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FAO FOOD AND NUTRITION PAPER

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Food and Agriculture Organization of the United Nations

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Report of an FAO technical consultation
Rome, 3-6 May 1993

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REPORT OF A FAO TECHNICAL CONSULTATION ON SAMPLING PLANS FOR AFLATOXIN ANALYSIS IN PEANUTS AND CORN

Rome, 3-6 May 1993

Background for the Consultation

Aflatoxin was first noted in the early 1960s and has been a significant health concern and trade problem since that time. There is an increasing worldwide awareness of the serious consequences which undesirable levels of mycotoxins may have on food and feed supplies and on human and animal health. Concerns regarding mycotoxin contamination have been the subject of many national and international meetings.

The major aflatoxin-producing fungi are *Aspergillus flavus* and *Aspergillus parasiticus*. The aflatoxins are secondary fungal metabolites for which there is no known function within the fundamental life processes of the organism. They are, however, extremely toxic to other forms of life and cause through fungal contamination of food and feedstuffs mycotoxicosis in both man and animals.

Toxic moulds may invade agricultural products during plant growth, and during storage and processing. The aflatoxins occur in the sub μg/kg to the mg/kg range in groundnuts and groundnut products, corn and other grains, such as rice, wheat, sorghum and millet. In many countries, tolerance levels for aflatoxins in foodstuffs are in the range of 5-50 μg/kg.

To rationally enforce these tolerance levels reliable analytical procedures are needed. Validated methods of analysis exist but there are no internationally accepted sampling plans. Results of analyses are somewhat dependent on the method of analysis used, but are always dependent on the type of sampling plan applied, because the aflatoxins are found to be heterogeneously distributed in or on foodstuffs. When one single aflatoxin contaminated nut finds its way into a sample representative of a lot, the entire lot may be rejected for human consumption.

FAO's programmes are designated to ameliorate the adverse effects of mycotoxins on food availability and food self sufficiency, and on health and trade. Climatic conditions and poor storage conditions, frequently encountered in many developing countries, favour the occurrence of mycotoxins. For those countries that rely on the export of food and agricultural products, food containing even low levels of aflatoxins may mean rejection of consignments on import. Such rejected consignments almost invariably end up in other markets posing health hazards elsewhere.

The setting of internationally agreed upon tolerance levels for mycotoxins in food and feed is of global importance. However, the setting of such levels is encountering difficulties despite years of work carried out by various Codex Committees on this subject. Divergent views still exist between importing and exporting countries on the levels to adopt. FAO does hope that a suitable agreement can be reached on this important matter in the near future; compounding factors are the analytical methods used and the sampling plans followed.

Although actions to prevent formation of mycotoxins in food commodities should receive priority attention, there will always be a need for simple, accurate and reliable analytical methods to enforce existing regulatory limits. However, in the case of mycotoxins the Contamination level of a lot can only be determined when a representative sample is taken and analyzed.

The Joint FAO/UNEP Conference on mycotoxins held in Nairobi, Kenya in September 1977, identified as a major area of work the designing of sampling plans that would provide samples representative of lots of aflatoxin contaminated commodities. The Second Joint FAO/WHO/UNEP Conference on mycotoxins held in Bangkok, Thailand, in 1987 recommended among other things, that "international harmonization and agreement should be secured through organizations like the Joint FAO/WHO Codex Alimentarius Commission on the design of sampling procedures and analytical methodologies as well as on internationally agreed limits for mycotoxins in food and feedstuffs". It is clear from this 10 year gap that virtually no agreement was made, and that remains the case today.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) had been requested by the FAO and WHO to evaluate aflatoxins from the viewpoint of their impact on health when present as food contaminants. The JECFA discussed the aflatoxins at its 31st session in February 1987 and reiterated the view expressed at the 22nd meeting of the Committee: "A health hazard can be determined only by taking into account toxicological knowledge and information about potential exposure. However, in the case of potent carcinogens, for example certain mycotoxins, the Committee believed that efforts should be made to limit their presence in food to irreducible level". It defined an irreducible level as that concentration of a substance which cannot be eliminated from a food without involving the discarding of that food altogether, severely compromising the ultimate availability of major food supply. JECFA did not established any tolerable intake for aflatoxins.

Since the 31st meeting the aflatoxins have not been on the Agenda of the subsequent meetings of JECFA.

Aflatoxin sampling plans were discussed by the Codex Committee on Food Additives and Contaminants (CCFAC) at its 19th, 20th, 21st, 22nd, 23rd and 24th sessions (1987-1992), by the Codex Committee on Cereals, Pulses and Legumes (CCCPL) at its 6th, 7th and 8th sessions (1988-1992) as well as at the 8th session of the Codex Coordinating Committee for Asia (1992). Discussions about sampling plans at the meetings of the Codex Committee on Methods of Analysis and Sampling (CCMAS) were of a more general nature.

Specifically, the CCCPL and CCFAC reaffirmed their earlier conclusion that any proposed guideline level for aflatoxins would be linked to a specific commodity (i.e., as opposed to a general level applicable to all foods) as well as to specific sampling plans and methods of analysis. In this regard, it was noted that aflatoxin levels and their measurements varied extensively in different countries of the world.

These Committees also noted that sampling plans and guideline levels should be established for specific commodities based on available data rather than applying specific data to cover all foods, and that aflatoxins should be kept at the lowest practical level while protecting the health of consumers and avoiding technical barriers to international trade.

In view of these discussions, the CCCPL and CCFAC agreed to recommend to the FAO and WHO the convening of an Expert Consultation on sampling plans for aflatoxins to examine the various issues concerning this subject.

The CCFAC, at its 24th Session, suggested the following general terms of reference for the Expert Consultation:

- to examine the scientific basis of sampling plans and procedures for aflatoxins, and to establish the mathematical model(s) of the distribution of aflatoxins in the commodities on which the sampling plan is to be based;

- to establish guidelines for the development of sampling plans, i.e. the required operating characteristics of the sampling plan (specified acceptance and rejection probabilities for commodity lots containing a certain level of aflatoxin);

- to specify the type of commodity and its characteristics as it moved through international trade (i.e. intermediate products such as corn, raw peanuts, corn meal, processed peanut products, etc.) and to specify parameters needed in the mathematical model(s) which described the distribution of aflatoxin in the commodity;

- to evaluate the effects of sample collection and sample preparation procedures on the overall results of the analysis; specifications should include representative samples, representative sub-samples, and appropriate preparation of homogeneous composites;

- to indicate, as far as possible, the percentage of the commodity which would be rejected after harvest when the proposed sampling plan is applied;

- to include recommendations for sampling plans used in export control as well as import control;

- to consider specific sampling problems submitted to it by the Commission and any of its Committees.

To enable such a consultation to arrive at a decision concerning sampling plans linked to a specific level, the CCCPL agreed to suggested levels of 10 μg/kg total aflatoxins in processed peanuts and 15 μg/kg in raw peanuts, as well as the sampling plans previously discussed (Appendix II to Alinorm 91/29) for adoption at Step 5 by the 20th Session of the Codex Alimentarius Commission (CAC) in July 1993 on a provisional basis. The CCCPL also requested advice in establishing sampling plans based on higher or lower levels than those currently proposed.

Introduction

In view of suggestions made by the Codex Alimentarius Commission (CAC) a FAO Technical Consultation on Sampling Plans for Aflatoxin Analysis in Peanuts and Corn was held in Rome from 3 to 6 May, 1993. The programme of the Consultation is attached as Annex I. The membership of the Consultation is given in Annex II.

Mr. John R. Lupien, Director, Food Policy and Nutrition Division, Food and Agriculture Organization of the United Nations (FAO), opened the Consultation and welcomed the experts on behalf of the Director-General of FAO, Dr. Edouard Saouma. He expressed FAO's gratitude to the Government of Italy and, in particular, to Professor Antonio Manzoli, Director of the "Istituto Superiore di Sanita", for their cooperation in hosting this consultation. He also thanked the U.S. Agricultural Research Institute, and through it all those who have made financial and technical contributions toward the convening of this important Consultation. Mr. Lupien stressed the economic importance of aflatoxin contamination of certain foods, especially corn and peanut products moving in international trade, and the public health concerns as well. He indicated the need for both the producing and importing countries to harmonize regulatory levels and sampling plans as a follow-up to similar efforts in harmonizing analytical methodologies and quality assurance practices.

Dr. Marina Miraglia, Senior Researcher, Food Laboratory, Istituto Superiore di Sanità, welcomed the participants to the Institute on behalf of the Director of the Food Laboratory of the Institute, Prof. Angelo Stacchini.

Dr. E. Boutrif of the FAO made a brief introduction in which he reminded the participants of the objectives of the meeting, which were:

- to specify the type of major aflatoxin-susceptible peanut and corn commodities and their characteristics, including statistics on occurrence and levels of aflatoxins in these commodities;
- to establish the mathematical models of the distribution of aflatoxins in the above commodities;
- to establish guidelines for the development of sampling plans for peanuts and corn;
- to recommend sampling plans for export control as well as import control of the above commodities;
- to evaluate the effects of sample collection and sample preparation on the results of analysis;
- to indicate the percentage of the commodity which would be rejected when the proposed sampling plan is applied.

The Consultation elected Dr. A. Pohland as Chairman and Dr. Marina Miraglia as Vice-Chairman.

At the subsequent Plenary Session major working documents and oral reports were presented and discussed. The Consultation then, broke into three Working Groups, the first on Occurrence of Aflatoxin in Peanut and Corn Products Moving in International Trade, the second on Development and Evaluation of Aflatoxin Sampling Plans for Peanuts and Corn, and the third on Practical Issues Associated with Application of Sampling and Sample Preparation Procedures. The respective tasks and composition of each Working Group are included in Annex III.

Reports of the Working Groups were discussed and adopted at the final Plenary Session. They are presented in the following sections of this report.

OCCURRENCE OF AFLATOXIN IN PEANUT AND CORN PRODUCTS MOVING IN INTERNATIONAL TRADE

Introduction

Aflatoxins characterized as aflatoxin B_1, B_2, G_1 and G_2, are potent hepatotoxic and carcinogenic metabolites of Aspergillus flavus and A. parasiticus that grow on agricultural commodities. Structures of the most common aflatoxins are shown in Figure 1. Aflatoxin formation can occur while the product is in the field or after harvesting. Improper post-harvest treatment of the commodity, i.e. insufficient drying, improper storage conditions, etc., can result in increased levels of aflatoxins. Aflatoxin formation during the growing stages of the crop can increase due to environmental factors, i.e. drought or excess rain, insect infestation, etc. Although current agronomic practices will not eliminate the possibility of aflatoxin formation, the varying ability of the producer to utilize proper post-harvest handling and storage procedures can also result in an increase in aflatoxin contamination levels. The primary commodities associated with aflatoxin contamination are corn, peanuts (ground nuts), tree nuts, cottonseed, rice, and dried fruit.

The risk to humans can be through direct exposure, i.e., consumption of aflatoxin contaminated product, or indirectly through consumption of foods such as milk, eggs and liver from food-producing animals which have been fed aflatoxin-contaminated feed. Aflatoxin metabolites/reaction products including aflatoxin M_1, a metabolite of aflatoxin B_1 found in milk of dairy cows exposed to aflatoxin contaminated feed, also have demonstrated toxic potentials. These events highlight the importance of clearly understanding the fate of aflatoxin and/or metabolites in the food supply.

Incidence and Patterns of Aflatoxin Contamination

Human exposure to aflatoxin contamination occurs in two general patterns, i.e., acute and chronic. Acute aflatoxicosis, with reported major poisonings in India where 74 people died and Kenya with 12 deaths (Park and Stoloff, 1989), demonstrate that humans are not immune to the toxic effects of aflatoxin. These events also show that poisonings can occur through exposure to high levels of the toxins over relatively short time periods. Although a direct cause/effect relationship has not been demonstrated, there is a general correlation between the incidence of liver cancer in humans in specific areas of Africa and Asia and dietary exposure to aflatoxins (Shank et al., 1972; Peers and Linsell, 1973; Van Rensberg et al., 1985; Peers et al., 1976; Hsieh, 1986). The International Agency for Research on Cancer (IARC) has classified aflatoxin as a probable human carcinogen (IARC, 1993). JECFA, in its evaluation of aflatoxin intake levels, did not identify a tolerable intake value. They did recommend, however, that chronic exposure to aflatoxin contamination be reduced to the lowest practical level.

Many organizations worldwide continually monitor aflatoxin susceptible commodities for incidence and levels of aflatoxin contamination. Although, as indicated earlier, multiple commodities are susceptible to aflatoxin contamination, the CCCPL and CCFAC recommended that any review of proposed sampling programmes concentrate on corn, corn-based products, peanuts for human consumption, and peanut products subject to international trade. The terms corn/maize and peanuts/ground nuts are interchangeable and are used throughout this report.

Frequency of aflatoxin contamination in corn (maize) and corn-based commodities in Latin America and the Caribbean are presented in Table 1. These data show significant aflatoxin contamination for this region of the world. Table 2 presents similar data from Canada, France, United Kingdom, Senegal and Japan. Again higher incidence levels are evident for tropical regions (Latin America, Caribbean, Central Africa). Relatively lower levels of aflatoxin contamination occur in temperate regions. Table 3 presents the results of the FAO/WHO/UNEP GEMS Food Contamination Monitoring Programme for years 1976-83 (Ellis et al. 1991). Again higher levels of aflatoxin are evident in tropical regions. The U.S. Food and Drug Administration compliance data for milled corn products (meal, grits, hominy, and hominy grits) obtained from retail outlets from 1986-1991 are presented in Table 4 (Wood, 1989 and 1992; Wood and Pohland, 1992; Wood, G.E., Personal Communication). These data show that aflatoxin contamination for these products in the southeastern states was significantly higher than for other states. Data for shelled corn destined for human consumption for the U.S. corn crop for the same time period showed significant contamination levels (Table 5). Earlier surveys of aflatoxins in corn and dry-milled products from the corn belt areas outside the South and Southeast, showed products virtually aflatoxin-free. U.S. corn destined for animal feed (Table 6) showed much higher levels. Again southern and southeastern regions of the United States have the highest contamination levels. Although aflatoxin contamination can occur at significant levels in the raw product, processing procedures such as screening and milling can reduce these levels. This is evident by U.S. Department of Agriculture aflatoxin test results of processed corn products for 1989-1991 which revealed negative screening results (<20 μg/kg) for 14 013 analyses (Table 7). This may not, however, be the situation for lesser developed countries.

