin assays as well as mycological enumerations.

NAPHIRE researchers indicated an interest in studying the toxigenicity of Fusarium since F. moniliforme and F. graminearum have been isolated frequently from maize and rice. Further training in fungal identification and analytical methods were identified as essential in increasing research capabilities. It was also felt that a study of the economic implications of mycotoxins in grains would be valuable.

Mycotoxin standards available at NAPHIRE include aflatoxin, zearalenone, deoxynivalenol, sterigmatocystin, ochratoxin A, citrinin and T-2 toxin. Although NAPHIRE possesses a GC, which could be used for mycotoxin analyses, columns and column packing materials are lacking. Qualitative toxigenicity assays are conducted using the agar plug method in which an agar plug is taken from a culture isolated and placed on a TLC plate. A drop of solvent is placed on the plug and the TLC plate

is run. Spray reagents, Rf and observation under UV light provide qualitative toxigenicity results. Tests for citrinin production have yielded negative results in the past but researchers suspect that this may be due to difficulties with the agar plug methodology.

The chemistry laboratory is furnished with general laboratory equipment as well as safety hood, rotary evaporator, densitometer with integrator, UV chamber, and GC with FPD and ECD detectors. A laminar flow hood, microscopes (stereo and light) and incubator are available in the microbiology laboratory. NAPHIRE is also equipped with an entomology laboratory, although no studies have been conducted on the relationship between insect and fungal infestation in grain.

Four researchers at NAPHIRE have undergone training in Australia, three in the analysis of mycotoxins by TLC and HPLC and one in food mycology and the identification of common fungal genera to species. In addition, a staff chemist has participated in a course offered by the Bogor Research Institute for Veterinary Sciences in Indonesia for ELISA analysis of aflatoxin as well as the course on mycotoxin analysis conducted at the National Resources Institute in the United Kingdom.

FAO is organizing a series of training courses in the Philippines, Thailand and India in which several areas of mycotoxins will be addressed. The session in the Philippines will be conducted at NAPHIRE and will cover training and management (see Section 6.4.2).

In the past, NAPHIRE has accepted university students in the undertaking of thesis research.

3.4.7 DEPARTMENT OF SCIENCE AND TECHNOLOGY •FOOD AND NUTRITION RESEARCH INSTITUTE FOOD QUALITY AND SAFETY SECTION

FOOD AND NUTRITION RESEARCH INSTITUTE:
The Food and Nutrition Research Institute (FNRI) was the first institution in the Philippines to carry out research on fungi and mycotoxins. An aflatoxin laboratory was established in 1967 with USAID funding. An American aflatoxin consultant was also provided. The FNRI laboratory was initially engaged in an epidemiological study of the role of aflatoxin in primary liver cancer in collaboration with the University of the Philippines College of Medicine. FNRI conducted surveys and aflatoxin analyses on maize consumed by communities as a staple whereas the College of Medicine was responsible for clinical and biochemical studies on autopsied livers of primary liver cancer patients. Dietary intake of aflatoxins and correlative studies on aflatoxin, alcohol consumption, hepatitis B and primary liver cancer were also conducted. These studies suggested that hepatitis B infection and aflatoxin are co-carcinogens in primary liver cancer in humans.

In order to have a more accurate index of aflatoxin exposure, the feasibility of measuring excretion of aflatoxin or its metabolites, instead of intake was investigated by sampling urine and faeces from persons known to have consumed foods highly susceptible to aflatoxin contamination.

An extensive fifteen-year survey of cereals, root crops, peanuts, other nuts, beans, coconuts, fish, meat, alcoholic beverages, cocoa and coffee as well as their respective food products revealed cassava as potentially the most dangerous food based both on levels of aflatoxin and the number of positive samples. Maize and peanuts were also identified as principal vectors for aflatoxin exposure.

Monitoring of aflatoxin load in agricultural products from the different regions was started at FNRI in October, 1975 for the purpose of obtaining information regarding environmental variables (ie. geographic and seasonal) that influence mycotoxin contamination in foodstuffs.

Several acute toxicity studies on intubated duckling have been conducted using extracts from test materials that were either economically important or significant from the point of view of public health (peanut butter, maize, cassava). In addition, histopathological examinations were conducted on the livers of duckling administered extracts of urine from human subjects known to have consumed maize contaminated with aflatoxin.

Between 1980 and 1982, a multi-mycotoxin method was used to survey maize and peanuts for contamination by sterigmatocystin, zearalenone and ochratoxin A. Of the 400 samples tested, only three contained ochratoxin A or zearalenone.

Scientists at FNRI have conducted a comparison of three methods for the determination of aflatoxin in copra meal. Precision, accuracy, cost, time for analysis, skill and equipment requirements were considered. The EEC and AOCS methods were found to be more appropriate under present FNRI conditions than the AOAC method.

Research activities at FNRI have recently been concentrated on the development of a low-cost, rapid field test kit for aflatoxin. In this regard, a minicolumn semi-quantitative method for copra and peanuts is in the process of being perfected and undergoing inter-laboratory testing. This method has been adapted from both the EEC and AOAC methods with UV chamber for detection and it is hoped that similar rapid screening methods for maize and other commodities will be developed in the near future.

Facilities include basic TLC equipment, HPLC, rotary evaporator, spectrophotometer, safety hood, UV chamber for sterile work, GC (not operational) and adequate general laboratory equipment. It was indicated that some equipment in the laboratory was either dated or required repairs and that it may be necessary to acquire some equipment if FNRI were to increase their capacity in mycotoxin research.

The Food Quality and Safety Section has been instructed to investigate decontamination methods in the future. Researchers feel that their strengths lie in mycology and analytical methods development. One of the scientists at FNRI has undergone two training courses in mycology in Japan and another scientist has participated in the mycotoxin analysis course offered by the National Resources Institute in the United Kingdom. Researchers felt it important to learn analytical methods for mycotoxins other than aflatoxin.

Scientists at FNRI are familiar with a number of different methods of aflatoxin analysis including TLC, biological assays using day old ducklings, and HPLC. FNRI has conducted training courses for government and university sector scientists in analytical methods. The FNRI analytical precision and accuracy is periodically evaluated through the IARC maize, peanut and milk check samples programs for aflatoxin. In addition, internal aflatoxin reference materials from local food/feed materials are currently being developed for use in method development and evaluation. One such in-house feed reference material from naturally contaminated copra meal has been the subject of an inter-laboratory comparison with a local laboratory and another in the U.K.

Filipino researchers are extremely concerned about occupational exposure to mycotoxins, particularly aflatoxin.

Researchers at FNRI expressed the concern that low quality consignments which may have been rejected by other countries may be imported into the Philippines due to a lack of enforceable regulations. They felt that an integrated surveillance system to oversee the aflatoxin monitoring efforts of the various responsible institutions is necessary in ensuring that this does not happen.

Databases for the organization of fungi and mycotoxin contacts, bibliographic material, and scientific data was identified as an essential component of an effective ASEAN mycotoxin research effort. Coordination

of research activities on fungi and mycotoxins within the Philippines was also identified as essential. FNRI is compiling a food composition database that will be compatible with similar international databases and includes information on toxicants.

A comprehensive list of suggestions for research and development activities for the development of an integrated control strategy for fungi and mycotoxins were identified by a researcher from FNRI at the February Workshop in Manila (see Section 3.4.2). These will be presented in the Workshop proceedings. A consensus report for such a strategy will also be generated in the proceedings.

3.4.8 UNIVERSITY OF SOUTHERN MINDANAO

A physiological study conducted at the University of Southern Mindanao in the 1980's confirmed the data of other investigators that moisture content and length of storage period play vital roles in the growth of Aspergillus and Penicillium in maize kernels.

3.4.9 UNIVERSITY OF THE PHILIPPINES AT LOS BANOS

- **INSTITUTE OF CHEMISTRY**
- **INSTITUTE OF BIOTECHNOLOGY**
- **INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY**
- **INSTITUTE OF BIOLOGICAL SCIENCES**
- **INSTITUTE OF PLANT BREEDING**
- **NATIONAL CROP PROTECTION CENTRE**
- **COLLEGE OF VETERINARY MEDICINE**
- **INSTITUTE OF ANIMAL SCIENCES**

INSTITUTE OF CHEMISTRY:
No mycotoxin research is currently conducted at the Institute of Chemistry. However, past research since 1973 has included:

- a study of aflatoxin production in copra by Aspergillus flavus as a function of time, moisture content and temperature;
- thiabendazole as seed treatment to control infection by A. flavus and aflatoxin contamination in maize;
- the effect of washing and cooking on aflatoxin content in maize grits; and
- surveys of aflatoxins in food grains, legumes and copra.

All aflatoxin analyses were conducted by TLC donated by USAID. Other laboratory equipment includes HPLC, dual wavelength spectrophotometer (UV-vis out of order), IR, 40 MHz NMR and safety hood.

INSTITUTE OF BIOTECHNOLOGY:
The Institute of Biotechnology initiated research on the production of polyclonal antibodies to aflatoxin for the purpose of screening for plant pathogens in foods and feeds in 1985. The Institute feels that antibody-based analytical methods are preferable to chemically-based methods since they are very sensitive and need not involve lengthy extraction procedures. This makes them safer from an occupational health and safety perspective. In addition, the technology can be applied to the early detection of plant pathogens in the field.

In the last six months, the Institute has been involved in the development of a monoclonal antibody (mab) based rapid screening kit for aflatoxin B_1. It is hoped that the kit will provide an alternative to purchasing costly commercially available immunocolumn kits. They feel that their mab aflatoxin kit would be useful not only for routine screening but also for research activities to determine at what postharvest stage aflatoxin is produced. Work has progressed rapidly since aflatoxin B_1-Bovine Serum Albumin conjugate

is purchased rather than produced at the Institute. Antibodies are produced by immunizing BALB/c mice. A relatively stable fusion product with myeloma cells has already been obtained. The Institute claims that 2 ppb levels of aflatoxin B_1 or 10^2 aflatoxigenic microorganisms (Aspergillus flavus or A. parasiticus) can be detected. Studies to correlate the number of aflatoxin producing organisms to levels of aflatoxins in food and feedstuffs are intended. The Food and Nutrition Research Institute and the Bureau of Animal Industry will participate in an inter-laboratory test of the efficacy and suitability of this mab kit for use as a field test kit. They hope to eventually commercialize the kit.

The International Development Research Centre has provided funding for projects to produce other mab-based diagnostic kits for such plant pathogens as Abaca (Manila hemp) mosaic virus, banana bract mosaic virus and banana bunchy top virus.

Researchers at the Institute plan to initiate mab production to aflatoxin M_1 in the near future and are enthusiastic to develop mab methods for the detection of mycotoxins other than aflatoxin. Human resource capabilities include a Ph.D. level principal researcher in the area of food science, biochemistry and microbiology. She has studied and worked in the United States for ten years. Technicians are trained in the plant pathology, microbial biochemistry and microbiology.

Laboratory equipment at the Institute includes gel electrophoretic equipment, spectrophotometer, CO_2 incubator, shakers, ELISA reader, UV inoculation chamber and laminar flow hood. A considerable amount of equipment, including safety hood and ultracentrifuge, were in disrepair and therefore not useable. Further constraints to the effective use of available equipment were the unreliability of electric power and the expense of purchasing interruptive power sources necessary for sensitive equipment. Scientists identified a difficulty in obtaining Specific Pathogen Free animals necessary to conduct immunological research.

INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY:

In the 1970's, mycological surveys for aflatoxigenic fungi were conducted at the Institute of Food Science and Technology (IFST) on peanuts, maize, soybean, copra and rice. Scientists have also conducted studies to determine the most favourable temperature for aflatoxin production by fungi isolated from rice and maize. Aflatoxin B_1 was found to be concentrated in the bran layers of brown rice, whereas polished rice was found to contain only traces of aflatoxin.

For several years, researchers have investigated the effects of processing (eg. boiling, roasting, oil extraction, shelling) on aflatoxin levels in peanut and peanut products. Studies involving the monitoring of aflatoxin through various postharvest stages have determined levels to significantly increase in peanuts during storage. For this reason, scientists feel it is important to develop postharvest technologies for drying peanuts. Variations in aflatoxin levels of peanuts harvested in different seasons have also been examined.

As part of a five year program, aflatoxin surveys have been conducted on peanuts and peanut products. This research is supported by USAID through the Collaborative Research Support Project (CRSP). Aflatoxin is determined mainly by TLC but has also been analyzed by HPLC and minicolumn with spectrophotometric detection.

Modified atmosphere storage of peanuts in plastic bags have been carried out to determine the levels of CO_2 that either deter or prevent aflatoxin production.

The detoxification of aflatoxin in a variety of grains, sorghum, peanut, copra and copra meal has been investigated at IFST using such chemicals as ammonia and sodium hypochlorite, calcium hydroxide, sodium hydroxide, ammonium hydroxide, propionic acid thiabenzadole, methylamine and sodium bicarbonate. These studies were initiated partly in response to aflatoxin regulatory reformation in the

European market and partly in response to reports of suspected aflatoxicoses in poultry in the Philippines. Research was also conducted on the effect of Sevin, Thiodan, Benlate, Bithane M-45, propionic acid, benzoic acid and ammonia on the growth of storage fungi, including Aspergillus and Penicillium, both in culture an and grains.

Ongoing detoxification studies include the biological decontamination of aflatoxin in peanuts, copra and copra meal using Rhizopus oryzae, Candida and Cladosporium sp. One such Cladosporium isolate has shown some inhibitory effects on the growth of Aspergillus parasiticus. Scientists suspect that the inhibitory effect may be related to production of a certain pigment which has shown to inhibit hyphal growth and spore germination and promote spore lysis in Aspergillus parasiticus. The Cladosporium isolate has been tested for safety using chick embryo and mouse assays. Testing of Cladosporium isolates for their activity against A. flavus and Fusarium are also planned. Such biological detoxification strategies are intended for eventual field use.

Scientists are planning to initiate genetic research to develop non-toxigenic strains of Aspergillus. These strains could be used to inoculate stored commodities to prevent colonization by toxigenic strains of Aspergillus.

Interest in the development of non-destructive methods for aflatoxin detoxification in peanuts and copra during processing was indicated. In addition, scientists feel an important research task is the development of inexpensive, rapid methods for aflatoxin detection for use by manufacturers of peanut products.

A paper recently presented in Japan by a researcher from IFST, identified the following directions and needs for Asian countries in fungi and mycotoxin research and development:

- harmonization of national mycotoxin regulations;
- basic surveys to determine the extent of mycotoxin problems;
- establishment of collaborative regional monitoring programs organized by appropriate international agencies;
- establishment of standardized methods for detection and analysis including availability of reference organisms and test samples;
- networking activities and exchange of information and personnel for training purposes;
- establishment of a database for mycotoxin prevention and control;
- breeding of plant cultivars resistant to toxigenic fungi;
- genetic engineering of non-toxigenic strains of fungi for field use.

IFST is able to conduct collaborative research with other universities as well as with government research laboratories.

INSTITUTE OF BIOLOGICAL SCIENCES:
The Institute of Biological Sciences at UPLB is currently involved in a study with the Bogor Agricultural University in Indonesia to investigate aflatoxin resistance factors in peanuts using germplasm from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

INSTITUTE OF PLANT BREEDING:
At the Institute of Plant Breeding (IPB) research on selection for field and postharvest resistance of peanut cultivars to infection by A. flavus has been conducted in the past. Resistance was found to be related to tannin content of the seed coat. Some collaborative research in the breeding of maize for resistance to aflatoxin has been conducted with Isabela State University.

Experimental plots are available at IPB and at Isabela State University.

NATIONAL CROP PROTECTION CENTRE:

A study by the National Crop Protection Centre (NCPC) investigated the effects of benomyl and captan on the growth, sporulation and conidial germination of Aspergillus and Penicillium isolates on solid culture medium. Benomyl was also evaluated at to whether it afforded adequate protective and eradicative effects on the growth of Aspergillus and Penicillium isolates in maize during storage. Aflatoxins B_1, B_2, G_1, and G_2 were determined by TLC with UV fluorescence viewing.

Another study involved the identification of Aspergillus and Penicillium species associated with rice and maize from warehouses and market places in various parts of the country. Aflatoxigenicity of Aspergillus and ochratoxigenicity of Penicillium isolates was determined by TLC.

Many chemicals, plant extracts and Neem tree derivatives have been evaluated for their efficacy in controlling the growth, sporulation and spore germination of Aspergillus spp., Penicillium and some rice pathogens including Fusarium moniliforme. Aflatoxin production by A. flavus and A. parasiticus was also investigated in Neem treated agar plates. Chemicals investigated to control aflatoxin and ochratoxin contamination in stored maize include benlate, captan, ammonia, propionic acid. Some of these chemicals have also been evaluated for their efficacy in controlling the growth of toxigenic fungi in maize. Ammonia is currently being investigated for its effect on growth, sporulation and toxigenicity of various mycotoxin producing isolates from maize and copra.

