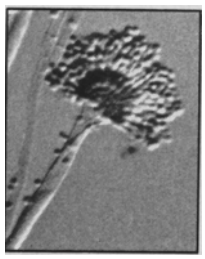
is free of oil, gossypol, and aflatoxin and high in nutritive value. The oil should be light. Hron and Kuk are making exciting strides toward this goal. Again, NCPA provides financial support and intense interest.

Increased funding at SRRC in the 1960s was generously supplied by Congress for aflatoxin research. Internal funds were shifted in the late 1970s to give a boost to biological research at SRRC. That shift in funds, with Alex Ciegler in charge of Food & Feed Safety, allowed for the hiring of two young scientists (Mellon and Klich). New funds came later in the mid-1980s to add to SRRC's brain power (Bhatnagar, Cleveland and Cotty). They were brought on board by Eivind Lillehoj. It is these plant biochemists, mycologists and plant pathologists who are doing the different, innovative aflatoxin research at SRRC. All research in Food & Feed Safety at SRRC has the solution of the aflatoxin problem as its ultimate goal. Even though the engineers have a more diverse program, aflatoxin removal is an important segment of that program.

Partly because of last year's drought and increased toxin levels detected, the NCPA, the corn industry and the peanut industry are asking for new funds for USDA research to eliminate the aflatoxin problem. They are confident that one of the many new approaches will be successful. The young scientists at SRRC are giving these new molecular biology techniques a real chance to solve a problem that has plagued us for nearly 30 years.



Contamination issues, technology

Aflatoxin made major news in U.S. agricultural circles last year as growing conditions led to outbreaks in various crops. In this report, Douglas L. Park and Henry Njapau of the Department of Nutrition and Food Science at the University of Arizona, Tucson, describe recent history on aflatoxin and talk about methods and instruments used to detect its presence in raw materials.

The scientific literature is replete with reports concerning the actual or probable occurrence of mycotoxin in foods and acute and subacute poisonings of man and animals after the ingestion of such foods. Aflatoxins, potent carcinogenic and toxic metabolites produced by the fungal species *Aspergillus flavus* and *A. parasiticus*, can contaminate animal feeds as a result of the currently unavoidable invasion by the molds before and during harvest, or because of improper storage of feeds. The need to limit aflatoxin in feeds is based on two major concerns: (a) the adverse effects of aflatoxin-contaminated feeds on animal health and productivity, and (b) the presence of aflatoxin residues or toxic metabolites in animal tissues used as human foods. U.S. crops most susceptible to unavoidable aflatoxin contamination are corn, peanuts and cottonseed. Although the overall incidence and levels are low, numerous surveys of animal feeds in the U.S. have shown that specific regions consistently have high preharvest aflatoxin contamination. The Southwest with cottonseed and the Southeast with corn are the areas in the United States most adversely affected by aflatoxin contamination. The 1988 corn crop from the Midwest and South, however, has shown unusually high levels of aflatoxin. This contamination has highlighted the importance of having a good program to monitor aflatoxin levels in agricultural products and of having adequate analytical tools. Also, the high aflatoxin contamination levels prompted the Food and Drug Administration (FDA) to re-evaluate current action levels for aflatoxins for corn. The primary goal of an effective food safety monitoring program is the protection of human health and the enhancement of food resources.

TABLE 1

Current Aflatoxin Action Levels Established by the Food and Drug Administration (μg/kg, ppb)

Human foods (except milk)	20.0
Milk	0.5
Animal feeds (except cottonseed meal)	20.0
Cottonseed meal (used for mature beef, swine and poultry rations)	300.0

Regulatory programs

The manner in which the FDA managed risks from aflatoxin is well documented. Current aflatoxin action levels for human foods and animal feeds are presented in Table 1. Through the years, however, when unusually high levels of aflatoxin occurred, the agency was required to revise the action levels previously established to minimize aflatoxin risks associated with interstate commerce or shipment of aflatoxin-contaminated products of the new crop and preserve an adequate food/feed supply for that particular year. As a result of the high levels of aflatoxin in the 1988 corn crop, FDA revised the action levels for that crop and has also in-