The incidence of aflatoxin contamination in raw peanuts from specific areas of the world is significant. Significant contamination levels are evident from the Latin America and the Caribbean regions (Table 8) and West Africa (Table 9). Canada, France and Japan, which are importing countries, show comparatively low levels for aflatoxins in finished products. Table 10 shows comparative aflatoxin contamination levels for various regions of the world (Ellis et al., 1991). Haydar et al., (1990) reported aflatoxin B_1, and B_2 levels of less than 3 μg/kg for raw and roasted peanut products from Syria. The results of 188 analyses for aflatoxin in edible peanuts imported into the U.K. during 1982-84 show 74% had aflatoxin B_1 levels ≤ 5 μg/kg (Kershaw, 1985). Again, although relatively high levels can be found in the raw product, processing techniques significantly reduce these levels for the product presented to the consumer (Table 11). Kamimura (1989) demonstrated that peanut processing procedures, i.e., steep tanks, coat splitting, sorting, and frying, can reduce aflatoxins to non-detectable levels in the finished product. The incidence of measurable aflatoxins in consumer peanut products in the U.S. for the time period 1980-84 and 1986 is presented in Tables 12, 13 and 14, respectively. These data show a variation from year to year; however, the overall incidence of products containing violative levels (total aflatoxins >20 μg/kg) is low.

Peanut and Corn Global Trade Patterns

With respect to aflatoxin susceptible commodities, raw peanuts and corn including processed products, comprise the largest percentage of products introduced in international exchange. Tables 15 and 16 present global and U.S. exports for selected commodities. A significant portion of corn and corn products (approximately 70%) moving in international trade originates from the U.S. as well as approximately 50% of the peanut in shell products. These values include figures for peanuts destined for non-edible purposes i.e., oil stock.

Major exporting countries for raw peanuts destined for human consumption are the U.S., China and Argentina. Major importers are the European Community, Japan and Canada. In 1991, global trade for corn and peanut commodities was around $9 billion and $1 billion, respectively. European Community imports and exports of corn (maize) and inshell peanuts for 1992 are presented in Table 17. France is a major European exporter of corn (4.7 million metric tons); however, over 9 million metric tons of corn are imported into the European Community.

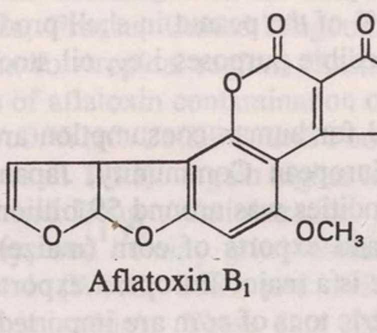
International shipments of these products are usually in bulk such as ship or truck. Peanuts are shipped usually in bags either containerized or break bulk (stored in bags in the hold of the ship). The final purpose of the product (edible or human food stuffs, or animal feeds) is usually known during shipment. With corn, however, the end use is not necessarily known. Corn is shipped primarily as bulk.

Although controlled experimental studies have not been performed, conditions of product storage during shipment could result in increased aflatoxin levels in the commodity. If the product were exposed to added moisture, i.e., condensation, leakage, etc., and holding temperatures were optimal for mold growth (A. flavus or A. parasiticus), increased levels of aflatoxins could occur.

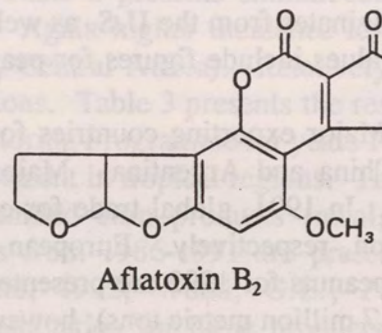
Commentary and Recommendations for International Trade

For a number of years, the CCFAC and CCCPL have discussed aflatoxin levels which could be acceptable for international trade. In these discussions, levels have largely reflected importer requirements for internal market purposes. In recommending levels for raw peanuts as well as consumer-ready products, Codex recognized the ability of processors to improve the quality of finished goods through manufacturing techniques (e.g. blanching, additional sorting, roasting, etc.) and the need to remove unnecessary technical barriers to international trade. As an example of this situation, over 95% of processed peanut products samples evaluated in the U.S. during the late 1980s by manufacturers showed levels below 5 ppb. This was also the situation described by a number of European processors. Corn also exhibits similar results from manufacturers.

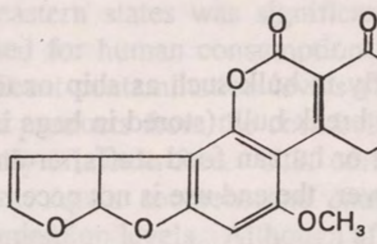
The establishment of uniform regulatory limits, i.e., international harmonization, for raw products would be beneficial, provided the limit does not penalize unfairly the exporting country. This is of particular significance for countries in the process of economic development, where procedures to reduce aflatoxin levels in the product being exported may divert the contaminated product to the local consumers. It is recommended that uniform international regulatory limits applicable at all stages of commerce be established on the basis of raw product.



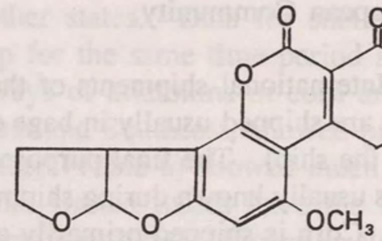
Aflatoxin B_1



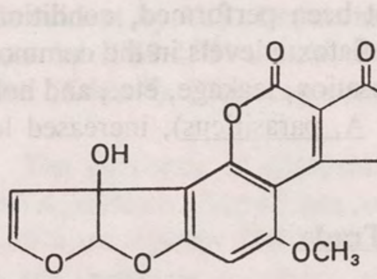
Aflatoxin B_2



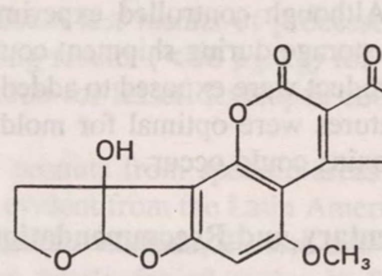
Aflatoxin G_1



Aflatoxin G_2



Aflatoxin M_1



Aflatoxin M_2

Figure 1. Structures of common aflatoxins

Table 1. Aflatoxin Contamination in Maize and Maize-based Commodities from Latin America and the Caribbean

Country	Years	Frequency (%)	Total Number of Samples	Mean	Range ($\mu g/kg$) or Specific level	Product
Argentina	1983-84	33.4	87	-	0-150	maize
	1986-89	21.3	644	1.1	2-230	maize
Brazil - south	-	18.0	165	14.3	5-900	maize
- southeast	-	8.5	163	3.0	5-148	maize
Costa Rica	1985-87	50.0	3000	156.1	(>20)	maize
	1979-90	17.0	3000	50.0	20-200	maize
Cuba	-	25.3	443	-	10-95	maize
Guatemala	1987	0	103	0	0	corn on cob
	1987	1.0	100	0.04	4	corn drink
	1987 group 1	2.1	50	11	>20	tortilla
	1987 group 2	16.0	50	6	>20	tortilla
	1987 group 3	26.7	52	10	>20	tortilla
	1987 group 5	54.0	57	51	>20	tortilla
- dry season	1976	16.7	18	-	21-130	corn
- wet season	1976	23.8	42	-	21-1650	corn
Mexico	-	87.8	41	-	5-465 (B_1)	Corn, kernel
			41		2-57 (G_1)	Corn, kernel

(Resnik, S. and Ferro Fontan, C., Personal Communication)

Table 2. Aflatoxin Contamination in Corn and Corn Products in Canada, France, Senegal and Japan

Country	Year(s)	Frequency (%)	Total numbers of samples	Mean	Range or specific level ($\mu g/kg$)	Commodity
Canada[a]	1992	0	39	<0.5	<0.5-<1.3	corn, all products
France[b]	1992	0	78	-	>200	corn gluten, raw
		23.0	78	-	>20	corn gluten, feed
Senegal[c]	1988	60.0	58	90 (B_1)	-	yellow corn
		40	60	119 (B_1)	-	white corn
Japan[d]	1972-89	0.5 (10)	371	6.4 (B_1)	0.7-52	corn
Italy[e]	1982-84	45.0	111	-	0.02-1.2 (B_1)	corn
United Kingdom[f]	-	3.4	29	-	>10	corn
		0	13	-	>10	corn flour
		0	2	-	>10	flakes

[a] Canadian Comments on CL 1992/8 - FAC, Paras 85 & 92, Alinorm 93/12, Codex Alimentarius
[b] Annual report (1992) from Lab. DDCCRF in Rennes, Ministry of Economy, RA 135-1992, p 35/96, with permission.
[c] Kane, A., personal communication, 1993.
[d] Aibara, K., personal communication, 1993.
[e] Micco, et al. (1986) Rev.Soc.Ital.Sci.Aliment., 15(3), 113-116.
[f] Jarvis, B. (1982) Occurrence of mycotoxins in UK foods. Food Tech Australia 34 (11) 508-514.

Table 3. Concentration of Aflatoxins in Corn

Country	Year	No. of samples	90th percentile μg/kg*
Brazil	1981	228	<8.0
Canada	1976	25	<4.0
Guatemala	1976-79	231	4-360
Kenya	1978-79	78	30-1920
Mexico	1979-80	96	<2.5-30
U.S.	1978-83	2633	10-700
U.K.	1978	29	8.0
Soviet Union	1981-82	219	<1-662

*Level below which 90% of findings occur in a given survey.

FAO/WHO/UNEP Monitoring Programme data (1976-83).

Table 4. Aflatoxins in milled corn products in U.S.

Area of U.S.	No. of products examined	Determinable aflatoxins, μg/kg: Percentage of products < 20	Percentage of products > 20	Maximum level
		1986		
Southeast[a]	61	18.0	9.8	53
Corn belt[b]	22	9.0	0.0	8
AR-OK-TX	8	0.0	0.0	0
VA-MD	4	0.0	0.0	0
Rest of U.S.	44	0.0	0.0	0
		1987		
Southeast[a]	94	11.7	3.1	45
Corn belt[b]	45	4.4	0.0	8
AR-OK-TX	24	0.0	0.0	0
VA-MD	4	0.0	0.0	0
Rest of U.S.	52	0.0	0.0	0
		1988		
Southeast[a]	206	1.9	1.4	34
Corn belt[b]	180	0.0	0.0	0
AR-OK-TX	40	2.5	0.0	6
VA-MD	40	2.5	0.0	10
Rest of U.S.	288	0.0	0.0	0
		1989		
Southeast[a]	155	0.6	0.6	24
Corn belt[b]	112	3.5	0.0	2
AR-OK-TX	64	1.5	0.0	7
VA-MD	16	0.0	0.0	0
Rest of U.S.	435	9.4	0.0	20
		1990		
Southeast[a]	44	18.1	0.0	6
Corn belt[b]	95	8.4	0.0	12
AR-OK-TX	14	28.6	0.0	8
VA-MD	1	0.0	0.0	0
Rest of U.S.	47	6.3	2.1	27
		1991		
Southeast[a]	43	21.0	11.6	69
Corn belt[b]	92	3.2	0.0	18
AR-OK-TX	15	6.6	0.0	6
VA-MD	11	0.0	0.0	0
Rest of U.S.	27	0.0	3.7	50

[a]AL, FL, GA, KY, LA, MS, NC, SC, TN.
[b]IA, IL, IN, KS, MI, MN, MO, NE, OH, SD, WI.
Wood, 1989 and 1992; Wood and Pohland, 1992; Wood, G.E., Personal Communication

Table 5. Aflatoxins in shelled corn designated for human consumption

Area of U.S.	No. of products examined	Determinable aflatoxins, µg/kg Percentage of products < 20	Percentage of products > 20	Maximum level
		1986		
Southeast[a]	59	15.2	16.9	364
Corn belt[b]	31	0.0	0.0	0
AR-OK-TX	23	13.0	0.0	12
VA-MD	8	25.0	0.0	20
Rest of U.S.	27	0.0	0.0	0
		1987		
Southeast[a]	105	9.5	20.9	125
Corn belt[b]	49	12.2	0.0	12
AR-OK-TX	49	12.2	2.0	36
VA-MD	5	0.0	0.0	0
Rest of U.S.	32	12.5	0.0	10
		1988		
Southeast[a]	299	2.0	7.0	398
Corn belt[b]	100	0.0	1.0	21
AR-OK-TX	115	4.3	5.2	96
VA-MD	44	4.5	2.2	30
Rest of U.S.	224	0.0	0.0	0
		1989		
Southeast[a]	262	4.1	3.4	219
Corn belt[b]	736	9.8	4.3	304
AR-OK-TX	252	7.1	12.3	582
VA-MD	8	0.0	0.0	0
Rest of U.S.	261	3.0	8.0	623
		1990		
Southeast[a]	24	8.0	4.0	323
Corn belt[b]	70	4.2	0.0	9
AR-OK-TX	82	42.6	23.1	392
VA-MD	0	0.0	0.0	0
Rest of U.S.	37	21.6	2.7	39
		1991		
Southeast[a]	62	24.1	20.9	157
Corn belt[b]	89	3.4	0.0	16
AR-OK-TX	49	22.4	26.5	109
VA-MD	0	0.0	0.0	0
Rest of U.S.	19	5.2	5.2	30

[a]AL, FL, GA, KY, LA, MS, NC, SC, TN.
[b]IA, IL, IN, KS, MI, MN, MO, NE, OH, SD, WI.
Wood, 1989 and 1992; Wood and Pohland, 1992; Wood, G.E., Personal Communication

Table 6. Aflatoxins in shelled corn designated for animal feed

Area of U.S.	No. of products examined	Determinable aflatoxins, µg/kg: Percentage of products < 20	Percentage of products > 20	Maximum level
		1986		
Southeast[a]	70	20.0	27.1	3872
Corn belt[b]	74	1.3	1.3	133
AR-OK-TX	39	15.4	59.0	2839
VA-MD	4	0.0	0.0	0
Rest of U.S.	16	6.2	0.0	2
		1987		
Southeast[a]	17	11.8	35.2	164
Corn belt[b]	75	8.0	0.0	20
AR-OK-TX	13	23.0	7.6	43
VA-MD	4	0.0	0.0	0
Rest of U.S.	7	0.0	0.0	0
		1988		
Southeast[a]	74	5.4	8.1	400
Corn belt[b]	15	0.0	0.0	0
AR-OK-TX	78	3.8	25.6	661
VA-MD	28	3.6	0.0	8
Rest of U.S.	71	0.0	0.0	0
		1989		
Southeast[a]	159	5.0	8.1	329
Corn belt[b]	784	11.4	3.4	199
AR-OK-TX	25	8.0	32.0	700
VA-MD	16	0.0	0.0	0
Rest of U.S.	28	0.0	0.0	0
		1990		
Southeast[a]	15	33.3	0.0	10
Corn belt[b]	136	39.7	2.9	92
AR-OK-TX	9	0.0	55.5	589
VA-MD	6	33.3	0.0	8
Rest of U.S.	7	0.0	0.0	0
		1991		
Southeast[a]	17	11.7	64.7	347
Corn belt[b]	127	33.8	22.0	660
AR-OK-TX	21	28.6	23.6	482
VA-MD	9	33.3	55.5	101
Rest of U.S.	12	58.3	0.0	6

