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STRENGTHENING QUALITY CONTROL AND TESTING FACILITIES OF NON-ALCOHOLIC LIQUID FOODS INCLUDING FISH SAUCE AND SOY-BEAN SAUCE

DP/VIE/87/009/11-52

VIET NAM

Technical report: Consultancy visit in food microbiology and technology*

Prepared for the Government of Viet Nam
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Programme

Based on the work of Alan Reilly,
expert in food microbiology/technology

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* This document has not been edited.

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SUMMARY

A Food Micrcbiology/Technology Consultancy visit to Vietnam was undertaken from 9 September to 8 November 1992, as part of UNIDO's technical assistance project DP/VIE/87/009 at the Department for Quality Control of Goods and Metrology (DTQC), Ministry of Trade, Hanoi. Included in this mission was a two day (10-11 September) visit to Bangkok to obtain a Vietnamese visa and a one day visit (6 November) to Vienna for debriefing at UNIDO's Headquarters.

The objectives of the visit were to assist with the installation and testing of equipment donated to the DTQC as part of the project, and to conduct training courses in food mycology and food bacteriology, as well as conducting a one day seminar on food quality control.

All of the equipment provided by the project for the microbiology laboratory was tested and most items were found to be in working order. The two incubators with purchase order (PO) Number (PO No. 15-0-1018P) are not suitable for use in a microbiology laboratory because of poor temperature control. The stereomicroscrope (PO No. 15-0-1018P) has the occular lenses missing and cannot be used. The Wearing Blender (PO No. 15-0-1018P) is not suitable for use in the microbiology laboratory as it cannot be sterilized. Various items of equipment are recommended for purchase by the DTQC and these are described in paragraph 4 and Annex 1 of this report.

Advice on laboratory safety regarding the disposal of contaminated material, cleaning schedules and hazards associated with handling pathogenic organisms and dehydrated enrichment media was given to laboratory staff.

A training course on the isolation and identification of fungi, and their recognition and enumeration in foods was conducted from 18 September to 3 October. Pre-prepared lecture notes and laboratory schedules were provided for five participants and seven observers who attended the course.

A second training course on the isolation and identification of food spoilage and pathogenic bacteria was conducted from 5 to 31 October. This was attended by five participants and two observers and these were also provided with pre-prepared lecture and laboratory notes.

A seminar on Food Quality Control was conducted on 2 November and attended by approximately 50 guests from the food industry and research organisations in Hanoi. The seminar focused on problems relating to control food safety and quality in an open market system and on mycotoxins in the food supply. The seminar was well received and generated much discussion.

The major outputs from this visit are -

(i) Five persons have been trained in methods of mycological examination of foods;

(ii) Five persons have been trained in methods of evaluating foods for foodborne bacterial pathogens;

(iii) A functioning food microbiology laboratory which is capable of undertaking analyses of foods for microbiological quality;

(iv) the laboratory has the capability of evaluating foods for the principal mycotoxin producing fungi and pathogenic bacteria responsible for foodborne disease.

INTRODUCTION

1. A two month consultancy visit in the specialist area of food microbiology and technology was undertaken at the Department of Quality Control of Goods and Metrology (DTQC), Ministry of Trade, Hanoi, Vietnam. This visit was made as part of the United Nations Industrial Development Organisation's (UNIDO) technical assistance project, DP/VIE/87/009. The purpose of this technical assistance project is to upgrade capabilities of the DTQC in order to enable it to implement more effective quality control programmes by strengthening its analytical expertise in assessing the quality and safety of foods, as well as upgrading its extension and advisory services.

2. The consultancy was undertaken by P.J.A. Reilly, Head of Food Safety, Natural Resources Institute (NRI), UK and the specific objectives of the mission were to:

(a) Test and commission equipment provided by the project for the food microbiology laboratory;

(b) Conduct a training course on the isolation and identification of fungi of importance in foods;

(c) Conduct a training course on foodborne pathogenic bacteria and their enumeration and identification in foods;

(d) Present a one-day seminar on food quality control;

(e) Prepare a terminal report on visit findings and recommendations.

3. The consultancy took place at the Food Microbiology Laboratory and the Training Room of the DTQC, with field assessment visits to State-owned food processing plants,

and a 3-day working visit to Ho Chi Minh City Branch of the DTQC.

Equipment in the Food Microbiology Laboratory

4. All major items of equipment supplied by the project for the food microbiology laboratory were tested and most were functioning according to specifications. A voltage regulator was required for the Laminar Flow Cabinet (PO No. 15-0-1018P) which was purchased and installed. This equipment provides a sterile working area for microbiological analysis that is essential for most of the analytical techniques used in the food microbiology laboratory.

The two general purpose incubators which were supplied by the project (PO No. 15-0-1018P) do not have precision temperature control (ie. operational efficiency of $\pm$1°C) and are therefore unsuitable for microbiological analysis. The laboratory needs precision temperature controlled incubators and these were included in the original equipment list as priority equipment. It is recommended that the DTQC replaces the two incubators supplied by the project with two precision controlled models. Detailed specifications, suppliers and cost of this equipment are given in Annex 1 of this report.

It is also recommended that the DTQC purchases a temperature control water bath which was listed as a priority in the original equipment list. There is a need to acquire this item for enumerating faecal coliforms and *E.coli* using internationally recognised standard methods. These organisms are important indicator bacteria which are included in most bacteriological standards for processed foods. The model, supplier and cost of this equipment is provided in Appendex 1 of this report.

At present the laboratory does not have facilities for isolating anaerobic organisms which are important in the spoilage of canned foods. Additionally, anaerobic conditions are required for the isolation and enumeration of such pathogenic bacteria as *Clostridium perfringens*. This organism is included in the Vietnamese Bacteriological Standards for various foods and the DTQC food microbiology laboratory should have the capability to isolate and enumerate this organism in foods. It is recommended that the DTQC purchase an Anaeorobic System and the suggested model, specifications and costs are given in Annex 1 of this report.

A compound microscrope was included as a priority item in the original equipment list. The laboratory is currently using a simple microscrope which was ordered for the mobile laboratory. Although the simple microscope was used during the training courses with the aid of a light source from the stereomicroscope, the system is far from ideal. It is recommended that the DTQC purchases a compound microscope and the suggested model, supplier and cost is supplied in Annex 1 of this report. It is also recommended that the supplier of the Cambridge Instruments stereomicroscope (PO No. 15-0-1018P) be contacted and requested to supply the missing lenses.

All these items of equipment were considered necessary when the initial project documents were drafted and are still necessary if DTQC is to perform some of its key functions and responsibilties, such as acting as the official government arbiter for microbiological quality problems of canned foods that may arise between customer and suppliers; and also for supplying official government certificates for canned foods.

Safety in the Food Microbiological Laboratory

5. Many of the microbiological procedures at the DTQC involve a significant amplification of pathogenic microorganisms in food samples in order to detect them in routine analyses, for example in order to detect a few *Salmonella* or *Staphylococcae* in a food samples, the numbers of these organisms are deliberately increased to many millions. Thus, the laboratory can become a major source of pathogens and it is extremely important to minimise the opportunity for release of these pathogens into the environment. In order to avoid this risk at the food microbiology laboratory of the DTQC, two new bench top autoclaves (pressure cookers) were purchased for the training courses and these were used for the decontamination of all materials from the food microbiology laboratory prior to disposal. This procedure should replace the old method of de-contamination which was practiced at the laboratory and should become part of standard operational practice.

It is extremely important to maintain good hygienic conditions in the food microbiology laboratory because there are severe risks associated with handling high numbers of pathogenic microorganisms. A cleaning schedule was devised, tested, and is now part of the standard operational practice in the laboratory in order to reduce the hazards associated with handling high numbers of pathogens.

Additionally there are potential hazards associated with handling and weighing dehydrated enrichment media and for some pathogenic bacteria, such as Baird Parkar Agar and XLD Agar. These potential hazards were explained to staff and precautions for handling dehydrated media discussed. Health and safety information supplied by one of the major UK producers of dehydrated media was provided for staff at the food microbiology laboratory,

and it is recommended that the necessary precautions are taken when handling dehydrated media.

Training Course in Food Mycology

6. The training course on the isolation and identification of spoilage and mycotoxin producing moulds was held from 18 September until 3 October. It was attended by five participants and seven observers. The course consisted of lectures which were held every morning from 08.30-09.45 and laboratory work from 10.15-12.00 and 13.30-16.30. Lecture notes and laboratory schedules which were prepared in the UK were provided for all attending the course. These notes form the basis of the NRI Food Microbiology Course which is held biennially in the UK. Details of the course content and schedule are provided in Annex 2. During the course participants evaluated the following foods for mould contamination:

> Chilli sauce, fermented soya sauce, stored and freshly harvested groundnuts, soya beans, dried fish, rice, maize and candy.

Participants were instructed in the identification of both field and storage moulds, with special emphasis on the identification of aflatoxin producing moulds. Pure cultures of important storage moulds were provided for the food laboratory for future reference. A full set of lecture notes describing the details of isolating, enumeration and identification of moulds and yeasts from foods were provided for the DTQC and are not included in this report. In addition a copy of the NRI Mycotoxins Manual was provided for DTQC staff. A full set of lecture notes describing the details of isolating, enumeration and identification of moulds and yeasts from foods were provided for the DTQC and are not included in this report. In addition, a copy of the NRI Mycotoxins Manual was provided for DTQC staff.

Training Course in Food Bacteriology

7. The training course on the isolation and identification of food spoilage and pathogenic bacteria was held from 5 - 31 October and was attended by five participants and two observers. The course followed a similar timetable as the Food Mycology Course, with lectures and laboratory sessions. The training course focused on foodborne pathogens and how to evaluate foods for important species, using internationally recognised bacteriological methods. The pathogens included *Salmonella*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Escherichia coli*, *Listeria monocytogenes*, *Bacillus cereus* and *Clostridium perfringenes*. In addition partipants were instructed in methods to enumerate bacteria in foods, such as standard plate counts of mesophiles and thermophiles, and in most probable number techniques for coliforms and faecal coliforms. Many different foods were tested throughout this course, examples of which are mineral water, raw and processed meats, seafoods, canned foods, eggs and ice cream. The details of the course content and schedule are provided in Annex 3. A full set of lecture notes describing the isolation, enumeration and identification of the principle food spoilage and pathogenic bacteria were provided for the DTQC and are not included as part of this report.

Rapid Microbiological Methods

8. Traditional microbiological methods have a number of shortcomings which include:

(a) the time it takes to obtain results;

(b) the costs which are especially important in countries like Vietnam who must import all media and chemicals;

(c) they are labour intensive and require capital investment in laboratories and equipment.

Because of these disadvantages a great deal of effort has gone into developing rapid methods and producing apparatus and reagents in order to speed up microbiological analyses. During this consultancy visit rapid microbiological methods were discussed with staff at DTQC and an assessment made of whether such techniques would be appropriate for the routine work of their Food Microbiology Laboratory.

9. Five types of procedures have been developed for rapid microbiological analysis and these are used by various food industries and regulatory agencies. These procedures include techniques that produce colony counts more rapidly than traditional methods; those that rely on direct detection of microbes by microscopy; those that assess the concentration of some particular component of microbes and assumes the value obtained is proportional to the numbers present in a food; those that exploit some physiological property of an organism; and those that assay products of metabolism. The advantages and disadvantages of these methods will be briefly discussed and their use by the DTQC assessed.

(a) Various techniques have been used to speed up the colony count procedure such as improvements in homogenization methods using the Colworth Stomacher. This equipment has been supplied by UNIDO and is a considerable improvement over the methods previously used at DTQC. Other equipment for speeding up enumeration of microorganisms include automatic pipettes for delivering small quantities and the

Spiral Plater System. Both these items of equipment are very expensive. Simple ideas which save time and cost include the Agar Slide and Contact Plate Methcds. The latter was introduced during the training courses. For routine quality control in food processing plants the use of the Coli-Strip (sterile paper impregnated with selective media for *E.coli*) would have a number of advantages and these were discussed with DTQC staff. The principle disadvantages are the expense involved in importing all media and chemicals and the limited storage life of some of the more senstitive chemicals.

(b) The principle improvement to direct enumeration by microscopy has been the development of the Direct Epifluorescent Filter Technique (DEFT). This technique has been found particularly useful in the milk industry. Fluoroescent microscopy is also used for identification of such pathogens as the 01 serotypes of *Vibrio cholerae* in seafcods. For these techniques a fluoroescent microscope which provides illumination with ultraviolet light is required and these are expensive, and would not be suitable for use in DTQC.