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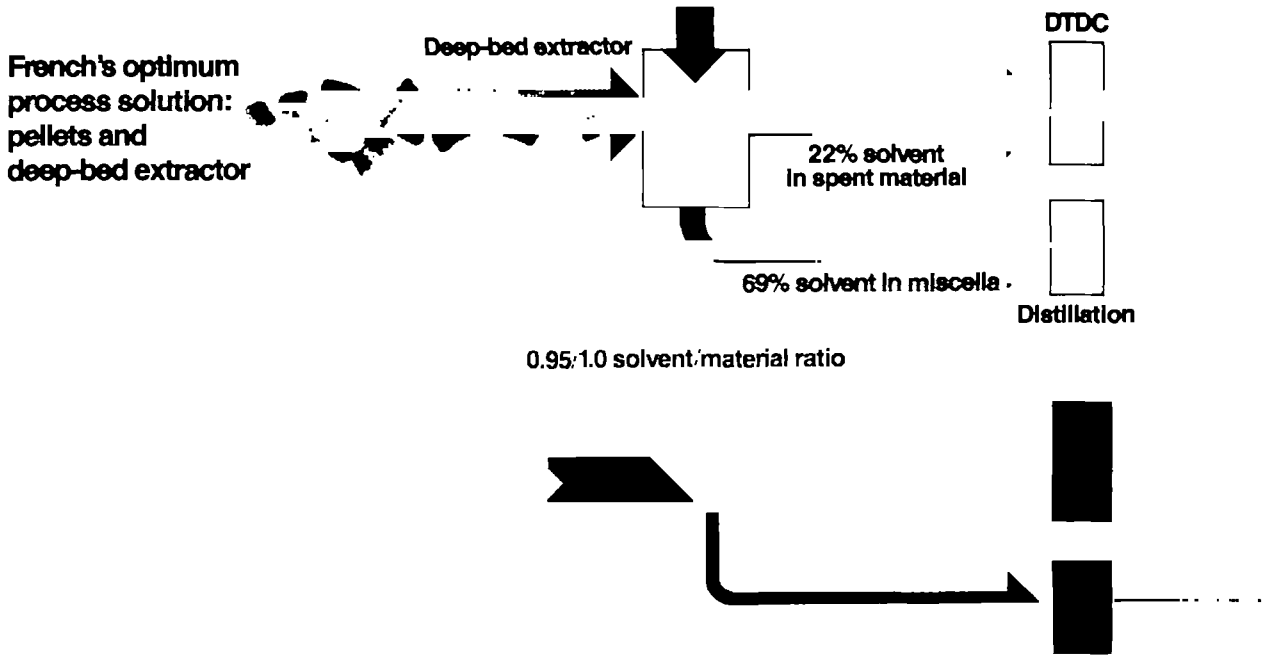

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vited comment concerning a proposed revision of its compliance policy guide for aflatoxin-contaminated corn (54 FR 22622). The final date for receipt of comments was July 24, 1989. The proposed revised action levels will be 100 parts per billion (ppb) aflatoxins for corn intended for breeding beef cattle, breeding swine or mature poultry. Two-hundred ppb aflatoxins will be permitted in corn intended for finishing swine, i.e., 100 pounds or greater, and 300 ppb aflatoxins for corn intended for feedlot beef cattle. The 20 ppb aflatoxin remains unchanged for corn intended for use by humans, for aflatoxins in corn for use by immature animals including immature poultry and by dairy animals, and for corn for which the intended use is not known. There is no change in the action level for residues of aflatoxins in fluid milk products. For the 1988 corn crop, FDA is permitting the blending of aflatoxin-contaminated corn with noncontaminated corn to levels permitted for intended animal feed use. As a result of a consumer action suit (Community Nutrition Institute) and resulting court decisions, the FDA issued a notice in the *Federal Register,* Feb. 19, 1988, (53 FR 5043) which explained that the agency's current action levels are not binding on the courts. In other words, situations can occur where enforcement actions at aflatoxin levels below an action level may be warranted or where enforcement action is not pursued even though the action level is exceeded. FDA plans to use the proposed revised action levels as a basis for guiding its enforcement of Section 402(1)(1) of the Food, Drug and Cosmetic Act, provided the corn containing more than 20 ppb which is shipped in interstate commerce will be fed to the appropriate group of animals.