[a]AL, FL, GA, KY, LA, MS, NC, SC, TN.
[b]IA, IL, IN, KS, MI, MN, MO, NE, OH, SD, WI.
Wood, 1989 and 1992; Wood and Pohland, 1992; Wood, G.E., Personal Communication

Table 7. **Distribution of the number of aflatoxin tests by type of processed corn products. All analyses showed negative results (<20 μg/kg) with screening techniques**

Products	01/01/88-09/30/89	10/01/89-09/30/90	10/01/90-09/30/91	01/01/89-09/30/91
Corn Soya Blend-Export	905	3,736	3,074	7,715
Corn meal-Export	324	450	745	1,519
Corn meal-Soya Fortified-Export	180	335	355	870
Corn Soya Milk-Export	-	-	455	455
Flour, Corn Soya Masa Instant-Export	36	89	120	245
Corn meal-Domestic	476	1,319	1,251	3,046
Grits, Corn, Fine-Domestic	15	61	52	128
Flour, Corn, Masa Instant-Domestic	1	16	18	35
Total	**1,937**	**6,006**	**6,070**	**14,013**

(J. Wu, personal communication)

Table 8. **Aflatoxin contamination in peanut and peanut products in Latin America and Caribbean**

Country	Year(s)	Frequency (%)	Total number of samples	Mean	Range (μg/kg)	Product
Argentina	1977-80	21.7,5.4,30.0	797	-	ND-10000	Peanut, peanut commodities
Brazil						
-West (rainy)	1981	61.2	152	1462.9	ND-30000	Peanuts
-West (dry)	1981	27.3	55	1242.3	ND-33500	Peanuts
-North west (rainy)	1981	60.0	50	1182.6	ND-12832	Peanuts
-North west (dry)	1981	15.4	13	10.1	ND-67	Peanuts
-North east (rainy)	1981	44.1	111	1176.0	ND-20423	Peanuts
-North east (dry)	1981	13.3	15	1.3	ND-10	Peanuts
Columbia	1984-85	38	-		5-766	Peanuts
Costa Rica	1989	42.8	87	42	4-228	Peanuts
Cuba	-	51.5	532	-	2-12000	Peanuts
Jamaica/St. Vincent	1984	0.05	160	82.8	8-7526	Peanuts

(Resnik, S. and Ferro Fontan, C., Personal Communication)

Table 9. **Aflatoxin contamination in peanuts and peanut products in Canada, France, Senegal and Japan.**

Country	Year(s)	Frequency (%)	Total number of samples	Mean	Range or specific level	Commodity
Canada	1992[a]	0.5 (>15)	202	<1	248	Peanuts processed
	1992[a]	6.2 (>15)	65	<1.3	18-52	Peanuts unprocessed
	1992[a]	0.7 (>15)	278	<1.9	18-22	Peanut batter
France	1992[b]	6.7	16	-	>200	Peanut cakes, raw
	1992[b]	31.3	16	-	>20	Peanut cakes, feeds
	1989-1990[c]		23	-	>1.0	Table peanuts
	1989-1990[c]	50.0	8	-	>1.0	Peanut butter
Senegal	1976-1985[d]	30.0	1475	403 (B_1)	-	Peanut kernels
	1989	5.0	50	5 (B_1)	-	Table peanuts
	1990	55.0	102	62 (B_1)	-	Peanut butter
	1990-1991	92.0	135	42 (B_1)	-	Crude peanut oil
	1982	90.0	125	490 (B_1)	-	Peanut cake
Japan	1972-89[e]	1.3 (>10)	17635	182 (B_1)	01-8070	Shelled peanuts, raw spanish
	1972-89	0.0 (>10)	5154	71 (B_1)	0.4-608	Shelled peanuts, raw Virginia
	1990	1.6 (>10)	1088	558 (B_1)	11-2250	Shelled peanuts, raw, spanish
	1990	0.0 (>10)	280	-	-	Shelled peanuts, raw, Virginia
	1990	0.0 (>10)	155	-	-	Peanut Butter
	1990	3.2 (>10)	93	26 (B_1)	10-56	Processed except peanut butter
	1987-90	1.3 (>10)	45	1.4 (B_1)	0.1-110	Peanut butter

[a] Canadian comments on CL 1992/8-FAC Paras. 85 & 92. Alinorm 93/12, Codex Alimentarius.
[b] Annual report from Lab. DDCCRF in Rennes, RA 135-1992, p. 35/96. Ministry of Economy, 1992, with permission.
[c] Herry, M.P., and Lemetayer, L., Microbiol. Alim & Nutrition, 10, p. 261-266, 1992.
[d] Kane, A., personal communication.
[e] Aibara, K., personal communication.

Table 10. Concentration of Aflatoxins in Peanut and Peanut Butter

Country	Year	No. of samples	90th percentile $\mu g/kg^{a}$
Brazil	1979-83	1044	30-5000
Guatemala	1977	13	150
Ireland	1977-82	61	300-4000
Mexico	1980	29	700
Switzerland	1980	11	338
U.K.	1978	159	75
U.S.	1983	120	24
Soviet Union	1982	21	329
U.K.[b]	1982-83	77	38-535

[a] Level below which 90% of findings occur in a given survey.
[b] Data for peanut butter.

FAO/WHO/UNEP Monitoring Programme (1977-83).

Table 11. Effectiveness of Postharvest Aflatoxin Management Strategies at the Processing Level*.

Technology	Aflatoxin Level (ppb)	Reduction (%)	Cumulative Reduction (%)
Farmers stock	217	--	--
Belt separator	140	35	35
Shelling plant**	100	29	54
Colour sorting**	30	70	86
Gravity table**	25	16	88
Blanching/colour sorting	2.2	91	99.0
Re-colour sorting**	1.6	27	99.3

* Results from the processing of a 40000 kg lot of contaminated peanuts.
** Data based on medium category peanuts only.

(R.S. Cole, personal communication).

Table 12. Distribution of consumer peanut products for the U.S. by fiscal year according to level of aflatoxin found (From FDA compliance reports).

Fiscal year	No. samples examined	Percentage of samples examined with total aflatoxin, ppb						
		ND[a]	>T	>5	>10	>15	>20	>50
1980	297	82.2	17.8	13.8	6.4	4.7	4.7	1
1981	202	84	16	11.5	7.5	4.5	4.5	0.5
1982	272	91.2	8.8	7.7	3.7	2.2	2.2	0.4
1983	312	86.9	13.1	12.5	9.3	6.4	5.4	1.9
1984	207	95.1	4.8	4.3	2.4	1.9	1.9	1.4

[a] ND = none detectable; limit of determination about 1 ppb; T = traces

Pohland, A. and Wood, G., 1987.

Table 13. Peanut products - incidence of determinable and violative levels of aflatoxins in specific U.S. commodities sampled and analyzed under FDA compliance programmes, by fiscal year.

Commodity	Fiscal year	No. of products	Samples with aflatoxins (%)	
			Determinable[a]	Violative[b]
Peanut butter	1980	220	17.2	3.2
	1981	140	17.8	4.2
	1982	187	9.6	2.1
	1983	177	10.7	2.2
	1984	122	4.9	2.4
Shelled, roasted	1980	72	22.27	9.7
	1981	56	7.4	5.4
	1982	81	18.3	1.2
	1983	120	4.9	10.8
	1984	81	0	1.2
In-shell, roasted	1980	5	33.3	0
	1981	6	0	0
	1982	4	0	0
	1983	15	0	0
	1984	4	0	0

[a] Limit of determination about 1 ppb.
[b] Total aflatoxins 20 ppb.

Pohland, A. and Wood, G., 1987.

Table 14. U.S. peanut products examined for aflatoxin and levels (1986)

		Determinable aflatoxins				
Peanut product	Total[a]	Number of products[b]	Percent of products	Average μg/kg	Maximum μg/kg	Number 20 μg/kg
Peanut butter	104	17	16	14	27	4
Shelled, roasted	55	6	11	68	329	2
In/shell, roasted	9	0	0	ND[c]	ND	0

[a] Total number of products examined.
[b] Total number of products with detectable levels of aflatoxins.
[c] ND, not detectable.

Wood, G. 1989.

Table 15. Selected U.S. and Global Agricultural Exports in Metric Tons - FAO Statistics

	1989			1990			1991		
Products	**World**	**U.S.**	**U.S.%**	**World**	**U.S.**	**U.S. %**	**World**	**U.S.**	**U.S. %**
Maize	77,553,504	56,513,328	72.9	72,210,320	52,172,320	72.3	66,602,128	44,558,240	66.9
Gluten Feed & Meal	6,408,758	5,181,893	80.9	7,026,888	5,814,068	82.7	7,516,516	6,350,104	84,5
Maize Cake	2,540,911	1,473,541	58.0	1,757,493	1,036,849	59.0	1,424,838	914,834	62.2
Maize Flour	792,857	237,832	30.0	825,376	197,240	23.9	1,070,755	326,059	30.5
Maize Bran	250,901	56,418	22.5	221,079	52,249	23.6	305,219	91,739	30.1
Maize Starch	509,814	61,568	12.1	495,413	51,792	10.5	454,076	50,329	11.1
Maize Germ	105,756	2,953	2.8	115,927	1,556	1.3	131,367	2,780	2.1
Forage & Silage	10,496	0	0.0	7,766	0	0.0	11,300	0	0.0
Grdnuts Shelled	821,283	210,264	25.6	1,017,815	236,792	23.3	1,003,853	173,389	17.3
Grdnuts in Shell	126,578	50,593	40.0	133,689	58,941	44.1	101,469	677,707	66.7
Cake of Grdnuts	889,302	4,966	0.6	644,967	20,772	3.2	786,852	42,820	5.4
Cottonseed Cake	1,419,794	11,934	0.8	1,136,054	14,478	1.3	1,103,500	37,700	3.4
Palm Kernels Cake	1,380,599	0	0.0	1,504,425	168	0.0	1,510,377	0	0.0
Coconut Cake	938,428	17	0.0	1,247,122	3,571	0.3	1,256,231	0	0.0
Desic. Coconut	197,300	0	0.0	192,703	0	0.0	190,982	0	0.0

Wu, J., personal communication.

Table 16. Selected U.S. and Global Agricultural Exports in US $1,000 - FAO Statistics

		1989			1990			1991	
Products	World	U.S.	U.S. %	World	U.S.	U.S. %	World	U.S.	U.S. %
Maize	10,207,531	6,691,131	65.6	9,827,489	6,205,804	63.1	8,923,151	5,146,692	57.7
Gluten Feed & Meal	956,353	755,789	79.0	991,796	793,789	80.0	1,063,824	880,282	82.7
Maize Cake	338,100	203,305	60.1	260,985	172,340	66.0	243,605	179,655	73.8
Maize Flour	169,101	56,681	33.5	165,134	40,981	24.8	193,427	60,347	31.2
Maize Bran	34,542	7,851	22.7	35,344	8,615	24.4	42,302	14,003	33.1
Maize Starch	188,469	31,361	16.6	216,297	28,926	13.4	212,019	30,170	14.2
Maize Germ	30,152	860	2.9	38,414	940	2.4	42,792	1,620	3.8
Forage & Sillage	644	0	0.0	593	0	0.0	1,021	0	0.0
Grdnuts Shelled	505,606	123,442	24.4	673,752	136,882	20.3	776,471	106,819	13.8
Grdnuts in Shell	95,957	37,566	39.1	92,137	40.226	43.7	78,253	42,919	54.8
Cake of Grdnuts	153,893	1,431	0.9	105,011	3,724	3.5	126,620	5,463	4.3
Cottonseed Cake	180,823	3,449	1.9	127,279	4,559	3.6	118,265	7,328	6.2
Palm Kernels Cake	132,023	0	0.0	133,028	27	0.0	142,272	0	0.0
Coconut Cake	112,031	2	0.0	118,523	295	0.2	127,872	0	0.0
Desic. Coconut	142,358	0	0.0	138,712	0	0.0	147,084	0	0.0

Wu J., personal communication

Table 17. Imports and exports of Corn and inshell peanuts for the European Community for 1992 (10^3 tons).

Commodity	European Community	Belgium/ Luxembourg	Denmark	Germany	Greece	Spain	France	Ireland	Italy	Netherlands	Portugal	UK
					IMPORTS							
Corn (maize)	9431	1287	61	1093	170	1518	321	46	999	1872	553	1510
Peanuts (in shell)	95	0.98	0.08	27	0.01	15	6.4	0.45	23	12	6.2	4.8
					EXPORTS							
Corn (maize)	5762	96	0.18	268	338	282	4735	0.18	14	9.3	3.6	14
Peanuts (in shell)	9.7	0.15	0.14	1.7	-	0.09	0.15	-	0.14	6.4	-	0.89

Eurostat - COMEXT (24.02.1993) and J.M. Fremy, personal communication.

Table 14. Selected U.S. and Global Agricultural Exports in US $1,000 - FAO Statistics

Products	1989 World	1989 U.S.	1989 U.S. %	1990 World	1990 U.S.	1990 U.S. %	1991 World	1991 U.S.	1991 U.S. %
Maize	10,207,531	6,691,134	65.6	9,727,439	6,205,804	63.1	8,923,151	5,146,692	57.7
Gluten Feed & Meal	956,353	755,789	79.0	991,796	793,789	80.0	1,063,824	880,282	82.7
Maize Cake	[illegible]	[illegible]	80.1	260,985	172,340	66.0	243,605	179,655	73.8
Maize Flour	169,168	56,521	33.5	165,134	40,981	24.8	193,427	60,347	31.2
Maize Oil	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	42,302	[illegible]	[illegible]
Maize Starch	188,489	31,361	16.6	216,297	28,926	13.4	212,019	30,170	14.2
Maize Germ	[illegible]	[illegible]	2.9	[illegible]	[illegible]	2.4	42,792	1,620	3.8
Forage & Sillage	644	0	0.0	893	0	0.0	1,021	0	0.0
Grdnuts Shelled	[illegible]	[illegible]	24.4	673,752	136,882	20.3	776,471	106,819	13.8
Grdnuts in Shell	95,257	37,388	39.1	92,137	40,226	43.7	78,252	42,919	54.8
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	3.5	126,620	5,463	4.3
Cottonseed Cake	180,823	3,440	1.9	127,279	4,559	3.6	118,265	7,328	6.2
Palm Kernels Cake	132,023	0	0.0	133,028	27	0.0	142,272	0	0.0
Coconut Cake	112,031	2	0.0	118,523	295	0.2	127,872	0	0.0
Desic. Coconut	142,358	0	0.0	138,712	0	0.0	147,084	0	0.0

Wu J., personal communication

DEVELOPMENT AND EVALUATION OF AFLATOXIN SAMPLING PLANS FOR PEANUTS AND CORN

Introduction

The first step associated with an aflatoxin sampling plan is the selection of a sample from a bulk shipment (lot). It is assumed in this section that recommended procedures, as outlined in the following section, for selecting a sample from a lot are followed. All kernels in the lot have an equal chance of being selected in the sample. As a result, the sample is correctly collected (Pitard, 1989) and no biases are introduced in the sampling procedure (Park and Pohland 1989).