(c) The assessment of the microbial load by using specific cellular components such as adenosine triphosphate (ATP), lipopolysaccharide (LPS) and deoxyribonucleic acid (DNA) have been used experimentally and some commercial kits are available. Although these techniques are very rapid, they require extensive experience in their use and interpreation of results. Chemicals involved in the assays are expensive and must be constantly stored under chilled temperature. These methods are not recommended for DTQC.

(d) Impedance/conductance methods are the most widely used of these techniques which rely on physiological properties for assessment of microbial numbers in foods. These involve the detection of changes in electrical properties of growth media as microorganisms grow. Food samples are added to an appropriate liquid medium in a special tube which contains electrodes. As the microorganisms grow the electrical properties of the liquid medium are measured and recorded by microcomputer, and the changes in impedance/conductance are proportional to the number of microorganisms in the food sample. The equipment is very expensive and is only used by large food companies or regulatory authorities who analyse large numbers of food samples. This equipment is not recommended for DTQC because of the capital and running costs.

(e) There are a range of methods that are gaining in popularity in the food industry which are based on antibodies and the use of the enzyme-linked immunosorbant assays (ELISA). These usually detect the presence of a microorganism or a microbial toxin by a colour change if the toxin or the microbe are present in a food. Commercial kits are available for a range of pathogens such as *Salmonella* and toxins, like aflatoxin and staphylococcal toxins, and some are in use in Vietnam by the Seaprodex Company, who produce seafoods for export. Such commercial kits could be used by DTQC if they can pass the cost of purchase and importation fees to their customers. At present the DTQC does not charge full economic costs to their customers for the analytical services which they supply. If the analytical services provided by the DTQC are to be sustainable, full economic costs must be charged.

Recommended Methods of Food Analyses

10. Staff at the Food Microbiology Laboratory were trained in internationally recognised methods of microbiological evaluations of foods during both training courses. This complemented the earlier training received by some of the participants at the Food Development Centre, Philippines, as part of the UNIDO Project. It is recommended that the DTQC use the methods for microbiological evaluation of foods which were introduced during the training courses. These methods, are based on current internationally recognised methodology recommended by such bodies as the International Commission for the Microbiological Specification for Foods (ICMSF) and the United States Food and Drug Administration in their Bacteriological Analytical Manual.

Two new techniques are recommended in order to reduce costs and speed up analysis at the DTQC Food Microbiology Laboratory. These are the use of the Drop Plate Technique for enumeration of bacteria in foods and the Modified Semi-solid Rappaport - Vassiliadis (MSRV)technique for the detection of *Salmonella* from foods.

(a) Standard Plate Counts

Counts of viable bacteria are commonly based on the number of colonies that develop in Plate Count Agar plates which have been innoculated with known amounts of diluted food and then incubated under prescribed environmental conditions. A wide variety of conditions can be obtained by changing the composition of the agar medium, gaseous environment, and the time and temperature of incubation. For example, incubation at temperatures of 30° - 37°C favours the growth of mesophilic organisms which

include both pathogenic and spoilage bacteria. Incubation at temperatures in the region of 55°C allows the development of thermophilic organisms and inhibits other groups.

In order to reduce operational costs the DTQC should consider using the Drop Plate Method for evaluating total counts in foods. Details of this technique were supplied for the DTQC during the training courses. The cost of the Drop Plate Method is less than 50% of the costs of Pour Plate and Spread Plate techniques, which are currently used at the DTQC.

(b) MSRV technique for detection of Salmonella from foods.

Conventional techniques used for the isolation and identification of *Salmonella* from foods involve a test period of 5-7 days.

Recently much attention has been given to the development of rapid and reliable techniques for the detection of *Salmonella* due to the inconvenience resulting from delays in issuing reports of analyses. Recent advances have been made which involve techniques based on serological specificity and motility of *Salmonella*. The stages of this new method involves non-selective enrichment of the food (12 hours); selective enrichment for *Salmonella* (12 - 18 hours); motility enrichment in Modified Semi-solid Rappaport-Vassiliadis medium (MSRV) (8 - 12 hours) and finally serological identification (2 hours). This method offers the advantage of completing all tests within 48 hours rather than the test period of 5 - 7 days using conventional techniques. Details of this method for the isolation of *Salmonella* from foods are to be found in Annex 5 of this report.

Field Assessment Visits

11. A range of State-owned food processing and storage companies were visited during training courses, and participants were instructed in the methods to evaluate process control and assessment of hygiene and sanitation during food processing. An example of how a sanitation audit should be carried out in premises processing food for sale is given in Table 1. It is recommended that DTQC staff use this or a similar auditing system when inspecting food processing plants. The attention paid to process control in all plants processing food for the domestic market was minimal and standards of hygiene and sanitation were basic. The DTQC has a very challenging function to carry out in the future in assisting food companies to improve basic processing standards and quality control. The most successful method of controlling food quality in an open market society is to introduce methods of process control which can be applied to foods from the point of production through to consumption. This concept was discussed during the seminar on food quality control.

Seminar on Food Quality Control

12. Three technical presentations were given by the consultant during this seminar, as well as a video on Food Safety Laws in the UK (the programme of the seminar is provided at Annex 4). The seminar was attended by about 50 participants from Food Research Institutes and the Food Industry in Hanoi, and the technical presentations that were given were:

(a) Food Control Programmes for Food Safety;

(b) New Concepts in the Food Quality Control;

(c) Occurrence and Significance of Mycotoxins in Foods.

Key issues discussed in this seminar were new approaches to food quality control; and the control of mycotoxin contamination of foods in the Vietnamese food supply.

Table 1: Sanitation Audit in Food Processing Plants

Name and Address of Processing Plant
Facility Owner (Company or Individual)
Products Concerned:
Date of Visit:

Facility Sanitation

1. PEST CONTROL	MIN	MAJ	SER	CR
1.1 Harborage and attractant areas present				
1.2 Pest control measures not effective 1.2.1 Exclusion				
1.2.2 Extermination				

2. STRUCTURE AND LAYOUT	MIN	MAJ	SER	CR
2.1 Grounds condition can permit contamination to enter the facility				
2.2 Facility 2.2.1 Design, layout or materials used cannot be readily cleaned or sanitized; does not preclude contamination.				
2.2.2 Insufficient separation by space or other means allows product to be adulterated or contaminated.				
2.3 Equipment and utensils' design, construction, location or materials cannot be readily cleaned or sanitized; does not preclude product contamination.				
3. MAINTENANCE	**MIN**	**MAJ**	**SER**	**CR**
3.1 Condition of roof, ceilings, walls, floors, or lighting not maintained; lights not protected 3.1.1 Areas directly affecting product or primary packaging material.				
3.1.2 Other.				
3.2 Insufficent lighting.				
3.3 Equipment and utensils not maintained in proper repair or removed when necessary.				
3.3.2 Other.				

Table 1/continued

4. CLEANING AND SANITIZING	MIN	MAJ	SER	CR
4.1 Product contact surfaces not cleaned and sanitized before use.				
4.2 Non-product contact surfaces not cleaned before use.				
4.3 Inadequate housekeeping.				
5. PERSONNEL	**MIN**	**MAJ**	**SER**	**CR**
5.1 Processing or food handling personnel do not maintain a high degree of personal cleanliness.				
5.2 Processing or food handling personnel do not take necessary precautions to prevent contamination of food.				
5.3 Controls.				
5.3.1 Facility management does not have in effect measures to restrict people with known disease from contaminating the product.				
5.3.2 Handwashing and hand sanitizing stations not present or conveniently located.				
6. RESTROOMS	**MIN**	**MAJ**	**SER**	**CR**
6.1 Insufficient number of functional toilets.				
6.2 Inadequate supplies.				
7. WATER SUPPLY	**MIN**	**MAJ**	**SER**	**CR**
7.1 Unsafe water supply.				
7.2 No protection against backflow, back-siphonage or other sources of contamination.				
7.3 Inadequate supply of hot water.				
8. ICE	**MIN**	**MAJ**	**SER**	**CR**
8.1 Not manufactured, handled or used in a sanitary manner.				
9. CHEMICALS	**MIN**	**MAJ**	**SER**	**CR**
9.1 Chemical(s) improperly used or handled.				
9.2 Chemical(s) improperly labelled.				
9.3 Chemical(s) improperly stored.				

Table 1/continued

10. VENTILATION	MIN	MAJ	SER	CR
10.1 Condensation				
10.1.1 Area directly affecting product or packaging material.				
10.1.2 Other				
11. WASTE DISPOSAL	**MIN**	**MAJ**	**SER**	**CR**
11.1 Improper disposal of:				
11.1.1 Sewage.				
11.1.2 Processing waste				

SUMMARY	MIN	MAJ	SER	CR
Total Deficiences				

Table 1/continued

Final Facility Rating
Inspector Signature and Date
Supervisor Signature and Date

Systems Audit Frequency Schedule					
Facility Rating	Audit Frequency	Number of Deficiencies			
		Minor	Major	Serious	Critical
Level I	One visit every two months	0-6	0-5	0	0
Level II	One visit per month	≥ 7	6-10	1-2	0
Level III	Two visits per month	NA*	≥ 11	3-4	0
Level IV	Daily	NA	NA	≥ 5	≥ 1

Note: For a facility rating of Level II,no more than 10 combined "Major" and "Serious" deficiences can exist. If the combination of "Major" and "Serious" deficiences exceeds "10", then the facility will be rated as a Level III.

Food Process Control

13. Changes are recommended to the strategy of the DTQC in their approach to food surveillance and quality control. The DTQC relies on the traditional practice of end product inspection of food samples for assessment of quality and safety. Food samples are taken by the DTQC staff during routine inspection of premises or markets, and these are analysed by the laboratory. Sampling plans are not used and the samples are generally not representative. The practice of relying on inspection and surveillance of final products has many shortcomings and experience within the food industry has shown that even the most careful and thorough inspection programme and end product testing scheme does not lead to good management of risks. The main limitations to end product testing are the difficulties of collecting and examining sufficient samples to obtain meaningful information; the time required to get results, which can be several days; and the high cost of analytical procedures, particularly those for microbiological testing of foods.

14. More recently, food processing companies are adapting the guidelines of the International Organisation for Standardisation (ISO) on quality management systems and quality assurance standards, referred to as the ISO 9000 series. These specify the need to establish procedures which can lead to national and international confidence in quality standards for foods moving in the international trade as well as domestic trade. Under the ISO 9000 series modern food companies must put in place systems to monitor quality of raw materials, production, packaging, storage and distribution procedures. The system must deal with the organisational structure of the company, responsibilities, procedures, processors and methods for implementing quality management.

15. It is recommended that the DTQC take a more pro-active role in food quality control and apply the principals of the Hazard Analysis Critical Control Point concept (HACCP) to food process control. This is a systematic approach to the identification of hazards associated with food processing and the putting in place of a system to control these hazards. The HACCP approach focuses attention on factors which directly affect safety of foods, it eliminates wasteful use of resources while assuring that the desired levels of safety and quality are met and maintained. As an example for DTQC staff the HACCP system for a moderate size food company involves the following stages:

* The formation of a HACCP team or group of people who know and understand the food companies' products and how they are used. Employees of the company in the team should include, but should not be limited to, the Production Manager, Quality Control Manager, Sales Manager and advice should be saught from experts in food microbiology and technology, and other relevant disciplines.

* The identification of hazards and the assessment of the severity of these hazards (hazards analysis) and their risks, associated with production, harvesting, processing/ manufacture, distribution, marketing, preparation and/or use of a raw material or final product. A hazard means the unacceptable contamination of a food product by microorganisms which may affect its safety or lead to spoilage; it also includes factors which lead to economic fraud (e.g. adulteration, species substitution, addition of prohibited chemicals etc.). Hazard analysis includes the identification of all procedures in the process where hazards of potential significance occur; listing all identified hazards associated

with each step and listing all preventive measures to control hazards.

* The determination of Critical Control Points (CCPs) at which identified hazards can be controlled. A CCP is defined as an operation where control can be exercised over one or more factors to eliminate, prevent or minimize a hazard. In some food processors, control of a single operation (CCP) can completely eliminate one or more microbial hazards, such as pasteurisation in milk processing.