FDA's position of not permitting ammoniation as a method for reducing aflatoxin levels in feedstuffs has not changed and the process is still not permitted. Although data from research conducted over the past 20 years support, as a whole, the safety and efficacy of ammonia as a process for inactivating aflatoxin in feeds, approval of the process by FDA is being withheld primarily due to concern over potential toxicity and possible carcinogenicity of ammonia/aflatoxin reaction products which could occur in human foods derived from animals fed the ammoniated aflatoxin-contaminated product.

Proposed aflatoxin action levels range from 300 to 20 ppb

Goldblatt's distinction between PPM and PPB

The late Leo Goldblatt once was telling an AOCS audience about the small amounts of aflatoxin—parts per billion—that may be involved in analytical work. To illustrate the difference between detecting parts per million (PPM) and parts per billion (PPB), Goldblatt used the following illustration:

If you put one jigger of vermouth in a railroad tank car of gin, that's one part per million. If you distribute that same jigger of vermouth among 1,000 tank cars, that's one part per billion.

Analytical methods

Suitable analytical methods for the detection and quantitation of the aflatoxins are necessary for an adequate food safety monitoring program. These methods could be used under a variety of applications, i.e., screening, survey, regulatory control, etc. Various methods based on either biological responses or on the chemical characteristics of the toxins have been developed and validated by an interlaboratory collaborative study. Organizations such as the Association of Official Analytical Chemists (AOAC), American Oil Chemists' Society (AOCS), American Association of Cereal Chemists (AACC) and International Union of Pure and Applied Chemistry (IUPAC) have method validation programs and work closely with each other to coordinate their activities. A committee, represented by members of each organization, meets each year during the annual AOAC meeting. This Joint AOAC-AOCS-AACC-IUPAC Mycotoxin Committee serves as a focal point for coordinating mycotoxin-related problems. The method validation program administered by AOAC involves subjecting the candidate method to an interlaboratory collaborative study to determine method performance characteristics. The report of the study, usually prepared by the Associate Referee, is then submitted to the AOAC for adoption of the method. Methods that have successfully met performance characteristics as determined by in-depth reviews by the General Referee, Committee Statistician, Methods Committee for that particular commodity/analyte, and Official Methods Board are granted interim Official First Action. The final stage in the approval process is a vote of acceptance by the Association membership at the annual meeting. At this point the method is Official First Action.

Several test systems have been developed based on an emerging technology of immunochemistry using antibodies with specificity to aflatoxins. These methods can be divided into two categories: enzyme-linked immunosorbent assay (ELISA) and affinity chromatography. Some of these methods have been evaluated through joint AOAC/IUPAC collaborative studies. The ELISA method using microtiter wells (AgriScreen) has been adopted Official First Action by AOAC for screening for aflatoxin B_1 in cottonseed products and mixed feeds

ANNOUNCEMENT

AVAILABILITY OF FISH OIL TEST MATERIALS

Test Materials Currently Available:

- n-3 ethyl ester concentrate, prepared from menhaden oil, bulk packed or soft-gel encapsulated (80% n-3 fatty acids including EPA and DHA)
- ethyl esters of olive oil (70% oleic), bulk packed or soft-gel encapsulated
- deodorized menhaden oil, bulk packed or soft-gel encapsulated
- commercial preparations of corn, olive, or safflower oil, soft-gel encapsulated only

Processing and Specifications of Biomedical Test Materials:

■ n-3 Ethyl Ester Concentrate

The n-3 ethyl ester concentrate is prepared from vacuum-deodorized menhaden oil using transesterification, urea adduction and short-path distillation. The concentrate contains approximately 80% n-3 fatty acid ethyl esters (44% EPA, 24% DHA, 10-12% other n-3 fatty acid ethyl esters), 3% C18 (other than n-3), 6% C16 and the remainder as other esters. It contains 0.2 mg/g TBHQ as antioxidant, 2 mg/g tocopherols and 2.0 mg/g cholesterol. The concentrate is available in 1 g soft-gel capsules (100 capsules/bottle) or packaged bulk in quantities suitable to investigators needs.