The Japanese agency, TARC, was approached to sponsor a five year project to investigate the control of Aspergillus infection and aflatoxin formation in maize using microbial isolates. Whether this project has been completed or if will be funded in the future is unknown.

The biological control of Aspergillus infection and aflatoxin contamination in maize and peanuts is currently being undertaken at NCPC. In addition, NCPC plans to evaluate the fungicidal activity of indigenous forest botanicals at on-farm levels of maize storage. Fusarium moniliforme is one of the fungi under investigation.

Several graduate students are currently involved in fungi and mycotoxin studies. These include:

- the effect of varying moisture content on the mycoflora of stored rice;
- aflatoxin contamination in cassava to determine the relative degrees of contamination prior to harvest, soon after harvest, during processing and in storage; and
- the effect of aflatoxin on the growth and survival of juvenile jumbo tiger shrimp.

The incidence of ochratoxin A, citrinin, penicillic acid, zearalenone and cyclopiazonic acid in copra as determined by the multi-mycotoxin TLC method is currently being investigated. Preliminary results show zearalenone as a common contaminant. Fusarium moniliforme and F. graminearum occur more frequently in copra than Aspergillus. A proposal to investigate Fusarium incidence was submitted to the Government of the Philippines but was rejected.

Production of toxins by Fusarium spp. associated with various major agricultural commodities was investigated in collaboration with the University of Minnesota. Thirty-two isolates of Fusarium from various agricultural commodities were screened for toxicity when crude extracts were injected into rats. Four were found to be toxic. The collaboration is continuing with a study of the cytotoxicity of mycotoxins in HeLa cells.

A three year USAID project entitled "Determination of the mode of transfer of Aspergillus flavus from the soil and maize using heterokaryon compatibility grouping" was commenced in 1992. Studies under this project include:

- determining the heterokaryon compatibility group pattern in fields containing an endemic population of A. flavus;
- assaying numerous soils in which maize is cultivated to determine whether a correlation exists between the soil population of A. flavus and the aflatoxin contamination of maize;
- developing and utilizing a marked strain of A. flavus that will allow for the tracking of movement from soil to grain; and
- ascertaining how the spores of A. flavus migrate from the soil to maize.

A marked strain of Aspergillus flavus has already been developed.

Researchers at the NCPC suggested that interdisciplinary research at three levels would be appropriate to developing control measures for aflatoxin contamination in agricultural commodities. These levels are:

- macrolevel, to view the overall physio-socio-economic environment in which the problem persists and where potential control strategies will be adopted;
- agricultural level, looking at the specific situation and context of the problem; and
- biological level, investigating the implications of nutritional toxicology on humans and animals.

The need for a mycotoxin coordinating committee was stressed.

NCPC scientists suggest that Philippine researchers would be able to contribute to the following areas of research if cooperative projects were to be developed:

- germplasm screening of agricultural crops for resistance to mould infection and aflatoxin contamination;
- chemical decontamination; and
- biological control of fungal growth and aflatoxin production.

Insufficient project funding, the cost of solvents and chemicals were indicated by NCPC scientists to be constraints to fungi and mycotoxin research. Import regulations at customs are reported to decrease Philippine purchasing power by 50%.

A second mycotoxin laboratory facility is currently being built at NCPC, partly with USAID funding. Equipment includes: UV inoculation chamber, safety hood, UV chamber for TLC plate reading, incubator, laminar flow and autoclave.

COLLEGE OF VETERINARY MEDICINE:
The College of Veterinary Medicine in collaboration with the Philippine Poultry Raisers Association, the Bureau of Animal Industry and the Food and Nutrition Research Institute undertook a study in the late 1970's to examine the incidence of mycotoxins and toxigenic fungi in mixed poultry feeds and feed ingredients. Their toxicological effects on poultry were also studied.

Aflatoxins in feed ingredients (maize, copra, sorghum and soybeans) and mixed feeds (layer, broiler and breeder mash) were quantitatively measured by TLC. Ochratoxin, sterigmatocystin and zearalenone in feed ingredients were qualitatively determined by the multi-mycotoxin TLC method.

Mycological examination of the feeds resulted in identification of Aspergillus, Trichoderma, Penicillium, Mucor and Verticillium sp. A. parasiticus and A. flavus isolates were cultured and their respective extracts fed to day-old ducklings to examine histopathological changes. The effects of aflatoxin on growth rate, protein efficiency ratio and feed utilization efficiency of broiler chicks were also investigated.

INSTITUTE OF ANIMAL SCIENCES:

The Institute of Animal Sciences (IAS) provides analytical services to industry for protein, proximate, fibre and mineral analyses etc. in feed. They are particularly well situated for this since there is a central broiler production facility located 30 km from the Institute. No mycotoxin analyses are conducted due to lack of analytical equipment. However the IAS does undertake collaborative mycotoxin studies with other institutes at UPLB that are able to conduct mycotoxin analyses. One such study with IFST determined aflatoxin to be a significant contaminant of maize in feeds.

Researchers at the IAS feel that mycotoxin problems in feeds warrant further research due to frequent reports of reproductive failures in swine; stunted growth, sudden death and leg problems in poultry; enteric disorders; vaccination failures and poor feed conversion rates. They feel that the development of an aflatoxin field test kit for feeds is imperative and have submitted a proposal to the EEC this effect (see Section 6.5.7).

The IAS has large animal housing facilities. The National Swine Performance Testing Centre is based at the IAS with a total sow population of 450. A hatchery with an incubation capacity of 10,000 and a small feed mill are also available.

Laboratory facilities include adequate general laboratory equipment, GC (no column) and rotary evaporator.

Researchers at the IAS feel that they possess the facilities and manpower (animal scientists, nutritionists, biochemists) necessary to conduct feeding trials on the effects of mycotoxins on animal health and production.

3.4.10 INTERNATIONAL RICE RESEARCH INSTITUTE

The International Rice Research Institute conducts no research on mycotoxins in the Philippines.

3.4.11 LADIES CHOICE PEANUT BUTTER COMPANY

The Philippines is second only to the United States in peanut butter consumption.

Ladies Choice Peanut Butter Company screens for aflatoxin in raw peanuts by TLC. Most peanut butter manufacturers in the Philippines do not screen for aflatoxins.

3.4.12 PRIVATE FEEDMILL COMPANY

Commercially available immunoaffinity column are used to screen for aflatoxin B_1 in raw maize, copra and rice bran by this particular feedmill company. Finished feeds are also tested for aflatoxin B_1 once a month.

3.4.13 PRIVATE MYCOTOXIN BINDER MANUFACTURING COMPANY

The manufacturers of a detoxification product called "Anti-tox Plus" indicated an interest in conducting, and partially funding, pharmacological studies in collaboration with the Bureau of Animal Industry (BAI) of the Philippines. The company, which has marketing representatives in Malaysia and Taiwan, is interested in proving the efficacy of its product for marketing purposes. BAI has already conducted efficacy trials on pigs using another mycotoxin binder.

This particular company had excellent access to international literature through its head office in Europe. Furthermore, the company is able to obtain data on the occurrence of mycotoxins in ASEAN grains by sending feed and feed ingredient samples to its European laboratories for analysis. In this fashion, survey data on the occurrence of toxins that are not assayed for in ASEAN countries, such as T-2, HT-2 and nivalenol has been collected and compiled. Veterinarians at local 'Anti-tox Plus' offices are extremely well educated in the potential adverse effects of mycotoxins, and because they are in contact with the farmers, are able to pass on this knowledge to the field level. This contact with the farmers provides a mechanism for a proactive linkage between clinical field problems and levels of mycotoxins in grains. The mycotoxin binder manufacturing industry represents a means through which the *results* of mycotoxin research can be utilized to directly benefit the farmer and therefore the consumer.

Previous studies by the "Anti-tox Plus" manufacturers have shown nivalenol and deoxynivalenol in Filipino maize. Finished Filipino feeds have been found to contain T-2 toxin, HT-2 toxin, zearalenone, nivalenol and deoxynivalenol. Clinical symptoms such as reproductive failures in pigs, stunted growth and leg problems in broilers have been observed by both the Anti-tox Plus representative and the Institute of Animal Sciences at the University of the Philippines at Los Banos.

Incorporation of an industry component, relating to the manufacture of detoxification products in feeds, into future mycotoxin research projects would benefit the general status of mycotoxin research since:

- this industry has direct contact with farmers and will thereby ensure that a *knowledge* of the potential adverse effects of mycotoxins in the field reach the farmer;
- this industry represents a means through which the *results* of mycotoxin research can be utilized to directly benefit the farmer and therefore the consumer; and
- manufacturers of detoxification products in feeds have a realistic knowledge of actual field problems and have veterinarians that can recognize mycotoxin induced clinical symptoms.

3.5 THAILAND

Crop production:
Maize production in Thailand is second only to rice in cereal grain production. Sorghum is produced for local consumption only. Copra, cassava and peanut meal is used locally and also exported. Raw peanuts are produced locally but are also imported from China, Burma and India.

Committees:
The National Committee on Mycotoxin Control in Agricultural Commodities was established in 1985 and is divided into three Sub-committees, each responsible for one of the three areas: Research and Development, Extension and Marketing. The Committee is composed of representatives from various government organizations, universities and the private sectors. The Committee is also responsible for the coordination of mycotoxin research.

Regulations:
The tolerance level established by the Food and Drug Administration of Thailand is 20 ppb in foods.

3.5.1 MINISTRY OF AGRICULTURE AND COOPERATIVES
DEPARTMENT OF LIVESTOCK DEVELOPMENT
•NATIONAL ANIMAL HEALTH AND PRODUCTION INSTITUTE
TOXICOLOGY AND BIOCHEMISTRY SECTION
•VETERINARY PUBLIC HEALTH DIVISION
•FEED QUALITY CONTROL DIVISION
REGULATION AND STANDARDIZATION SECTION

NATIONAL ANIMAL HEALTH AND PRODUCTION INSTITUTE:
The National Animal Health and Production Institute (NAPHI) was founded in 1986 largely with funding from the Japanese International Cooperation Agency (JICA), in order to conduct research and diagnostic activities to improve animal health and productivity. Since then, NAPHI has participated in a Thai-Japanese Technical Cooperation Program which has provided counterpart training in Japan, as well as long and short term Japanese consultancies.

A system of nine provincial veterinary diagnostic centres in Thailand is currently being reorganized to fall under NAPHI. When this is finalized, NAPHI will diminish its diagnostic service responsibilities and assume research as a priority. In addition, NAPHI will take on the role of a reference centre for national livestock and will act as a centre for national and international technology transfer.

NAPHI currently operates a service arm wherein farmers or provincial veterinarians can send in feed and tissue samples for aflatoxin analysis. Feed samples are analyzed first by TLC and subsequently by HPLC in positive samples. In collaboration with the Feed Quality Control Division of the Department of Livestock Services, researchers at NAPHI have conducted a comparative analysis of AOAC column clean up and sep-pak silica cartridge methods with subsequent detection by TLC and HPLC.

Aflatoxin B_1 antibodies have been produced at NAPHI by injecting rabbits with Bovine Serum Albumin conjugate. In feeds the binding efficiency for aflatoxin B_1 is as low as 0.1 ppb but less efficient for aflatoxin B_2. The system has been used to determine aflatoxin B_1 in broiler and cattle feed.

NAPHI conducted a comparative determination of feeds spiked with aflatoxin B_1 using HPLC, ELISA and TLC densitometer. Recoveries for ELISA, HPLC and TLC were 98%, 95% and 82% respectively. This was a collaborative study with the Japanese.

A recent collaborative study with the Japanese involved the detection of aflatoxin B_1 in the plasma of fowl consuming feed naturally contaminated with aflatoxin B_1. HPLC and ELISA methods were compared. The ELISA method was found to have a lower limit of detection.

The Toxicology and Biochemistry Section of NAPHI has recently developed an ELISA method for the detection of aflatoxin B_1 in cattle and buffalo sera by using microtitre plates. The limit of detection was found to be lower than 10 ppb.

A collaborative study with the Feed Quality Control Division and the Disease Control Division, both of the Department of Livestock Development, was conducted in which 300 samples of chicken livers, kidneys, breast and leg muscle were collected and analyzed for aflatoxins. Levels in positive samples were all lower than the five ppb allowed in human food.

A major thrust of aflatoxin research at NAPHI has involved studies on the effects of a variety of binding agents, mycoinhibitors and detoxification agents on aflatoxin residues in chickens and ducks administered feeds containing these agents. Residues in tissues (muscle, eggs and liver), histopathological changes, serum glutamic oxaloacetic transaminase (SGOT) activity, serum glutamic pyruvic transaminase (SGPT) activity and egg production were studied. Propionic acid, calcium

propionate, ammonium carbonate, diatomaceous earth, polyvinyl pyrolidone, polyplasdon XL-DE and Anti-tox Plus were the agents used in these feeding trials. Most of these studies have been collaborative efforts with the Department of Animal Sciences at Kasetsart University and the Feed Quality Control Division of the Department of Livestock Development.

The Systematic Diagnosis Research Division of the National Institute of Animal Health in Japan has also conducted collaborative research with the Toxicology and Biochemistry Section of NAPHI. Studies have examined the activity of cytochrome b_5, NADPH cytochrome *c* reductase, aniline hydroxylase and NADH ferricyanide reductase in liver microsomes of ducklings injected with aflatoxin B_1. In another study with the Japanese, histopathological changes in the livers of duckling intramuscularly administered aflatoxin B_1 were examined.

NAPHI considers the study of aflatoxins and aflatoxin residues as a priority for Thailand. Researchers indicated a desire to further research on the carcinogenicity of aflatoxins and their metabolites. Reports of animal illnesses from veterinarians in Thailand lead NAPHI researchers to believe that zearalenone and trichothecene investigations in feeds may be a valuable exercise.

The NAPHI laboratories are well outfitted with new equipment that was generously donated by JICA when the Institute was established in 1986. Equipment in the Toxicology and Biochemistry Section laboratory includes TLC and densitometer, HPLC, spectrofluorometer, spectrophotometer, densitometer for blood serum electrophoresis, rotary evaporators, safety hoods, zone inhibition reader for microtitre plates and excellent general laboratory equipment. Animal housing facilities for cattle, sheep and poultry and a pathology laboratory are also available.

Scientists emphasized the expense of chemical reagents and standards as inhibitory to the conduct of mycotoxin research. Human resources were indicated as a constraint due to the length of time required to perform extractions. Additional safety hoods and equipment such as a more sensitive HPLC, that would detect aflatoxin to 0.01 ppb, would facilitate future research.

NAPHI claims to have the most complete library for human and animal mycotoxin information in Thailand. NAPHI also has access to the Mahidol University Library which is apparently the best for plant mycotoxin information.

NAPHI acts as a training centre and is currently hosting trainees from six different developing countries who are funded by organizations such as FAO or UNDP. It has a capacity to take on an advisory role for university students and can provide training sessions for the private sector. NAPHI can also interact with universities, other government departments and international institutions in collaborative research.

VETERINARY PUBLIC HEALTH DIVISION:

Although this Division does not conduct mycotoxin analyses, it does conduct routine mycological inspections of meat. Because Aspergillus flavus, A. fumigatus and A. bracus(?) are frequently isolated, the Division feels it would be useful to screen for mycotoxins in meat. An HPLC with fluorescence detection is available for this purpose but the Division would need to obtain training for mycotoxin detection in tissues if this were to be undertaken.

FEED QUALITY CONTROL DIVISION:

The Regulation and Standardization Section of the Feed Quality Control Division is responsible for the routine screening of raw and complete feed for aflatoxins B_1, B_2, G_1 and G_2. Maize, groundnut and soybean are the most important feed ingredients. Analyses are conducted first by TLC and followed by HPLC in positive samples.

The Director of this Section sits on the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in which the subject of mycotoxins is often discussed.

Researchers have had reports of decreased milk production in dairy cattle and therefore would like to obtain training in the analysis of ochratoxin A, zearalenone, T-2 toxin and mycological training.

The Regulation and Standardization Section has a microbiology laboratory and a biochemistry laboratory. Equipment available in these laboratories includes a laminar flow hood, shaker for culture flasks, incubator, safety hood, rotary evaporator, TLC UV light chamber, densitometer and integrator, GC with FID detector and integrator, GCMS, HPLC with UV and fluorescent detector, temperature controlled ultracentrifuge.

The expense and difficulty in importing standards were identified as constraints to mycotoxin analyses.

Due to a high demand for analytical equipment within the section, university students have not been accepted for training in the past.

3.5.2 MINISTRY OF AGRICULTURE AND COOPERATIVES
DEPARTMENT OF AGRICULTURE
•PLANT PATHOLOGY AND MICROBIOLOGY DIVISION
•DIVISION OF AGRICULTURAL CHEMISTRY

PLANT PATHOLOGY AND MICROBIOLOGY DIVISION:
The Department of Agriculture has been involved in a number of international projects related to fungi and mycotoxin research in which the Plant Pathology and Microbiology Division has been active.