Even with a correctly collected sample, it is difficult to estimate the true aflatoxin concentration of a lot because of the large variability associated with the sampling plan. Typically the sampling plan consists of three independent steps: a sampling step where a sample (sometimes called a laboratory sample) is collected from the lot, a sample preparation step where the entire sample is comminuted in a mill to reduce particle size and a subsample or test portion is removed from the comminuted sample, and finally an analytical step where aflatoxin is extracted from the test portion and the aflatoxin in the extract is quantified using defined quantification procedures.

The total variability associated with a sampling plan, as measured by the statistical variance, is the sum of sampling, sample preparation, and analytical variances.

Sampling - Sampling variance is large because the distribution among aflatoxin contaminated kernels in a lot is typically skewed. Only a small percentage of the kernels is contaminated, i.e. 0.03 percent at a lot concentration of 5 μg/kg, and the concentration on a single peanut kernel has been reported as high as 1,100 μg (Cucullu et al., 1966). For small sample sizes the sampling step is usually the largest source of variation (Whitaker et al., 1974, 1979, and 1992). By increasing the size of the sampe that is comminuted, the sampling variance can be reduced.

Sample preparation - Comminution of the sample breaks the kernels into many smaller particles. The number of particles per unit mass depends on the type of milling equipment. The aflatoxin concentration among comminuted particles will also vary greatly as in the case of the kernels in the sample. The concentration among replicate test portions will vary about the true sample concentration. Typically the sample preparation variance is lower than the sample variance because of the large number of comminuted particles in the subsample (Whitaker et al., 1974, 1979, and 1992; Dorner et al., 1993)

The sample preparation variance can be reduced by increasing the size of the test portion from which aflatoxin is extracted and/or increasing the degree of comminution by increasing the number of particles per unit mass (Campbell et al. 1988).

<u>Analysis</u> - Once the subsample is removed from the comminuted sample, aflatoxin is extracted and quantified. These methods are usually complicated involving several steps such as extraction, filtration, centrifugation, drying, dilution, and quantification. As a result there is variability among replicate analysis from the same extract (Whitaker and Dickens, 1981 and Horwitz, et al., 1993). Analytical variability can be reduced by increasing the number of aliquots quantified, using improved quantification technology, and improving the training of laboratory personnel.

<u>Classification Errors</u> - If the aflatoxin concentration, M, in a lot exceeds a defined lot guideline, M_c, then the lot should be rejected and diverted from food and feed usage. If the lot concentration, M, is less than or equal to the guideline, M_c, then the lot should be accepted and can be used for human consumption.

The lot aflatoxin concentration M is unknown in practice. The unknown lot aflatoxin concentration, 'M, is estimated by quantifying the aflatoxin in a random sample (called a sample test result) correctly collected from the lot. The sample test result, $\bar{x}$, is then used as an estimate of the lot concentration M.

As a consequence of an aflatoxin sampling plan, an export lot is classified as either acceptable or unacceptable. When the sample test result, $\bar{x}$, is less than or equal to a predefined sample acceptance level, $\bar{x}_a$, the lot is classified as acceptable. Otherwise, the lot is rejected. The sample acceptance level, $\bar{x}_a$, may or may not equal the guideline, M_c.

Because of the total variability among sample test results, two types of mistakes are associated with any aflatoxin sampling plan. First, some good lots (lots with $M \leq M_c$) will be rejected ($\bar{x} > \bar{x}_a$) by the sampling plan. This type of mistake is often termed false positives and the chance of committing this type of mistake is the exporter's risk. Secondly, some bad lots ($M > M_c$) will be accepted ($\bar{x} \leq \bar{x}_a$) by the sampling plan. This type of mistake is often termed false negatives and the chance of committing this type of mistake is the importer's risk.

For a given lot concentration, M, the frequency with which these mistakes occur depends on the sampling design as defined by sample size, sample preparation methods, analytical methods, and the sample acceptance level.

Operating Characteristic Curves

Lots with an aflatoxin concentration, M, will be accepted when using a specified sampling plan with probability P(M) which is the probability that a sample test result, $\bar{x}$, is less than or equal to the sample acceptance level, $\bar{x}_a$. A plot of P(M) against M is called an operating characteristic (OC) curve. Figure 1 depicts the general shape of an OC curve. A desirable OC curve is one where both the exporter's risk and the importer's risk have been minimized (minimize the shaded areas in Figure 1) to levels that are acceptable to both exporter and importer.

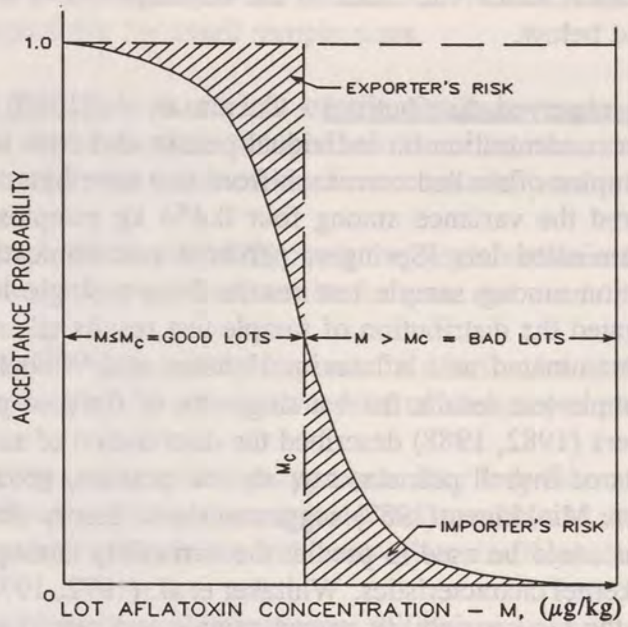


Figure 1. Typical operating characteristic (OC) curve for evaluating sampling plans.

The exact shape of the OC curve is uniquely defined for a given aflatoxin sampling plan with designated values of sample size, degree of comminution, subsample size, type analytical method, lot guideline level, sample acceptance level, and a probability distribution for aflatoxin within a lot.

<u>Lot Distribution</u> - The goodness of a sampling plan for inspecting a single lot with concentration M may be described by the OC curve. The goodness of a sampling plan for inspecting a collection of lots, such as lots produced during a crop year, requires the additional specification of a lot distribution. The lot distribution describes the frequency distribution of aflatoxin concentrations among a collection of lots.

From the accept probabilities P(M) (OC curve) and the lot distribution, the total number of lots accepted and rejected, the proportion of correct and incorrect decisions, and the average aflatoxin concentration among all lots accepted or rejected can be computed for a given sampling plan. Evaluating several sampling plans by this method allows for a quantitative comparison of the goodness of sampling plans.

Theoretical Probability Models

A critical requirement for computing the acceptance probability P(M) for an OC curve is the development and application of a mathematical model. The model should accurately describe the distribution of sample test results associated with sampling a lot with concentration M, given a sample size, sample preparation method, analytical method, lot guideline level, and sample acceptance level.

Several experimental studies have investigated the nature of the aflatoxin distribution among kernels and among sample test results within corn and peanut lots. These studies can provide useful information which can assist in the development of a mathematical model. These studies are listed below.

Experimentally observed distributions - Cucullu et al. (1966) and Lee et al. (1980) measured the aflatoxin concentration on individual peanut and corn kernels. Johnson et al. (1969) analyzed 72 samples of shelled corn each from two corn bins. A study sponsored by the U.S. FDA measured the variance among four 0.454 kg samples of shelled corn each taken from five contaminated lots (Springer, personal communication). Tiemstra (1969) measured the distribution among sample test results from a single lot of shelled peanuts. Brown (1984) investigated the distribution of sample test results taken from each of 22 lots of shelled peanuts contaminated with aflatoxin. Hanssen and Waibel (1978) measured the distribution among sample test results from a single lot of finished peanut kernel product. Coker (1989) and Jewers (1982, 1988) described the distribution of sample test results taken from contaminated lots of inshell peanuts, raw shelled peanuts, ground nut cakes, shelled corn, and peanut butter. Minkkinen (1987) suggested that a theory developed by Gy (1982) for mining applications could be used to predict the variability among aflatoxin sample test results using physical kernel characteristics. Whitaker et al. (1972, 1974, 1979, 1992, 1993a, and 1993b) measured the total variability among sample test results and also described the distribution among sample test results taken from contaminated lots of raw shelled peanuts, inshell peanuts, and shelled corn. Whitaker et al. also partitioned the total variability associated with sampling the above commodities for aflatoxin into a sampling variance, sample preparation variance, and analytical variance.

The above experimental studies suggest the variance associated with the various steps of a sampling plan for both peanuts and corn have the following characteristics:

(1) all variance components are a function of the aflatoxin concentration,

(2) the sampling, sample preparation, and analytical variances increase with an increase in aflatoxin concentration,

(3) the variance among kernel concentrations is numerically greater than the mean aflatoxin concentration in the lot, and

(4) the sampling variance associated with testing shelled corn appears to be less than the sampling variance associated with testing inshell and shelled peanuts after adjusting for sample size differences.

The observed distributions of sample test results taken from peanut and shelled corn lots suggest that a mathematical model should have the following characteristics:

(1) the model should allow for both a high percent of non-contaminated kernels and a small percent of contaminated kernels where kernel concentrations can vary from low to extremely high concentrations,

(2) the percent contaminated kernels increases with lot concentration, and

(3) the model should describe a distribution of sample test results that is positively skewed (more than 50% of the sample test results, $\bar{x}$, will have values less than the true lot concentration, M), especially for small sample sizes.

Mathematical Models - A review of the literature suggests that seven different mathematical models have been developed to describe the experimentally observed distributions among sample test results taken from corn and peanut lots. They are:

(1) Whitaker et al. (1969 and 1972) chose the Negative Binomial distribution to model the observed distribution of sample test results for corn and peanuts.

(2) Tiemstra (1968) compared the Exponential distribution to the observed distribution of sample test results for a single contaminated lot of shelled peanuts.

(3) Brown (1984) used a two step approach. The Log-Normal distribution was used to describe the distribution of non-zero sample test results and an empirical equation to describe the percent sample test results with zero aflatoxin for shelled peanuts.

(4) Waibel (1977) used a modification of the Gamma distribution (called the Waibel distribution) to describe the distribution of sample test results for peanuts.

(5) Jewers et al. (1986) investigated the suitability of the Waibel distribution to describe the distribution of sample test results for corn and peanut products.

(6) Resnik (personal communication) used the Gamma distribution to describe the observed distribution of sample test results for corn.

(7) Minkkinen (1987) used Gy's theory (1982) to predict the variability among sample test results. The predicted variability was compared to variability estimates measured by Whitaker (1972) and found the comparison to be reasonably close. No model assumptions were made. However, acceptance probabilities would be estimated using either the Normal or Poisson distributions.

Several skewed type models would most likely provide satisfactory fits to the observed distributions of sample test results. However, the development of seven different mathematical models, as described above, is evidence of how difficult it is to determine what is the most realistic probability model. In addition, the use of different methods to estimate the unknown model parameters makes it difficult to compare the adequacy of the above mathematical models. Finally, it is not clear from the available literature whether some of the models provided a satisfactory fit to the observed data.

Model Recommendations - The Consultation recommends use of the Negative Binomial distribution to develop OC curves for granular peanut and corn products and the Normal distribution to develop OC curves for peanut butter.

The Negative Binomial distribution is recommended because large amounts of information that have been accumulated concerning the model and the model parameters. The Negative Binomial model is a discrete distribution described by two parameters, the mean and a shape parameter (Anscombe, 1948). The shape parameter was found to be a function of the mean aflatoxin concentration for both corn and peanut products. Consequently, functional relationships were established to estimate the shape parameter for several products using the mean and variance from experimental sample test results and using the statistical method of moments. As a result, the OC curves developed from the Negative Binomial model can account for sample size, sample preparation, and analytical effects.

Goodness of fit test, (Whitaker et al., 1974 and 1993b), indicated that fits were acceptable for observed distributions where all sample test results were greater than zero μg/kg. However, the model always underestimated the percent samples with test results that measured zero aflatoxin (percent zeros). The failure to accurately estimate the percent zeros was probably due to a combination of fitting technique (method of moments) and the limit of detection of the analytical procedure (TLC) where a measured zero was about 2-3 μg/kg or less. However, when observed zero test results were assumed to equal the limit of detection, the negative binomial model accurately predicted the percent samples at the limit of detection.

The Consultation recommends the use of the Normal distribution to develop operating characteristic curves for peanut butter. The Normal model is a continuous distribution that is described by two parameters, the mean and the variance (National Bureau of Standards, 1967).

Because of the homogeneous nature of a thoroughly blended lot of peanut butter, the major source of error associated with testing peanut butter for aflatoxin should be analytical variability. If analysis is the largest source of variability, the distribution of sample test results are expected to be normally distributed (Quesenbury et al., 1976).

Since a peanut butter lot may not meet the "thoroughly blended" criteria, many small peanut butter increments, as described in the following section, must be composited (Coker, 1989). The composite portion should be thoroughly blended before a sample is removed for analysis.

Development and Evaluation of Sampling Plans

As suggested by FAO Food Control Series No. 4, "Mycotoxin Surveillance", several critical factors must be defined before an OC curve describing a sampling plan can be developed. These and other factors are:

(1) type commodity,
(2) lot guideline level, in μg/kg,
(3) sample acceptance level, in μg/kg,
(4) sample size,
(5) sample preparation method, and
(6) analytical method.

The Consultation made explicit choices for each of these critical factor which are shown in Appendix I.

Type Commodity - The Consultation decided that sufficient information existed to develop OC curves for raw shelled peanuts, inshell peanuts, peanut butter, and shelled corn.

Guidelines - Five lot guidelines 5, 10, 15, 20, and 30 μg/kg total aflatoxin were chosen by the Consultation. These guidelines ranged below and above guidelines suggested for peanuts by CCFAC and CCCPL. The above guidelines also cover the guideline of 20 μg/kg that is currently used by the United States for export corn.

Sample Acceptance Level - To maintain simplicity and practicality when testing corn and peanuts in international trade, the sample acceptance level was set equal to the guideline ($\bar{x}_a = M_c$) in all sample designs. Therefore, the chances of a false positive occurring approximately balances the chances of a false negative occurring.

The balancing of importer's and exporter's risk is a key factor in the design of an aflatoxin sampling plan. The seller is concerned about the economic loss associated with rejecting good lots while the buyer is concerned with the economic loss and health risks associated with accepting a bad lot. The buyer can possibly tolerate a higher risk than the seller when sampling raw product since additional processing steps such as electronic color sorting and blanching will reduce the aflatoxin concentration of a processed lot.

Sample Size - The Consultation only considered the use of a single sample taken from a lot when testing corn or peanuts for aflatoxin. It was also assumed that there will be no reinspection or appeals.