■ Placebo Ethyl Esters

The ethyl esters of virgin olive oil are prepared by transesterification. The preparation contains approximately 70% oleic acid, 13% C16, and 15% C18 (<1% n-3) fatty acid ethyl esters. It contains 0.2 mg/g TBHQ as antioxidant and 2 mg/g tocopherols. The preparation is available in 1 g soft-gel capsules (100 capsules/bottle) or packaged in bulk in quantities suitable to investigators needs.

■ Deodorized Menhaden Oil

Deodorized menhaden oil is prepared from oil that has been winterized and alkali refined; it is processed through a two-stage wiped-film evaporator to remove cholesterol, volatile oxidation products and any traces of organic contaminants. The oil contains approximately 30% n-3 fatty acids in the triglyceride form; 14% EPA, 8% DHA, 8% other n-3. It contains 0.2 mg/g TBHQ as antioxidant, 2 mg/g tocopherols and 2.0 mg/g cholesterol. The deodorized oil is available in 1 g soft-gel capsules (100 capsules/bottle) or is packaged in bulk quantities suitable to investigators needs. Special requests for antioxidant free oil will be considered.

■ Placebo Oils

Commercial preparations of corn, olive, and safflower oil have been soft-gel encapsulated to serve as placebos for studies involving encapsulated menhaden oil. These oils contain 0.2 mg/g TBHQ as antioxidant and 2 mg/g tocopherols. The major fatty acids for each oil are: corn (58% 18:2n-6, 26% 18:1n-9), olive (17% 18:2n-6, 57% 18:1n-9), safflower (80% 18:2n-6, 9% 18:1n-9). They are available in 1 g soft-gel capsules (100 capsules/bottle). Although vegetable oils will not be supplied in bulk form, investigators may request analysis of antioxidant and tocopherol levels in vegetable oils that they purchase.

FISH OIL TEST MATERIALS PROGRAM

The Fish Oil Test Materials Program is administered by the Division of Nutrition Research Coordination in the Office of Disease Prevention, NIH. It was established in 1986 through the cooperation of the National Institutes of Health (NIH), the Alcohol, Drug Abuse, and Mental Health Administration (ADAMHA), and the National Oceanic and Atmospheric Administration/ Department of Commerce (NOAA/DOC). This program has been designed to provide a long-term, consistent supply of quality-assured/quality-controlled test materials to researchers in order to facilitate the evaluation of the role that omega-3 fatty acids play in health and disease.

Fish Oil Test Materials Advisory Committee:

A Fish Oil Test Materials Advisory Committee (FOTMAC) is cochaired by scientific staff from ADAMHA and NIH and is composed of scientists representing the funding agencies (NIH, ADAMHA), the research community, Department of Commerce (DOC) and the Food and Drug Administration (FDA). The FOTMAC provides scientific advice to the DOC regarding the types of materials needed by research scientists, shipping procedures for the materials, and additional quality control and production issues. The committee is advisory to the Fish Oil Test Materials Program on general programmatic issues such as future directions and has produced a Good Lab Practices for Polyunsaturate Handling Manual. In addition, the committee provided guidance to DOC during the production of the Drug Master File submitted to the FDA by the FOTMAC. A manual on Analytical Methods for the Quality Assurance of Fish Oil was produced by the DOC.

Fish Oil Test Materials Distribution Committee:

A Fish Oil Test Materials Distribution Committee (FOTMDC) is composed of NIH and other Federal sciences that do not use these products. The Distribution Committee processes the applications received from investigators and advises the DOC of applications that have fulfilled the application process and makes recommendations regarding the distribution of requested materials.

The awarded materials are provided to investigators free of charge. Availability of materials are contingent on DOC/NOAA production capabilities. When prioritization is necessary, the order will be: 1) NIH/ADAMHA funded, 2) other government funded, 3) peer-reviewed, privately funded, 4) NIH/ADAMHA approved, not funded, and 5) other.