The UNDP and the Government of Thailand initiated in 1984 a joint project on the Reduction of Post-Harvest Losses in Stored Grains. Several international consultants were involved to provide expertise in warehouse management, microbiology/mycology, inspection, sampling and analysis of aflatoxin. One such consultancy provided in depth training to Thai personnel in the methods for isolation, identification, enumeration, handling and disposal of food borne toxigenic molds. More than 24 species of Aspergillus, 12 species of Penicillium and ten other mold genera were isolated from maize, beans, vegetables, flour and soil. Stressed in the identification process were the ability to recognize the macroscopic, the microscopic, and the diagnostic characteristics of each species. Limited training was also provided in the areas of laboratory quality assurance and safety.

A second consultancy involved the set up of studies to assess the resident aflatoxigenic fungal populations in and around maize from crop maturity in the field through storage in warehouses of regional traders. Maize samples were collected from shops, warehouses, drying platforms, processing stations, silos, fields (cobs, stalks, debris and soil), hill farm maize fields and storage areas. Fungal spore populations in the air of fields during harvest and around the C warehouses were assessed as were population of mycotoxin producing fungi carried by the maize weevil from infested maize. Representative pure cultures of each fungus species detected in the various isolations were prepared for inclusion in the Departments fungal culture collection. Diplodia zeae was the major pathogen attacking Thai maize. Fusarium moniliforme, F. semitectum, and Penicillium citrinum were found in all maize samples and it was suggested that they may present a greater potential contamination problem than Aspergillus flavus. A. flavus in the air was detected on and around stored maize, in fields near piles of ears but not in harvested fields. Weevils carried a significant body burden of A. flavus and F. moniliforme spores.

A third consultant undertook a review of the extent of aflatoxin contamination in maize and groundnut in various geographic locations and farming systems. Harvesting, drying and storage practices leading to aflatoxin contamination were assessed and control measures including training, handling, sampling, inspection and analysis recommended.

An operational manual was developed for aflatoxin analysis during a fourth consultancy. In it were specified guidelines for safe handling and proper decontamination procedures, sampling procedures, sample size, criteria for handling and safe storage of samples, preparation of storage and aflatoxin standards, sample documentation and quality control techniques that are essential in conducting large scale surveys in a safe, efficient and reliable manner. In addition, an assessment of Thai maize marketing systems revealed several major factors influencing mold growth and aflatoxin production at the farm with respect to harvesting, shelling, storage and drying. Guidelines for the prevention of mold growth and aflatoxin contamination in high moisture maize were recommended for the early stages of the marketing system.

Phase I of a three phase project known as the Thai-British Maize Aflatoxin Project was completed in 1984. The aim of this project was the development and evaluation of methods of drying maize to control aflatoxin, especially during the high risk rainy season. Three drying systems incorporating mechanical driers were evaluated and their effectiveness was compared with sun drying. The following results were obtained:

- aflatoxin contamination in maize from Lopburi Province was low at harvest but increased significantly during temporary and longer term storage;
- mechanical drying was capable of "freezing" aflatoxin levels provided that the drying period does not exceed 48 hours; and
- aflatoxin monitoring of maize at 14% moisture content during storage established that both mechanically dried maize and sun dried maize can be stored for at least two months without any increase in aflatoxin over its initial level, provided that the storage conditions are good.

In a second phase of the Thai-British Maize Aflatoxin Project the overall objective remained to find a practical and economic solution to the incidence of aflatoxin in maize in Thailand. Further work was undertaken on the incidence of aflatoxin and its control in order to confirm the findings of Phase I and to relate them to another maize producing area of Thailand. Work on developing and evaluating the potential of the BGYF test as an aflatoxin quality control check was also undertaken. In a program of field drying trials, specific drying systems were designed, tested and evaluated for use by both farmers cooperatives and primary merchants. Whether maize, at moisture contents greater than 14%, could be stored for a short period of time without an unacceptable increase in aflatoxin content before final drying was determined. Studies were also undertaken to assess the economics of maize drying in the early post harvest period and to suggest what premiums would be necessary to encourage the production of high quality, low aflatoxin maize at all levels of the marketing chain.

The third and final phase of the project aimed to apply the findings of the first two phases within the production and marketing system of the Thai maize industry. The specific objectives were to:

- produce dry maize that is low in aflatoxin content;
- demonstrate that investment in drying equipment up country can be profitable;
- develop marketing systems that will promote quick release of maize after harvest; and
- demonstrate that farmers and merchants who cooperate to produce high quality, low aflatoxin maize will obtain higher rewards than obtainable from traditional systems.

The following approach was taken:

- selected merchants and buyers with suitable grain dryers were invited to participate;
- farmers and merchants were interviewed to establish the difficulties in changing their current practices;

- work was undertaken at the Asian Institute of Technology to develop a farm level sprayer to apply a chemical fungal inhibitor (propionic acid) to maize cobs in locations which are inaccessible in the rainy season;
- the economics of early selling of maize in a falling market and the attitude of farmers to early selling of maize were considered; and
- small laboratories were set up at one export silo and one up-country merchants godown to monitor aflatoxin levels (as supervised by the Thai-British project team).

Conclusions were drawn as to the price incentives existing for farmers, merchants and feedmillers, adequacy of sampling methods, drying and storage technologies, reliability of the BGYF test, efficacy of propionic acid as a fungicide in maize.

A five year USAID project was commenced in 1985 in collaboration with the one of the Thai Field Crops Research Institutes on the control of aflatoxin in maize, sorghum and peanuts. A maize crib designed for use in Africa was evaluated for appropriateness in Thailand but was found unsuitable. The use of minerals for heat absorption was tested for their ability to create aeration in a second solar crib dryer for the purpose of preventing aflatoxin formation. This having failed, ammonium polypropionate was used to treat maize for the control of aflatoxin and insect infestation during storage. Eleven other chemicals were tested for their efficiency in detoxifying or preventing aflatoxin production in stored maize. The project also involved the comparison of aflatoxin in maize dried using different methods (field drying, solar drying on concrete platform and mechanical drying). Operational costs of various fuels used in mechanical dryers were considered. The BGYF test was further examined for its potential as a presumptive test in indicating aflatoxin levels in grains. In addition, analytical methods for aflatoxin detection were improved to minimize costs of reagents and solvents. Varietal differences in aflatoxin formation in sorghum were studied. No differences in susceptibility were observed. Ammonium polypropionate and thiabendazole were shown to prevent aflatoxin formation. Control of aflatoxin in peanuts was investigated through improved storage and handling. A technology transfer component was incorporated into the project in which personnel from other government departments, private industry and universities were trained in mycotoxin detection using a variety of methods. A training program was also offered to extension officers in technology transfer.

A collaborative project with the Tropical Agriculture Research Centre of Japan between 1985-1990 emphasized ecology and control of aflatoxigenic fungi by determining when and how aflatoxin is produced. Aspergillus flavus and aflatoxin contamination in maize were surveyed in the field and at different stages of postharvest handling and processing. Aflatoxigenicity and sclerotium formation were compared in isolates from different locations. The regional distribution and seasonal changes of A. flavus in air and soil were also investigated in the field, warehouses and drying locations. Responses of growth and survival of A. flavus to changes in various environmental conditions (temperature, water activity) were studied. Inoculations were conducted in field maize to determine the relative susceptibility of maize at various stages of development.

An extensive project funded by the Japan International Cooperation Agency was undertaken by the Thai Department of Agriculture between 1986 and 1992 and aimed at expanding the technology to improve maize quality by preventing aflatoxin contamination. Long and short-term consultancies were arranged with staff from the Tropical Agriculture Research Centre in Japan. A very well equipped facility, known as the Thai Maize Quality Improvement Centre, was established in which studies were conducted to clarify the factors influencing aflatoxin contamination in maize and to develop technologies to eliminate these factors. Research was conducted in three components, namely, Microbe, Agronomy, and Postharvest. Research reports have been prepared describing in detail the studies undertaken in each component. Only the titles of the studies are listed below but more information can be obtained from the Plant Pathology and Microbiology Division of the Department of Agriculture.

Microbial studies:

- Study on the growth and aflatoxin production by Aspergillus flavus from maize, air and soil in the maize field;
- Water activity of Thai maize and growth of Aspergillus flavus;
- Equilibrium moisture content of Thai maize and growth of Aspergillus flavus;
- Aspergillus flavus infection and aflatoxin contamination of maize in Thailand;
- Changes of aflatoxin content in stored wet maize;
- Field infection and contamination of maize by Aspergillus flavus;
- Studies on Aspergillus flavus and aflatoxin contamination during sun-drying in the middleman and laboratory scale;
- Aspergillus flavus in insect damaged cobs;
- Relation between BGYF (Bright Greenish Yellow Fluorescence) and aflatoxin B_1 content in maize;
- Simplified rapid screening method for aflatoxin in maize "Improvement of minicolumn method";
- Application of coconut agar media to determine aflatoxin production ability of Aspergillus flavus;
- Control of Aspergillus flavus infection and aflatoxin content in high moisture content maize by ammonia treatment;
- Control of Aspergillus flavus and aflatoxin contamination by high moisture maize by airtight storage;
- Control of aflatoxin in maize with various moisture contents by airtight storage and the population dynamics of microorganisms involved; and
- Cultivation conditions and simple analysis methods for the detection of aflatoxigenic fungi.

Agronomy:

- Study on the relationship between environmental conditions and aflatoxin incidence in maize;
- Effect of different harvesting methods, moisture conditions and storage periods on aflatoxin contamination;
- Effect of plant density and nitrogen application on aflatoxin contamination in maize;
- Effect of nitrogen regarding prevention of aflatoxin contamination by the inoculation method;
- Effect of crop rotation on Aspergillus spp. in the soil;
- Large scale trial of harvesting methods on aflatoxin occurrence in storage;
- Varietal comparison maize kernel moisture and its variation according to different times of harvest; and
- Identification of insect role on aflatoxin occurrence on maize in Thailand.

Postharvest:

- Study on the relationship between damage on kernel, kernel moisture content and aflatoxin content;
- Study on the effect of grading ear maize prior to storage (estimation of increase of damaged kernel ratio during handling);
- Allowable duration for delay of drying in the post-harvest process of maize;
- Analysis of the internal environment of farmer's storage;
- Analysis of drying performance with blower;
- Stimulation of drying ear maize under ambient air ventilation;
- Survey on the present situation of farmer's and middleman's postharvest practices regarding maize in Thailand;
- Standardization of standard oven method (for drying);
- Calibration test of established moisture meter;
- Improvement and development of moisture meter;
- Performance test of established shellers for high moisture maize;
- Analysis of the relation between mechanical damage to kernel, machinery design, operational condition and moisture content;

- Maize sheller improvement;
- Experiment of dryer for drying ear maize in a solar house using engine-blower;
- Research on drying ear maize with vinyl plastic house;
- Study on the effect of in-store drying;
- Ammonia treatment of maize to control Aspergillus spp. and to prevent aflatoxin contamination;
- Urea treatment of maize to control Aspergillus spp. and to prevent aflatoxin contamination;
- Ammonia and sulphur dioxide supplemented ambient air drying of high moisture maize;
- Preliminary test of sulphur dioxide treatment with sulphur cake;
- In-store treatment of high moisture maize with sulphur dioxide; and
- Improvement of storage for high moisture ear maize.

Research on fungi and mycotoxins at the Thai Maize Quality Improvement Centre will continue by staff from the Department of Agriculture with an emphasis on the following crops in the following order of priority:

1- maize, groundnut, sorghum, copra
2- cassava, mung bean, dried chili
3- soybean, rice.

The following mycotoxins will be studied in the following order of priority:

1- aflatoxin
2- ochratoxin A
3- fusarium toxins, particularly zearalenone.

A collaborative project with the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Australia from 1989-1992 undertook an extensive survey of the mycological status of durable commodities in Thailand, with respect to both the fungal flora and mycotoxins. This study was funded by the Australian Centre for International Agricultural Research (ACIAR). Fungi were isolated, enumerated and identified from both high and low grade commodities and their toxigenicity determined. Thai food samples analyzed include maize, sorghum, mung beans, soybeans, wheat, paddy rice, cashews, peanuts, and copra. Duplicate samples were examined for fungi and mycotoxins in Australia. The following mycotoxins were analyzed initially by TLC and subsequently by HPLC in positive samples: aflatoxins B_1, B_2, G_1, and G_2, sterigmatocystin, cyclopiazonic acid, ochratoxin A, penicillic acid, verruculogen, citrinin, zearalenone, T-2 toxin, diacetoxyscirpenol, deoxynivalenol, neosolariol, nivalenol, alternariol, alternariol monomethyl ether and tenuazonic acid. Similar studies are currently being carried out in the Philippines and Indonesia. An important component of the project was to develop a database of fungi significant in Asian commodities, including incidence, mycotoxins and factors influencing toxin production. Training was provided in Australia to a microbiologist and a chemist through the project (see below).

A second phase of this project was commenced in 1991 to investigate the factors affecting invasion by Aspergillus flavus and aflatoxin formation in Asian peanuts. This project is a collaborative effort between the CSIRO, the Division of Plant Pathology and Microbiology and three of the network of Thai Field Crops Research Institutes (also under the Department of Agriculture) in Chiang Mai, Khon Kaen and Suphan Buri. In order to clearly establish the main times of A. flavus entry into peanut plants and soil, peanut crops are monitored for the presence of the fungus from the time of planting, through nut development, harvest and storage. At the same time the development of aflatoxin development is assessed. The comparative distribution of A. flavus, A. parasiticus and A. nomius in Thai peanuts and soils used for peanut cultivation is being determined. The efficacy of techniques currently under development in Australia for reducing A. flavus invasion under Asian field conditions will be assessed. If they are successful, field trials will be conducted.

Research on mycotoxins at the Plant Pathology and Microbiology Division commenced in the 1970's. Since then research has continued under Department of Agriculture programs and funding. Maize, insect damaged maize, peanuts, chili, garlic, cassava, sorghum and copra have been surveyed for A. flavus and/or aflatoxin contamination. In some commodities, comparative analyses of contamination by the fungus and/or aflatoxin have been made at pre- and postharvest stages. Soil and air samples have also been examined for incidence of A. flavus and, in some cases, aflatoxigenicity of isolates. The occurrence of ochratoxin A and ochratoxigenic fungi in maize and peanuts has been investigated. Contamination in maize by zearalenone and zearalenone producing fungi has been determined. Scientists at the Division have been involved in studies on the effects of such factors as moisture content, relative humidity, temperature, stage of maturity, packaging, length of storage and anaerobicity on the growth and production of aflatoxin by fungi in maize. In addition, varietal differences in the accumulation of aflatoxin producing fungi and aflatoxin in field inoculated maize have been studied.

Several chemicals have been investigated for their potential to detoxify aflatoxin or to prevent growth and aflatoxin production in maize. These include methanol, ethanol, propionic acid, ammonium bis propionate and ammonia.

Ongoing research at the Plant Pathology and Microbiology Division includes:

- an investigation of chemicals that may be effective in the control of fungi and mycotoxin contamination;
- the factors influencing fungi and aflatoxin contamination in peanuts in the field;
- survey of fungi and mycotoxins associated with copra; and
- concurrent mycological and mycotoxin analyses in maize, peanuts and rice for some or all of the following mycotoxins: aflatoxins, ochratoxin A, zearalenone, citrinin, cyclopiazonic acid, moniliformin, nivalenol and deoxynivalenol.

Preliminary studies show the occurrence of Aspergillus, Penicillium and Fusarium in copra. Fusarium moniliforme is a common contaminant of maize.

Scientists at the Division are familiar with many analytical methods for mycotoxins and have been involved in the development of rapid screening methods for aflatoxins by minicolumn and BGYF in maize. The use of the minicolumn method for aflatoxin in maize developed there is currently being promoted for use by farmers and merchants. A field kit for aflatoxin analysis of peanuts is under development. A method for the production of aflatoxin antibodies in rabbits has also been developed. This was done with the view of starting commercial production of ELISA kits, however, human resources were limiting in this endeavour.

A training course is held every year at the Division on the control of mycotoxins and analytical methods for aflatoxin in maize using BGYF, minicolumn, TLC, HPLC and ELISA. The course has been developed for participants in various levels including researchers, extension workers, industry personnel and farmers. In addition, the Department of Agriculture will be the Thai counterpart for a series of training programs organized by FAO to be held in Thailand, the Philippines and India (see Section 6.4.2).

Through the various international research collaborations in which the Division has participated, researchers have obtained substantial expertise in various aspects of fungi and mycotoxin research methods. In addition to this, two scientists have undergone one month training courses in Australia, one in food mycology methods including identification of a wide range of food-borne fungi to species level, and the other in the analysis of zearalenone, ochratoxin A, citrinin, deoxynivalenol, nivalenol and cyclopiazonic acid by TLC and HPLC. The latter scientist has also had three months of training in Japan for identification of Aspergillus, Penicillium, and Fusarium.