Two sample sizes were chosen for all products except peanut butter where one size was chosen. The two sample sizes were not determined by statistical criteria. Instead, the two sample sizes reflect a minimum and a maximum size that were recommended by the Consultation based upon practical aspects associated with sample handling and sample preparation method.

Due to the assumed homogeneous nature of a well-blended composite sample of peanut butter that is accumulated from many different locations in the lot, a single small sample taken from the composite sample was chosen for peanut butter.

Sampling variability associated with different sample sizes is estimated using equations in Appendix II. A sample size larger than those specified will reduce the sampling variance.

Sample Preparation - A single sample preparation method was chosen by the Consultation for each product. A hammer mill with a #14 screen (3.1 mm diameter hole in the screen) similar to the type used by the U.S. Department of Agriculture to prepare samples for aflatoxin analysis was specified for peanuts. A hammer mill with a #20 screen (1 mm diameter hole in the screen) was chosen for corn. The choices represent a compromise in terms of cost and precision.

A minimal test portion size of 100 g was chosen for comminuted peanuts and 50 g was chosen for comminuted corn. The variability associated with the sample preparation step can be estimated using equations in Appendix II. If larger test portions or mills that produce a finer grind are used to prepare the sample, a lower sample preparation variance will result.

Analytical Methods - The Consultation chose TLC analytical methods to quantify aflatoxin in the subsample extract. An extensive survey by Horwitz et al. (1993) suggested that TLC represents the most common type analytical method used by analytical laboratories.

Horwitz et al. (1993) measured the precision associated with testing several different commodities using several different analytical methods from a large number of laboratories. The analytical variability, as measured by the coefficient of variation, ranged from about 9 to 82 percent. The variability associated with TLC methods can be estimated using equations in Appendix II and reflects a compromise in the precision capabilities of the various analytical laboratories. If different analytical methods are used or more aliquots are analyzed per extract, the analytical variability can be reduced.

OC Curves - A total of 35 sampling plans were evaluated. The OC curves for inshell peanuts, raw shelled peanuts, peanut butter, and shelled corn are shown in Figures 2-1 through 2-7 in Appendix III. A numerical representation of each OC curve is given in Tables III-1 through III-4 in Appendix III. No reinspection or appeal sampling is assumed in the computation of the OC curves.

Lot Distribution - To demonstrate how sampling plans perform for a collection of lots, a lot distribution was coupled to each of the 10 OC curves for raw shelled peanuts. The lot distribution was constructed from sample test results recorded in the 1980 crop year in the U.S. The crop averaged about 14 μg/kg which was three to four times higher than a normal crop produced in the U.S.

The constructed cumulative lot distribution is shown in Table IV-2 in Appendix IV. Tables IV-3 and IV-4 in Appendix IV, developed using equations IV-1 through IV-10, show for each 100 lots tested the number of lots accepted, the number of lots rejected, the number of correct decisions, the number of false positives, the number of false negatives, and the average aflatoxin in the accepted and rejected lots.

Equations in Appendix IV allow countries that maintain an aflatoxin data base to use their own lot distributions, instead of the U.S. example, to evaluate the individual sampling plans. However, crop distribution data do not generally exist for products to the same extent as that for shelled peanuts marketed in the United States. Regulatory agencies of both importing and exporting nations are encouraged to collect and maintain statistically valid survey data that will describe the incidence of contamination among the various products so that meaningful assessments of respective risks may be conducted.

Appendix I: Choices for Critical Design Factors

The Consultation made practical choices for critical factors that are needed to design aflatoxin sampling plans. Table I-1 shows the various commodities, guidelines, sample sizes, sample preparation methods, and analytical methods chosen. Aflatoxin concentrations are total μg/kg or the sum of B_1, B_2, G_1, and G_2. As a result of these choices, a total of 35 sampling plans for four commodities were developed and evaluated. The Negative Binomial distribution was chosen by the Consultation to describe the distribution of sample test results for raw shelled peanuts, inshell peanuts and corn. The Normal distribution was chosen to describe the distribution of sample test results for peanut butter.

Table I-1. Critical Factors for the Design of Aflatoxin Sampling Plans.

Raw Shelled Peanuts	Number Plans
Guidelines (μg/kg) - 5, 10, 15, 20, 30	10
Sample Size (kg) - 5, 20 (kernels)	
Comminution - Hammer Mill (#14 screen)	
Test Portion Size (g) - 100	
Analytical Method - TLC	
Inshell Peanuts	
Guidelines (μg/kg) - 5, 10, 15, 20, 30	10
Sample Size (kg) - 7, 27 (pods)	
Comminution - Hammer Mill (#14 screen)	
Test Portion Size (g) - 100	
Analytical Method - TLC	
Peanut Butter	
Guidelines (μg/kg) - 5, 10, 15, 20, 30	5
Sample Size (kg) - 0.10	
Comminution - none	
Test Portion Size (g) - none, entire sample extracted	
Analytical Method - TLC	
Shelled Corn	
Guidelines (μg/kg) - 5, 10, 15, 20, 30	10
Sample Size (kg) - 3, 10	
Comminution - Hammer Mill (#20 screen)	
Test Portion Size (g) - 50	
Analytical Method - TLC	
Total Number Plans	35

Appendix II: Variability Estimates

Tables II-1 through II-4 show the variances and coefficients of variation for each product, sample size, sample preparation, and analytical method. The total or sum of the three variances is also presented. Regression equations are also provided so that variances other than the values shown in the tables can be computed.

Definition of Terms:

s^2 = variance
cv = coefficient of variation (%)
M = aflatoxin concentration (μg/kg)
n = sample size (kg)
nss = comminuted test portion size (g)

Table II-1. Sampling, Sample Preparation, Analytical, and Total Variability Associated with Testing Raw Shelled Peanuts for Aflatoxin. Variance, s^2, and Coefficient of Variation, cv, Associated with Two Sample Sizes (n), Hammer Mill with a #14 Screen, 100 g Test Portion (nss), and TLC Analytical Methods.

Sampling

Aflatoxin Conc. M	Sample Size 5 kg		Sample Size 20 kg	
(µg/kg)	variance s^2	cv (%)	variance s^2	cv (%)
5	86.0	185.5	21.5	92.7
10	217.1	147.4	54.3	73.7
15	373.2	128.8	93.3	64.4
20	548.0	117.0	137.0	58.5
25	738.3	108.7	184.6	54.3
30	941.9	102.3	235.5	51.2
40	1383.2	93.0	345.8	46.5
50	1863.5	86.3	465.9	43.2
75	3202.8	75.5	800.7	37.7
100	4703.3	68.6	1175.8	34.3
150	8083.7	59.9	2020.9	30.0
200	11871.1	54.5	2967.8	27.2

$s^2|_s = (5.4533/n)*9.19*M^{1.3357}$
(Whitaker et al., 1974)

Sample Preparation

Aflatoxin Conc. M	Test Portion Size 100 g	
(µg/kg)	variance s^2	cv (%)
5	13.0	72.220
10	43.2	65.739
15	87.1	62.221
20	143.2	59.839
25	210.6	58.055
30	288.7	56.637
40	474.7	54.469
50	698.2	52.845
75	1407.2	50.017
100	2313.9	48.103
150	4663.9	45.529
200	7668.9	43.786

$s^2|_{ss} = (275/nss)*0.2935*M^{1.7287}$
(Whitaker et al., 1974)

Analytical

Aflatoxin Conc. M	TLC	
(µg/kg)	variance s^2	cv (%)
5	4.8	43.6
10	15.4	39.3
15	30.7	36.9
20	50.1	35.4
25	73.1	34.2
30	99.7	33.3
40	162.5	31.9
50	237.3	30.8
75	472.6	29.0
100	770.3	27.8
150	1533.8	26.1
200	2500.2	25.0

$s^2|_a = 0.3088*M^{1.6985}$
(Variance equation developed by Whitaker by sorting data published by Horwitz et al., 1993)

Total

Aflatoxin Conc. M	Sample Size 5 kg		Sample Size 20 kg	
(µg/kg)	variance s^2	cv (%)	variance s^2	cv (%)
5	103.8	203.8	39.3	125.4
10	275.8	166.1	112.9	106.3
15	491.0	147.7	211.1	96.9
20	741.3	136.1	330.3	90.9
25	1022.1	127.9	468.4	86.6
30	1330.2	121.6	623.8	83.3
40	2020.3	112.4	983.0	78.4
50	2799.0	105.8	1401.4	74.9
75	5082.5	95.1	2680.5	69.0
100	7787.5	88.2	4260.0	65.3
150	14281.4	79.7	8218.6	60.4
200	22040.2	74.2	13136.9	57.3

$s^2|_t = s^2|_s + s^2|_{ss} + s^2|_a$

Table II-2. Sampling, Sample Preparation, Analytical, and Total Variability Associated with Testing Inshell Peanuts for Aflatoxin. Variance, s^2, and Coefficient of Variation, cv, Associated with Two Sample Sizes (n), Hammer Mill with a #14 Screen, 100 g Test Portion (nss), and TLC Analytical Methods.

Sampling

Aflatoxin Conc. M (μg/kg)	Sample Size 7 kg variance s^2	Sample Size 7 kg cv (%)	Sample Size 27 kg variance s^2	Sample Size 27 kg cv (%)
5	45.7	135.2	11.8	68.8
10	120.4	109.7	31.2	55.9
15	212.3	97.1	55.0	49.5
20	317.4	89.1	82.1	45.4
25	433.6	83.3	112.4	42.4
30	559.5	78.9	145.1	40.2
40	836.5	72.3	216.9	36.8
50	1142.8	67.6	296.3	34.4
75	2014.5	59.8	522.3	30.5
100	3011.9	54.9	780.9	27.9
150	5309.3	48.6	1376.5	24.7
200	7938.1	44.6	2058.0	22.7

$s^2|_s = (9.5/n)*3.5483*M^{1.3981}$
(Whitaker et al., 1992)

Sample Preparation

Aflatoxin Conc. M (μg/kg)	Test Portion Size 100 g variance s^2	Test Portion Size 100 g cv (%)
5	13.0	72.220
10	43.2	65.739
15	87.1	62.221
20	143.2	59.839
25	210.6	58.055
30	288.7	56.637
40	474.7	54.469
50	698.2	52.845
75	1407.2	50.017
100	2313.9	48.103
150	4663.9	45.529
200	7668.9	43.786

$s^2|_{ss} = (275/nss)*0.2935*M^{1.7287}$
(Whitaker et al., 1974)

Analytical

Aflatoxin Conc. M (μg/kg)	TLC variance s^2	TLC cv (%)
5	4.8	43.6
10	15.4	39.3
15	30.7	36.9
20	50.1	35.4
25	73.1	34.2
30	99.7	33.3
40	162.5	31.9
50	237.3	30.8
75	472.6	29.0
100	770.3	27.8
150	1533.8	26.1
200	2500.2	25.0

$s^2|_a = 0.3088*M^{1.6985}$
(Variance equation developed by Whitaker by sorting data published by Horwitz et al., 1993)

Total

Aflatoxin Conc. M (μg/kg)	Sample Size 7 kg variance s^2	Sample Size 7 kg cv (%)	Sample Size 27 kg variance s^2	Sample Size 27 kg cv (%)
5	63.5	159.4	29.6	108.9
10	179.1	133.8	89.9	94.8
15	330.1	121.1	172.9	87.6
20	510.7	113.0	275.6	83.0
25	717.4	107.1	396.2	79.6
30	947.9	102.6	533.4	77.0
40	1473.7	96.0	854.1	73.1
50	2078.3	91.2	1231.8	70.2
75	3894.3	83.2	2402.1	65.3
100	6096.1	78.1	3865.1	62.2
150	11507.0	71.5	7574.2	58.0
200	18107.2	67.3	12227.1	55.3

$s^2|_t = s^2|_s + s^2|_{ss} + s^2|_a$

Table II-3. Sampling, Analytical, and Total Variability Associated with Testing Peanut Butter for Aflatoxin. Variance, s^2, and Coefficient of Variation, cv, Associated with 100 g Sample (n) and TLC Analytical Methods.

Sampling			Analytical			Total		
Aflatoxin Conc. M	Sample Size 0.1 kg		Aflatoxin Conc. M	TLC		Aflatoxin Conc. M	Sample Size 0.1 kg	
μg/kg	variance s^2	cv (%)	μg/kg	variance s^2	cv (%)	μg/kg	variance s^2	cv (%)
5	1.0	19.6	5	2.8	33.4	5	3.7	38.7
10	2.7	16.5	10	8.8	29.7	10	11.6	34.0
15	5.1	15.0	15	17.3	27.7	15	22.4	31.5
20	7.8	14.0	20	27.9	26.4	20	35.8	29.9
25	11.0	13.3	25	40.4	25.4	25	51.5	28.7
30	14.5	12.7	30	54.8	24.7	30	69.3	27.7
40	22.5	11.9	40	88.4	23.5	40	110.9	26.3
50	31.6	11.2	50	128.1	22.6	50	159.7	25.3
75	58.4	10.2	75	251.4	21.1	75	309.8	23.5
100	90.5	9.5	100	405.6	20.1	100	496.1	22.3
150	167.5	8.6	150	796.2	18.8	150	963.7	20.7
200	259.3	8.1	200	1284.7	17.9	200	1544.0	19.6
$s^2\|_s = (0.1/n)*0.0829*M^{1.5190}$ (Dorner et al., 1993)			$s^2\|_a = 0.1914*M^{1.6631}$ (Variance equation developed by Whitaker by sorting data published by Horwitz et al., 1993)			$s^2\|_t = s^2\|_s + s^2\|_a$		

Table II-4. Sampling, Sample Preparation, Analytical, and Total Variability Associated with Testing Shelled Corn for Aflatoxin. Variance, s^2, and Coefficient of Variation, cv, Associated with Two Sample Sizes (n), Hammer Mill with a #20 Screen, 50 g Test Portion (nss), and TLC Analytical Methods.