To qualify to receive materials described in this announcement the applicant must: 1) have peer-reviewed research, and 2) submit a correctly completed application form and a signed waiver of liability. The committee will not be responsible for assessing the scientific merit of the application. Regulations on human subjects and animal research apply. In accordance with federal regulations, an IND number will be required for the use of these materials in human studies. The FOTMAC has established a drug master file at the FDA which includes manufacturing, chemical composition and toxicological data relevant to these products. Investigators using DOC/NOAA materials may reference this file in order to expedite their IND requests.

Requests for materials of amounts greater than 500 kg of vacuum deodorized menhaden oil and/or 50 kg of n-3 ethyl ester concentrate should not be submitted without prior discussion with the NMFS - Charleston Laboratory. For further information contact Ms. Patricia Fair at (803) 762-1200.

Test Materials Available in the Future:

Test Materials and their relevant application process will be announced in the NIH Guide as new materials become available.

Other Information:

Additional information will be provided the investigator in the form of complete quality assurance data for each lot of test material shipped, general diet preparation information, and instructions for formulation of placebos containing antioxidants balanced to the level in the test material.

Investigators may obtain further information and apply for available fish oil test materials for relevant studies by requesting an application form from:

Ms. Melissa Workman
Program Assistant
Fish Oil Test Materials Program
Division of Nutrition Research Coordination
Building 31, Room 4B63
National Institutes of Health
Bethesda, Maryland 20892
(301) 496-2323

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TABLE 2

Commercially Available Aflatoxin Test Kits

Test Kit	Analyte(s)	Type of Test	Level of Detection (mg/kg)	Analysis Time[a] (min/sample)	Application	Manufacturer
Aflatest-P	B_1,B_2,G_1,G_2 M_1	affinity[b] column	2 0.1 (M_1)	7	instrumental, semiquantitative	VICAM 29 Mystic Ave. Somerville, MA 02145 (617) 623-0030 (800) 338-4381
AflaQuick	B_1,B_2	affinity[b] column	2	5		
AgriScreen[c]	B_1,B_2,G_1,M_1	ELISA[b], microtiter wells	1 0.2 (M_1)	12	visual and instrumental, semiquantitative, quantitative	Neogen Corp. 620 Lesher Place Lansing, MI 48912-1509 (517) 372-9200
Afla-20[d]	B_1,B_2,G_1	ELISA, cup	20	4	visual pass/fail	International Diagnostic System Corp. P.O. Box 799 St. Joseph, MI 49085 (616) 983-3122
Afla-10			10			
IDEXX-AFB	B_1,B_2,G_1	ELISA, micro titer wells	3 20	45	instrumental, semiquantitative	IDEXX 100 Fore Street Portland, ME 04101 (207) 774-4334 (800) 548-6733
CITE-Probe-Aflatoxin	B_1,B_1	ELISA, probe		3	visual, pass/fail	
EZ-SCREEN: Aflatoxin	B_1,B_2,G_1	ELISA, Card[e]	20,5	7 7 7	visual, pass/fail	Environmental Diagnostic Systems Corp. P.O. Box 908 2990 Anthony Road Burlington, NC 27215 (919) 226-6311 (800) 334-1116
Total aflatoxins	B_1,B_2,G_1,G_2	affinity column	1	30	visual (with UV viewer), semiquantitative	Oxoid U.S.A., Inc. 9017 Red Branch Rd. Columbia, MD 21045 (301) 997-2216 (800) 638-7638
Aflatoxin M_1	M_1	affinity column	<0.1	30	visual (with UV viewer), semiquantitative	
Aflatoxin test	B_1,B_2,G_1,G_2	ELISA, microtiter	1 ppb	30	semiquantitative	Transia 8, rue Saint-Jean-de-Dien 69007 Lyon, France 72-73-03-81
Aflatoxin M_1 test	M_1	ELISA, microtiter	10 ppt	40	semiquantitative	
SAM-A	B_1,B_2,G_1,G_2	Selective absorption[f]	10 ppb	10	Pass/fail	Raildon Diagnostics 3609 E. 29th St. Bryan, TX 77802 (409) 846-6202 (800) 888-5688
SAM-AZ	B_1,B_2,G_1,G_2 (zearalenone)	Selective absorption[f]	10 ppb (500 ppb)	10	Pass/fail	