Mycotoxin research by Plant Pathology and Microbiology Division scientists will be conducted mainly at the Thai Maize Quality Improvement Centre in the future. Facilities and equipment at the Thai Maize Quality Improvement Centre are outstanding. The Centre has been established as a research centre for research in mycotoxins and phytotoxins and therefore has all the equipment necessary to conduct up-stream research. Equipment includes: TLC with densitometer, HPLC with fluorescence and UV detection and integrator, 2 GCs, fluorescent and UV spectrophotometers, ELISA plate readers, shakers, rotary evaporators, safety hoods, photomicroscope, fluorescence microscope, phase contrast microscope, refrigerated ultracentrifuge, freeze drier with ampoule attachment, UV viewing chamber, -10 and -20 degree freezer, incubators, laminar flow hoods, cold room, media preparation room in addition to other excellent general laboratory equipment. Facilities and equipment available in the Plant Pathology and Microbiology Division laboratory are also excellent.

Researchers at the Division indicated that they felt it important to develop inexpensive drying technologies and rapid test kits for use by farmers and merchants. It was also felt that it is important to conduct surveys and to obtain additional training for the analysis of mycotoxins other than aflatoxin. In the future researchers would like to develop test kits mycotoxins other than aflatoxin.

The Department of Agriculture has an institutional mandate to interact with universities, other government departments and industry.

A fungal culture collection of approximately 200 isolates is maintained, mostly as oil submersed cultures. A freeze drier with ampoule attachment is available at the Thai Maize Quality Improvement Centre.

DIVISION OF AGRICULTURAL CHEMISTRY:
The Division of Agricultural Chemistry conducts routine screening for aflatoxins and ochratoxin A in raw and processed agricultural products for export, cereals, nuts, sorghum, soybean, and vegetable oils. Samples are either actively collected or sent in for analysis by manufacturing companies. Aflatoxins are first screened for by TLC and densitometer and then by HPLC in positive samples. TLC is also used in the analysis of ochratoxin A, T-2 toxin, zearalenone and patulin.

Research activities include:

- investigation of plant extracts for use as fungicides especially for Aspergillus flavus;
- determination of aflatoxin in medicinal plants;
- determination of aflatoxin in vegetable oil and oil seeds.

The Division of Agricultural Chemistry has a project with FAO on quality control of agricultural produce for export which has a small mycotoxin component.

It was felt that research in the Division should concentrate on aflatoxin but that the importance of T-2 toxin should also be investigated. The Division has not yet undertaken T-2 toxin research due to a lack of safety equipment.

3.5.3 MINISTRY OF PUBLIC HEALTH
•DEPARTMENT OF MEDICAL SCIENCES
FOOD ANALYSIS DIVISION

DEPARTMENT OF MEDICAL SCIENCES:
The Department of Medical Sciences of the Ministry of Public Health determines base line data for many residues and toxins in foods on which regulations can be based. They also compile daily intake charts for food contaminants.

The Food Analysis Division (FAD) of the Department of Medical Sciences is responsible for the monitoring and determination of base line levels of aflatoxins in food, feedstuffs, raw materials and medicinal plants as well as some animal products and tissues (eg. milk, chicken livers). Note that the responsibility for monitoring foods and feeds is not clearly defined between federal government departments in Thailand. Aflatoxins in foods and feedstuffs are analyzed by TLC whereas HPLC is used for milk and tissues.

Studies on cool season maize from Northern Thailand have revealed the existence of low levels of ochratoxin A and fungi commonly associated with European grains.

Surveys showed that feeds purchased from large feedmills were generally of better quality in terms of mycotoxin contamination than those feeds used by small farmers. Small farmers often produce their own feed or buy from small feedmills.

Feed samples associated with suspected mycotoxicoses in animals are analyzed at the FAD. Possible mycotoxin related problems have been reported in chickens and pigs. They include immunosuppression and fertility problems and tend to occur at the end of the rainy season and in the hot season.

FAD is currently conducting a study with the Faculty of Veterinary Science at Chulalongkorn University and the Department of Livestock Development to determine baseline levels of aflatoxin M_1 in milk. The study involves feeding trials in Dutch cows. They hope to determine good agricultural practices that can be passed on to small farmers. These practices will ensure low levels of aflatoxin M_1 in milk.

The FAD laboratory has experience in analyzing for mycotoxins other than aflatoxin (ochratoxin A, zearalenone, deoxynivalenol) by TLC in some commodities.

Researchers in the FAD laboratory have particular strength in analytical capabilities. They have modified many analytical methods (extraction solvents, mobile phase etc.) used in various commodities to the high temperature and humidity conditions in Thailand. A manual of modified analytical methods has been compiled for use in the FAD laboratory. In addition, the FAD laboratory has developed a rapid plastic minicolumn method that can be used in rural laboratories for determination of aflatoxin in food and agricultural products. FAD has conducted training courses in analytical methods for mycotoxins.

The FAD participates in the FAO/WHO crosscheck sample program for aflatoxins in food and milk and have submitted a request to participate in the ochratoxin A and deoxynivalenol crosscheck programs. They indicated that their aflatoxin M_1 analyses have not compared well with other participants and therefore feel that they would benefit from training in this mycotoxin as well as for Fusarium toxins and ochratoxin A. One technician is now in Japan undergoing a training course for the analysis of 'cool temperature' mycotoxins.

In the view of FAD researchers, aflatoxin research remains a priority for Thailand; however, they indicated the necessity in developing a further understanding of ochratoxin A contamination. Further research on Fusarium toxins pertaining to animal production would also be of interest. Researchers do not feel that Penicillium toxins are a problem in freshly harvested rice, but limited contamination may occur in rice that has been stored over long periods of time.

The FAD laboratory accepts graduate students every year for training in the analysis of aflatoxin and can also interact with other government departments. FAD keeps in close contact with the Thai Department of Agriculture.

Laboratory equipment includes a laboratory scale grinder, safety hoods, rotary evaporator, distillation apparatus, cold room, UV chamber for TLC with densitometer and integrator, two HPLCs with fluorescent detection and integrator.

3.5.4 MINISTRY OF SCIENCE, TECHNOLOGY AND ENVIRONMENT
THAILAND INSTITUTE OF SCIENTIFIC AND TECHNOLOGICAL RESEARCH

- **INDUSTRIAL METROLOGY AND TESTING SERVICE CENTRE**
  BIOCHEMICAL LABORATORY
  RESEARCH AND DEVELOPMENT GROUP
- **BIOTECHNOLOGY DIVISION**
  MICROBIAL RESOURCES CENTRE
- **INDUSTRIAL RESEARCH DIVISION**

INDUSTRIAL METROLOGY AND TESTING SERVICE CENTRE:
The Biochemical Labpratory of the Industrial Metrology and Testing Service Centre conducts aflatoxin analyses on processed foods and finished feeds for the purpose of issuing 'aflatoxin-free certification required by importing countries. Samples analyzed include honey and vegetable oil. This service is provided to manufacturers at a cost of approximately US$ 50 per sample.

Initial screening for aflatoxin is done by TLC or minicolumn with subsequent quantitative analyses by HPLC with UV detection in positive samples. The laboratory is equipped with good equipment including GC, TLC with densitometer, HPLC.

This laboratory participates in the FAO/WHO crosscheck sample program for aflatoxin.

The Biochemical Laboratory is not mandated to conduct training courses but has accepted university student for training in analyses other than aflatoxin. Researchers indicated that they would benefit greatly from training in zearalenone and Fusarium toxin analysis as well as sampling procedure.

BIOTECHNOLOGY DIVISION:
The Biotechnology Division of the Research and Development Group houses the Microbial Resources Centre (MIRCEN) which is one of the 24 members of a world network of MIRCENs partially funded by UNESCO and UNEP (see Section 4.5).

INDUSTRIAL RESEARCH DIVISION:
Reduction of aflatoxin in crude peanut and coconut oil has been successfully accomplished to less than 20 ppb with two types of bleaching earth (ie. AAA and Galleon).

3.5.5 NATIONAL CANCER INSTITUTE

The National Cancer Institute in collaboration with the Department of Biochemistry at Mahidol University has been involved in the determination of the effect of vitamin A deficiency on the *in vitro* metabolic activation of aflatoxin B_1.

The influence of diazepam on the carcinogenicity of aflatoxin B_1 was investigated in rats. The incidence of liver tumour development in rats treated with aflatoxin B_1 and diazepam was lower than in the group treated with aflatoxin B_1 alone.

3.5.6 KING MONGKUT'S INSTITUTE OF TECHNOLOGY
•FACULTY OF AGRICULTURAL TECHNOLOGY

FACULTY OF AGRICULTURAL TECHNOLOGY:
The Faculty of Agricultural Technology has investigated extracts of star anise in the growth inhibition in culture of seed-borne fungi isolated from soybean. Isolates tested included Aspergillus flavus, A. fumigatus, A. niger, Alternaria alternata, Fusarium solani, Penicillium terrestre and Curvularia lunata. The extracts were also tested for their activity in inhibiting growth and spore production of the isolated on inoculated soybean seeds.

3.5.7 •KASETSART UNIVERSITY
FACULTY OF VETERINARY MEDICINE
- •DEPARTMENT OF ANIMAL SCIENCE
- •DEPARTMENT OF VETERINARY PHARMACOLOGY
- •DEPARTMENT OF VETERINARY ANATOMY

FACULTY OF AGRICULTURE
- •DEPARTMENT OF PLANT PATHOLOGY
- •DEPARTMENT OF AGRONOMY

FACULTY OF AGRO-INDUSTRY
- •DEPARTMENT OF PRODUCT DEVELOPMENT
- •DEPARTMENT OF BIOTECHNOLOGY

FACULTY OF SCIENCE
- •DEPARTMENT OF BOTANY
- •DEPARTMENT OF GENETICS

•INSTITUTE OF RESEARCH AND DEVELOPMENT

KASETSART UNIVERSITY:
Kasetsart University has two campuses, one in Bangkhen in the outskirts of Bangkok and one in Kaphaeng Saen about 90 Km northwest of Bangkok.

DEPARTMENT OF ANIMAL SCIENCE:
Clinical studies are distributed in both campuses of Kasetsart University. Three animal hospitals are associated with the Faculty of Veterinary Medicine:

- the Small Animal Hospital at the Bangkhen Campus;
- the Large Animal Hospital at the Kamphaeng Saen Campus;
- the Large Animal Hospital in Nong Po District, Ratchaburi Province.

Survival rates were compared in broiler chick fed moldy maize that had been exposed to a variety of physical treatments including high temperatures, high pressures, washing and solar radiation. Solar radiation exposure was the most effective in detoxifying the moldy maize. Ammonia, hydrogen and oxygen were also found to be effective in the reduction of growth and feed conversion in broilers fed moldy maize. The Department has also found that supplementing broiler rations with 15% moldy maize had no adverse effect on growth, feed conversion or mortality.

A recent study by a graduate student at the Department of Animal Science examined the effects of dietary supplementation of aluminosilicates on the nutrient utilization and performance in broilers consuming aflatoxin contaminated feed.

DEPARTMENT OF VETERINARY PHARMACOLOGY:
A survey of aflatoxin in finished feeds, raw maize and raw soybean was recently conducted in collaboration with the Plant Pathology and Microbiology Division of the Department of Agriculture.

Farmers have sent feed samples to the Department for analysis when they have suspected mycotoxins to be implicated in animal problems. Farmers have reported symptoms such as reduced growth rates, diarrhea, jaundice, difficulty breathing and reproductive problems in livestock. Vaccine failures have been reported in chickens, ducks and pigs.

Researchers feel that aflatoxin analysis in maize and soybeans remains a priority. They would also like to commence analyses for aflatoxin in liver samples of animals.

A large animal veterinarian with the Department of Veterinary Pharmacology has studied in Germany where he conducted analyses for T-2 toxin, zearalenone and deoxynivalenol by HPLC. The Department of Veterinary Pharmacology will soon be obtaining an HPLC. Researchers would like to commence screening for T-2 toxin, zearalenone and deoxynivalenol in raw feeds. It was suggested that former students of the Department could facilitate in the collection of suspect samples from farmers since many now have jobs with pharmaceutical companies which provide for regular visits to farms.

Laboratory equipment consists of a safety hood, rotary evaporator and adequate general laboratory equipment. Researchers identified the cost of standards and HPLC columns as inhibitory to mycotoxin research.

DEPARTMENT OF VETERINARY ANATOMY:
Histopathological lesions in the kidneys and spleen in mice dosed with ochratoxin A either subcutaneously or orally were examined. In another experiment red blood cell and white blood cell counts were monitored in mice dosed subcutaneously and orally with ochratoxin A. The LD_{50} of ochratoxin A was determined in orally dosed mice. The pathology and pathogenesis in mice was also observed for sublethal doses and acute lethal doses.

DEPARTMENT OF PLANT PATHOLOGY:
A study at the Department of Plant Pathology compared the relative efficacy of three types of homemade solar dryers in reducing moisture content of maize to levels low enough to prevent contamination by A. flavus and aflatoxin. The maize was subjected to a number of primary processing procedures during testing.

A. flavus infestation and aflatoxin contamination was compared in maize kernels of various initial moisture contents that had been treated with imazalil. The duration of maize storage was also taken into account in the study. Bacterial and yeast isolates have also been investigated for their efficacy as biocontrol agents against A. flavus and aflatoxin contamination of maize kernels.

The International Development Agency (Ottawa: IDRC) supported research from 1986-1987 on the natural occurrence of aflatoxin in peanuts and its relative accumulation in various kernel parts. Peanut samples were collected from different parts of the country, both in the dry and rainy season, and analyzed for aflatoxin as well as for colonization by Aspergillus flavus. The results indicated a positive fungal correlation between colonization and aflatoxin content regardless of seed moisture content and sample origin. Colonization by A. flavus in the seed coat, cotyledon and embryo of peanuts varied greatly, whereas, most of the aflatoxin was concentrated in the embryo and cotyledon.

Also included in the IDRC supported studies was an evaluation of 61 groundnut lines for resistance to A. flavus. The appropriateness of several screening methods was determined. The role of A. flavus as a soil inoculum in the accumulation of aflatoxin in groundnut was also investigated. A positive correlation was found between the number of propagules in the soil and the level of aflatoxin in the seeds. Evaluation of the efficacy of chemical controls, crop rotation and solar soil sterilization in controlling the fungus showed that benomyl, both as seed and soil treatment was most effective. Five different concentrations of propionic acid, sodium pyrosulphite, potassium hydrogenate carbonate and sodium chloride were investigated for controlling the growth of A. flavus and reducing the level of aflatoxin in peanuts. Bisulphite salts of sodium were found to be effective. Some studies on the effect of chemicals in aflatoxin reduction have also been conducted in collaboration with private industry.

A follow up to the above studies aimed to examine the penetration of A. flavus from the pod to the kernel of peanuts. A. flavus infection of undamaged pods and pods that were artificially pierced were compared. In addition, pods from 29 cultivars of peanut were screened and evaluated for resistance to two strains of A. flavus after inoculation. Similar studies revealed that percent seed infection by A. flavus in seven groundnut cultivars was similar but that a strain of A. parasiticus was more virulent. When duration of seed storage was considered, the infection percentage increased with storage time. Older seeds had higher levels of aflatoxin B_1 than new seeds.

Field and laboratory techniques for screening for A. flavus infection and aflatoxin resistance in maize have been evaluated and are used in routine screening.

The Department has been involved in an effort to produce monoclonal antibodies to aflatoxin B_1 in rabbits. Because high titres were not obtained from in house experiments, anti-aflatoxin B_1 will be obtained from Japan for future studies.

Kasetsart University has field stations which can be used when experimental plots are needed. The Department of plant pathology has mycologists, plant pathologists, chemists and plant breeders on staff. Laboratory facilities were not visited but were described as modest. Laminar flow and media preparatory facilities are available. Analytical facilities and safety equipment are lacking. Research involving aflatoxin determinations, even by TLC, must be conducted in collaboration with institutions which have the necessary analytical capability or samples must be sent out for analysis at cost to the Department.

Researchers would like to increase their analytical capabilities through training. An interest in collaborative studies with international organizations was indicated in order to gain access to international expertise.

Kasetsart University is able to interact with other universities, government departments and private industry. The Department of Plant Pathology is interested in recruiting a graduate student to conduct studies in plant pathology with a minor in plant breeding.

DEPARTMENT OF AGRONOMY:

A Masters student conducted a study on the effect of planting date, harvesting date, shelling, moisture content and storage on aflatoxin B_1 contamination in maize.

Elite maize germplasm was screened for resistance to Aspergillus flavus and aflatoxin contamination in collaboration with the National Corn and Sorghum Research Centre (NCSRC) of Kasetsart University. Inoculation techniques were investigated during the study. In a further study from 1988-1990, also with the NCSRC, S_2 lines were bred and tested for yield. Superior resistance was reaffirmed.

DEPARTMENT OF PRODUCT DEVELOPMENT:
Tannin content in the seed coat of Thai peanut cultivars was investigated for its effect on growth inhibition of Aspergillus flavus on solid culture medium.