Sampling

Aflatoxin Conc. M (μg/kg)	Sample Size 3 kg variance s^2	Sample Size 3 kg cv (%)	Sample Size 10 kg variance s^2	Sample Size 10 kg cv (%)
5	6.6	51.3	2.0	28.1
10	13.2	36.3	4.0	19.9
15	19.8	29.6	5.9	16.2
20	26.3	25.7	7.9	14.1
25	32.9	23.0	9.9	12.6
30	39.5	21.0	11.9	11.5
40	52.7	18.1	15.8	9.9
50	65.9	16.2	19.8	8.9
75	98.8	13.3	29.6	7.3
100	131.7	11.5	39.5	6.3
150	197.6	9.4	59.3	5.1
200	263.4	8.1	79.0	4.4

$s^2|_s = (0.4997/n)*7.9078*M$
(Whitaker et al., 1979)

Sample Preparation

Aflatoxin Conc. M (μg/kg)	Test Portion Size 50 g variance s^2	Test Portion Size 50 g cv (%)
5	1.3	22.4
10	2.5	15.8
15	3.8	12.9
20	5.0	11.2
25	6.3	10.0
30	7.5	9.1
40	10.0	7.9
50	12.5	7.1
75	18.8	5.8
100	25.0	5.0
150	37.5	4.1
200	50.1	3.5

$s^2|_{ss} = (50/nss)*0.2503*M$
(Whitaker et al., 1979)

Analytical

Aflatoxin Conc. M (μg/kg)	TLC variance s^2	TLC cv (%)
5	5.2	45.8
10	17.5	41.9
15	35.6	39.8
20	58.7	38.3
25	86.7	37.2
30	119.1	36.4
40	196.7	35.1
50	290.2	34.1
75	588.6	32.3
100	972.1	31.2
150	1971.4	29.6
200	3255.6	28.5

$s^2|_a = 0.3088*M^{1.6985}$
(Variance equation developed by Whitaker by sorting data published by Horwitz et al., 1993)

Total

Aflatoxin Conc. M (μg/kg)	Sample Size 3 kg variance s^2	Sample Size 3 kg cv (%)	Sample Size 10 kg variance s^2	Sample Size 10 kg cv (%)
5	7.8	56.0	3.2	35.9
10	15.7	39.6	6.5	25.4
15	28.7	35.7	14.9	25.7
20	48.9	35.0	30.4	27.6
25	74.7	34.6	51.7	28.8
30	105.8	34.3	78.1	29.5
40	149.4	30.6	112.5	26.5
50	197.5	28.1	151.4	24.6
75	314.2	23.6	245.1	20.9
100	447.0	21.1	354.8	18.8
150	823.7	19.1	685.4	17.5
200	1285.6	17.9	1101.2	16.6

$s^2|_t = s^2|_s + s^2|_{ss} + s^2|_a$

Appendix III: Evaluation of Sampling Plans

A total of 35 sampling plans were evaluated. The operating characteristic curves describing sampling plans for raw shelled peanuts, inshell peanuts, peanut butter, and shelled corn are shown in Figures 2-1 through 2-7, respectively. Each curve shows the probability that a lot with a given aflatoxin concentration, M, will be accepted given the sample size, sample preparation method, analytical method, and sample acceptance level. A numerical representation of the 35 OC curves are shown in Tables III-1 through III-4.

The acceptance probabilities for sampling raw shelled peanuts, inshell peanuts, and corn (Tables III-1, III-2, and III-4) were computed using the Negative Binomial equation and the appropriate variance relationships in Appendix II (Whitaker and Wiser, 1969 and Whitaker et al., 1972).

The acceptance probabilities for sampling peanut butter (Table III-3) were computed using the Normal distribution and the appropriate variance relationships in Appendix II (National Bureau of Standards, 1967).

Negative Binomial Distribution - The Negative Binomial density function f(x) is

$$f(x) = (\Gamma(x+k)/(x!\Gamma(k)))\ (k/(k+M))^k\ (M/(k+M))^x, \qquad \text{(III-1)}$$

where x is the quantity of aflatoxin on a single kernel, Γ is the gamma function, M is the average quantity of aflatoxin among all kernels in the lot, and k is the shape parameter. The cumulative density function is

$$F(x) = \sum_{r=0}^{x} f(r) = \sum_{r=0}^{x} (\Gamma(r+k)/(r!\Gamma(k)))\ p^k q^r, \qquad (III\text{-}2)$$

where r is a summation index for x, p=(k/(k+M)) and q=(M/(k+M)). If the random variable x is described by the Negative Binomial distribution with mean M and shape parameter k, then the sum of n independent observations is Negative Binomial with mean nM and shape parameter nk. The sum of x in $n\bar{x}$ where $\bar{x}$ is the aflatoxin concentration in a sample of n kernels. Therefore, the cumulative distribution of the sum of n observation is

$$F(n\bar{x}) = \sum_{r=0}^{n\bar{x}} (\Gamma(r+nk)/(r!\Gamma(nk)))\ p^{nk} q^r, \qquad (III\text{-}3)$$

The cumulative distribution of aflatoxin concentration among replicate samples, $F(\bar{x})$, can be determined by a scale transformation of equation (III-3).

The moment estimate of the shape parameter, k, is

$$k = M*M\ /\ ((n * s^2) - M), \qquad \text{(III-4)}$$

where s^2 is the total variance given in Appendix II. The sample size n in Appendix II is in kg and must be converted to number of kernels using the appropriate kernel or pod count per unit mass.

Normal Distribution - The Normal probability density function is

$$f(z) = (1 / \sqrt{2\pi}) * \exp(-z^2/2). \qquad (III-5)$$

The cumulative density function is

$$F(z) = (1 / \sqrt{2\pi}) \int_{y=-\infty}^{z} \exp(-y^2/2)\,dy, \qquad (III-6)$$

where z is a normalized random variable.

$$z = (\bar{x}_a - M) / s, \qquad (III-7)$$

where s is the standard deviation or square root of the variance associated with the aflatoxin testing procedure given in Appendix II.

The cumulative distribution in equation (III-6) can be computed using approximation formulas given by National Bureau of Standards, edited by Abramowitz and Stegun (1967). For example,

$$F(z) = 1-0.5(1+c_1z+c_2z^2+c_3z^3+c_4z^4)^{-4}, \qquad (III-8)$$

where $c_1 = 0.196854$, $c_2 = 0.115194$, $c_3 = 0.000344$, and $c_4 = 0.019527$.

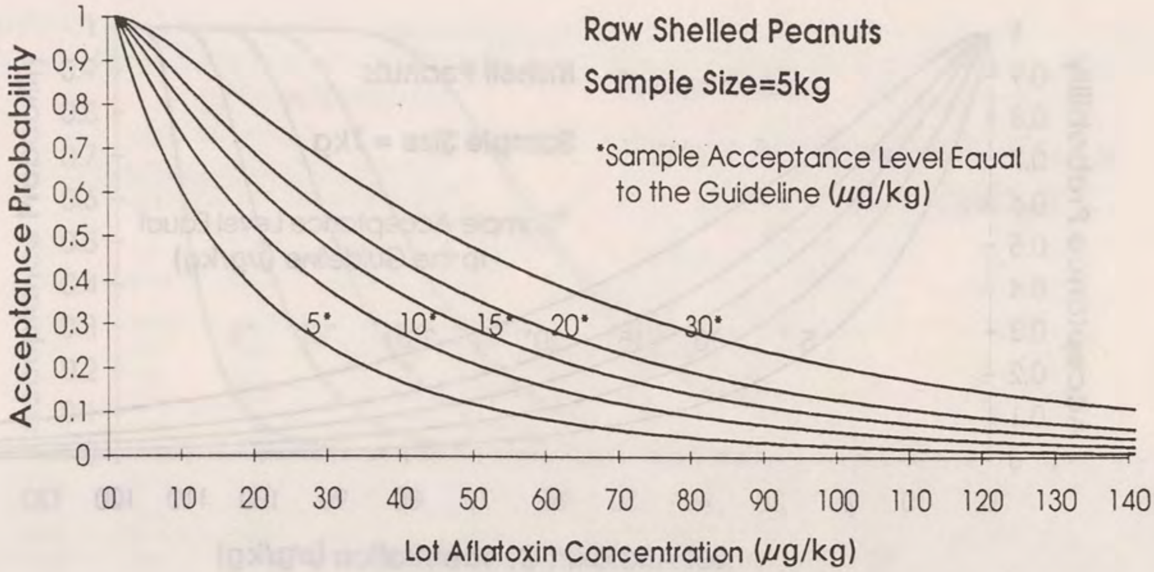


Figure 2-1. Five operating characteristic curves showing the probability of accepting raw shelled peanut lots when using 5 kg sample kernels, hammer mill for comminution, 100 g test portion, TLC analytical methods, and five sample acceptance levels.

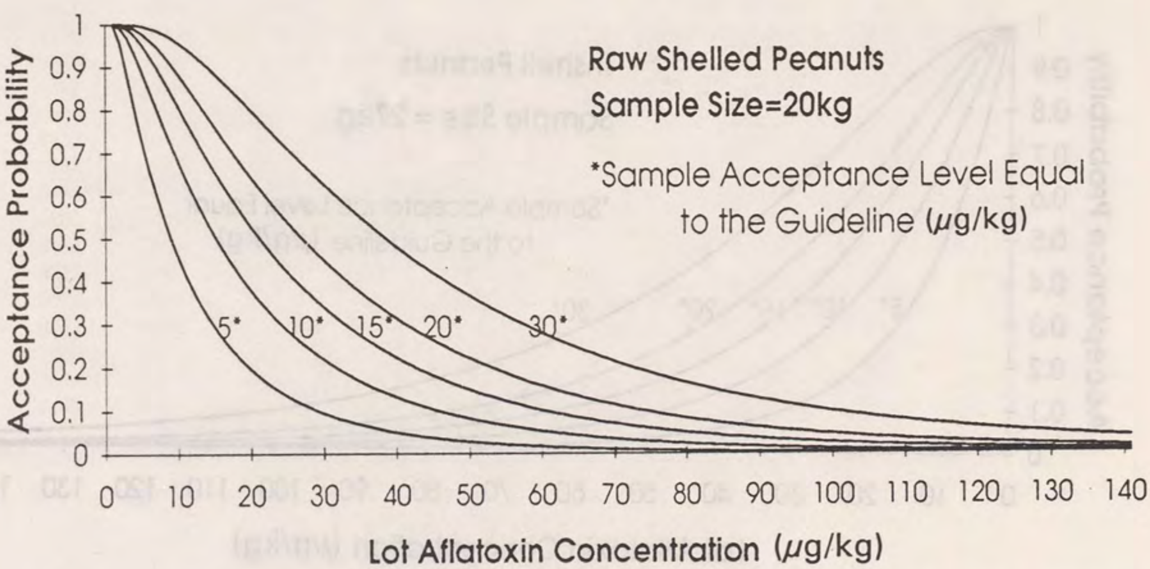


Figure 2-2. Five operating characteristic curves showing the probability of accepting raw shelled peanut lots when using 20 kg sample kernels, hammer mill for comminution, 100 g test portion, TLC analytical methods, and five sample acceptance levels.

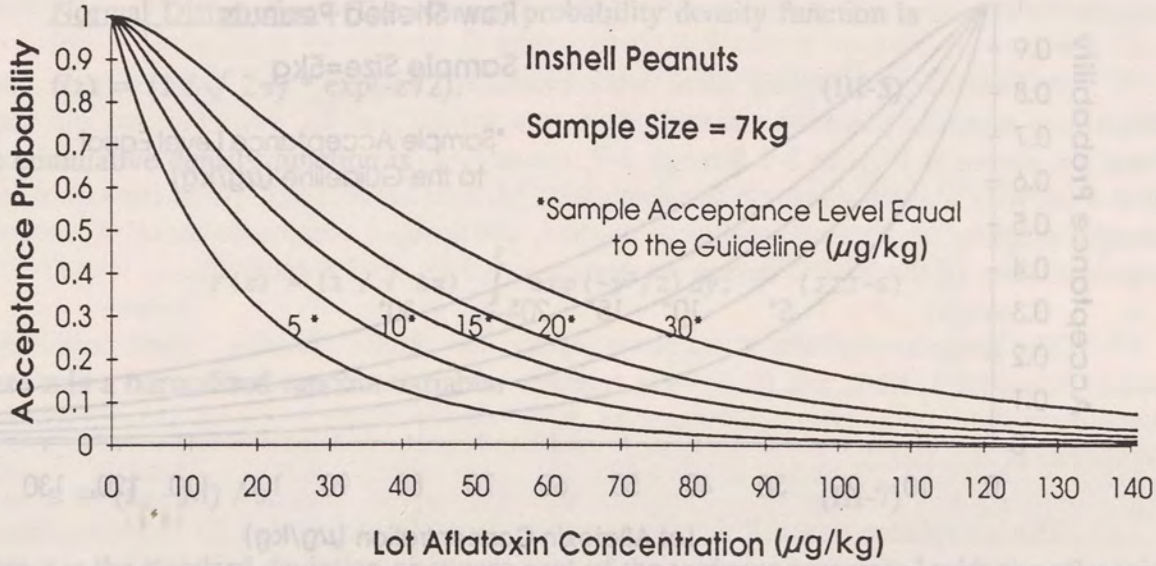


Figure 2-3. Five operating characteristic curves showing the probability of accepting inshell peanut lots when using 7 kg sample pods, hammer mill for comminution, 100 g test portion, TLC analytical methods, and five sample acceptance levels.

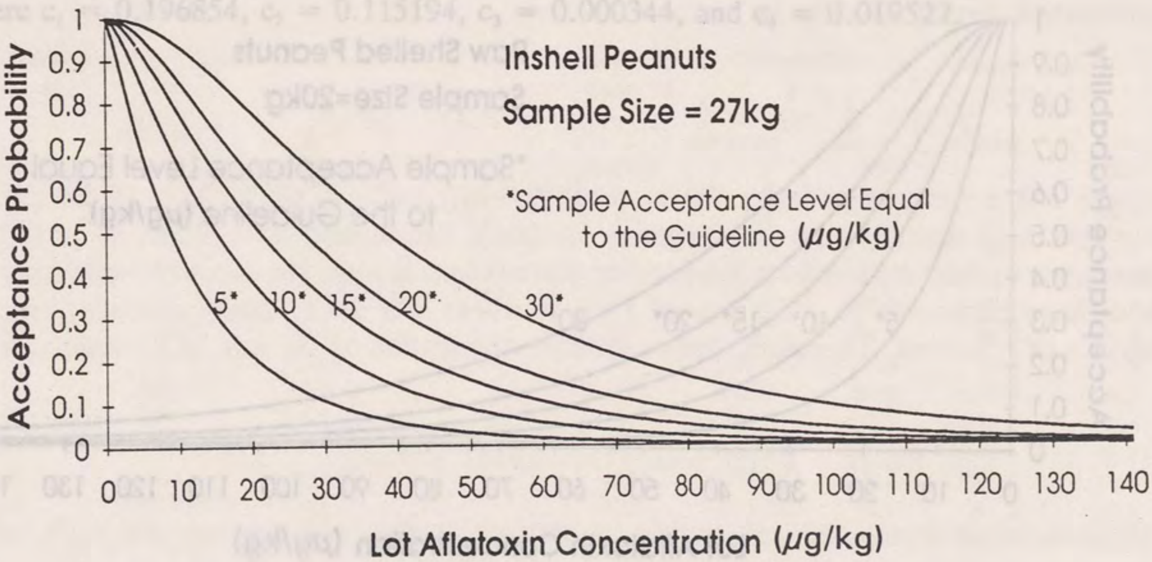


Figure 2-4. Five operating characteristic curves showing the probability of accepting inshell peanut lots when using 27 kg sample pods, hammer mill for comminution, 100 g test portion, TLC analytical methods, and five sample acceptance levels.