[a]Does not include sample preparation and extraction.
[b]Immunochemical methods; Affinity column or ELISA (enzyme-linked immunosorbent assay).
[c]Adopted AOAC Official 1st Action for screening for aflatoxin B_1 in cottonseed products and mixed feed; Adopted AOAC interim Official 1st Action for screening for aflatoxin B_1 in corn and peanut butter.
[d]Adopted AOAC interim Official 1st Action for screening for aflatoxins B_1, B_2 and G_1 in corn, peanut butter, poultry feed, cottonseed, and raw peanuts.
[e]Three card system available: one sample/card or five sample/card at 20 ppb or one sample/card at 5 ppb.
[f]Modified Holaday-Velasco minicolumn (AOAC method 26.020-26.025).

TABLE 3

Commercially Available Sample Cleanup Columns for Aflatoxin Analysis

Item	Analytes(s)	Column	Cleanup Time	Application	Manufacturer
224 Myco-Sep	B_1,B_2,G_1,G_2	selective column	10 sec	sample cleanup, TLC/HPLC	Romer Labs, Inc. P.O. 2095 Washington, MO 63090 (314) 239-2708
Aflatest-P	B_1,B_2,G_1,G_2,M_1	affinity[a] column	5 min	sample cleanup, HPLC	VICAM 29 Mystic Ave. Somerville, MA 02145 (617) 623-0030 (800) 338-4381
EASI-EXTRACT (Total aflatoxins)	B_1,B_2,G_1,G_2	affinity[a] column	17 min	sample cleanup, HPLC	Oxoid U.S.A., Inc. 9017 Red Branch Rd. Columbia, MD 21045 (301) 997-2216 (800) 638-7638
EASI-EXTRACT (Aflatoxin M_1)	M_1	affinity[a] column	17 min	sample cleanup, HPLC	

[a]Immunochemical.

and interim Official First Action for screening for aflatoxin B_1 in corn and peanut butter. Similarly, the cup method (Afla-20) has been adopted interim Official First Action by AOAC for corn, peanut butter, raw peanuts, poultry feed and cottonseed. Two collaborative studies evaluating the affinity column are under way. These studies are evaluating the use of the affinity column as the cleanup step for liquid chromatographic methods or a test kit using fluorescence for the determinative step. Several new commercially available methods are listed in Table 2.

Chemical methods, using thin layer (TLC) and liquid chromatography, have a major problem with sample cleanup of the extract prior to the determinative step. Several columns have been developed to alleviate this problem (Table 3). The Aflatest-P (Vicam) and EASI-EXTRACT (Oxoid U.S.A.) affinity columns are undergoing interlaboratory validation studies. With respect to other validation studies on chemical methods for aflatoxins, a liquid chromatographic method based on trifluoroacetic acid (TFA) derivatization of the toxins and a reverse-phase column with fluorescence detection has been adopted interim Official First Action by AOAC for the determination of aflatoxins B_1, B_2, G_1 and G_2 in peanut butter and corn at concentrations of >13 ng total aflatoxins/g. The results of a collaborative study for a solvent-efficient TLC method for aflatoxins are under review by AOAC. These studies were joint AOAC/IUPAC efforts.

The evolution of aflatoxin methods from nonspecific biological responses to highly specific chemical and biological methods demonstrates that analytical capabilities, i.e., level of detection, specificity, etc., are continuing processes. Consequently, as better methods become available, it is necessary to submit them to a collaborative study to truly evaluate performance characteristics of the method. Also, regardless of method performance attributes as demonstrated by the validation study, each analyst must demonstrate his or her own proficiency with that particular method. For this purpose check-sample and laboratory quality assurance programs have been developed and should be used. Finally, the importance sample collection and preparation play in the overall analytical picture cannot be overemphasized. We have not discussed this issue, however, the sample/test portion must represent the lot tested for the result to be of any meaning.