DEPARTMENT OF BIOTECHNOLOGY:
The Department of Biotechnology was involved in a mycological survey and enumeration of fungi associated with 177 local and exotic varieties of peanuts. A. flavus was found to be the most commonly occurring species.

DEPARTMENT OF BOTANY:
The Department of Botany in collaboration with the Department of Genetics are investigating the application of biotechnology to aflatoxin resistance breeding in maize. Plantlets were regenerated from cultured immature embryos but not from anther culture. Researchers are also familiar with protoplast isolation techniques.

DEPARTMENT OF GENETICS:
Yield, heterosis and heterobeltiosis were studied in F_1 hybrids of peanuts obtained by crossing cultivars resistant to seed infection by Aspergillus flavus.

INSTITUTE OF RESEARCH AND DEVELOPMENT:
Aflatoxin contamination was surveyed in agricultural products and animal feeds by TLC. 1,444 isolates of fungi were obtained from soil samples and agricultural products. These isolates were screened for ability to reduce aflatoxin contamination in ground maize samples that were either naturally contaminated or co-inoculated with aflatoxin-producing Aspergillus flavus. Rhizopus sp. and Trichoderma sp. were found to be effective in aflatoxin degradation.

3.5.8 CHIANG MAI UNIVERSITY

FACULTY OF MEDICINE
- **DEPARTMENT OF BIOCHEMISTRY**
- **DEPARTMENT OF MEDICAL MICROBIOLOGY**

FACULTY OF SCIENCE
- **DEPARTMENT OF BIOLOGY**

FACULTY OF ASSOCIATED MEDICAL SCIENCES
- **DEPARTMENT OF MICROBIOLOGY**

DEPARTMENT OF BIOCHEMISTRY:
Mycotoxin research has continued at the Department of Biochemistry of the Faculty of Medicine since 1969. Aflatoxin B_1 was surveyed in rice, peanuts, peanut products and chinese noodles by TLC and fluorometry. Aspergillus flavus isolates were assayed for toxigenicity. Some Fusarium species were also isolated from these samples. The Department of Biochemistry has a service component to test for aflatoxin in foods and feeds on request but not on a regular basis.

Chemicals, plant extracts and other factors have been investigated for their inhibitory effect on the growth, and/or aflatoxin production, and/or detoxification by Aspergillus spp. in culture in maize, mung bean and roasted peanuts. Chemicals and other factors tested include carbonate, calcium hydroxide, hydrogen peroxide, ammonium benzoate, ammonium bicarbonate, propionic acid, sorbic acid, benzoic

acid, gamma irradiation, sunlight, UV light and temperature. In some cases, extracts of treated and untreated samples were assayed for mutagenicity by the Ames test or for toxicity by chick embryo assay or rats' feeding trials. Inhibition of fungal growth was sometimes confirmed by the incorporation of ^{3}H-thymidine into mycelia.

Aqueous and organic extracts of several medicinal plants have been investigated for their capacity to inhibit aflatoxin B_1-induced mutagenesis in the Ames test. Researchers suggest that certain extracts may exert their inhibitory effect on aflatoxin B_1 mutagenesis by suppression of *in vitro* metabolic activation of the mutagen.

Metabolic studies on the urinary excretion patterns of aflatoxin and its metabolites in rats orally administered with aflatoxin B_1 have been conducted by graduate students at the Department of Biochemistry. Kinetic data generated from these studies may be useful for epidemiological studies of human and animal aflatoxin exposures from staple foods. In addition, the study resulted in the adaptation of a sensitive, economical TLC method of analysis for aflatoxins in urine.

Aflatoxins B_1 and M_1 in tissues and excreta of Thai children who died after consuming rice from acute encephalopathy and fatty degeneration of viscera have been determined by investigators from the United States at Massachusettes Institute of Technology. Chiang Mai University is associated with Maharaj Nakorn Chiang Mai Hospital from which clinical and specimens can be obtained. The Department of Biochemistry, in collaboration with the Department of Pathology, has also been active the study of the relationship between pathological changes and aflatoxin in human liver diseases. Pathomorphological changes in liver-autopsied tissue obtained from normal and liver-disease patients were studied for correlation to the presence of aflatoxin in nuclear DNA fractions of liver. Aflatoxin analyses were conducted by TLC. Primary liver cancer patients in the hospital were questioned as to their eating habits.

Aflatoxins in liver and urine samples of cattle that had died of suspected aflatoxicosis were analyzed. Aflatoxins were found in these tissues as well as feed samples. The outbreak was detected by the Huaykeo Livestock Animal Husbandry Station in Chiang Mai. The animals were autopsied for histopathological abnormalities and it was concluded that the probable cause of death was aflatoxin contaminated feed.

Biochemical studies on the effect of aflatoxin B_1 on haemoglobin synthesis and DNA synthesis in bone marrow cells and hepatic nucleated red blood cells from guinea pigs and rats suggest that metabolic activation for aflatoxin B_1 is necessary to elicit an erythropoietic effect.

In an effort to develop possible early diagnostic methods for aflatoxin exposure, aflatoxin B_1 has been investigated in the Department for its effects on plasma lipoproteins and erythrocyte morphology in sublethally dosed rats. Electrophoretic patterns of proteins in aflatoxin B_1 treated toad tadpoles have also been studied for this purpose.

The Department of Biochemistry has been involved in the collection of rice samples associated with 'yellow rain'. One of the biochemists in the Department will be going to study mycotoxin toxicology at the National Centre for Toxicological Research in the United States.

In 1992, a three year project to develop a rapid ELISA screening method for selecting Aspergillus resistant maize cultivar was commenced. This project is funded by the Thai Ministry of Science and Technology Development Agency. The project was undertaken in collaboration with the Faculties of Medical Technology, Agriculture, Science and the Chiang Mai Field Crops Research Centre. Fungal infection and aflatoxin contamination will be determined in an extensive number of maize cultivars that have been field inoculated with Aspergillus flavus and Aspergillus parasiticus. Simple, specific and

sensitive commercial ELISA kits will be used in the aflatoxin detection. The results will be compared with *in vitro* growth rates of Aspergillus flavus and Aspergillus parasiticus on maize seeds, and with the field infection tests. A quick, economical strip ELISA kit for aflatoxin B_1 will then be developed at the Department of Biochemistry.

The Department of Biochemistry is equipped with HPLC (no fluorescence detector), rotary evaporator, safety hood, fluorescence microscope, inverted microscope, NMR, ELISA equipment including plate reader and hybridoma production facilities, tissue culture facilities, laminar flow hood, scanning electron microscope, TLC and fluorotoximeter, spectrophotometer, lyophilizer with ampoule attachment, CO_2 incubator, -70 degree freezer, gamma counter and good general laboratory equipment.

The Department of Biochemistry has diverse mycotoxin expertise including the areas of biochemistry, molecular biology, detoxification methods, chemistry and toxicology.

Researchers identified the time required in obtaining reagents and lack of certain equipment (densitometer and HPLC fluorescence detector) as a barrier to research.

DEPARTMENT OF MEDICAL MICROBIOLOGY:
A small culture collection for fungi associated with human diseases is maintained by the Department of Medical Microbiology.

DEPARTMENT OF BIOLOGY:
In collaboration with the Department of Biochemistry a study was conducted to determine the effects of aflatoxin B_1 on implantation and parturition in orally dosed pregnant rats.

In collaboration with the Department of Biochemistry studies were conducted on intraperitoneally aflatoxin dosed rats to compare the change of protein and non-protein bound thiol groups to alkaline phosphatase activity. Changes in alkaline phosphatase activity was suggested to be an indicator of aflatoxicosis since its activity was significantly higher in aflatoxin treated rats.

DEPARTMENT OF MICROBIOLOGY:
A mycological study of molds present in ground, roasted peanuts revealed Aspergillus flavus as the commonly isolated fungus. Aflatoxin was also analyzed in the samples. Only six isolates of A. flavus were found to be aflatoxigenic. Other surveys have been conducted on market foods and feedstuffs collected from Chiang Mai. Aflatoxin production could be inhibited by extracts of clove, white and black pepper, garlic, Kaempfria Calonga and sodium benzoate. Aflatoxin analyses were conducted by TLC.

The capability of Aspergillus flavus to grow and produce aflatoxins in grapes, coconut, papaya, sapodilla and banana was studied. All were found to be good substrates for fungal growth with the exception of sapodilla.

3.5.9 CHULALONGKORN UNIVERSITY
FACULTY OF SCIENCE
•DEPARTMENT OF BOTANY
•DEPARTMENT OF PATHOBIOLOGY

DEPARTMENT OF BOTANY:
Graduate studies conducted at Chulalongkorn University include a determination of storage fungi causing dry rot of garlic bulbs. Of the twenty-eight isolates of fungi obtained from garlic in various parts of the

country, six were claimed to produce aflatoxins when tested by TLC. These included Aspergillus fumigatus, A. wentii, and A. cervinus. Other fungi isolated include Penicillium notatum, P. funiculosum, Fusarium solani, F. moniliforme and A. versicolor.

DEPARTMENT OF PATHOBIOLOGY:
Collaborative studies at the Department of Pathobiology were aimed to determine the effect of dietary protein and vitamin B_{12} on the lethality and carcinogenicity of aflatoxin in rats. In chronic aflatoxicosis, the livers of rats fed a low protein and aflatoxin B_1 contaminated diet exhibited more severe lesions than those fed a high protein, aflatoxin B_1 contaminated diets. For carcinogenicity of aflatoxin, the results showed a high incidence of aflatoxin-induced hepatoma in the high protein group compared to the low protein group. Vitamin B_{12} supplementation was found to enhance the induction of both hyperplastic nodules and hepatoma by aflatoxin in the high protein group.

3.5.10 MAHIDOL UNIVERSITY

FACULTY OF SCIENCE
- **•DEPARTMENT OF PATHOBIOLOGY**
- **•DEPARTMENT OF PHYSIOLOGY**
- **•DEPARTMENT OF MICROBIOLOGY**
- **•DEPARTMENT OF BIOCHEMISTRY**

FACULTY OF MEDICAL TECHNOLOGY
- **•DEPARTMENT OF CLINICAL MICROBIOLOGY**

RAMATHIBODI HOSPITAL
FACULTY OF MEDICINE
- **•RESEARCH CENTRE**

Mahidol University has a good library for plant mycotoxin information and has CD-ROM facilities.

DEPARTMENT OF PATHOBIOLOGY:
The Department of Pathobiology has been active in mycotoxin research since 1967 when a project was established with the aflatoxin researchers at the Massachusettes Institute of Technology. The first objective of the study was to determine the number of toxigenic fungi in foods destined for local consumption. The second purpose was to determine the distribution of aflatoxins in various foods and geographic regions of Thailand. The third task was to investigate a possible correlation between aflatoxin ingestion and the incidence of liver cancer in the population. This last study involved the sampling of foods (peanuts, maize, chili, millet, dried fish, mung beans and rice) from representative families and analyzing for aflatoxin. The estimated aflatoxin intake from food and crude liver cancer rate showed that an increased intake of aflatoxin may be associated with liver cancer incidence.

The Department of Pathobiology has also been active in the determination of levels of aflatoxins in feeds associated with outbreaks of suspected aflatoxicoses in domestic animals. Pathological studies were conducted on these animals where clinical evidence of aflatoxicosis and high levels of aflatoxin contamination in feeds were found. Field cases of suspected aflatoxicoses have been investigated in layers, dogs, cattle, ducks, pigs and horses.

Histopathological lesions and aflatoxin contamination in feed and tissue samples were investigated in two cases of suspected aflatoxicosis in horses. Lesions included encephalomalacia of cerebral hemispheres, fatty degeneration, necrosis, bile duct hyperplasia, fibrosis of the liver, fatty infiltration of the kidney, haemorrhagic enteritis and myocardial degeneration. Aflatoxin B_1 and B_2 were detected in feed samples, whereas only aflatoxin B_1 was detected in liver samples of these horses.

Various groups of agricultural commodities for export were surveyed for aflatoxin B_1 contamination including rice and rice products, food crops, garden crops and fruits, oil seed products and animal feed. Groundnut and maize were found most frequently and most highly contaminated with aflatoxin. Herbal drugs from Bangkok drug shops have also been analyzed for aflatoxins. Ochratoxin A has been analyzed in maize based duck feed. The department does not feel that ochratoxin A is an important mycotoxin in Thailand.

Much of the mycotoxin research at the Department of Pathobiology has involved feeding studies to determine the toxicological and histopathological effects of aflatoxins and other toxins in animals. Cultures of Aspergillus flavus, A. ochraceous, Penicillium species and Fusarium species have been grown in culture and their respective toxins extracted and used in the feeding trials. Much of the aflatoxin research has been published in international journals. The Department feels several constraints in the conduct of this work including danger to laboratory workers, expense, lack of support from the government and the time expenditure required.

Fungi that had been isolated in previous studies from market foods and foodstuffs were re-isolated, cultured on rice and screened for their toxicity and pathological changes produced in rats dosed intragastrically or intraperitoneally with culture extracts. Extracts from Aspergillus niger, A. niger, A. ochraceous, A. clavatus and Penicillium citrinum were found to be severely toxic. Histopathological changes in liver, kidney and blood vessels in rats treated with crude toxins from A. clavatus were similar to the effects reported on cytochalasin E. Other organs affected included liver, kidney, heart, lung, spleen and salivary gland depending on the species from which the extract was obtained.

A similar study was conducted using extracts from 40 strains of Aspergillus flavus. Aflatoxigenicity of the strains in rice culture was determined by TLC with blue and green fluorescent spot detection. Extracts were administered orally to rats by intubation and histopathological changes were noted.

Another experiment involved the determination of testicular degeneration in rats following dietary administration aflatoxin B_1.

Trichothecenes have been used in the conduct of feeding trials on rats. The Department feels it is important to develop animal models for trichothecenes (eg. shrimp bioassays and dermal applications).

The Department of Pathobiology has spent a considerable amount of time studying liver cancer induction in rats by aflatoxin. Many of these studies are cooperative efforts with the Faculty of Medicine at Ramathibodi Hospital, a hospital associated with Mahidol University. One such study conducted in collaborative with the Japanese aimed to determine the single oral dose-response effect of aflatoxin B_1 on rapid liver induction in two strains of rats. Levels of serum glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, albumin and protein were monitored along with the incidence of altered cell foci, neoplastic nodules and well differentiated hepatocellular carcinomas were monitored.

Several agents have been investigated in the Department for their potential in reducing aflatoxin induced liver cancer in experimental animals. Alpha benzene hexachloride (BHC) has been studied for its possible inhibitory effect on aflatoxin B_1 induced hepatocarcinogenesis in rats. Liver morphology, blood chemistry and haematology in rats fed BHC and aflatoxin B_1 diets were compared with rats fed aflatoxin B_1 diets only. The effects of Plasmodium berghei infection on the development of liver tumours induced in rats by aflatoxin B_1 has also been examined. These rats were intraperitoneally injected with parasitized red blood cells prior to administration of dietary aflatoxin B_1. Results suggested a reducing effect of P. berghei infection on aflatoxin induced liver tumours. A similar study showed that aflatoxin B_1 is less effective in the induction of cholangiocarcinoma in hamsters infected with Opisthorchis viverrini than controls.

Several studies have been conducted on the enhancing effects of several agents in aflatoxin induced liver cancer in rats. Some of these studies were conducted in collaboration with a number of other university Departments including the Department of Physiology at Mahidol University, the Department of Pathology at Ramathibodi Hospital and the Department of Clinical Microscopy at Khon Kaen University. Ultrastructural alterations in hepatocytes, acute hepatotoxicity and hepatic fat accumulation has been studied in rats pretreated with ethanol and administered with aflatoxin B_1. Another experiment suggested that diethylnitrosamine potentiates the hepatocarcinogenesis induced by aflatoxin B_1 in rats. The malarial parasite Plasmodium berghei was also suggested to facilitate liver tumour development initiated by aflatoxin B_1 in rats.

The mycotoxin research laboratory at the Department of Pathobiology has been established for over twenty years and is well equipped.

DEPARTMENT OF PHYSIOLOGY:

The Department of Physiology has been involved in several studies to determine the population of toxigenic fungi in market foods and foodstuffs. The occurrence of aflatoxins and ochratoxin A in some of these samples has been determined. In many cases the toxigenicity and toxicological evaluations of crude toxins produced by representative strains of isolates (Aspergillus niger, A. alliaceous, Claviceps sp., Penicillium sp.) were conducted.

Glucose was studied as a sole carbon source for the production of aflatoxins in liquid spore and mycelium cultures of Aspergillus flavus. Aflatoxin production increased with concentration of glucose.

Researchers at the Department of Physiology have been involved in some of the feeding trials using aflatoxin with the Department of Pathobiology. In addition to some of the studies on aflatoxin mentioned in the above section, the Department of Physiology has investigated the aflatoxigenicity of Aspergillus flavus var. columnaris. Toxicological studies have been conducted with aflatoxin to determine embryonic toxicity and behavioral changes in rats. The influence of pretreatment by a number of solvents and chemicals on the hepatotoxic effects of aflatoxin B_1 in rats has been investigated.