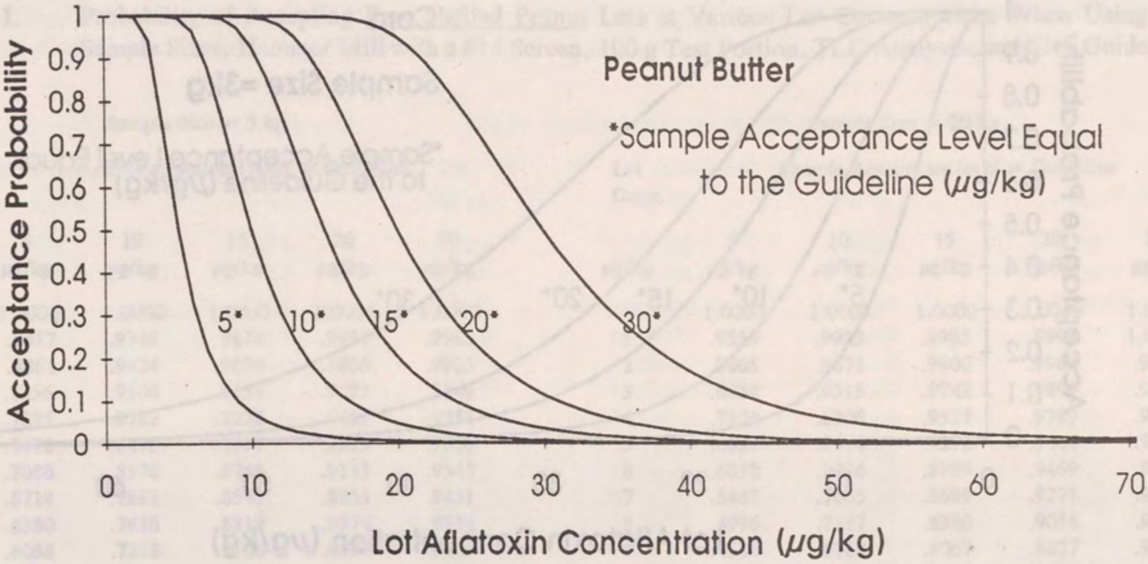


Figure 2-5. Five operating characteristic curves showing the probability of accepting peanut butter lots when using 100 g sample, TLC analytical methods, and five sample acceptance levels. The entire sample is extracted for aflatoxin.

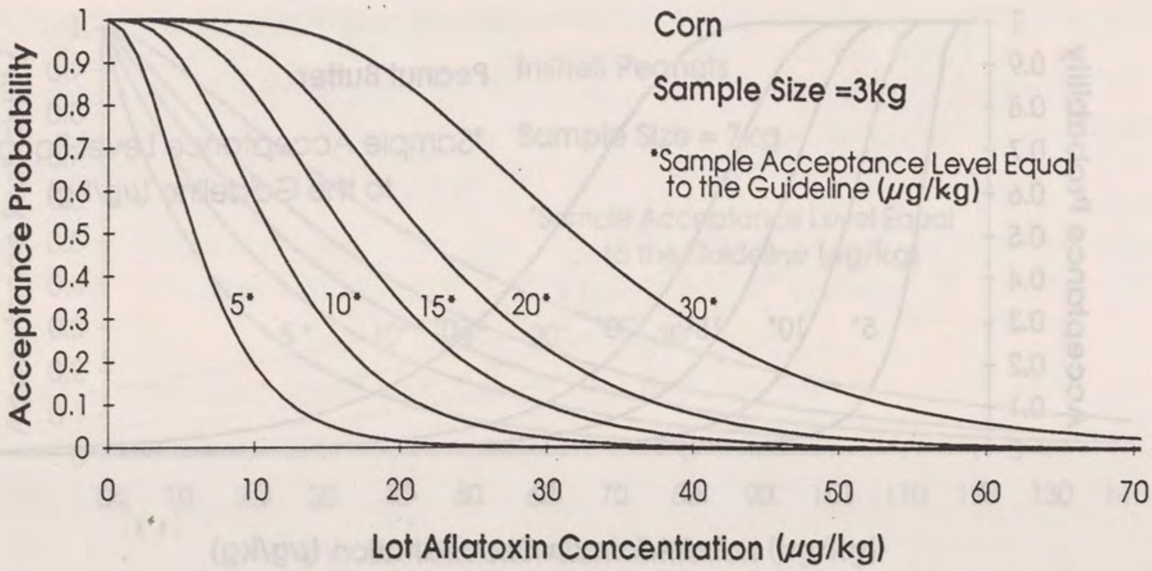


Figure 2-6. Five operating characteristic curves showing the probability of accepting shelled corn lots when using 3 kg sample kernels, hammer mill for comminution, 50 g test portion, TLC analytical methods, and five sample acceptance levels.

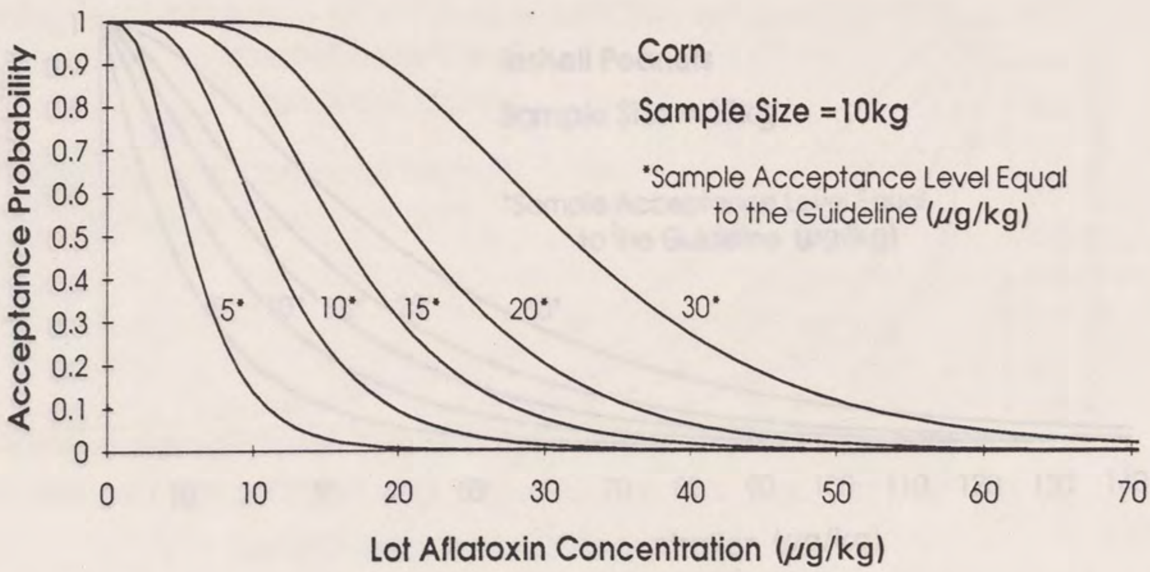


Figure 2-7. Five operating characteristic curves showing the probability of accepting shelled corn lots when using 10 kg sample kernels, hammer mill for comminution, 50 g test portion, TLC analytical methods, and five sample acceptance levels.

Table III-1. Probability of Accepting <u>Raw Shelled Peanut</u> Lots at Various Lot Concentrations When Using Two Sample Sizes, Hammer Mill with a #14 Screen, 100 g Test Portion, TLC Analysis, and Five Guidelines.

Sample Size = 5 kg

Lot Conc.	Sample acceptance level = Guideline				
μg/kg	5 μg/kg	10 μg/kg	15 μg/kg	20 μg/kg	30 μg/kg
0	1.0000	1.0000	1.0000	1.0000	1.0000
1	.9417	.9746	.9874	.9934	.9980
2	.8861	.9424	.9674	.9806	.9925
3	.8356	.9100	.9454	.9653	.9849
4	.7895	.8782	.9226	.9487	.9758
5	.7472	.8473	.8997	.9313	.9656
6	.7080	.8176	.8768	.9135	.9547
7	.6718	.7891	.8541	.8955	.9431
8	.6380	.7616	.8319	.8774	.9312
9	.6066	.7353	.8100	.8594	.9189
10	.5772	.7100	.7887	.8415	.9063
11	.5497	.6858	.7679	.8237	.8935
12	.5239	.6625	.7476	.8062	.8807
13	.4997	.6402	.7278	.7890	.8678
14	.4769	.6187	.7086	.7720	.8548
15	.4554	.5982	.6899	.7553	.8418
16	.4352	.5784	.6717	.7389	.8289
18	.3980	.5412	.6369	.7071	.8032
20	.3647	.5068	.6040	.6765	.7778
25	.2954	.4317	.5298	.6058	.7166
30	.2415	.3694	.4657	.5427	.6591
35	.1990	.3175	.4103	.4866	.6057
40	.1651	.2739	.3622	.4367	.5564
50	.1156	.2060	.2842	.3531	.4694
60	.0825	.1568	.2247	.2869	.3964
70	.0598	.1206	.1790	.2343	.3354
80	.0440	.0936	.1435	.1922	.2844
90	.0327	.0733	.1157	.1584	.2417
100	.0246	.0577	.0938	.1311	.2059
120	.0143	.0366	.0626	.0908	.1504
140	.0085	.0237	.0425	.0638	.1109
160	.0052	.0156	.0293	.0454	.0825
180	.0033	.0105	.0205	.0326	.0618
200	.0021	.0071	.0145	.0237	.0467
220	.0014	.0049	.0103	.0174	.0355
240	.0009	.0034	.0075	.0129	.0272
260	.0006	.0024	.0054	.0096	.0209
300	.0003	.0012	.0030	.0054	.0126
340	.0001	.0007	.0017	.0032	.0078
380	.0001	.0004	.0009	.0019	.0049
420	.0000	.0002	.0006	.0011	.0031

Sample Size = 20 kg

Lot Conc.	Sample Acceptance level = Guideline				
μg/kg	5 μg/kg	10 μg/kg	15 μg/kg	20 μg/kg	30 μg/kg
	1.0000	1.0000	1.0000	1.0000	1.0000
1	.9555	.9923	.9985	.9997	1.0000
2	.8805	.9671	.9900	.9968	.9997
3	.8034	.9315	.9742	.9899	.9984
4	.7306	.8906	.9527	.9789	.9956
5	.6637	.8474	.9272	.9644	.9911
6	.6032	.8036	.8989	.9469	.9849
7	.5487	.7605	.8689	.9271	.9769
8	.4996	.7187	.8380	.9056	.9673
9	.4556	.6784	.8067	.8827	.9562
10	.4161	.6401	.7754	.8590	.9438
11	.3805	.6037	.7445	.8346	.9303
12	.3484	.5692	.7142	.8100	.9158
13	.3195	.5368	.6846	.7853	.9005
14	.2934	.5062	.6559	.7606	.8845
15	.2697	.4774	.6281	.7362	.8679
16	.2483	.4503	.6013	.7121	.8510
18	.2112	.4011	.5507	.6652	.8162
20	.1805	.3577	.5041	.6204	.7809
25	.1240	.2705	.4023	.5191	.6932
30	.0870	.2066	.3250	.4333	.6105
35	.0623	.1592	.2623	.3618	.5351
40	.0453	.1239	.2127	.3025	.4678
50	.0249	.0768	.1419	.2130	.3564
60	.0144	.0490	.0964	.1516	.2718
70	.0086	.0320	.0666	.1092	.2080
80	.0053	.0214	.0468	.0795	.1600
90	.0033	.0145	.0333	.0585	.1237
100	.0021	.0100	.0240	.0435	.0962
120	.0009	.0050	.0129	.0247	.0592
140	.0004	.0026	.0072	.0144	.0372
160	.0002	.0014	.0041	.0087	.0239
180	.0001	.0008	.0024	.0053	.0156
200	.0001	.0005	.0015	.0034	.0103
220	.0000	.0003	.0009	.0022	.0069
240	.0000	.0002	.0006	.0014	.0047
260	.0000	.0001	.0004	.0009	.0033
300	.0000	.0000	.0002	.0004	.0016
340	.0000	.0000	.0001	.0002	.0008
380	.0000	.0000	.0000	.0001	.0004
420	.0000	.0000	.0000	.0001	.0002

Table III-2. Probability of Accepting Inshell Peanut Lots at Various Lot Concentration When Using Two Sample Sizes, Hammer Mill with a #14 screen, 100 g Test Portion, TLC Analysis, and Five Guidelines.

Lot Conc.	Sample Size = 7 kg Pods Sample acceptance level = Guideline				
µg/kg	5 µg/kg	10 µg/kg	15 µg/kg	20 µg/kg	30 µg/kg
0	1.0000	1.0000	1.0000	1.0000	1.0000
1	.9415	.9781	.9906	.9957	.9990
2	.8809	.9453	.9718	.9847	.9951
3	.8255	.9109	.9497	.9702	.9887
4	.7749	.8768	.9261	.9538	.9806
5	.7286	.8435	.9020	.9361	.9711
6	.6860	.8112	.8776	.9177	.9605
7	.6466	.7801	.8534	.8989	.9492
8	.6101	.7503	.8294	.8798	.9372
9	.5763	.7216	.8059	.8606	.9247
10	.5449	.6941	.7827	.8415	.9118
11	.5155	.6677	.7601	.8225	.8986
12	.4881	.6424	.7381	.8036	.8851
13	.4625	.6182	.7166	.7850	.8716
14	.4386	.5950	.6957	.7666	.8579
15	.4161	.5728	.6753	.7485	.8441
16	.3950	.5515	.6555	.7307	.8303
18	.3565	.5115	.6177	.6961	.8028
20	.3224	.4747	.5820	.6628	.7755
25	.2527	.3951	.5018	.5858	.7091
30	.1998	.3302	.4331	.5173	.6464
35	.1592	.2769	.3743	.4567	.5880
40	.1277	.2330	.3240	.4034	.5342
50	.0835	.1664	.2438	.3151	.4398
60	.0556	.1201	.1845	.2468	.3614
70	.0376	.0875	.1405	.1939	.2970
80	.0257	.0642	.1075	.1528	.2441
90	.0178	.0475	.0826	.1208	.2008
100	.0125	.0353	.0638	.0958	.1653
120	.0062	.0199	.0385	.0607	.1125
140	.0032	.0114	.0236	.0389	.0770
160	.0017	.0067	.0146	.0252	.0530
180	.0009	.0040	.0092	.0165	.0366
200	.0005	.0024	.0058	.0109	.0255
220	.0003	.0015	.0037	.0072	.0178
240	.0002	.0009	.0024	.0048	.0125
260	.0001	.0006	.0016	.0033	.0088
280	.0001	.0004	.0010	.0022	.0063
300	.0000	.0002	.0007	.0015	.0045
340	.0000	.0001	.0003	.0007	.0023
380	.0000	.0000	.0001	.0003	.0012
400	.0000	.0000	.0001	.0002	.0009
450	.0000	.0000	.0000	.0001	.0004
500	.0000	.0000	.0000	.0000	.0002