* The specification of criteria that indicate whether an operation is under control at a particular CCP. Criteria can be physical, chemical or biological factors. Examples are time and temperature in thermally processed foods; water activity in dried foods; and chlorine levels in water used in food processing. All criteria have to be clearly defined and documented.

* Establishing and implementing procedures to monitor each CCP to check that it is under control. Monitoring and control procedures should specify who is to act, what action is to be taken, when the action is to be taken, and the limits requiring further action. Monitoring parameters such as time, temperature and visual appearance are most useful because of their speed, simplicity and low cost. Others include measurement of chlorine concentration in water for food processing using a cheap and simple colour test kit. The measurement of salt concentration in fish sauce using a hand-held refractometer; and pH and salt levels in such products as pickled vegetables. It is most beneficial if the process is monitored by the actual operator and that this person receives training in

the process. Microbiological testing is of little value for monitoring most CCPs.

* Taking corrective action whenever monitoring includes that criteria are not met. All corrective actions must be taken by trained individuals and documented to report action taken.

* Verification of HACCP system. This may be carried out by quality control staff or personnel from government regulatory agencies such as DTQC. Regular reviews are necessary because of the evolving nature of food processing and it is necessary to ensure that all hazards have been identified, all CCPs detected, criteria are appropriate and that monitoring procedures are effective in evaluating operations.

Mycotoxins in Foods

16. The greatest interests by participants at the seminar were shown during discussions on the occurrence and significance of mycotoxins in foods. The principal reasons for this interest is that exports of groundnuts from Vietnam have been rejected in overseas markets because of contamination by aflatoxin. The issues discussed during the seminar on Food Quality Control were the extent of the mycotoxin problems, with emphasis on S.E. Asia, significance of mycotoxins in foods and feeds, aflatoxin control and detoxification, methods to detect mycotoxins and legislative aspects. The text of the seminar is included in Annex 8, which includes information on sampling plans and methods to detect aflatoxins in foods.

As a result of discussions during the seminar on Food Quality Control, the following work is suggested on mycotoxins in the food supply in Vietnam. This work

could be carried out by the DTQC in collaboration with the Ministry of Agriculture in order to ensure that food products offered for sale are safe with respect to mycotoxin contamination. This includes:

(a) Training and extension activities to promote at field level the prevention of mould contamination during harvest, storage and distribution stages in order to prevent post-harvest contamination.

(b) Strengthening food control systems in order to control mycotoxin contamination of marketed foods.

(c) Improve surveillance systems and strengthen laboratory capabilities in mycotoxin analysis.

(d) Collaboration at national, regional and international levels to improve the prevention of mycotoxin development as well as monitoring and control.

Visit to DTQC Branch, Ho Chi Minh City

17. A brief visit was made to the laboratory of the DTQC Branch in Ho Chi Minh City. During the visit an ad hoc seminar on rapid methods of microbiological analysis of foods, and food laws and standards was given to staff. The laboratories are poorly equipped and it is not possible to carry out chemical and microbiological analysis of foods. There is an urgent need to upgrade facilities for chemical, physical and microbiological testing of foods at these laboratories in order to strengthen the quality control network of the Ministry of Trade.

Information on Food Quality Control

18. There is a real need for the DTQC to obtain recent publications and up-to-date information on food quality and safety, food surveillance and control, and standards and methods of analysis. A recommended list of publications which should be purchased by the DTQC is provided at Annex 6, and those can be ordered through the FAO field office, Hanoi.

Recommendations

19. (a) In order to perform some of its key functions and responsibilities the DTQC should purchase the equipment listed in paragraph 4 and Annex 1 of this report for their Food Microbiology Laboratory. The total cost of this equipment is US$ 3616.

(b) In the interests of safety the DTQC should autoclave all contaminated material from the Food Microbiology Laboratory prior to disposal.

(c) Staff in the Food Microbiology Laboratory should observe all necessary precautions when handling potentially toxic dehydrated microbiological media.

(d) It is recommended that the DTQC use the drop-plate method for enumeration of bacteria in foods and that they use the modified semi-solid Rappaport-Vassiliadis technique for the detection of salmonella in foods.

(e) It is recommended that the DTQC use a structured system for auditing sanitation in food processing plants and details are provided in Table 1, paragraph 12.

(f) It is recommended that the DTQC purchase the list of publications included in Annex 6 of this report.

Acknowledgements

20. To all those who assisted during this visit and who showed much hospitality, both at the DTQC and the UNIDO Field Office, I am very grateful.

ANNEX 1

Equipment List to be Purchased by the DTQC

	Cost
1. Anaerobic System	
Anaerobic System including small jar, lid and 'O' ring gasket (BBL60465)	£ 98.30
Hydrogen/carbon dioxide generators (BBL7034), 50 units	£325.00
Indicators (BBL70504), 50 units	£ 65.00
Spare catalysts (BBL70303),	£ 11.80
	£500.10

For export details the Belgium office of BBL should be contacted. The fax numbers are 325 378 5665; 325 372 0200.

2. Water Bath	
Water bath (Grant Barrington series), 14 litre model B22-409. Tank dimensions 325 x 300 x 150 mm.	£110.00
Lid for water bath, B22-436.	£ 35.00
	£145.00

This water bath is available from Phillip Harris Scientific, 618 Western Avenue, Park Royal, London W3 OTE. Fax number 448 1993 8020.

3. Microscope

Compound Microscrope. Olympus Microscrope, model CHT213E. Fitted with a 45° binocular head, mechanical stage, 2 x 10 lb eye pieces, X4, X10, x40 and X100 oil immersion achromatic objectives £965.00

£965.00

This model is available from Olympus Optical (UK) Ltd., 2-8 Honduras Street, London EC1Y OTX. Fax number 447 1490 7880.

4. Incubators

Precision Incubators. 33 litre incubator, with internal dimensions of 350 x 310 x 300 mm and external dimensions of 600 x 420 x 430 mm. Temperature control accuracy ±0.25°C. £344.00

This model is available from Phillip Harris International, Model No. 15-140.
Fax No. 44 81 993 8020.
Two incubators should be purchased by the DTQC for maximum flexibility for microbiological analysis. £688.00

TOTAL £2,298.10

(Total cost of equipment recommended for purchase by DTQC = US$3616)

ANNEX 2

TRAINING COURSE ON THE IDENTIFICATION OF FOOD SPOILAGE MOULDS AT DTQC FROM 18 SEPTEMBER - 3 OCTOBER 1992

(L = Lecture; P = Laboratory Practical)

Friday, 18 September

Nature of filamentous fungi (L)

Preparation of general purpose microbiological media and low water activity media for mould isolation and identification (P)

Saturday, 19 September

Nature of yeast and yeast like organisms (L)
Plating out of mould pure cultures (P)

Monday, 21 September

Methods for detection of moulds and yeasts (L)
Identification techniques (P)

Tuesday, 22 September

Total viable counts for moulds and yeasts (L)
Enumeration of moulds in food samples (P)

Wednesday, 23 September

Field visit to collect samples

Thursday, 24 September

Principles of food spoilage (L)
Isolation and identification of moulds from field samples I (P)

Friday, 25 September

Significance of moulds in foods - guidelines and standards (L)
Isolation and identification of moulds from field samples II (P)

Saturday, 26 September

Fungi in foods - Sampling (L)
Isolation and identification of moulds from field samples III (P)

Monday, 28 September

Microbiology of dried foods (L)
Cultural and microbiological characteristics of *Aspergillus* spp. (P)

Tuesday, 29 September

Mycotoxins (L)
Cultural and morphological characteristics of *Penicillium* spp. (P)

Wednesday, 30 September

Control of mould spoilage (L)
Cultural and morphological characteristics of *Fusarium* spp. (P)

Thursday, 1 October

Aflatoxins in man and animals (L)
Laboratory demonstration of cultural and morphological characteristics of yeasts (P)

Friday, 2 October

Regulatory aspects of mycotoxins in foods (L)
Cultural and morphological characteristics of *Eurotium* spp.

Saturday, 3 October

End of course/exam.

ANNEX 3

TRAINING COURSE IN ISOLATION AND IDENTIFICATION OF FOOD SPOILAGE AND PATHOGENIC BACTERIA AT THE DTQC FROM 5 - 31 OCTOBER 1992

(L = Lecture, P = Laboratory practical)

Monday, 5 October

Methods for the Bacteriological Examination of foods (L)
Standard Plate Counts I. Mesophiles and thermophiles (P)

Tuesday, 6 October

Bacteria associated with foodborne diseases (L)
Standard Plate Count II. Spread and drop plate methods (P)

Wednesday, 7 October

Enteric indicator organisms (L)
Preparation of laboratory media (P)

Thursday, 8 October

Significance of coliforms, faecal coliforms and EEC in foods (L)
Enumeration of coliforms in water and milk (P)

Friday, 9 October

Salmonella in Foods (L)
Enumeration of faecal coliforms in water and milk (P)

Saturday, 10 October

Shigella in Foods (L)
Complete experimental work on coliforms (P)
Sterility testing of laminar flow hood

Monday, 13 October

The preparation of an audit form for field assessment (L).
Food laws (L).
Preparation of media for *S.aureus* (P).

Tuesday, 14 October

Significance and occurrence of *Staphylococcus aureus* in foods (L).
Isolation of *S.aureus* from the human body (P).

15-17 October

Field Visit to Ho Chi Minh City Branch of DTQC

19 October

Isolation of *S.aureus* from meat products (P).

20 October

Identification of pathogenic *S.aureus* from foods (L).
Completion of laboratory study on *S.aureus* (P).

21 October

Bacillus cereus in foods (L)
Examination of commercial food samples I (P)

22 October

Pathogenic *Clostridia* in foods (L)
Examination of commercial food samples II (P)

23 October

Isolation of C. *perfrigens* from foods (P)

26 October

Occurrence and significance of *Listeria monocytogenes* in foods (L)
Isolation of *Salmonella* from poultry, eggs and seafood.

27-28 October
Contamination of laboratory work on *Salmonella*

29 October

Pathogenic *Vibrios* in foods (L)
Continuation laboratory work on *Salmonella* (P)

30-31 October

Field assessment visit to Haiphong District to assess Q.C. in food processing.

2 November

Seminar on Food Quality Control

3 November

Awarding Certificates to training course participants.

ANNEX 4

PROGRAMME FOR THE SEMINAR ON FOOD QUALITY CONTROL WITH EMPHASIS ON MICROBIOLOGY AND MYCOTOXINS

Monday, November 2, 1992

8.30 Opening Session
Prof Dr Phan Duc Thang
Director of DTQC, Hanoi
- Welcome
- Introduction of Speakers
- The Importance of Food
- Quality Control in Vietnam

TECHNICAL SESSION

9.30 Mr Alan Reilly
Head, Food Safety,
Natural Resources Institute
Chatham Maritime
United Kingdom

Food Control Programmes for Food Safety
- Food Legislation
- Food Laws in UK
- Role of Food Industry
- Food Surveillance

10.15 Video on UK Food Safety Laws

10.45 Mr Alan Reilly

New Concepts in Food Quality Control

11.15 Discussions on technical sessions

12.00 Lunch

14.00 Mr Alan Reilly

Occurance and Significance of Mycotoxins in Foods
- Extent of Mycotoxin Problem in Foods and Feeds

- Methods to Control Contamination
- Methods of Detection and Decontamination
- Legislation

14.45 Prof Dr Bui Xuan Dong — The Problem of Mycotoxins in Vietnamese Agricultural Food Products

15.15 Discussions on technical sessions

16.00 Recommendations and Final Remarks.

ANNEX 5

DETECTION OF SALMONELLA IN FOODS USING THE MODIFIED SEMI-SOLID RAPPAPORT-VASSILIADIS (MSRV) MEDIUM

1. Homogenise in a stomacher 25g of food sample in 225ml Buffered Peptone Water (Oxoid CM509) and incubate at 35°C for 12-20 hrs.

2. Inoculate 0.1ml of the pre-enrichment Peptone Water culture to 10ml of Rappaport-Vassiliadis (RV) enrichment broth and incubate at 42°C for 12-24 hrs.

3. Inoculate 3 drops of enrichment culture in separate spots on the surface of MSRV medium plates (Oxoid CM910) plates.

4. Incubate plates in an upright position at 42°C for 8-20 hrs.

5. Examine the plates from a motile bacteria which will be shown by a halo of growth originating from the inoculated spot.