Research on cytochalasins was commenced in the mid-1970's. Cytochalasin E and two tremorgens were isolated and purified from Aspergillus clavatus. Subsequently, a series of studies have been conducted on experimental animals to determine the toxic effects of several of the cytochalasin mycotoxins. Effects studied include fluid balance changes; inhibition of intestinal phenylalanine, fructose, galactose, glucose, maltose and sucrose absorption; changes in plasma volume and protein concentration; inhibition of glucose transport in skeletal muscles; inhibition of glucose transport in red blood cells; H^+ and pepsin secretion; ultrastructural changes in the intestinal absorptive cells and dermal capillaries; inhibition of motility of epididymal spermatozoa; and stimulation of gastric mucus and protein secretion.

With the objective of developing a skin bioassay as a semiquantitative estimation for mycotoxins the dermal effects were observed in day-old mice injected with cytochalasins A, B, C, D, and E; patulin; roridin A; verucarin A; aflatoxin B_1; citrinin; diacetoxyscirpenol; leuteoskyrin; rubratoxin B; sterigmatocystin; T-2 toxin and zearalenone.

The principal mycotoxin researcher at the Department of Physiology has recently accepted a position at Rangsit University. Because Rangsit University is new, equipment for mycotoxin research is lacking. However, this particular scientist is very interested in continuing studies on the effect of ethanol pretreatment on aflatoxin B_1 induced hepatocarcinogenicity in rats.

DEPARTMENT OF MICROBIOLOGY:
Aflatoxigenicity of an isolate of Aspergillus flavus var. columnaris obtained from soysauce koji was found to be undetectable when cultured on glutinous rice or soybean substrate.

DEPARTMENT OF BIOCHEMISTRY:
The inhibitory effect of garlic extracts on the growth, spore germination and aflatoxin production of Aspergillus flavus was investigated by a graduate student at the Department of Biochemistry.

DEPARTMENT OF CLINICAL MICROBIOLOGY:
Crude extracts of toxins isolated from Fusarium sporotrichioides cultured on rye were investigated for their ability to inhibit the growth of Kluyermyces fragilis.

RESEARCH CENTRE:
In addition to some of the collaborative studies with the Department of Pathobiology, the Research Centre at Ramathibodi Hospital has conducted studies on the binding of aflatoxin B_1 to DNA in the colon of vitamin A deficient rats. The finding that DNA of colon mucosa in vitamin A deficient rats bound more aflatoxin B_1 than the normal vitamin A rats suggests that vitamin A may play an important role in the prevention of chemical carcinogenesis.

Researchers have also investigated the cytogenetic effect of aflatoxin on the life cycle of lymphocytes. Human lymphocytes in various phases were treated with aflatoxin and chromosomal aberrations were observed.

3.5.11 THAMMASAT UNIVERSITY
FACULTY OF ECONOMICS

FACULTY OF ECONOMICS:
A graduate student undertook a study aimed at evaluating the social costs and benefits of solving the aflatoxin problem in maize in the Thailand. The social costs of the various methods proposed for preventing or detoxifying aflatoxin were considered, namely, mechanical drying, modification of the cropping pattern and chemical control. The comparison of results showed that mechanical drying is the method which has the highest possibility from a social point of view, whereas, modification of the cropping pattern is not worthwhile under the existing technological frontier. Previous scientific studies on chemical control in Thailand were not found to confirm the effectiveness of this method. It was found that the most important constraints to solving the aflatoxin problem, were technological problems in methods suggested in the prevention and reduction of aflatoxin in maize. Therefore, the study suggested that research should concentrate on developing more effective, low cost methods and models than those proposed at the present. Also recommended was the development of aflatoxin detection methods which could be applied easily by traders.

3.5.12 OFFICE OF ATOMIC ENERGY FOR PEACE

The Office of Atomic Energy for Peace (OAEP) has investigated the aflatoxigenicity of variants of Aspergillus parasiticus produced by gamma irradiation. Suppression or enhancement of toxigenicity of variants was investigated in sterile and non-sterile rice cultures that had been co-inoculated with parent spores. The toxicity of a high aflatoxin producing variant was significantly suppressed by even one percent of the parent spore in irradiated rice as determined by mammalian cell bioassay. In another experiment, it was found that competing flora play a role in aflatoxin production by A. parasiticus in rice.

The OAEP has also been involved in international collaborative research to determine the effects of irradiation on ochratoxin production in toxigenic fungi.

3.5.13 CHAROEN POKHPAND FEEDMILL COMPANY

Aflatoxin in screened for by BGYF test or TLC in maize, peanut, cassava, sorghum, sesame and copra. Systematic screening is done in high risk commodities and randomly in others. Charoen Pokhpand laboratories in other Southeast Asian countries also screen for aflatoxin by BGYF. Technicians at the laboratory in Thailand have received training in minicolumn methods for detection of some other mycotoxins. They are familiar with TLC and minicolumn methods of analyses for T-2 toxin, zearalenone, deoxynivalenol and ochratoxin A. The laboratory is equipped with an HPLC with UV, florescence and refractive index detectors but it is not used for mycotoxin analyses due to the amount of time necessary to conduct HPLC analyses.

Veterinarians with Charoen Pokhpand report problems in animals that may be associated with mycotoxins in feeds (eg. oral lesions in chickens, abortions in pigs and deoxynivalenol related problems).

The development of sampling protocols was indicated to improve screening procedures.

4. CULTURE COLLECTIONS

The importance of maintaining a central fungal culture collection to act as a reference centre and depository was recognized throughout the region. Some researchers expressed the opinion that an internationally recognized culture collection would be useful to ensure strain authenticity.

4.1 MALAYSIA

Location: Institute of Medical Research, Division of Microbiology.
Microorganisms: microorganisms of medical importance including fungi.

Preservation: fresh agar cultures, oil submersion, freeze dried.

Location: Universiti Pertanian Malaysia, Department of Biology.
Microorganisms: plant pathogenic fungi, mostly Penicillium, Aspergillus and Trichoderma.

Preservation: fresh agar slants.

Location: Universiti Sains Malaysia, School of Biological Sciences.
Microorganisms: storage and plant and animal pathogenic fungi, mostly Fusarium.

Size: approximately 2,500.

Preservation: liquid nitrogen, sterile soil.

4.2 SINGAPORE

Location: National University of Singapore, Botany Department.
Microorganisms: storage and plant pathogenic fungi.
Size: approximately 2,500.
Preservation: fresh agar slants, freeze dried.

4.3 INDONESIA

Location: Bogor Research Institute for Veterinary Sciences, Mycology Division.

Microorganisms: fungi.
Size: approximately 60.
Preservation: mostly freeze dried.

Location: Food and Nutrition Development Research Centre.
Microorganisms: storage and plant pathogenic fungi.
Size: approximately 500.
Preservation: freeze dried and -80 degree freezer.

Location: Southeast Asian Regional Centre for Tropical Biology.

Microorganisms: storage fungi and plant pathogens.
Preservation: fresh agar slants.

Location: Bogor Agricultural University, Integrated Laboratory.

Microorganisms: bacteria and fungi of biotechnological importance, storage and plant pathogenic fungi.
Size: 237 fungi, 287 bacteria.
Preservation: fresh cultures.

4.4 PHILIPPINES

Location: Department of Agriculture, Bureau of Plant Industry.

Microorganisms: fungal plant pathogens.
Size: approximately 50 isolates.
Preservation: fresh agar slants.

Location: Bureau of Food and Drug.

Microorganisms: some Aspergillus, Fusarium, Alternaria isolated from processed foods.
Size: approximately 40 isolates.
Preservation: freeze dried.

Location: National Postharvest Institute for Research and Extension.

Microorganisms: storage and plant pathogenic fungi.
Preservation: fresh agar slants.

4.5 THAILAND

Location: Thailand Department of Agriculture, Plant Pathology and Microbiology Division.

Microorganisms: storage and plant pathogenic fungi.
Size: approximately 200 isolates.
Preservation: oil submersed and freeze dried.

Location: Chiang Mai University, Department of Medical Microbiology.

Microorganisms: microorganisms including fungi of medical importance, no food contaminants.

Location: Thailand Institute of Scientific and Technological Research, Microbial Resource Centre (MIRCEN).

Microorganisms: bacteria, fungi, yeast, algae.
Size: approximately 1,800 including fungi.
Preservation: freeze dried, liquid nitrogen, -80 freezer, fresh agar.

Comments:
Bangkok MIRCEN was established in 1976 at the Thailand Institute of Scientific and Technological Research (TISTR). It is part of a worldwide network of Microbial Resource Centres in environmental, applied microbiological and biotechnological research which was established within the framework of UNESCO and UNEP with the following objectives:

- to provide the infrastructure for the building of a world network which incorporates regional and inter-regional cooperating laboratories geared to the management, distribution and utilization of the microbial gene pool
- to foster the development of new indigenous technologies considered appropriate for the region
- to promote the application of microbial technology in strengthening the rural economy
- to serve as focal centres for the training of manpower and the diffusion of microbiological knowledge

At present, Bangkok MIRCEN is operating with seven cooperating laboratories: University of the Philippines, University of Indonesia, National University of Singapore, National University of Malaysia, Chinese University of Hong Kong, Thai Department of Agriculture and Kasetsart University (Thailand). These laboratories interact with Bangkok MIRCEN in the promotion of MIRCEN activities at the national level and cooperate in the dissemination of information. Bangkok MIRCEN helps to strengthen their roles by providing technical assistance, especially in the field of culture collections, funding for maintenance of culture collections and research grants.

Financial support for Bangkok MIRCEN has largely been contributed by UNEP and UNESCO. Additional funds have been provided from various local, regional and international sources and by the Government of Thailand which through TISTR has also contributed in the form of technical personnel.

Bangkok MIRCEN preserves microbial gene pools vital to agriculture and industry to make them accessible to the Southeast Asian region. At present it maintains over 1,800 strains of bacteria, yeast, algae and fungi. Catalogues of its holding have been prepared and extensively distributed. Cultures are distributed, free of charge, to various universities and research institutions both within and outside the region. Attempts have also been made to acquire economically important cultures from the collections in the region for deposition.

Bangkok MIRCEN has all the facilities and equipment necessary to maintain a modern culture collection including laminar flow hoods, light and phase contrast microscopes, electron microscope, GC. Most cultures are preserved freeze dried in ampoules although some fresh cultures are maintained. Liquid nitrogen is also available.

Personnel at Bangkok MIRCEN do not currently have expertise in the maintenance of plant pathogenic fungi; however, researchers indicated that this would be possible if some training and funding were provided. It may also be possible to provide a service for the freeze drying of cultures but that there would be an associated cost.

Bangkok MIRCEN is also actively involved in research in widening the scope of usefulness of microorganisms for industrial and agricultural applications, including the testing of new strains. Additional funds are obtainable from other sources for foreign scientists to carry out joint cooperative research programs at Bangkok MIRCEN and vice versa.

Holding regional, national and international workshops and training courses in the field of applied microbiology, including culture collections forms one of the main activities of MIRCEN.

5. RECOMMENDATIONS

Participation from ASEAN governments is imperative to maintain an environment conducive to the sustained implementation of mycotoxin control programs. This requires the formulation of policies that will regulate mycotoxins in agricultural commodities. In support of this, grades and standards must be established for each commodity and a pricing system must be worked out so that incentives are provided to the farmers and processors to encourage them to produce good quality grains. Policy makers in the ASEAN region should be made aware of the adverse health, animal production and economic implications of mycotoxins.

5.1 FACILITIES, EQUIPMENT AND METHODS

Reference laboratories

National reference laboratories for mycotoxin analysis should be established within ASEAN.

Regional Culture Collections

A regional culture collection for toxigenic fungi should be established as a reference centre and central depository. A catalogue of the collection could be compiled to facilitate access to cultures in such a central depository. The collection could be developed within one of the existing collections in a facility that has equipment and a thorough knowledge of preservation techniques, perhaps MIRCEN. Training agreements could be developed with appropriate international laboratories or international depository authorities to ensure curator competence in correct species identification. The possibility of establishing informal agreements with international mycologists for species verification could be investigated. Once established, the culture collection facility could act as a regional training centre for the identification of toxigenic fungi.

Existing facilities and high tech equipment should be required, if necessary, and utilize before introducing new high tech equipment into the region. Fungal and mycotoxin projects in ASEAN which include the supply of equipment should also include a provision for maintenance of the equipment.

Appropriate Analytical Methods

Research efforts should be directed towards the development of inexpensive, reliable mycotoxin analytical methods that are appropriate to the climactic, environmental and financial conditions in ASEAN. Improved sampling methods for different commodities in ASEAN need be developed. Regional workshops should be held to facilitate the standardization of analytical and sampling methods.

That TLC and minicolumn methods for quantitative or semi-quantitative mycotoxin analyses should be utilized in laboratories conducting routine analyses and other methods requiring more sophisticated equipment should be confined to core reference laboratories. The cost of solvent and maintenance of equipment is a constraining factor particularly in Indonesia and the Philippines.

The development of mycotoxin field test kits within ASEAN, and particularly in the Philippines, be coordinated. Commercially available field test kits are too expensive to be adopted by small food processing industries, merchants and farmers in developing countries. As a result, a number of institutes in the Philippines and Thailand are undertaking independently to develop more affordable aflatoxin test kits. This work may already have been duplicated within the Philippines or within the region.

Standards of required mycotoxins should be made available.

5.2 HUMAN RESOURCES AND TRAINING

Human resources projects

Future international collaborative projects should attempt to develop trilateral cooperative agreements both with institutions advanced in mycotoxin research and with institutions conducting applied research. This strategy best utilizes human resource capabilities already existing in the region and at the same time develops expertise in institutions that may not otherwise have the opportunity to further their mycotoxin research capabilities. In addition, communication linkages would be established in the region and may lead to increased stimulation of independent ASEAN collaborative mycotoxin research projects.

The general level of mycological and analytical capability should be increased through short term training programs.

The level of general knowledge of the effects of mycotoxins on animal health and production should be increased. This could be achieved through the participation of ASEAN nationals in graduate studies programs in veterinary sciences, toxicology and pathology.

The number of fungal and mycotoxin researchers should be increased in the region through graduate studies programs abroad. This would alleviate the current situation in which existing expertise is not optimally utilized because mycotoxin researchers take on several areas of study. This is a result of the lack of highly educated personnel within the region.

Technical workshops within the region should be encouraged. This would not only fulfill the objective of increasing technical capability within the region but would also bring together regional mycotoxin researchers to discuss common research challenges. In addition, it would facilitate the standardization of analytical methods and sampling procedures.

5.3 INFORMATION DISSEMINATION AND COMMUNICATIONS

ASEAN Food Handling Bureau

The ASEAN Food Post-Production Information Exchange Project (APEX) could be developed as a regional bibliographic reference centre:

- the AFHB should be approached to expand the APEX Technical Information Service to include information on fungi and mycotoxin research as a specific objective;
- that any international research agreements struck should include a provision for the deposition of proposals, progress reports and final reports to APEX as well as to all national fungi and mycotoxin research coordinators;
- the AFHB should be approached to develop a searchable fungi and mycotoxin database and make available hard copies of contained reference materials at cost - this database would include the reference material indicated in the above item;
- that existing fungi and mycotoxin course material and manuals relevant to developing countries be deposited in APEX (eg. NRI, FAO, Japanese, Australia).

Spreading existing information

The following information should be distributed to the heads of all organizations involved in fungi and mycotoxin research:

- brochure on AFHB APEX Technical Information Service with special attention to the Selective Dissemination of Information Service;
- how to subscribe to the Australian Mycotoxin Newsletter since this is the only no cost semi-selective service which provides information on international fungal and mycotoxin research publications;
- how to subscribe to the ACIAR Postharvest Newsletter and Swedish Food Laboratory Newsletter;
- a list of available reference material including proceedings of past conferences, workshops etc. relevant to fungi and mycotoxin research;
- a list of the titles of all on-going fungi and mycotoxin research projects within the region;
- availability of existing reference material pertaining to occupational health and safety of mycotoxin researchers - this information has been frequently requested, particularly in the Philippines.

Communication of results

ASEAN researchers should be encouraged to publish their results in international journals whenever possible. Aagreements should be made before the commencement of international research projects as to results that ASEAN researchers do not wish to be disclosed.

Proposal budgets should incorporate a provision for the cost of photocopying and communications as well as communications plan.

A directory should be compiled of regional, if not international, mycotoxin researchers' addresses and their respective areas of expertise. This could be distributed to encourage inter-regional networking and facilitate entry into the international mycotoxin research community.