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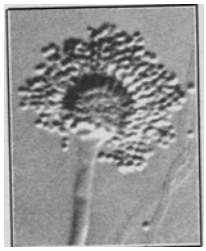
FGIS seeks bids on test kits

The USDA's Federal Grain Inspection Service is seeking competitive bids on three types of aflatoxin test

(Continued)

kits after preliminary evaluations showed five kits would meet requirements for testing corn. Tentative plans are to be using the kits to test the 1989 corn crop, if bidding and acquisition procedures can be completed in time.

FGIS announced in mid-July it would seek bids on the EZ-Screen, Aflatest and Afla-20-Cup kits. Two other kits, SAM-A and OXOID, also passed FGIS test requirements to indicate excess of 20 ppb aflatoxin. The kits are to be used at 38 FGIS service point, replacing minicolumn and TLC methods. Another 13 kits are to be used at ten export field offices and three other offices that receive requests to test corn for aflatoxin.



European perspective on aflatoxin

The European Economic Community established severe limits in 1988 on aflatoxin contamination in raw materials imported for use as feedstuffs or for other purposes. When the new rules were announced, many traditional suppliers of such materials complained the rules would drastically cut their trade with Europe. This study of U.K. and EC legislation on aflatoxin was prepared by the U.K. Ministry of Agriculture, Fisheries and Food in London to explain the background and previous regulatory steps that led to current legislation on aflatoxin.

Mycotoxins will be a familiar subject to many. Others may like to be reminded that mycotoxins are toxic compounds produced by molds. Aflatoxins, in turn, are a group of mycotoxins, generated by some strains of *Aspergillus flavus* and *A. parasiticus*.

Aflatoxins have been found in many crops. But the known problems are more acute in tropical and subtropical products—corn, cotton and peanuts, for example, have been cited. The problem is often exacerbated by storage in hot or humid conditions.

Aflatoxins have been put forward as causes of liver cancer and acute liver damage in Third World countries. No clear-cut link has yet been established between man and contaminant, but the connection forms the basis of governmental risk management strategies.

U.K. approach

Aflatoxin contamination affects both animal feeds and some human food and is a subject which is taken seriously in the United Kingdom and in the European Community. The legislation on animal feeds aims to protect the food chain—that is, to preserve the quality of the ultimate products consumed by humans. Legislators also must consider the impact on the farm animal, both from the point of view of the animal's own welfare, and the implications for the farmer.

Attention has focused particularly on contamination in animal feeds by aflatoxin B_1. This is the most common of the aflatoxins, and the most carcinogenic in animal tests. It was first identified in the 1960s, in the U.K., following the death of over 100,000 young turkeys and ducks who had fed on peanut meal. And we now know of the possibility of onward contamination of milk by the metabolism of aflatoxin B_1 to aflatoxin M_1 in dairy cattle.

Controls of final feeds

In 1973, the European Community passed legislation prescribing maximum permissible levels of aflatoxin B_1 in various types of animal feeds. This coincided with the U.K.'s entry to the EC and therefore applies in the U.K. There have been some amendments and additions but the basic framework remains unchanged and applies across all of the 12 member countries of the EEC. Examples of the upper limits allowed are 0.01 mg/kg for complete and complementary dairy feeds and 0.05 mg/kg for straights (each with reference to a moisture content in the feed of 12%).

Controls on raw materials

There are also controls on some raw materials, used in the manufacture of animal feeds. There have been changes recently but the basic aim remains the same—to reinforce the controls on animal feeds generally, and so to protect the safety and quality of animal feed and of the human food chain.

In the late 1970s it was clear that the U.K. had a problem with milk. The monitoring program of milk showed that too many samples contained aflatoxin. It was thought that the culprit was the presence of aflatoxin B_1 in dairy feeds: when dairy cattle are given rations contaminated with aflatoxin B_1, then the metabolite aflatoxin M_1 is excreted into the milk. Peanuts and cottonseed—raw materials used in the manufacture of dairy feeds—were thought to be the sources of the contamination. The U.K. therefore introduced a ban on the import of peanuts or cottonseed containing detectable traces of aflatoxin B_1. This was shortly adapted to a ban on imports of peanuts when contaminated at a level above 0.05 mg/kg.

The policy of controlling the raw material was highly successful: aflatoxin contamination of milk was reduced dramatically. Of sam-