6. Sub-cultures can be taken from the outside edge of the halo to confirm purity and for further biochemical and serological testing (as described in laboratory schedule used during the training courses).

ANNEX 6

LIST OF RECOMMENDED PUBLICATIONS FOR THE DTQC

The following publications can be purchased/ordered from the FAO, Field Office, Hanoi.

CAC/RCP 1-1969	Revision 1-1979, General Principles of Food Hygiene
Alinorm 79/13A annex to Appendix II	Annex 1, "Cleaning and Disinfection" General Principles of Food Hygiene
CAC/RCP 2-1969	Code of Hygienic Practice for Canned Fruit and Vegetable Products
CAC/RCP 3-1969	Code of Hygienic Practice for Dried Fruits
CAC/RCP 9-1976	Code of Practice for Fresh Fish
CAC/RCP10-1976	Code of Practice for Canned Fish
CAC/RCP11-1976	Code of Hygienic Practice for Fresh Meat
CAC/RCP21-1979	Code of Hygienic Practice for Foods for Infants and Children
CAC/RCP23-1979	Code of Hygienic Practice for Low-Acid and Acidified Low-Acid Canned Foods
CAC/RCP24-1979	Code of Practice for Lobsters
CAC/ACCEPTANCES	Summary of Acceptances of Recommended
CAC/FAL 1-1973	List of Additives Evaluated for their Safety-in-Use in Foods
CAC/FAL 5-1979	Guide to Safe Use of Food Additives
CAC/PR 1-1978	Guide to Codex Maximum Limits for Pesticide Residues
Alinorm 79/24A Relating to Appendix VII	Problems in Developing Countries Pesticide Residues
CX/PR 79/17	Guidelines for the Regulation of Pesticide Residues in Food

Codex Alimentarius - Volume VI

Codex Standards and Guidelines for the Labelling of Foods and Food Additives

CODEX STAN 1-1981	General Standard for the Labelling of Prepackaged Foods
Guidelines for Date marking of Prepackaged Foods for the Use of Codex Committees	
CODEX STAN 20-1981	Edible Soya Bean Oil
CODEX STAN 22-1981	Edible Cottonseed Oil
CODEX STAN 23-1981	Edible Sunflowerseed Oil
CODEX STAN 25-1981	Edible Maize Oil
CODEX STAN 26-1981	Edible Sesameseed Oil
CODEX STAN108-1981	Natural Mineral Waters
CODEX STAN137-1981	Edible Ices
CODEX STAN 65-1981	Maximum Limits for Pesticide Residues: 4th Series

ANNEX 7

List of People Met

1. Department of Quality Control of Goods and Metrology, Ministry of Trade

Dr. Phan Duc Thang, Director, DTQC.

Dr. Le Xuan Dich, Deputy Director, DTQC.

Mr Ngo Dinh Co, Secretary of Project and Head of Quality Control of Agriculture and Foodstuff Section.

Mr Phan Quoc Dong, Chief of Quality Control Laboratory.

Mrs Huynh Le Tam, Food Microbiologist.

Mrs Thai Thi Tuat, Quality Control Section.

Mr Lai Van Ty, Quality Control Section.

2. UNIDO Field Office, Hanoi.

Dr M.J. Meixner, Country Director.

Mr P. Hjortlund, Field Officer (Deputy to Country Director).

Miss A.C. Latacz, Programme Officer.

3. DTQC Branch, Ho Chi Minh City

Dr Pham Thi Be Nan, Director.

Mr Le Minh Tam, Deputy Director.

ANNEX 8

Occurance and Significance of Mycotoxins in Foods

Paper presented by A Reilly at the DTQC Seminar on Food Quality Control, 3 November 1992, Hanoi.

THE OCCURANCE AND SIGNIFICANCE OF MYCOTOXIN IN FOODS

Introduction

It is now widely appreciated that the presence of mycotoxins in food and feeds can represent serious health risks for both man and animals alike. This appreciation arose from the discovery in 1961 by workers in the UK at the Tropical Products Institute and at Central Veterinary Laboratory that a toxin-producing fungus could be isolated from groundnut kernals (Sargeant, *et al* 1961; Spensley, 1963) following the outbreak of Turkey X disease. The concern about health and safety of the consumer in European countries has led to the introduction of legislation by the European Commission, which restricts the level of aflatoxin in animal feeds and this has substantially reduced the importation of feeding stuffs of developing country origin. Rejection of consigments involves economic losses for exporting countries and health hazards to its population if rejected food is redistributed on the national market. The health risks due to mycotoxins are seriously increased when the good quality commodities are sold abroad, while substandard or contaminated products are retained for domestic use. The commodities identified as most susceptable to contamination by aflatoxins include groundnuts, cottonseed, copra, oil palm and maize. In this seminar I would like to discuss the extent of the mycotoxin problem in foods and feeds, as well as methods to control contamination, techniques for detoxification and methods of detection. I will rely heavily on the research and development work which has been carried out at the Natural Resources Institute and it's predecesors in the UK, as we have been active in the field since the discovery of aflatoxin in 1961 .

Extent of the mycotoxin problem

Enviromental conditions leading to mycotoxin production exist nearly ever/where in the world. The problem is most serious in warm humid tropical countries where harvest and post-harvest technologies which would prevent mould growth and mycotoxin production can occur throughout the food chain; in the field, during harvest, processing, storage, and transport; and are influenced by many different ecological factors such as temperature, moisture and substrate. Mycotoxins may be present in foods long after the toxic species of moulds which produced the toxin have died. The major mycotoxins implicated in mycotoxicoses are:

Aflatoxin

The aflatoxins (B_1, B_2, M_1, M_2, G_1, G_2) appear to constitute a contamination problem mainly in groundnut products, cottonseed meal, and corn. The occurance of aflatoxins is usually associated with poor storage conditions, although recent evidence indicate that these compounds are also produced in the field.

A.flavus and *A.parasiticus* species of the *A.flavus-oryzae* group were isolated from groundnuts in 1961 and shown to produce aflatoxins. These fungi are commonly associated with stored products, and are prevalent in soil and on decaying matter particularly in tropical and sub-tropical countries. These fungi can also cause heating and decay in stored grain with an equilibrium relative humidity (ERH) of 85% or more. The optimum temperature for growth is between 27-33°C.

Aflatoxins cannot be destroyed by cooking temperatures, and can only be inactivated or detoxified by some microbiological or chemical processes, which are costly but, may be commercially feasible. The best method for prevention of aflatoxin production is to inhibit the growth of *A.flavus* by careful harvesting, handling, processing, and storage of the food or feedingstuff. However, if toxic batches of feed constituents are detected it is sometimes possible to dilute them to a presumed safe level with non-toxic meal for animal feed. Meat products from animals fed on diets containing aflatoxins have been found to be free from the mycotoxin although it is known that lactating mammals secrete a modified aflatoxin (M_1) in the milk. Maximum acceptance levels of aflatoxin in animal feedingstuff as laid down by EEC regulations are 10-15 ppb depending on age and animal species.

Ochratoxin A and Citrinin

Fungi associated with Ochratoxin A production are *Aspergillus ochraceus* and *Penicillium viridicatum*, and for Citrinin production *Penicillium citrinum*, and *P.viridicatum*. Ochratoxin A was first isolated from maize in South Africa on which *A.ochraceus* had grown (Merwe, *et al.*, 1965). The toxin has been found to occur naturally in wheat, oats, barley, groundnuts, white beans, mixed feed grain, and coffee. Ochratoxin A is highly toxic metabolite that produces both liver and kidney damage as chronic toxicity studies with both poultry and swine have demonstrated.

Citrinin

Citrinin was originally isolated from *P.citrinum* and was considered responsible for the yellow colour of rice imported into Japan from Thailand around 1951 (Scott,P.M, 1973). However, the occurence of Citrinin as a contaminant of feedstuff has been associated with *P.viridicatum*. Ochratoxin A and Citrinin may have involved in the mycotoxicoses which recently affected 7% of pigs in Denmark. Reports of naturally occurring citrinin in wheat, rye, barley and oats have occured in America, Canada and Denmark.

Zearalenone or F-2

Fungi asscociated with Zearalenone are species of *Fusarium*.The occurence of Zearalenone is related to low temperatures and the invasion of grain by various species of *Fusarium*, particularly *F.graminearum, and F.roseum (Gibberella zeae)*. These fungi invade developing corn at the silking stage in periods of heavy rainfall and proliferate on mature grains that have not dried because of wet weather or, because the grains have been stored under wet conditions. Zearalenone causes an estrogenic syndrome in swine and is associated with consumption of mouldy corn in the U.S. Because F-2 has been found to occur naturally in feeds in sufficient amounts to cause disease it is one of the best understood of the mycotoxicoses problems. Usually, levels of 1-5 ppm Zearalenone in feeds are enough to induce physiological responses in swine associated with estrogenic syndrome. At lower levels, Zearalenone and various derivatives actually stimulate growth in farm animals (Hesseltine, 1976).

Trichothecenes or T-2

Fungi associated with the group of compounds called trichothecenes are *Fusarium tricinctum, F graminearum*, and *Gibberella zeae* (perfect stage of *F roseum*).

The trichothecenes have been implicated in a variety of mycotoxicoses involving both humans and animals on a large scale; diseases include alimentary toxic aleukia, mouldy corn toxicosis, and refusal or vomiting phenomenon (Ciegler,1975).

Significance of mycotoxins in foods and feeds

Mycotoxins have be shown to be aetiological agents in a wide range of diseases in animals. Circumstantial evidence exists which supports the view that fungal toxins, in particular aflatoxin, may be responsible for a wide range of diseases man, including primary liver cancer. Reports from Africa and India link aflatoxin contaminated foods with fatalities (Hannsen,1970; Yadgiri *et al*, 1970). The incidence of Reye's Syndrome, characterised by vomiting, hypoglycemia, convulsions, presence of ammonia in the blood, coma and death within 1-2 days, has been reported in North-East Thailand. This syndrome only effects children up to adolescence and the incidence of the disease corresponds with seasonal and geographical variations in dietary aflatoxin (Harwig *et al* 1975). Analysis of the livers from victims of this disease has shown the presence of aflatoxins B_1 and G_1. Results of various studies summarised in Table 1 show a significant correlation between the level of aflatoxin in the diet and the incidence of liver cancer. The intake of aflatoxin was determined by the collection and analysis of "plate samples" of foods from homes and from market samples of foods.

Aflatoxins are acutely toxic to some animals and are known potent liver carcinogens. Toxicity varies with animal species and with such factors as age, sex, and general condition of the animal, composition of diet and route of administration. Some mycotoxins are more potent when administered in the presence of certain other mycotoxins or when administered to animals with certain nutrient deficiencies (e.g.the synergistic affect of aflatoxin and rubratoxin). Toxicological studies indicate that farm animals will vary considerably in their susceptibility to aflatoxicosis. Some animals, notably rabbits, ducklings, trout, pigs, calves and turkeys are susceptable to the acute toxicity of aflatoxin, and their aflatoxin intake must be controlled very carefully if heavy losses are to be prevented. Other animals are more resistant, but can suffer chronic effects which manifest themselves as loss in productivity and poor growth.

Aflatoxin M_1 is particularly important as this is the toxic metabolite found in milk of lactating animals ingesting aflatoxin B_1. When feeds containing 100ppb aflatoxin B_1 are fed to cows, milk produced by these cows will contain 1ppb aflatoxin M_1. This is one of the most likely ways aflatoxins enter the human food chain (i.e. from animal products), and it is particularly worrying because babies whose diet consits mainly of milk are particularly vulnerable. Aflatoxin M_1 has a similar acute toxicity to aflatoxin B_1 and is also a very active carcinogen in some animals, being at least one tenth as potent as aflatoxin B_1. It is therefore, very important to monitor milk, and milk products for aflatoxin M_1.

Although many farm animals are susceptable to the carcinogenic effects of aflatoxin, this is rarely of importance as they are usually sacrificed before such effects manifest themselves. However it has been shown that ducks can develop hepatic carcinoma within 14 month when fed a diet containing as low as 30 ppb aflatoxin B_1 (Carnaghan,1965). This of importance when laying ducks are kept for this length of time. In experimental studies when chickens were fed high levels of aflatoxin in their diet (6000ppb), results showed stunting in growth and affected birds have been shown to bruise very easily after slaughter and this greatly reduces their market value.