6. APPENDIX

6.1 ADDRESSES OF MYCOTOXIN PERSONNEL

A preliminary address list of ASEAN mycotoxin research personnel is available from:

Dr. J. David Miller
Plant Research Centre
Agriculture Canada
Ottawa, Ontario
Canada
K1A 0C6
FAX: 1-613-992-7909

6.2 COMMONLY ENCOUNTERED ACRONYMS

AAECP	ASEAN-Australian Economic Cooperation Program
AARD	Agency for Agricultural Research and Development (Indonesia)
ACIAR	Australian Centre for International Agricultural Research
ADB	Asia Development Bank
AED	Agricultural Engineering Division (Thailand)
AFHB	ASEAN Food Handling Bureau
AFSRB	ASEAN Food Security Reserve Board

AGMARIS	Agricultural Marketing Information System
AGPP	ASEAN Grain Postharvest Program
AGPTC	ASEAN Grain Posthavest Technology Centre (proposed continuation of AGPP)
AMAF	ASEAN Ministers of Agriculture and Forestry
APEX	ASEAN Food Post-Production Information Exchange Project
ASEAN	Association of South East Asian Nations
ASPEMTI	Asosiasi Produsen dan Eksportir Makanan Ternak Indonesia (Indonesian Animal Feedstuff Producers and Exporters Association)
AUSTRADE	Australian Trade Commission
BAAC	Bank of Agriculture and Agricultural Cooperative (Thailand)
BAI	Bureau of Animal Industries (Philippines)
BALITVET	Balai Penelitian Veteriner (Research Institute for Veterinary Sciences) (Bogor, Indonesia) (under Ministry of Agriculture)
BIOTROP	SEAMEO Regional Centre for Tropical Biology
BORIF	Bogor Research Institute for Food Crops (Indonesia) (under AARD)
BPI	Bureau of Plant Industries (Philippines)
BULOG	Badan Urusan Logistik (Indonesia)- National Logistics Agency
CABI	Commonwealth Agricultural Bureau International
CBS	Centraalbureau voor Schimmelcultures (Baarn, Netherlands)
CGIAR	Consultative Group on International Agricultural Research
CHA	Country Host Agency (AGPP)
CIDA	Canadian International Development Agency
CIRAD	International Centre for Research and Development
CNEVA	Centre National Etudes Veterinaires de Paris (France)
COFAF	ASEAN Committee on Food, Agriculture and Forestry
COFM	Community and Occupational Family Medicine (Singapore)
CRIAS	Central Research Institute for Animal Sciences (Indonesia) (BALITVET under CRIAS)
CRIFC	Central Research Institute for Food Crops (Indonesia)
CSIRO	Commonwealth Scientific and Industrial Research Organization
DFCEP	Director of Food Crop Economics and Processing (indonesia)
DGFA	Director General of Food Crops Agriculture (Indonesia)
DIC	Disease Investigation Centre (Yogyakarta, Indonesia)
DOLOG	regional BULOG agencies
Drs.	Bachelor (Indonesia)
ELISA	Enzyme-linked immunosorbent assay
FAMA	Federation of Agricultural Marketing Authority (Malaysia)
FANDARC	Food and Nutrition Development and Research Centre (Gadjah Mada University, Indonesia)
FAVA	Federation of Asian Veterinary Associations
FKUI	Fakultas Kedokteran Universitas Indonesia (Faculty of Medicine, University of Indonesia)
FNCC	Food and Nutrition Culture Collection (Gadjah Mada University, Indonesia)
FNRI	Food and Nutrition Research Institute (Manila)
FOMCA	Federation of Malaysian Consumers Associations
FTDC	Food Technology Development Centre, Research Institute, Bogor Agricultural University
GASGA	Group For Assistance on Systems relating to Grain After- harvest
GTZ	Deutsche Gesellschaft Fur Technische Zusammenarbeit
GWG	Grains Working Group - ASEAN Food Handling Project
HPLC	High pressure liquid chromatography
HPTLC	High performance thin layer chromatography
IARC	International Agency for Research on Cancer (Lyon, France)
ICDS	Institutional Cooperation and Development Services Branch (CIDA, Canada)
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics

IDRC	International Development Research Centre (Canada)
IFIS	International Food Information Service
IFRPD	Institute of Food Research and Product Development
IGR	Institut Gustave Roussy de Villejuif (France)
IMCB	Institute for Molecular and Cell Biology (Singapore)
IMR	Institute of Medical Research (Malaysia)
INI ANSREDEF	Indonesia International Animal Science Research and Development Foundation
INNOTECH	SEAMEO Regional Centre for Educational Innovation and Technologies
IPB	Bogor Agricultural University
IPB	Institute of Plant Breeding (UPLB - Philippines)
IPO	Institute for Plant Protection (Wageningen, Netherlands)
Ir.	Insignorio (Engineer) (Indonesia)
IRHO	Institut de Recherche pour les Huiles et Oleagineaux (CIRAD, France)
IRPA	Intensified Research on Priority Areas Program (University Pertanian Malaysia) (under UNESCO ROSTSEA)
IRRI	International Rice Research Institute (Philippines)
ISFM	Institute of Science and Forensic Medicine (Singapore)
ISHAM	Indonesian Society for Human and Animal Mycology
ITB	Bandung Institute of Technology (Bandung, Indonesia)
IUC	Inter University Centre (Gadjah Mada University, Indonesia)
IUMS	International Union of Microbiological Societies
IWCSPP	International Working Conference on Stored-product Protection
JICA	Thai-Japan International Cooperation Agency
JPH	Jabalan Perkhidmalan Halwan (Department of Veterinary Services, Malaysia)
KSU	Kansas State University - Food and Feed Grain Institute
KUD	village cooperatives
LBN	National Biological Institute (Bogor, Indonesia - under LIPI)
LCHA	Laboratoire Toxicologie Alimentaires (France)
LIPI	Lembaga Ilmu Pengetahuan Indonesia (Indonesian Institute of Science) (see also LBN)
LKN	National Institute of Chemistry (Indonesia) (under LIPI)
LPN	Lembaga Padi dan Beras Negara (Malaysia) (National Rice and Paddy Board)
MARDI	Malaysian Agricultural Research and Development Institute
MARIF	Malang Institute for Food Crops (Malang, Indonesia)
MIRCEN	Microbial Resource Centre (Bangkok)
MSTE	Ministry of Science, Technology and Energy
NAPHIRE	National Post Harvest Institute for Research and Extension (Philippines)
NCGEB	National Centre for Genetic Engineering and Biotechnology (Thailand)
NCPC	National Crop Protection Centre (UPLB - Philippines)
NFA	National Food Authority (Philippines)
NIHRD-MOH	National Institute for Health Research and Development, Ministry of Health (Indonesia)
NPC	National Postharvest Committee (Indonesia, Malaysia, Philippines, Singapore, Thailand)
NPC/LA	National Postharvest Committees Lead Agencies
NRCC	National Research Coordinating Committee (for veterinary sciences only, Indonesia)
NRDC	Nutrition Research and Development Centre (Indonesia - under Ministry of Health)
NRDI	National Research and Development Institutions
NRI	Natural Resources Institute (U.K.)
NSTP	National Science and Technology Priority (Singapore)
NUFFIC	Netherlands University Foundation for International Cooperation
ODA	Overseas Development Agency (U.K.)
ORSTOM	Institut Francais Scientifique pour le Developpement en Cooperation
PAB	Policy Advisory Board (ASEAN?)
PCA	Philippine Coconut Authority

PCARRD	Philippine Council for Agriculture Forestry and Natural Resources Research and Development
PLANTI	ASEAN Plant Quarantine Centre and Training Institute (Malaysia)
PSC	Programme Steering Committee (AGPP)
QDPI	Queensland Department of Primary Industries
RECSAM	SEAMEO Regional Centre for Education in Science and Mathematics
REGNET	Regional Network Inter-Country Cooperation on Postharvest Technology and Quality Control of Foodgrains (UNDP/FAO)
RIVM	National Institute of Public Health and Environmental Protection (Netherlands)
ROSTSEA	UNESCO Regional Office for Science and Technology for Southeast Asia
RUT	Integrated Selective Research Program (Indonesia government)
SCOFH	ASEAN Sub-Committee of Food Handling
SDI	Selective Disseminatiio of Information Service (APEX)
SEAMEC	Southeast Asian Ministers of Education Council
SEAMEO	Southeast Asian Ministers of Education Organization
SEAMES	Southeast Asian Ministers of Education Secretariat
SEARCA	SEAMEO Regional Centre for Graduate Study and Research in Agriculture
SIRIM	Standards and Industrial Research Institute of Malaysia
STTC	Science Teachers Training Centre (University of the Philippines - Diliman, Quezon City, Philippines)
SURIF	Sukamandi Research Institute for Food Crops (Sukamandi, Indonesia)
TAC	Technical Advisory Committee (AGPP)
TDRI	Tropical Development Research Institute (U.K.) (now NRI)
TISTR	Thailand Institute of Scientific and Technological Research (Bangkhen)
TLC	Thin layer chromatography
TNSTDA	Thailand National Science and Technology Development Agency
TRDD	Technology Resource Development Department (NFA - Philippines)
UNDP	United Nations Development Program
UNESCO	United Nations Educational, Scientific and Cultural Organization
UNICEF	United Nations International Childrens Emergency Fund (now called United Nations Childrens Fund)
UPLB	University of the Philippines at Los Banos
UPM	Universiti Pertanian Malaysia (Agricultural University Malaysia)
USAID	United States Agency for International Development
USDA-ARS	United States Department of Agriculture - Agriculture Research Service
USM	Universiti Sains Malaysia (University of Science Malaysia)
VUC	Village Unit Cooperative (Indonesia)
WAU	Wageningen Agricultural University (Netherlands)

6.3 ASEAN PUBLICATIONS AND RESOURCES AVAILABLE

6.3.1 NEWSLETTERS

The Swedish Food Laboratory Newsletter

ASEAN Food Handling Newsletter

ASEAN Food Journal

Australian Mycotoxin Newsletter

ACIAR Postharvest Newsletter

6.3.2 CONFERENCE PROCEEDINGS, WORKSHOPS, SEMINARS, REPORTS

Report of the Joint FAO/WHO/UNEP Conference on Mycotoxins. 1977. Nairobi, Kenya. FAO Food and Nutrition Paper No. 2.

Prevention of Mycotoxins. 1979. FAO, Rome. FAO Food and Nutrition Paper No. 10.

Perspective on Mycotoxins. 1979. FAO, Rome. FAO Food and Nutrition Paper No. 13.

Microbiological Analysis. 1979. FAO, Rome. FAO Manuals for Food Quality Control 14/4.

Mycotoxin Surveillance a Guideline. 1982. FAO, Rome. FAO Food and Nutrition Paper No. 21.

Post-Harvest losses in quality of Foodgrains. 1983. FAO, Rome. FAO Food and Nutrition Paper No. 29.

Proceedings of the Workshop on Mycotoxins in Thailand. January 13-14, 1983. Bangkok, Thailand.

Proceedings of the Regional Workshop on Mycotoxins. 23-36 March, 1983. Bangkok, Thailand.

Proceedings of the Workshop on Toxic Substances in Thailand. June 6-8, 1983. Bangkok, Thailand.

Proceedings of the First Annual Meeting of the Toxicological Society of Thailand. Toxic Substances in Thailand: Trichothecenes. June 13-15, 1984. Bangkok. Thailand.

Food Analysis: General Techniques, Additives, contaminants and Composition. 1986. FAO, Rome. FAO Manuals for Food Quality Control 14/7.

Summary 1984-85 Monitoring Data, GEMS Food- FAO/WHO-EHE/FOS/884. 1987. WHO, Geneva.

Proceedings of the First Asia-Pacific Congress on Animal, Plant and Microbial Toxins. National University of Singapore. June 24-27, 1987.

Nairobi +10, Report of the Second Joint FAO/WHO/UNEP International Conference on Mycotoxins. Bangkok, Thailand. September 28-October 3, 1987.

The role of epidemiological and clinical studies in the evaluation of aflatoxin health risks in SEAMIC countries. 1987. Bungay, Alice Alma C.

Introduction to Food Sampling. 1988. FAO, Rome. FAO Manuals for Food Quality Control 14/9.

Utilization of Tropical Foods, Cereals. 1989. FAO, Rome. FAO Food and Nutrition Paper No. 47/1.

Utilization of Tropical Foods, Tropical Oil Seeds. 1989. FAO, Rome. FAO Food and Nutrition Paper No. 47/5.

Mycotoxin Analysis. 1990. FAO, Rome. FAO Food and Nutrition Paper No. 14/10.

Report of the Regional Workshop on Control of aflatoxins in Asia. Chiang Mai, Thailand. February, 1990.

Proceedings of the Seminar-Workshop on Aflatoxin Analytical Methodologies. 28 May, 1990. Diliman, Quezon City, Philippines.

Proceedings of the First Asian Conference on Food Safety. Kuala Lumpur, Malaysia. September, 1990.

Proceedings of the First Asia-Pacific Regional Seminar on Analysis of Trace Constituents in Food. Penang, Malaysia. November, 1990.

Proceedings of the Symposium on Pest in Stored Products. SEAMEO-BIOTROP, Bogor, Indonesia. January 29-31, 1991.

Proceedings of the Conference on Fungi and Mycotoxins in Stored Products. ACIAR Proceedings No. 36. April 23-26, 1991. Bangkok, Thailand.

Proceedings of the Sixth Asian Congress of Nutrition. Kuala Lumpur, Malaysia. September, 1991.

Proceedings of the 14th ASEAN Seminar on Grain Postharvest Technology. Manila, Philippines. November 5-8, 1991.

Semple, R.L.; Frio, A.S.; Hicks, P.A.; Lozare, J.V. 1991. Mycotoxin Prevention and Control of Foodgrains. UNDP/FAO/REGNET.

Proceedings of the Fourth ASEAN Food Conference and Food Technology Exhibition. Jakarta, Indonesia. February 17-21, 1992.

Proceedings of the 15th ASEAN Seminar on Grain Postharvest Technology. Singapore. September 8-11, 1992.

Proceedings of the Second Asia-Pacific Regional Seminar on Food Analysis. Kuala Lumpur, Malaysia. November, 1992.

6.4 TRAINING PROGRAMS

6.4.1 NATIONAL RESOURCES INSTITUTE

The Natural Resources Institute of the United Kingdom offers a 13-week Mycotoxins Training Course annually to eight participants. The course emphasizes the following:

- sampling and sample preparation;
- qualitative and quantitative analysis;
- health and safety in the laboratory; and
- prevention and detoxification of mycotoxins.

Knowledge training includes bioassay, chromatography, proper laboratory practice, proper storage practices, isolation, mycology, prevention and detoxification of mycotoxins, sampling and sample preparation, statistics and toxicology of mycotoxins. Skills training includes sample preparation methods, basic analysis techniques, preparation of standard solutions, thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC), enzyme-linked immunosorbent assay (ELISA), bonded-phase and immunoaffinity clean-up methods, method development, validation and design and implementation of a short project.

At the end of the course participants are able to obtain representative samples from bulk commodities, select the most appropriate analytical methods, analyze a range of commodities of interest for aflatoxins and other selected mycotoxins, objectively assess the performance of the analytical methods, carry out analyses safely and efficiently, and report the results concisely and clearly.

Funding for participation in the course by personnel from developing countries may be available through the British Technical Co-operation Training Program (TCTP).

6.4.2 FOOD AND AGRICULTURE ORGANIZATION

A series of training workshops on fungi and mycotoxins is being organized by FAO and UNEP to take place in 1993-1994 in India, Thailand and the Philippines. Laboratory methods for the analysis of aflatoxins and some other mycotoxins will be held in India, while a course on technology transfer will be held in the Philippines and directed towards extension workers. The Thai Department of Agriculture will host the component regarding sampling methods directed at food and feed inspectors. Personnel trained as part of the program activities will later become trainers of other personnel at the country level. The training programs are not restricted to laboratory staff. They are also targeting decision-makers, planners and senior administrators, so as to increase awareness in the importance of mycotoxins in human and animal health, trade and economic losses.

6.5 OTHER MYCOTOXIN PROPOSALS IN ASEAN

This section is included to indicate those areas of mycotoxin research which ASEAN scientists feel important to pursue. Proposals have been prepared to express this, some of which have already been submitted to potential funding bodies.

6.5.1 1984 PROPOSED ASEAN AFLATOXIN CONTROL PROGRAM

In response to an expressed regional need for a concerted and coordinated effort to contain the aflatoxin problem and minimize aflatoxin contamination, a Technical Consultation of a group of ASEAN Mycotoxin Experts was convened from December 3-8, 1984 in Kuala Lumpur, with the principal task of formulating a comprehensive aflatoxin control program for ASEAN.

In designing such a program, the Expert Group identified two essential components for control: i) a collaborative surveillance effort and ii) the initiation of research and development on prevention and control. The Expert Group, in placing priority on aflatoxin during the initial stages of the control program, agreed that the program could be extended to cover other mycotoxins later. Thus, for all intents, the control program would be referred to initially as the "ASEAN Aflatoxin Control Program".

Of the array of food commodities and feedstuffs on which the Aflatoxin Control Program would initially focus, the Expert Group identified and assigned priority to two commodities, maize and groundnut, as high-risk commodities, highly susceptible to aflatoxin contamination and of economic importance to the ASEAN region.