Table 1- INCIDENCE OF PRIMARY LIVER CANCER AND AFLATOXIN INTAKE

Locality	Estimated Aflatoxin Intake (μg/Kg)	Aflatoxin in Food (μg/Kg)	Cancer Rate (per 100,000 per year)
Kenya-High Altitude	0.2	0.1	1.2
Thailand-Songkhla	0.3	0.2	2.0
Swaziland-High Veld	0.4	0.2	2.2
Kenya-Middle Alt.	0.4	0.2	2.5
UK	?	?	2.9
Swaziland-Mid. Veld	0.6	0.3	3.8
Kenya-Low Altitude	0.7	0.3	4.0
Thailand-Ratburi	3.2	1.6	4.0
Swaziland-Low Veld	3.0	1.5	9.2
Mozambique	15.5	7.8	13.0

From : Coker, (1979).

Farmed fish are also affected when fed diets containing aflatoxin. Rainbow trout are the most susceptable animals to aflatoxin, and carcinogenic effects can result in death before the animals can be utilized. It has been shown that levels as low as 0.5ppb in the trout feed can produce significant incidence of liver cancer (Sinnhuber and Wales 1978). Studies from the Philippines show reduced growth and a disease of the hepatopancreas in cultured shrimp (*P.monodon*) when feeds contain aflatoxin, and this can lead high mortalities even complete loss of stock.

Contamination of foods and feeds by mycotoxins also results in economic losses. Estimation of all losses due to mycotoxins is difficult, but one study in the USA put losses at USD 200 million due to aflatoxin contamination of maize in one particular state (Hagler,1983). Chronic mycotoxicosis in animals can lead to decreased production, and these losses often remain undetected or unexplained, so the scale of the real problem is unknown, and financial losses are a matter of conjecture. Financial losses associated with the downgrading of food and feed commodities, because of high mycotoxin content, are more easily quantified. Poor mycotoxin surveillance in some countries often means that contaminated produce remains undetected. In fact, many countries have only become aware of the problem when export markets for their agricultural products are much reduced, or even lost completely.

Results of survey work on the contamination of the food supply in two SE Asian countries are shown in Tables 2, 3 and 4.

Table 2. AFLATOXIN CONTENT OF MAIZE SAMPLES FROM 5 PROVINCES OF INDONESIA

Province	No of Samples	Percentage Positive	Total aflatoxin content (μg/kg)	
			Mean	Highest
EAST JAVA	15	100	149	890
WEST NUSA TENGGARA	12	83	180	1140
LAM PUNG	12	92	144	350
NORTH SULAWESI	12	100	464	790
SOUTH SULAWESI	12	100	108	250

Table 3. THE INCIDENCE OF AFLATOXIN IN MAIZE AT DIFFERENT POINTS IN THE MARKETING CHAIN

Sampling Point	No of Sample	Percentage of Positive samples %	Aflatoxin content μg/kg mean (min-max)	Moisture Content % mean
Preharvest	130	4.6	0.6 (0-33)	23.1 (15.0-28.8)
On Farm				
After 1month	16	25.0	7 (0-120)	17.6 (13.4-24.7)
After 2month	8	62.5	21 (0-75)	18.7 (13.4-24.7)
Middlemen in store				
After 1month	22	86.4	196 (0-750)	19.2 (14.0-29.3)
After 3month	28	78.6	104 16.2 (0-435)	(11.5-23.8)
Silos	40	97.5	93 (0-362)	14.0 (21.1-18.2)

Table 4. AFLATOXIN CONTAMINATION OF MARKET FOODS IN THAILAND

Foodstuff	No Contaminated (%)	Mean (all samples) (µg/kg)	Mean (Contaminated) (µg/kg)	Maximum Total aflatoxin (µg/kg)
Groundnuts	49	750	1,530	12,256
Maize	35	140	400	2,730
Chili peppers	11	14	125	966
Millet	11	7	67	248
Dried fish	5	8	166	772
Mung beans	5	1	16	112
Other beans	3	6	213	1,620
Sesame	3	<1	<1	<10
Sago/Cassava	3	5	150	294
Garlic/onions	3	2	67	60
Rice	2	<1	20	98
Fresh vegetables	1	<1	30	46
Prepared foods	6	31	510	3,904

Aflatoxin control and detoxification

Because of the possible risks to human and animal health associated with the contamination of foods and feedingtuffs by aflatoxins much time and effort has been expended on seeking methods for the removal of or the destruction of these toxins in contaminated products. The best approach to the control of aflatoxin is prevention. However, contamination by aflatoxin is sometimes unavoidable, and if prevention fails, other alternatives must be considered. Techniques for the " detoxification " of aflatoxin in various commodities include removal physically or biologically and chemical inactivation.

In the long-term, prevention of contamination would appear to offer the most effective and profitable control measure to avoid aflatoxin infection; it would also reduce the large foods losses incurred due to fungal damage. Unfortunately, such measures are the most difficult to realise in practice since they depend on climatic conditions and also necessitate changes in agricultural and storage practices that are traditional and commonplace in many areas where problems arise. Even where advanced agricultural methods are practiced low level contamination of some commodities by aflatoxin is unavoidable (Wilson and Flowers, 1978).

The most common method to control fungi and mycotoxins in stored products is by drying to "safe" moisture contents and maintaining products at this level. Table 5 shows the moisture level at which mould growth is inhibited for important stored commodities.

Table 5

Commodity	Moisture level to prevent mould growth (~ 25°C)
Maize	13.5 %
Groundnut	7.0 %
Cotton seed	11.0 %
Soybean	9.0 %

* In equilibrium with relative humidity ≤ 70 % .

Other methods of preventing fungal damage of stored commodities are to harvest crops when fully mature - unripe grain takes longer to dry and is therefore more susceptible to mould damage. Crops left unharvested for long period may be subject to rain damage. Care should be taken to avoid physical damage of products at all times, for instance, damaged grains are more susceptable to fungal invasion and mycotoxin contamination. Controlling insect infestation in store is also very important to avoid both losses and spread of fungal contamination.

Development of commercially acceptable varieties that would resist toxin-producing moulds or completely inhibit toxin production would be an ideal solution, and genetic approaches that may result in resistance to elaboration of aflatoxin have been and are being investigated. Laboratory and field studies have indicated broad varietal differences in the resistance of maize to *A.flavus* and its production of aflatoxin . Impermeable seed coat cotton-seed, so-called "hard seed", has been reported (Lillehoj and Zuber,1975) to have less tendency to allow *A.flavus* to grow on it and produce aflatoxins than seeds without this "hard coat" trait (Mayne *et al.*,1969). Research to identify groundnut varieties with resistance to aflatoxin producing fungi has continued for many years with limited success.

Other methods of control involve physical sorting. Since prevention of aflatoxin contamination is extremely difficult to realise in practice, it has been necessary to seek other types of "control"measures to obtain aflatoxin-free edible foods and animal feeds. The most widely used method involves the selective removal of contaminated portions of the commodity. The extent to which this is applicable depends mainly upon the physical nature of the material involved. Sorting procedures based on characteristics such as colour, size and shrivalled kernels, have been used successfully for edible groundnuts (Patterson *et al.*, 1968; Dickens and Whitaker, 1975).These methods rely upon the localisation of aflatoxin in a relatively small proportion of kernels with easily recognised defects in size or appearance. However, due to so-called "hidden damage", even these measures cannot be said to be 100 per cent effective (Dickens and Whitaker, 1975). Similar processes based on fluorescence have been suggested for pistachio nuts (Farsaie *et al.*, 1978), and cottonseed (Lee *et al.*, 1977).

A great deal of time and effort has been extended in studying possible methods for the detoxification of aflatoxin - contaminated products. Chemical methods which have been successfully employed in detoxification of aflatoxin contaminated products include the use of hydrogen peroxide, hypochlorite and ammonia. A number of methods have been published and patented for the detoxification of aflatoxin contaminated oilseed residues with ammonia (Manabe *et al*, 1978 ; Lesieur, 1977; Beckke *et al*, 1979). These methods vary mainly in the percentage of ammonia used, the moisture content of the cake and the temperature and pressure at which the reaction is carried out. Results of experimental work showed a batch of cottonseed which initially contained 550 μg/kg aflatoxin B_1 was detoxified (undetectable levels of aflatoxin) after exposure to ammonia at 46psi, 110 °C for 30 minutes. Feeding trials with ducks using the detoxified material showed no adverse effects (Hoelscher, 1979).

At NRI we have designed an experimental detoxification plant which uses a combination of ammonia gas and steam to destroy aflatoxin in feedingstuffs; contaminated groundnut cake, cottonseed cake and corn have been successfully detoxified using this process. In Senegal groundnut cake contaminated with aflatoxin is detoxified using the ammonia process. The detoxified material is considered suitable only for animal feed purposes and not for human consumption.

There is much discussion worldwide regarding the acceptability of detoxified products for animal feeds. One of the major constraints for the general use of detoxification methodology is likely to economic. The economic feasibility of any detoxification process will depend heavily upon the world market prices for other acceptable food or feed materials as against the cost of extra processing involved in detoxification.

Detection of mycotoxins

There are two principle types of assays which have been develop for detecting mycotoxins - chemical and biological. Bioassays are usually qualitative and are useful for indicating the presence of unknown toxins. Chemical assays are used for quantification of toxins in routine analysis. More recently immunoassays have been developed, which are a combination of chemical and biological methods.

A flow diagram indicating the various steps involved in analysis of mycotoxins is shown in Appendix 1. Sampling procedures are one of the most important steps in mycotoxin analysis. The importance of taking representative samples cannot be over-emphasized. Aflatoxin is known to occur in "pockets" within products. Therefore as many small units should be selected from as many locations as possible throughout a batch of food or lot. It has been reported that 90 % of the aflatoxin in a sample of maize can be restricted to less than 0.5 % of the kernals. A similar situation exits for groundnuts, where as much as 1,000 mkg aflatoxin has been found in a single kernal (Coker, 1979). Such distribution patterns make it very difficult to draw a sample from a given batch of food or food which will allow a close estimate to be made of the true average quantity of aflatoxin present. Additionally when a sample has been taken, its reduction in size for assay purpose, must be performed in such a way so that the final aliquot is representative of the original sample.

An example of a sampling plan is one which was designed at NRI,which enables an assessment to be made of the aflatoxin content of a 20 tonne batch (approx 40 bags) of groundnuts :

a) Systematically collect at least 100 x 105g subsamples from the batch under test, e.g. if 300 sacks are accessable then collect a 105g subsample from every third sack.

b) divide the sample of at least 10.5 kg into 3 samples of equal weight

c) grind and assay one or more of those samples.

d) If any sample contains 10.0ppb or less of aflatoxin the 20 tonne batch is acceptable.

e) If no sample contains 10.0ppb or less of aflatoxin the 20 tonne batch is rejected.

Different standard sampling plans exits and currently the EEC is considering the preparation of a new sampling directive. Readers are refered to a recent FAO publication on Management of Food Control Programmes p122-136, which gives examples of sampling specifications for Asian products. Also to the NRI Mycotoxins Manual for additional information on sampling and sample preparation.

Analysis of mycotoxins involves the extraction of the toxin from the sample using organic solvents (Chloroform, methenol, acetone or accetonitrile) which are usually mixed with polar solvents to aid extraction. The clean-up procedures may be chemical or chromatographic. The qualitative assessment of the extract (i.e. deciding upon the presence or absence of aflatoxin) can be performed using a minicolumn (Romer, 1975). Examples of chromatographic procedures for quantification of aflatoxins are given in Table 6. (Coker, 1979). Deatils for qualitative and quantitive analysis of aflatoxin, which have been taken from the NRI Mycotoxins Manual, can be found in Appendix 1 of this seminar paper.

Table 6. CHROMATOGRAPHIC QUANTIFICATION OF AFLATOXIN

Technique	Detector	Detection limit of aflatoxin B_1 (μg)
1.Thin-layer	(a)Human eye	0.4
chromatography(TLC)	(b)Densitometer	0.4
2.High performanceTLC	Densitometer	0.01
3.High performance	(a)UV detector	1.0
liquid chromatography	(b)Fluorescence detector	1.0
	(c)Mass spectrometer	0.03
	(d)Laser fluorescence	0.05

Legislation

Aflatoxin is the mycotoxin most widely controlled under national law and international regulations but legislation is beginning to be introduced for other mycotoxins. For instance, the EEC now controls zearalenone in animal feeds (pigs 500ppb, poultry 2,000 ppb, others 10,000 ppb). Sweden has fixed a provisional limit of 50 ppb for patulin in apple juice and India has fixed a provisional limit of 0.05% for ergot in millet.