The proposed ASEAN Aflatoxin Control Program recommended an integrated and coordinated regional approach with the following five-point strategy:

1) To increase the number and capability of aflatoxin research scientists, assistants and technicians, and by providing training opportunities in surveillance and control work, and research and development projects.

2) To upgrade facilities in food control, and monitoring laboratories to provide surveillance and control activities, and service research and development projects.

3) To develop and standardize suitable sampling, as well as to sample preparative and analytical methodologies to determine aflatoxin levels in commodities produced and consumed in the ASEAN region.

4) To provide research and development efforts in aflatoxin prevention and control measures during pre- and post-harvest activities, including food processing by manufacturers and households.

5) To develop an effective extension component to the Aflatoxin Control Program.

The Expert Group recommended the following specific projects for immediate implementation during the initial five years of the ASEAN Aflatoxin Control Program:

i) Establishment of an ASEAN Mycotoxin Regional Training Centre and Reference Laboratory.

ii) Development of National Mycotoxin Training and Reference Laboratories.

iii) Upgrading of facilities in selected surveillance laboratories.

iv) Out-of-Region Training Project on Mycotoxins for professionals and technicians.

v) Development of standardized sampling, sample preparations and analytical methodologies using consultants.

vi) Conduct research and development studies on prevention and control measures in the following areas:

   a) Pre-harvest, harvest and post-harvest handling practices (eg. harvesting time and planting practices);
   b) Drying and storage practices; and
   c) Food processing activities by manufacturers and households.

vii) Development of an effective Extension Project on a national and Regional basis, including the dissemination of technical information on mycotoxins through the ASEAN Food Handling Bureau.

The ASEAN Food Handling Bureau was proposed to implement systems for Program administration, facilitate administrative arrangements related to acquisition and purchase of project equipment and vehicles, operate routine accounting, maintain consolidated expenditure records, undertake consultancy arrangements, arrange travel for meeting and participation in project activities and liaise with funding bodies on disbursement and reimbursement of project funds.

The total program cost for the five year ASEAN Aflatoxin Control Program was estimated at US $14,055,000. Of this, 44% was identified as an input contribution by ASEAN to the Program. The remaining sum of US $ 7,836,900 was requested from the EEC to finance the Program but was later refused.

6.5.2 1992 DRAFT PROPOSAL - INVESTIGATION AND ALLEVIATION OF THE EFFECT OF AFLATOXIN ON FOOD SAFETY, QUALITY AND RESULTANT HEALTH ECONOMICS IN INDONESIA

This proposal was to be submitted to the EEC for funding, however, due to political problems, submission has been suspended.

The overall objective of the proposal is to reduce the negative economic impact of aflatoxin-related illness by making practical improvements in food legume and food supply, and to develop techniques for production and storage of groundnuts which can be applied to other commodities.

The project consists of a Human Health Component and an Agronomic Component which propose to carry out four years of activities related to aflatoxin in groundnuts in West, Central and East Java, Indonesia. The link between the Medical and Agronomic Components is established through the use of data obtained from agronomic field surveys. The National Institute of Health Research and Development under the Indonesian Ministry of Health (NIHRD-MOH) will select rural areas with high and low levels of aflatoxin contamination in peanuts. This will be related to aflatoxin levels in the blood of individuals residing in these areas.

i) Human Health Component - The relationship of aflatoxin levels in the human body and intake of peanuts in Indonesia.

Medical coordination is to be based at the NIHRD-MOH where they will conduct dietary and serological surveys. In addition, the Faculty of Medicine at the University of Indonesia (FKUI), regional hospitals in Yogyakarta and Semarang, and regional medical agencies and staff will carry out the medical investigation required. FKUI will focus its research activities in the hospital on the relationship of aflatoxin and primary hepatocellular carcinoma. Collaborating European medical staff, particularly epidemiologists, will cooperate with survey agencies.

In order to achieve the main objective of collecting baseline data of aflatoxin levels in the human body and intake of peanuts in the community, the health component undertakes to:

a) determine quantitative levels of aflatoxin in the body of Indonesian individuals;
b) study food intake habits of the community;
c) study the relationship of aflatoxin levels in the body and intake of peanuts;
d) develop simple but accurate methods for determining aflatoxin levels in the human body.
e) establish a preservation system for storing laboratory specimens in good condition for indefinite periods of time;
f) develop a national reference laboratory for aflatoxin determination.

ii) Agronomic Component - Improvement of cultural practice and postharvest handling to minimize aflatoxin contamination of peanuts.

Both general management of the project and coordination of agronomic studies will be centred at the Bogor Research Institute for Food Crops (BORIF). Short- and long-term foreign visiting agronomists and a socio-economic study team will be located at BORIF. Mycotoxin surveys and analysis will be conducted at BORIF. Technical and field studies will be carried out using equipment and experimental facilities at BORIF, the Malang Research Institute for Food Crops (MARIF), and Sukamandi Research Institute for Food Crops (SURIF). The economic and social science team will work in collaboration with local populations in areas identified by epidemiological studies as having a high incidence of mycotoxins.

The specific objectives of the Agronomic Component are:

a) to collect data on the current peanut production areas, production and marketing system, use of peanut as food and feed, agro-climactic factors in peanut production, socio-economic and cultural background of peanut growers, as well as socio-demographic and dietary habits of the farmers and other peanut consumers;
b) to identify aflatoxin producing fungi and agro-climactic conditions conducive to aflatoxin production and aflatoxin contamination in peanut and peanut products;

c) to study the relationship between the aflatoxin producing fungi with other biotic factors such as insect pests and nematodes on peanut production and aflatoxin contamination;
d) to determine the effect of various cultural practices, harvest, processing, storage and handling on damage by aflatoxigenic fungi and levels of aflatoxin contamination;
e) to obtain high yielding peanut cultivars which are resistant or tolerant to damage by aflatoxin producing fungi as well as aflatoxin contamination;
f) to develop economical, accurate, simple methods for sampling, detection of aflatoxin and control of aflatoxin contamination in peanut and its products which are practical for researchers, extension workers, farmers and distributors;
g) to conduct workshops and training courses to improve knowledge and awareness of peanut growers, distributors and consumers on aflatoxin and how to reduce associated risks;
h) to publish results of the project in national and international journals and to use the results as a basis for further studies on the aflatoxin problem in Indonesia.

The direct total cost of the proposed project is estimated at 4.0 million ECU. Funding for the project will be sought from the EEC in the form of a grant. Counterpart contributions will be made by the various Indonesian agencies and research centres involved.

European Community contributions for expatriate expertise (for which travel and per diem will be supported by the general EEC budget) will be provided as follows:

a) the Institut Francais de Recherche Scientifique pour le Developpement en Cooperation (ORSTOM - France) will contribute 120 man months;
b) the Natural Resources Institute will contribute 48 man months;
c) the Institut Gustave Roussy (France) will contribute two man months;
d) the Centre National des Etudes Veterinaires (France) will contribute two man months;
e) the Institut de Recherche pour les Huiles et Oleagineaux (France) will contribute four man months.

Indonesian Government contributions to be added to EEC funds will include:

a) 800 man months from Indonesian scientists and technicians;
b) offices, technical facilities, supplies, housing, equipment installation and maintenance.

Final drafting of the proposal and submission to EEC has been temporarily suspended due to political problems.

6.5.3 1990 - DEVELOPMENT OF A CENTRAL FACILITY FOR AN INDONESIAN CULTURE COLLECTION OF MICROORGANISMS FOR AGRICULTURAL BIOTECHNOLOGY

This project was to be a collaborative effort between three universities in Indonesia (Bogor Agricultural University, University Gadjah Mada, University of Indonesia) and three Dutch Institutes (Centraalbureau voor Schimmelcultures, Institute for Plant Protection, Agricultural University of Wageningen). Although an agreement in principal was reached and the Indonesian funding contribution granted, in the final stages of approval by the Dutch Ministry of Foreign Affairs, the proposal was suspended due to political problems.

The objectives of this proposal are to i) establish a central facility for agricultural biotechnology at the Bogor Agricultural University (IPB) in cooperation with the University of Indonesia (UI) and University Gadjah Mada (UGM) and ii) support the culture collection with basic equipment and skilled staff which in turn will support the development of agricultural biotechnology in Indonesia. The implementation of the

activities will be divided into two four year phases. The first phase will emphasize the training of staff and technicians with the gradual building up of the culture collection. The second phase will focus on the consolidation and strengthening of the culture collection in order to become the central facility for agricultural biotechnology in Indonesia.

The first phase activities will include:

i) short term training of technicians and staff members at Centraalbureau voor Schimmelcultures (CBS) in the Netherlands to acquire skills in handling and preservation of microorganisms to maintain the stability of biochemical properties of the microorganisms;
ii) short term training of all junior staff members and technicians through two courses to be given in Indonesia by Dutch scientists: Microbial Genetics and Advanced Mycology;
iii) procurement of reference material and equipment for the maintenance of the culture collection;
iv) research by senior staff members and Masters students in the fields of fungi in foods and the development of pesticides of microbial origin. This would involve additional training at appropriate Dutch laboratories. Specific research topics include:
    a) Isolation and identification of mycotoxigenic fungi;
    b) Isolation and identification of thermophilic fungi;
    c) Distribution of Bacillus thuringensis in Indonesian soils and their insect toxicity;
    d) Diversity within serogroups of Bradyrhizobium sp. as indicated by plasmid profile analysis, protein profiles, intrinsic antibiotic resistance and symbiotic effectiveness;
    e) Biological and ecological studies of Bongkrek bacteria;
v) the publication and presentation of research results at national and international workshops and seminars;
vi) the compilation and publication of a culture collection catalogue for access to research and training institutions and industry.

The second phase of the project proposes:

i) courses focused on selected properties or productivity of microorganisms to be determined later;
ii) the development of identification services and culture accessibility to scientific institutions and industry in order to acquire external funds.

Funding:
Project costs were estimated at Dfl. 1,652,100. The financial contributions to be made by the Dutch Ministry of Foreign Affairs (of Dfl. 1,376,750) were to include training and technical assistance, equipment and reference material procurement, administration, catalogue development, travel, and research programs. Contributions by the Government of Indonesia (of Dfl. 275,350) include facilities, available equipment, manpower and indirect funding.

Current status:
Dr. Robert A. Samson of CBS visited Indonesia several times to act as a technical consultant over the last five years. A letter of agreement to establish a central facility for an Indonesian culture collection was signed by the Rector of IPB and Dr. Samson in March 1990. Project approval was granted by the Indonesian Directorate General for Higher Eduction in January 1991. Counter funding of Rp. 16,000,000 (approx. US $7,800) for 1990/1991 and Rp. 19,300,000 (approx. US $9,400) for 1991/1992 was provided by IPB. In addition, the construction of a new laboratory at IPB to house the culture collection is nearing completion.

In May 1991, the project was in the final stage of approval by the Dutch Ministry of Foreign Affairs. Funding was not granted.

Dr. Ratna Siri Hadioetomo, the proposed Indonesian Project Coordinator, visited the Netherlands to identify Dutch scientists to be involved in the project and discuss with them detailed project planning. Institutes visited included the Institute for Plant Protection, Wageningen Agricultural University, Delft Technical University, National Institute of Public Health and Environmental Protection and the Centraalbureau voor Schimmelculture.

The culture collection currently consists of 237 strains of fungi and 287 bacterial strains. These are maintained as fresh cultures as no freeze drying facilities are available. A limited number of the fungi are plant pathogens or toxigenic.

6.5.4 1993 - IDENTIFICATION OF FUNGI AND MYCOTOXINS OTHER THAN AFLATOXIN IN RICE AND MAIZE

A draft outline proposal was presented, for consideration by the GASGA Working Party, by the Food Technology Development Centre (FTDC) of Bogor Agricultural University (IPB). The draft proposes a coordinated study of fungi and mycotoxin occurrence to be conducted at research stations in Bogor, West Java, Cental Java and East Java. No time frame or budget for the proposal was presented.
Objectives:

i) determination and identification of fungi and mycotoxins other than aflatoxin in rice and maize in Indonesia;
ii) to collect information on research activities and development on fungal and mycotoxins and the existing results obtained in Indonesia;
iii) to establish a reference laboratory for fungi and mycotoxins
iv) to study and establish standardized procedures for fungi and mycotoxins to be used in Indonesia.

Scope:
- research activities on fungi and mycotoxins;
- advanced training (graduate and post-graduate) to study laboratory techniques and procedures on fungi and mycotoxins;
- to promote networking and information sharing between research laboratories at FTDC-IPB, Indonesia and other institutions abroad in fungi and mycotoxins.

This proposal has not been submitted to any other potential funding body.

6.5.5 MYCOTOXIN INFORMATION SERVICE CENTRE

Recognizing the need for an efficient documentation system, the Food Technology Development Centre of Bogor Agricultural University presented a proposal for consideration through the GASGA initiative - see Appendix I.

Objectives:
- to develop a documentation system (DS) on mycotoxins, related to management information system practices;
- to identify the necessary equipment to support the DS and training of personnel;
- to formulate the type of training needed including course content, practical equipment to increase ability of personnel and future development of the DS;
- to set up a bibliographic database on mycotoxins as a "pilot project" (approximately 500 records).

6.5.6 THE IMPROVEMENT OF POST HARVEST HANDLING OF SECONDARY CROPS TO REDUCE AFLATOXIN PRODUCTION

An Indonesian proposal has been formulated with the Directorate of Food Crop Economics and Processing as the executing agency. The Central Research Institute for Food Crops, the Directorate General of Foreign Trade, SEAMEO-BIOTROP, and the Faculty of Agricultural Technology of Bogor Agricultural University are proposed as cooperating institutions.

The immediate objectives of the project are to:
- identify post harvest handling problems at farmer and trader levels;
- establish pilot projects as a model for future systems in improving post harvest handling;
- improve post harvest handling at farmer and traders levels.

The long range objectives are to:
- obtain integrated improvement of post harvest handling in order to reduce aflatoxin in production of secondary crops;
- reduce economic losses and to increase farmer income;
- increase the volume of secondary crops export.

Project activities include:
- survey/observation and gathering information on existing secondary crops post harvest and marketing conditions;
- identification and determination of critical handling that causes aflatoxin contamination;
- identification and determination of technology and equipment appropriate for improving secondary crops post harvest handling;
- establishment of an aflatoxin laboratory;
- training of trainers and training user on applied postharvest technology;
- procurement of equipment, machinery and facilities necessary for the program;
- preparation of supporting system for the program including equipment procurement and credit systems;
- establishment of pilot project.

This four year project proposal requires foreign aid input in the amount of $US 5,000,000 with counterpart funding by the Government of Indonesia in the amount of $US 500,000. It is unknown whether this project has been submitted to international agencies for funding.

6.5.7 PREVENTION AND CONTROL OF AFLATOXINS AND INCREASED UTILIZATION OF COCONUT PRODUCT AND BY-PRODUCTS FOR POULTRY AND LIVESTOCK FEEDING

This proposal was submitted by the Institute of Animal Science of the University of the Philippines at Los Banos to the EEC for funding in 1988 but was not granted. Proposed activities were to be conducted at the University of the Philippines at Los Banos. The proposal included studies on:
- effect of chemicals on aflatoxin production in vitro;
- control of Aspergillus growth and aflatoxin on coconut product and its by-products;
- isolation and collection of microbial isolates;
- screening of plant extracts;
- evaluation of plant extracts;
- detoxification of aflatoxins by chemicals;
- aflatoxin reduction in coconut products and by-products by heat treatment;
- biological tests to confirm removal of aflatoxins;
- feeding trials on poultry and swine to assay acceptability of the treated samples;
- clinicopathological studies to assess effects on animal health.

6.5.8 MONITORING AND FORECASTING ON AFLATOXIN CONTAMINATION IN RAW AGRICULTURAL CROPS

This 5 year proposal, by the Bureau of Plant Industry, was submitted to the Philippine Department of Agriculture for funding in May, 1992 but was not granted. Foreign funding that would have to be obtained was estimated at US $576,900.

Objectives:

- to establish a comprehensive network of aflatoxin scouting and monitoring systems in the Philippines - activities would include periodic surveys of aflatoxin contamination in agricultural crops;
- to organize massive aflatoxin control operation in outbreak areas;

Project Strategy:

- establish the technical skills necessary to implement monitoring programs;
- increase analytical capability and laboratory facilities;
- conduct a survey to determine the incidence of aflatoxin contamination in selected domestic foods;
- conduct periodic survey of aflatoxin contamination in different regions in the country;
- test selected imported commodities;
- test selected exported commodities;
- furnish report copies to farmers and regulatory bodies;
- organization of massive aflatoxin control operations through farmers cooperatives;
- continued aflatoxin monitoring programs in susceptible crops for regulatory purposes;
- expand domestic monitoring in other agricultural crops not known to be susceptible to contamination but which should be examined because of their importance in the life and economy of the country.