The criteria used in setting limits are for aflatoxin in animal feeds that the allowed levels should not:

(a) impair the health of the animal, and hence its productivity.

(b) be sufficient to enter the human food chain via animal products.

The European Economic Community (EEC) limits were introduced in 1976 and are now incorporated into the legislation of all the member states.These limits were set employing criteria (a) and (b).

Table 7. EEC AFLATOXIN LIMITS FOR FEEDINGSTUFFS

Feedingstuff	Maximum Permissible Aflatoxin Content (ppb)
Straight feedingstuffs	50
Whole feedingstuffs for cattle, sheep and goats (except dairy cattle, calves, lambs and kids)	50
Whole feedingstuffs for pigs and poultry (except piglets and chicks)	20
Other whole feedingstuffs	10
Complementary feedingstuffs	20

It was criteria (b) that lowered the limits to those in force today. Had the limits been based solely on criteria (a) then they would certainly be higher. Most animals have a tolerance of 200 ppb aflatoxin with no ill effects, the main exceptions being: young animals generally, ducks, turkey, rainbow, trout, rabbits and possible dairy cattle (decreased milk yield). The EEC limits were set bearing in mind aflatoxin B_1 in feed/aflatoxin B_1 (or metabolite) in tissue conversion ratios, as illustrated in Table 8.

Table 8. FEED/TISSUE CONVERSION RATIOS FOR AFLATOXIN IN CERTAIN ANIMALS

Animal	Feed/Tissue Conversion Ratios		
Beef cattle	14,000	Feed	B_1/liver B_1
Dairy cattle	100	Feed	B_1/milk M_1
Pigs	800	Feed	B_1/liver B_1
Layer hens	2,200	Feed	B_1/egg B_1
Broiler chickens	1,200	Feed	B_1/liver B_1

The levels were set such that no aflatoxin (0.1 ppb) would be present in the tissue or product. Hence the EEC limit of 20 ppb for aflatoxin B_1 in dairy cattle cake. Countries vary in the actual limits which they have set for the maximum allowed aflatoxin content in animal feed (Table 9).

Table 9. AFLATOXIN LIMITS FOR FEED IN VARIOUS COUNTRIES

COUNTRY	TYPE OF CONTROL	COMMODIT	AFLATOXIN LIMIT (μg/Kg)	REMARK
Brazil	Govt.Decree	Groundnut meal	50	Export control
EEC	Council Directive	All mixed feeds & dairy supplement	10 - 50	Dependent on animal
Japan	Regulation	Groundnut meal	1000	0% Inclusion (chicks, calves pigs. 3% dairy cattle, 5%other livestock)
Norway	Regulation (Min.of Agric.)	Oilseed meals	600	8% in feed concentrates
Sweden	Advisory Stand	Groundnut meal	600	Dairy feed 15%
Zimbabwe	Voluntary Code	Mixed feeds	50 - 400	Dependent on animal

Ideally no aflatoxin should be present in human food and some countries, like Japan have legislated for a zero limit, while other countries have compromised and set a "practical" limit.Table 10.

Table 10. MAXIMUM PERMITTED LEVELS FOR AFLATOXIN IN FOODSTUFFS

Country	Maximum Permitted Level (μg/kg)	Product(s)
Australia	15 B_1	Groundnuts and Groundnut Products
	5 B_1	Other Foods
Canada	15(total aflatoxins)	Edible Nuts and Nut Products
India	60 B_1	Groundnut Flour
Japan	0(detection limit of method 10 μg/kg B_1)	All Foods
Poland	0	All Foods
Singapore	0	All Foods
Sweden	5 (total aflatoxins)	All Foods
Switzerland	6 (total aflatoxins)	Roasted Peanuts
	0.05 aflatoxin M_1	Milk, Specified Milk Products
USA	25(total aflatoxins)	Raw Unprocessed Groundnuts
	20(total aflatoxins)	Edible Nuts and Nut Products
	0.5	Milk

A list of limits adopted by various countries is given in Table 10. This list is provided as a sample of legislation which exits in some countries. The UK has a guideline limit of 30 ppb aflatoxin in imported nuts and nut products, but there is no specific limit for aflatoxin in prosessed foods. A manufacturer found to be selling food containing aflatoxin may be liable to prosecution under general food hygiene laws that forbid the sale of any food contaminated with a known poison. No such cases have been brought before the courts, to date.

Future work

Some suggestion for future work on mycotoxins in the food supply in Vietnam, in order to ensure that food products offered for sale are safe, with respect to mycotoxin contamination:

- Training and extension activities to promote at field level the prevention of mould contamination at harvest, storage and distribution stages for prevention of contamination of harveted products.
- Strenghtening food control systems in order to control mycotoxin contamination of marketed foods.
- Improve surveillance systems and strenghten laboratory capibilities in mycotoxin analysis.
- Collaboration at national, regional, and international levels to improve the prevention of mycotoxin development, as well as monitoring and control.

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APPENDIX

METHODS OF ANALYSIS OF AFLATOXIN

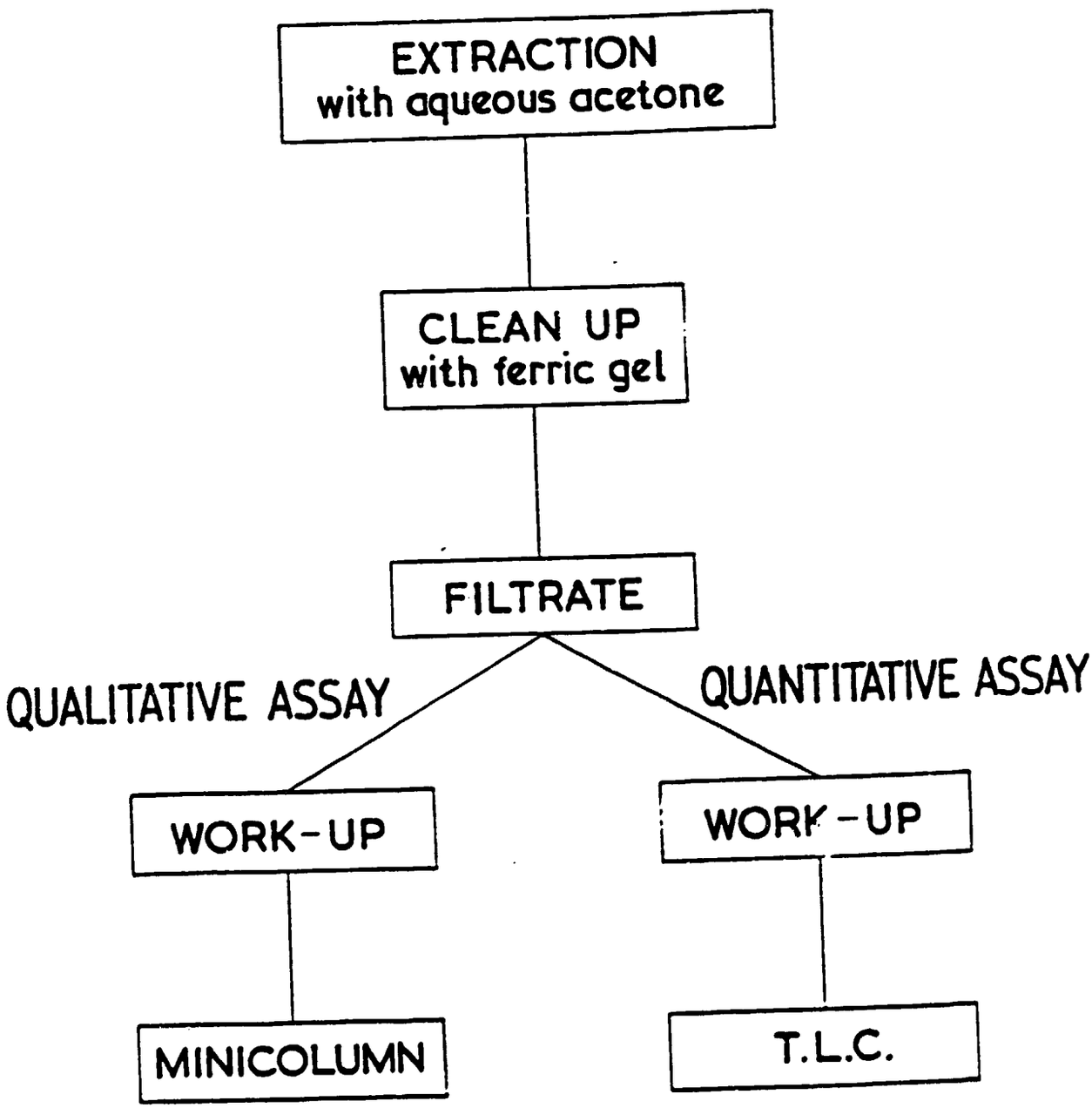
SECTION B:13

AFLATOXIN ANALYSIS OF GROUNDNUT OIL

SOURCE: Romer, J. Ass. Off. Anal. Chem., 1975, 58, 500-506.

Groundnut oil may be analysed by the Romer minicolumn method (see Section B:4). A simple modification of the extraction procedure is required as detailed below.

ANALYSIS OUTLINE



METHOD

Extraction

Weigh 30g of oil into a 250ml measuring cylinder and add acetone:water (85:15 v/v) to make up the volume to 150ml. Transfer the solution to a 500ml conical flask with stopper, stopper the flask and shake for 30 seconds.

Clean-up

Add 3g of cupric carbonate to the solution and swirl the "sample" flask to mix. Prepare ferric gel by measuring out accurately 170ml of sodium hydroxide solution and 30ml of ferric chloride solution (see Appendix:2) into a 500ml conical flask. Swirl the brown gel to mix and IMMEDIATELY add to the "sample" flask. Let the "sample" flask stand for 2 minutes, with occasional swirling and then filter through a fluted Whatman No. 1 filter paper into a measuring cylinder and collect the first 150ml of filtrate either for qualitative assay or for quantitative assay, whichever is required.

QUALITATIVE ASSAY

Work-up

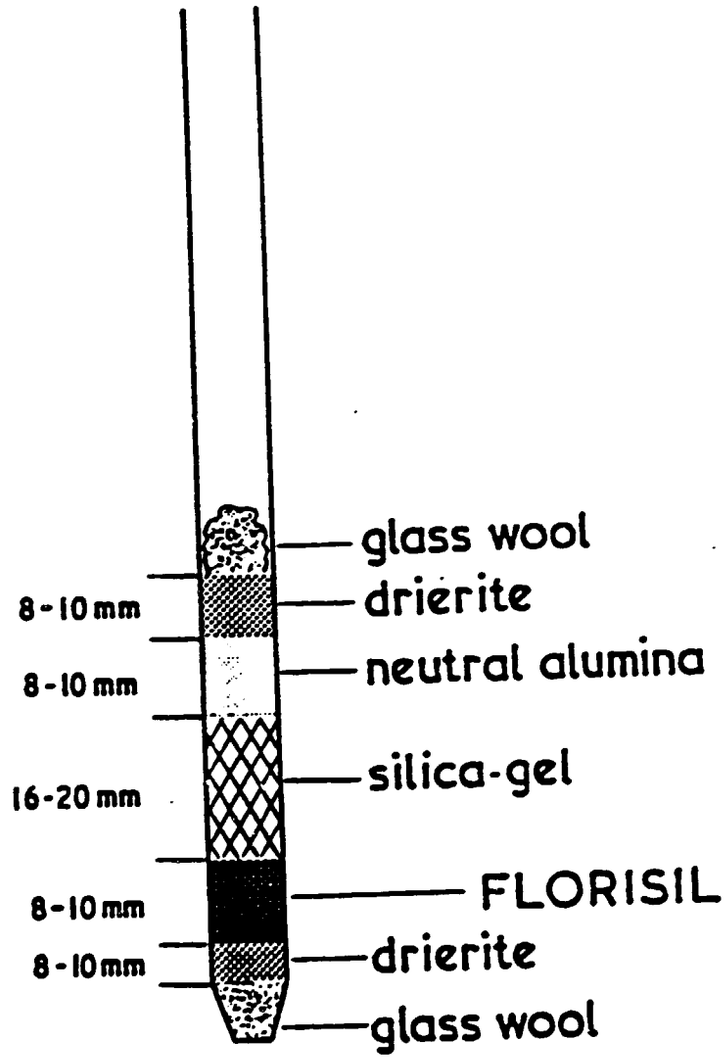
Transfer the 150ml of extract to a 500ml separating funnel, add 150ml of 0.03% sulphuric acid (see Appendix: 2) and 10ml of chloroform and shake for 2 minutes. Allow the layers to separate and run off the lower chloroform layer into a 250ml separating funnel containing 100ml of potassium hydroxide/potassium chloride wash solution (see Appendix:2). Swirl the funnel gently for 15 seconds and allow the layers to separate. Run off the lower layer through a bed of anhydrous sodium sulphate contained in a filter paper into a 25ml conical flask.

Minicolumn

Using a 1ml syringe transfer 2ml of the filtrate onto a minicolumn prepared as described in Appendix:1. Allow this to drain through (slight positive pressure may be applied using a rubber teat), then elute with 3ml of chloroform:acetone (9:1 v/v). When the meniscus of the solvent just reaches the top of the adsorbent the column is ready to read. Do not allow the column to go dry. View the minicolumn under a 365 nm UV light; a blue fluorescent band at the top of the florisil layer indicates the presence of aflatoxin. Other coloured fluorescences should be disregarded. With experience, the intensity of the fluorescence can be used to estimate the approximate level

Column Preparation

Tamp a small plug of glass wool into the tapered end of the column and then add the packing materials as indicated in the diagram below. Tap the column between addition of the materials to ensure even packing. Lengths of column glass can be cut to 8-10mm and 16-20mm and mounted to a piece of microscope slide to act as a measuring scoop; this makes column packing easier and quicker.



Appendix:2

APPARATUS AND REAGENTS

METHOD 1

Reagents

Sodium hydroxide 0.2 M - dissolve 80g of sodium hydroxide pellets in distilled water, make up to 10 litres and store in an aspirator.

Ferric chloride 0.41 M - dissolve 200g of anhydrous ferric chloride or 330g of ferric chloride hexahydrate in water, make up to 3 litres and store in an aspirator.

Sulphuric acid 0.03% - add 3ml of concentrated sulphuric acid to 1 litre of distilled water, make up to 10 litres and store in an aspirator.

Potassium hydroxide 0.02 M and potassium chloride 1% - dissolve 5.6g of potassium hydroxide and 50g of potassium chloride in distilled water, make up to 5 litres and store in an aspirator.

Chloroform A.R.

Acetone A.R.

Cupric carbonate - basic.

Sodium sulphate - anhydrous, granular.

Diatomaceous earth - "Hyflosupercel" or equivalent.

Minicolumn packing materials (see Appendix:1).

Apparatus

Explosion-proof blender - 1 litre or flask shaker and 500ml conical flasks with stoppers.

Measuring cylinders - 10, 50, 250ml.

Conical flasks - 25, 500ml.

Filter funnels - 5, 15cm.

Filter papers (Whatman No. 4 or equivalent) - 24cm.

Filter papers (Whatman No. 1 or equivalent) - 9cm.

Buchner flasks - 250ml (optional).

Buchner funnels - 10cm (optional).

Separating funnels - 250, 500ml.

Minicolumns (see Appendix:1).

Minicolumn rack.

Syringes - 1 and 5ml graduated syringes with large bore needles.

UV light - 365 nm, preferably in an enclosed viewing cabinet.

SECTION B:14

RAPID SCREENING METHODS FOR DETECTION AND QUALITATIVE ESTIMATION OF AFLATOXIN

SOURCE: Official Methods of Analysis of the AOAC, Association of Official Analytical Chemists, Washington, D.C., 13th Ed., 1980.

METHOD I (ROMER ALL PURPOSE METHOD)

This method has been officially adopted for the qualitative detection of aflatoxin in almonds (limit of detection 5ug/kg total aflatoxins), maize, groundnuts and groundnut products and cottonseed meal (limit of detection 10ug/kg total alfatoxins) and pistachio nuts (limit of detection 15ug/kg total aflatoxins).

Method

Extraction

Blend 50g of sample with 250ml of acetone:water (85:15 v/v) in a 1 litre blender jar for 3 min. Filter through a fluted, 24cm, Whatman No. 4 filter paper and collect 150ml of filtrate.

Alternatively, if blenders are not available, weigh out 50g of sample into a 500ml conical flask with stopper and add 250ml of acetone:water (85:15 v/v). Secure the stopper with tape, shake on a wrist-action shaker for 45 min and filter as above.

Clean-up

Transfer 150ml of filtrate into a 500ml conical flask, add 3g of cupric carbonate and swirl the "sample" flask to mix. Prepare ferric gel by measuring out accurately 170 ml of sodium hydroxide soluion and 30ml of ferric chloride solution (see Appendix:2) into a 500ml conical flask. Mix well and add to the "sample" flask. Add ca. 50g of diatomaceous earth, mix well and filter through a Whatman No. 4 filter paper using a 5cm funnel or a 10 cm Buchner funnel.

Work-up

Quantitatively transfer 150ml of filtrate to a 500ml separating funnel containing 150ml of 0.03% sulphuric acid (see Appendix:2). Add 10ml of chloroform and shake vigorously for about 2 min. Allow the layers to

separate and run off the lower chloroform layer into a 250ml separating funnel containing 100ml of potassium hydroxide/potassium chloride wash solution (see Appendix:2). Swirl gently for 30 sec, allow the layers to separate and run off the lower chloroform layer. If the layers do not separate and an emulsion is formed run off the emulsion into a conical flask containing about 1g of anhydrous sodium sulphate. Swirl the flask and filter through a fluted, 9cm Whatman No. 1 filter paper.

Minicolumn Assay

Using a 5ml syringe transfer 2ml of the above filtrate onto a minicolumn prepared as described in Appendix:1. Allow this to drain through (slight positive pressure may be applied with a rubber teat) until the solvent reaches the top of the adsorbent; then elute with 3ml of chloroform:acetone (9:1 v/v). When the meniscus of the solvent just reaches the top adsorbent the column is ready to read. Do not allow the column to go dry. View the column under a 365 nm UV lamp; a blue fluorescent band at the top of the florisil layer indicates the presence of aflatoxin. Some aflatoxin negative samples show faint white, yellow or brown fluorescent bands at the top of the florisil but if the band does not have a definite bluish tint the sample is negative. The analysis can be performed with an aflatoxin negative sample and a 'spiked' sample in order to obtain columns for comparison purposes.

METHOD 2 (HOLADAY-VELASCO METHOD FOR MAIZE)

This method has been officialy adopted for the qualitative detection of aflatoxin in maize (limit of detection 10ug/kg total aflatoxins).

Method

Blend 50g of sample with 100ml of methanol:water (4:1 v/v) in a 1 litre blender jar for 1 min at high speed. Filter through a fluted, 24cm Whatman No. 1 filter paper into a graduated tube with stopper and collect 15ml of filtrate. Add 15ml of sodium chloride/zinc acetate solution (see Appendix: 2), stopper the tube and shake vigorously for 10 sec. Filter through a fluted, 9cm Whatman No. 1 filter paper into a second graduated tube and collect 15ml of filtrate. Add 3ml of benzene, stopper the tube, shake gently for 10 sec and allow the layers to separate.

Minicolumn Assay

Pipette 1ml of the upper benzene layer onto the top of a minicolumn prepared as described in Appendix:1. Allow the extract to drain to the top of the

adsorbent then add 3ml of chloroform:acetone (9:1 v/v) and allow to drain through. When the meniscus of the solvent just reaches the top of the adsorbent the column is ready to read. Do not allow the column to go dry. Examine the column for the presence of aflatoxin as described for Method 1 above.

METHOD 3 (QUALITATIVE 'CB' METHOD)

This method has been officially adopted for the rapid screening of aflatoxins in maize. However, it is also possible to use this method for the analysis of commodities with low oil contents, which either do not require extensive clean-ups eg cassava, or are likely to contain high levels of aflatoxin eg groundnut cake/meal.

Method

Weigh 50g of sample into a 500ml conical flask with stopper and add 25ml of distilled water, 25g of diatomaceous earth and 250ml of chloroform. Stopper the flask, secure the stopper with tape, and shake for 30 min on a wrist-action shaker. Filter through a fluted Whatman No. 1 filter paper or, if filtration is slow, transfer to a Buchner funnel precoated with a layer of diatomaceous earth (ca. 5mm) and filter under slightly reduced pressure and collect 100ml of extract.

Evaporate 50ml of this extract to dryness on a water bath and immediately spot 5, 10 and 10ul of the warm corn oil residue onto a prewarmed TLC plate. The silica gel will absorb the corn oil more easily if both the TLC plate and oil are warm and if the oil is spotted as soon as the evaporation of solvent is complete. On the same plate spot 5ul of a standard containing all four aflatoxins and spot another 5ul of this standard on top of one of the 10ul extract spots to act as internal standard. Scribe a horizontal line across the centre of the TLC plate for half-plate development and develop the plate in anhydrous diethyl ether in an unequilibrated tank and then dry the plate. Redevelop the plate in the same direction in chloroform:acetone (9:1 v/v). View the plate under UV light (365 nm) and note the presence or absence of spot originating from the sample extracts with the same appearance and R_f as the aflatoxin B_1 standard.

If the extract proves to be positive take the remaining 50ml of filtrate obtained above and proceed with the column clean-up and quantitation as outlined in Section B:5.

Appendix:1

MINICOLUMN PREPARATION

Apparatus

Minicolumn - glass column that transmits 365 nm UV light, 6mm id x 190mm.
One end tapering to 2mm.

Rack - to hold minicolumn in a vertical position.

Syringes - 1ml graduated glass syringes, with large bore needles.

Column Packing Materials

Florisil - 100-200 mesh.

Silica-gel - 60-240 mesh.

Alumina (neutral) - 80-100 mesh.

Drierite (non-indicating) - 20-40 mesh.

Glass wool.

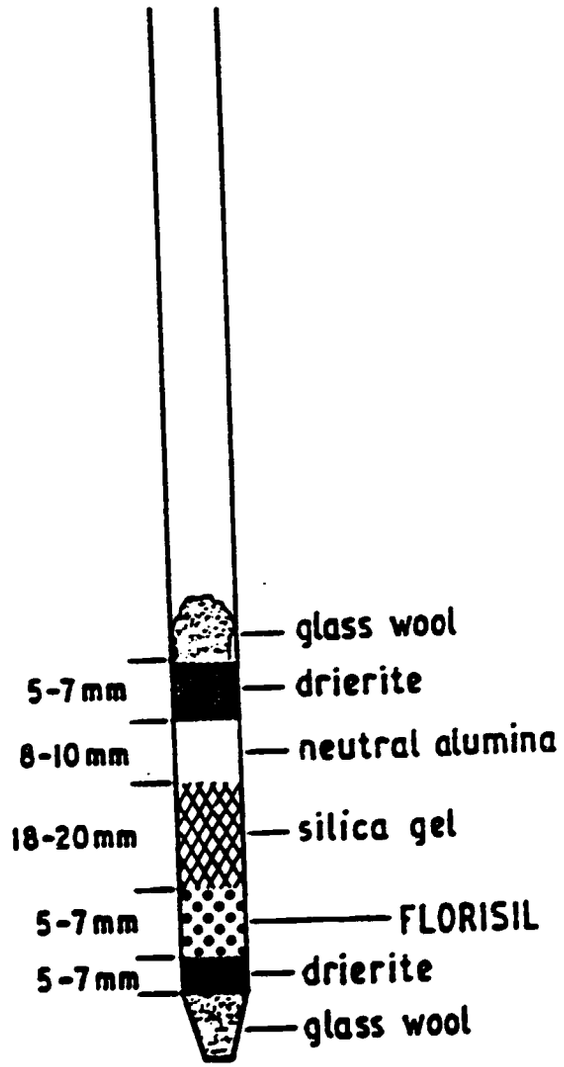
(These packing materials should be dried at 110°C for 1-2 hours twice a week and stored in a desiccator over "tell-tale" silica-gel.

Elution Solvent

Chloroform:acetone (90:10 v/v); make up 100ml and store in a stoppered flask.

Column Preparation

Tamp a small plug of glass wool into the tapered end of the column and then add the packing materials as in the diagram below. Tap the column between addition of each material to ensure even packing. Lengths of column glass can be cut to 5-7mm, 8-10mm and 18-20mm and mounted to a piece of microscope slide to act as a measuring scoop; this makes column packing easier and quicker.



Appendix:2

APPARATUS AND REAGENTS

Reagents

Sodium hydroxide 0.2 M - dissolve 80g sodium hydroxide pellets in distilled water, make up to 10 litres and store in an aspirator (170ml per assay).

Ferric chloride 0.41 M - dissolve 200g anhydrous ferric chloride or 330g ferric chloride hexahydrate in water, make up to 3 litres and store in an aspirator (30ml per assay).

Sulphuric acid 0.03% - add 3ml concentrated sulphuric acid to 1 litre of distilled water, make up to 10 litres and store in an aspirator (250ml per assay).

Potassium hydroxide 0.02 M and potassium chloride 1% - dissolve 5.6g potassium hydroxide and 50g potassium chloride in distilled water, make up to 5 litres and store in an aspirator (100ml per assay).

Chloroform A.R. (70ml per assay).

Acetone A.R. (200ml per assay).

Minicolumn packing materials (see Appendix:1).

Sodium sulphate-anhydrous, granular (10g per assay).

Cupric carbonate - basic (3g per assay).

Nitrogen.

Apparatus

Stoppered concial flasks - 500ml.

Measuring cylinders - 25, 250ml.

conical flasks - 25, 100, 500ml.

Filter funnels - 5, 15cm.

Filter papers (Whatman No. 1 or equivalent) - 9, 32cm.

Separating funnels - 250, 250ml.

Kieselgel 'G' precoated plates and other TLC equipment.

UV light - 365 nm, preferably in an enclosed viewing cabinet.

Vials - 4 dram.

Sample concentrator or water bath.

Minicolumns.

Syringes - 1ml graduated syringes, with large bore needles.

Minicolumn rack.

METHOD 2

Reagents

Methanol A.R.

Chloroform A.R.

Acetone A.R.

Benzene A.R.

Sodium chloride/zinc acetate solution - dissolve 300g of sodium chloride, 300g of zinc acetate and 7.5ml of acetic acid in 2 litres of water.

Minicolumn packing materials (see Appendix:1).

Apparatus

Explosion-proof blender - 1 litre.

Filter funnels - 5, 15cm.

Measuring cylinders - 25, 100ml.

Filter papers (Whatman No. 1 or equivalent) - 9, 24cm.

Graduated tubes with stoppers - 30ml.

Pipettes - 1, 3ml.

Minicolumns (see Appendix:1).

Minicolumn rack.

UV light - 365 nm, preferably in an enclosed viewing cabinet.

METHOD 3

Reagents

Chloroform A.R.

Diatomaceous earth - "Hyflosupercel" or equivalent.

Silicon carbide anti-bump granules.

Acetone A.R.

Diethyl ether - anhydrous, peroxide free.

Apparatus

Stoppered conical flasks - 500ml.

Measuring cylinders - 25, 50, 100ml.

Buchner funnels - 32cm diameter.

Buchner flasks - 250ml.

Filter papers (Whatman No. 1 or equivalent) - 32cm.

Filter funnels - 15cm.

Wrist-action shaker.

Conical flasks or beakers - 100ml.

Water bath.

Precoated Kieselgel 'G' plates and other TLC equipment.

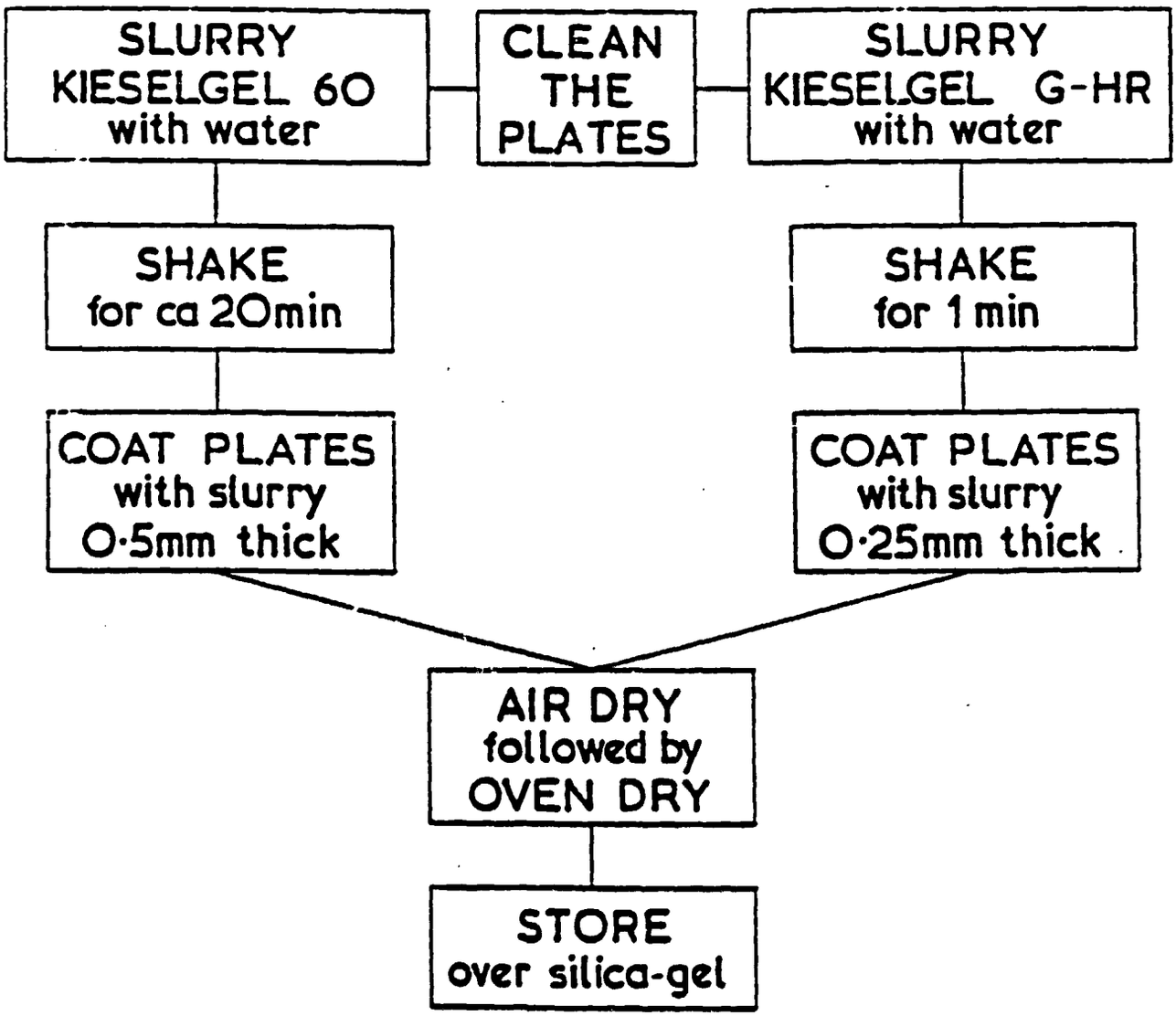
UV lamp - 365 nm, preferably in an enclosed viewing cabinet.

SECTION B:15

PREPARATION OF THIN-LAYER CHROMATOGRAPHY PLATES

Precoated Kieselgel 'G' chromatoplates are routinely used at TDRI for aflatoxin quantitation and these are preferred. However, it is possible to prepare TLC plates in the laboratory. Two types of silica gel adsorbent can be used; viz Kieselgel 60 Merck or silica-gel G-HR. The former plates will only allow separation of the B toxins from the G toxins but not B_1 from B_2 or G_1 from G_2 and thus only an estimation of the total aflatoxin B and total aflatoxin G content can be made. The individual B and G toxins can, however, be resolved on silica-gel G-HR plates using an appropriate developing solvent.

METHOD OUTLINE



METHOD

1. KIESELGEL 'G' CHROMATOPLATES

Cleaning the Plates

Thoroughly clean 15 glass plates (20 x 20 x 0.25cm) with acetone, taking particular care to remove grease marks, finger prints etc. Clamp the plates securely in a plate leveller.

Slurry

Weigh out 100g Kieselgel G60 (Merck) into a 500ml conical flask, add 220ml of distilled water, stopper the flask securely and shake by hand for about 20 min until the mixture has gelled. This should be sufficient to coat about 12 to 14 plates.

Coating the Plates

Adjust the plate spreader to 0.5mm (500u), pour in about half the slurry and with a firm but smooth action draw the spreader across the plates to coat them. Allow the plates to semi-dry in a dust-free atmosphere for 1 hour. Repeat the process with the remainder of the slurry.

Drying

Transfer the plates to a forced-draught oven at 100°C for a further 1 hour. Cool the plates in a dust-free atmosphere for 30 min.

Storage

Store the plates over "tell-tale" silica-gel in a desiccator or plate cabinet.

2. KIESELGEL G-HR CHROMATOPLATES

Cleaning the Plates

Thoroughly clean 5 glass plates (20 x 20 x 0.25cm) as in 1 above and clamp the plates securely in a plate leveller.

Slurry

Weigh out 30g of Kieselgel G-HR, or equivalent, into a 250ml conical flask, add 60ml of distilled water, stopper the flask securely and shake by hand for not longer than 1 min. The resulting slurry should be sufficient to coat 5 plates.

Coating the Plates

Adjust the plate spreader to 0.25cm (250u), pour in the slurry and with a

Appendix:1

APPARATUS AND REAGENTS

Reagents

Acetone A.R.

Kieselgel G60 (Merck, Darmstadt, West Germany).

Kieselgel G-HR (Machery Nagel and Co., Duren, West Germany).

Apparatus

Stoppered conical flasks - 250, 500ml.

Plate leveller (Camag or Shandon).

Plate spreader (Camag or Shandon).

Appendix:2

APPARATUS AND REAGENTS

Reagents

Sodium hydroxide 0.2 M - dissolve 80g sodium hydroxide pellets in distilled water, make up to 10 litres and store in an aspirator (170ml per assay).

Ferric chloride 0.41 M - dissolve 200g anhydrous ferric chloride or 330g ferric chloride hexahydrate in water, make up to 3 litres and store in an aspirator (30ml per assay).

Sulphuric acid 0.03% - add 3ml concentrated sulphuric acid to 1 litre of distilled water, make up to 10 litres and store in an aspirator (250ml per assay).

Potassium hydroxide 0.02 M and potassium chloride 1% - dissolve 5.6g potassium hydroxide and 50g potassium chloride in distilled water, make up to 5 litres and store in an aspirator (100ml per assay).

Chloroform A.R. (70ml per assay).

Acetone A.R. (200ml per assay).

Minicolumn packing materials (see Appendix:1).

Sodium sulphate-anhydrous, granular (10g per assay).

Cupric carbonate - basic (3g per assay).

Nitrogen.

Apparatus

Stoppered concial flasks - 500ml.

Measuring cylinders - 25, 250ml.

conical flasks - 25, 100, 500ml.

Filter funnels - 5, 15cm.

Filter papers (Whatman No. 1 or equivalent) - 9, 32cm.

Separating funnels - 250, 250ml.

Kieselgel 'G' precoated plates and other TLC equipment.

UV light - 365 nm, preferably in an enclosed viewing cabinet.

Vials - 4 dram.

Sample concentrator or water bath.

Minicolumns.

Syringes - 1ml graduated syringes, with large bore needles.

Minicolumn rack.

COMMENTS BY THE PROJECT BACKSTOPPING OFFICER

In his capacity as food microbiologist Mr. Alan Reilly provided to the project very valuable and important expertise in the following areas of food quality control laboratory activities: (a) Assessment of the food microbiology laboratory equipment (b) Safety procedures in a food microbiology laboratory (c) Application of rapid microbiological methods (d) Mycotoxins in food and (e) Food process control.

The expert carried out several training workshops in the following areas: (a) Food mycology (b) Food bacteriology (c) Food quality control. He also provided know-how on general routine work in a microbiological laboratory. He gave to the staff of the laboratory advice and recommendations with regard to the different laboratory activities and on the appropriate equipment, chemicals, media and literature.

Mr. Reilly's report contains all major information required on the functions of a microbiological laboratory. The expertise he provided is modern, highly professional and up-to-date. Mr. Reilly's services have been very much appreciated by the project management and by the staff of the laboratory.