Lot Conc.	Sample Size = 27 kg Pods Sample Acceptance level = Guideline				
µg/kg	5 µg/kg	10 µg/kg	15 µg/kg	20 µg/kg	30 µg/kg
0	1.0000	1.0000	1.0000	1.0000	1.0000
1	.9479	.9873	.9964	.9989	.9999
2	.8770	.9576	.9837	.9934	.9988
3	.8079	.9213	.9647	.9834	.9961
4	.7436	.8823	.9416	.9699	.9915
5	.6843	.8425	.9160	.9537	.9852
6	.6300	.8028	.8888	.9355	.9773
7	.5804	.7640	.8606	.9157	.9679
8	.5351	.7263	.8320	.8948	.9572
9	.4937	.6900	.8034	.8730	.9455
10	.4558	.6552	.7748	.8507	.9327
11	.4212	.6218	.7467	.8281	.9192
12	.3895	.5900	.7190	.8054	.9049
13	.3604	.5598	.6919	.7826	.8901
14	.3338	.5310	.6656	.7599	.8748
15	.3093	.5037	.6399	.7374	.8591
16	.2868	.4777	.6150	.7151	.8431
18	.2471	.4298	.5675	.6717	.8105
20	.2134	.3868	.5233	.6299	.7775
25	.1494	.2978	.4261	.5337	.6953
30	.1058	.2300	.3464	.4501	.6169
35	.0758	.1783	.2817	.3785	.5441
40	.0548	.1388	.2292	.3179	.4780
50	.0293	.0850	.1523	.2239	.3658
60	.0161	.0529	.1018	.1579	.2781
70	.0091	.0333	.0686	.1116	.2107
80	.0052	.0212	.0465	.0791	.1595
90	.0030	.0137	.0317	.0564	.1207
100	.0018	.0089	.0218	.0403	.0914
120	.0007	.0039	.0105	.0209	.0526
140	.0003	.0017	.0052	.0110	.0305
160	.0001	.0008	.0026	.0059	.0178
180	.0000	.0004	.0013	.0032	.0105
200	.0000	.0002	.0007	.0017	.0062
220	.0000	.0001	.0004	.0010	.0037
240	.0000	.0000	.0002	.0005	.0023
260	.0000	.0000	.0001	.0003	.0014
280	.0000	.0000	.0001	.0002	.0008
300	.0000	.0000	.0000	.0001	.0005
340	.0000	.0000	.0000	.0000	.0002
380	.0000	.0000	.0000	.0000	.0001
400	.0000	.0000	.0000	.0000	.0001
450	.0000	.0000	.0000	.0000	.0000
500	.0000	.0000	.0000	.0000	.0000

Table III-3. Probability of Accepting a Peanut Butter Lot at Various Concentrations When Using a 100 g Sample, TLC Analysis, and Five Guidelines.

Lot Conc.	Sample Acceptance Level = Guideline				
	5	10	15	20	30
μg/kg	μg/kg	μg/kg	μg/kg	μg/kg	μg/kg
0	1.0000	1.0000	1.0000	1.0000	1.0000
1	1.0000	1.0000	1.0000	1.0000	1.0000
2	.9993	1.0000	1.0000	1.0000	1.0000
3	.9416	1.0000	1.0000	1.0000	1.0000
4	.7326	.9998	1.0000	1.0000	1.0000
5	.5000	.9950	1.0000	1.0000	1.0000
6	.3276	.9630	.9999	1.0000	1.0000
7	.2157	.8809	.9990	1.0000	1.0000
8	.1451	.7599	.9931	1.0000	1.0000
9	.0999	.6259	.9730	.9997	1.0000
10	.0705	.5000	.9295	.9981	1.0000
11	.0510	.3927	.8617	.9928	1.0000
12	.0377	.3057	.7767	.9790	1.0000
13	.0284	.2379	.6829	.9521	.9999
14	.0219	.1856	.5884	.9102	.9997
15	.0171	.1454	.5000	.8546	.9990
16	.0136	.1145	.4206	.7888	.9973
18	.0090	.0724	.2920	.6424	.9857
20	.0062	.0470	.2016	.5000	.9530
22	.0044	.0314	.1396	.3784	.8920
24	.0033	.0216	.0974	.2819	.8063
25	.0029	.0181	.0817	.2428	.7572
26	.0025	.0153	.0687	.2090	.7057
28	.0020	.0111	.0490	.1548	.6003
30	.0016	.0082	.0355	.1150	.5000
32	.0013	.0062	.0261	.0858	.4099
34	.0010	.0048	.0195	.0643	.3320
36	.0009	.0038	.0148	.0486	.2671
38	.0007	.0030	.0114	.0371	.2141
40	.0006	.0024	.0088	.0285	.1713
42	.0005	.0020	.0070	.0221	.1369
44	.0005	.0016	.0056	.0174	.1095
46	.0004	.0014	.0045	.0137	.0877
48	.0004	.0012	.0037	.0110	.0703
50	.0003	.0010	.0030	.0088	.0566
52	.0003	.0008	.0025	.0072	.0457
54	.0002	.0007	.0021	.0059	.0370
56	.0002	.0006	.0018	.0049	.0301
58	.0002	.0006	.0015	.0041	.0246
60	.0002	.0005	.0013	.0034	.0202
65	.0001	.0004	.0009	.0023	.0127
70	.0001	.0003	.0007	.0016	.0082
75	.0001	.0002	.0005	.0011	.0054
80	.0001	.0002	.0004	.0008	.0037
85	.0001	.0001	.0003	.0006	.0026
90	.0001	.0001	.0002	.0005	.0019

Table III-4. Probability of Accepting a Shelled Corn Lot at Various Lot Concentrations When Using Two Sample Sizes, Hammer Mill with #20 Screen, 50 g Test Portion, TLC Analysis, and Five Guidelines.

	Sample Size = 3 kg				
Lot Conc.	Sample acceptance level = Guideline				
	5	10	15	20	30
µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg
0	1.0000	1.0000	1.0000	1.0000	1.0000
1	.9766	.9987	.9999	1.0000	1.0000
2	.9152	.9924	.9993	.9999	1.0000
3	.8216	.9763	.9971	.9996	1.0000
4	.7106	.9475	.9915	.9987	1.0000
5	.5961	.9052	.9810	.9965	.9999
6	.4884	.8509	.9641	.9921	.9997
7	.3929	.7876	.9400	.9848	.9992
8	.3117	.7186	.9086	.9737	.9982
9	.2447	.6474	.8705	.9583	.9965
10	.1906	.5767	.8267	.9383	.9938
11	.1476	.5087	.7784	.9136	.9898
12	.1138	.4449	.7272	.8844	.9841
13	.0875	.3864	.6742	.8513	.9764
14	.0672	.3335	.6209	.8148	.9666
15	.0515	.2863	.5682	.7755	.9545
16	.0395	.2447	.5171	.7343	.9398
18	.0232	.1769	.4219	.6487	.9033
20	.0137	.1265	.3387	.5632	.8576
22	.0082	.0899	.2684	.4816	.8043
24	.0049	.0635	.2105	.4065	.7454
26	.0029	.0448	.1638	.3396	.6833
28	.0018	.0316	.1267	.2811	.6199
30	.0011	.0223	.0975	.2309	.5572
32	.0007	.0157	.0748	.1886	.4966
34	.0004	.0111	.0573	.1532	.4394
36	.0003	.0078	.0438	.1240	.3861
38	.0002	.0056	.0334	.1000	.3373
40	.0001	.0040	.0255	.0804	.2932
44	.0000	.0020	.0149	.0517	.2186
48	.0000	.0010	.0087	.0331	.1607
52	.0000	.0006	.0051	.0212	.1169
56	.0000	.0003	.0030	.0136	.0844
60	.0000	.0002	.0018	.0087	.0606
70	.0000	.0000	.0005	.0029	.0261
80	.0000	.0000	.0002	.0010	.0112
90	.0000	.0000	.0000	.0003	.0048
100	.0000	.0000	.0000	.0001	.0021
110	.0000	.0000	.0000	.0001	.0009
120	.0000	.0000	.0000	.0000	.0004
130	.0000	.0000	.0000	.0000	.0002

	Sample Size = 10 kg				
Lot Conc.	Sample Acceptance level = Guideline				
	5	10	15	20	30
µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg
	1.0000	1.0000	1.0000	1.0000	1.0000
1	.9939	1.0000	1.0000	1.0000	1.0000
2	.9501	.9989	1.0000	1.0000	1.0000
3	.8520	.9925	.9997	1.0000	1.0000
4	.7185	.9735	.9982	.9999	1.0000
5	.5774	.9369	.9935	.9994	1.0000
6	.4481	.8819	.9829	.9980	1.0000
7	.3394	.8118	.9643	.9945	.9999
8	.2528	.7320	.9362	.9878	.9997
9	.1863	.6482	.8985	.9767	.9992
10	.1363	.5652	.8522	.9602	.9981
11	.0994	.4865	.7991	.9378	.9960
12	.0723	.4143	.7413	.9095	.9927
13	.0526	.3498	.6809	.8755	.9876
14	.0383	.2932	.6198	.8367	.9803
15	.0279	.2444	.5597	.7940	.9705
16	.0204	.2028	.5018	.7483	.9578
18	.0110	.1382	.3963	.6523	.9237
20	.0060	.0934	.3072	.5563	.8778
22	.0033	.0628	.2347	.4658	.8219
24	.0019	.0422	.1775	.3843	.7584
26	.0011	.0284	.1332	.3132	.6905
28	.0006	.0191	.0994	.2527	.6209
30	.0004	.0129	.0740	.2024	.5522
32	.0002	.0087	.0549	.1611	.4863
34	.0001	.0060	.0407	.1276	.4246
36	.0001	.0041	.0302	.1007	.3680
38	.0001	.0028	.0224	.0793	.3169
40	.0000	.0019	.0166	.0623	.2714
44	.0000	.0009	.0092	.0383	.1963
48	.0000	.0005	.0051	.0235	.1400
52	.0000	.0002	.0029	.0145	.0989
56	.0000	.0001	.0016	.0089	.0694
60	.0000	.0001	.0009	.0055	.0484
70	.0000	.0000	.0002	.0017	.0196
80	.0000	.0000	.0001	.0006	.0079
90	.0000	.0000	.0000	.0002	.0032
100	.0000	.0000	.0000	.0001	.0013
110	.0000	.0000	.0000	.0000	.0006
120	.0000	.0000	.0000	.0000	.0002
130	.0000	.0000	.0000	.0000	.0001

Appendix IV: Distribution of Lot Aflatoxin Concentrations

The acceptance probability, P(M), and rejection probability, (1-P(M)), associated with OC curves in Appendix III can be converted to number of lots accepted and rejected if a lot distribution can be established. The lot distribution describes the frequency distribution of aflatoxin concentrations among a collection of lots that will be tested over a given period of time such as a crop year.

The total number of lots having a specific lot aflatoxin concentration M is L*f(M) where L is the total number of lots tested in a given time period and f(M) is the fraction of L lots with concentration M obtained from the lot distribution. For a given sampling plan, the expected number of lots accepted can then be computed with equation IV-1.

$$L_a = \sum_{M=0}^{M_x} L*f(M)*P(M), \qquad (IV\text{-}1)$$

where P(M) is the acceptance probability obtained from the OC curve and M_x is the largest lot concentration in the lot distribution.

The expected number of lots rejected is

$$L_r = \sum_{M=0}^{M_x} L*f(M)*(1-P(M)), \qquad (IV\text{-}2)$$

or

$$L_r = L - L_a . \qquad (IV\text{-}3)$$

The correct decisions made with the sampling plan are good lots with $M \leq M_c$ that are accepted

$$L_{ga} = \sum_{M=0}^{M_c} L*f(M)*P(M) \qquad (IV\text{-}4)$$

and bad lots with $M > M_c$ that are rejected

$$L_{br} = \sum_{M>M_c}^{M_x} L*f(M)*(1-P(M)) \qquad (IV\text{-}5)$$

by the sampling plan.

The correct decision associated with a sampling plan is the sum of equations IV-4 and IV-5.

$$CD = \left(\sum_{M=0}^{M_c} L*f(M)*P(M)\right) + \left(\sum_{M>M_c}^{M_x} L*f(M)*(1-P(M)\right). \qquad (IV\text{-}6)$$

The number of unacceptable lots ($M > M_c$) that are accepted (importer's risk) by the sampling plan is

$$L_{ba} = \sum_{M>M_c}^{M_x} L*f(M)*P(M) \qquad (IV\text{-}7)$$

The number of acceptable lots ($M \leq M_c$) that are rejected (exporter's risk) by the sampling plan is

$$L_{gr} = \sum_{M=0}^{M_c} L*f(M)*(1-P(M)). \qquad (IV\text{-}8)$$

The average aflatoxin concentration among all lots accepted by the sampling plan is

$$A_a = \sum_{M=0}^{M_x} M*f(M)*P(M) \;/\; \sum_{M=0}^{M_x} f(M)*P(M). \qquad (IV\text{-}9)$$

The average aflatoxin concentration among all lots rejected bythe sampling plan is

$$A_r = \sum_{M=0}^{M_x} M*f(M)*(1-P(M)) \;/\; \sum_{M=0}^{M_x} f(M)*(1-P(M)). \qquad (IV\text{-}10)$$

The relationships between the different lot proportions is summarized in the two-way table below.

Table IV-1. Two-way table showing relationship between good and bad lots accepted and rejected.

	Good Lots	Bad Lots	Total
Accepted Lots	L_{ga}	L_{ba}	$L_a = L_{ga} + L_{ba}$
Rejected Lots	L_{gr}	L_{br}	$L_r = L_{gr} + L_{br}$
Total	L_g	L_b	$L = L_a + L_r$

$L_g = L_{ga} + L_{gr}$; $L_b = L_{ba} + L_{br}$; $L = L_g + L_b$

Table IV-2 shown below is an example of a distribution of lot aflatoxin concentrations for raw shelled peanuts. The lot distribution was constructed from aflatoxin test results on about 20,000 lots of raw shelled peanuts marketed from peanuts produced during the 1980 crop season in the U.S. The 1980 crop was purposely chosen because of the high degree of contamination that resulted from extreme drought conditions experienced that crop year. The shelled lots averaged about 14 μg/kg which was three to four times higher than a normal crop produced in the U.S.

Table IV-2. Cumulative Distribution of Lot Aflatoxin Concentrations for Raw Shelled Peanuts.

Lot Concentration (μg/kg)	Cumulative Distribution (%)
0	30.8
5	56.9
10	71.3
15	80.0
20	85.3
25	88.8
30	91.2
40	94.3
50	95.9
60	96.8
70	97.5
80	98.2
100	98.6
150	98.8
200	99.1
300	99.2

All lots averaged 14 μg/kg total aflatoxin

Using equations IV-1 through IV-10, the lot distribution in Table IV-2 and the accept probabilities in Appendix III, the number of raw shelled peanut lots accepted and rejected at five different guidelines and two sample sizes are shown in Tables IV-3 and IV-4.

Table IV-3. Comparison of Five Sampling Plans for Raw Shelled Peanuts Using Five Different Sample Accept Levels and a 5 kg Sample, Hammer mill, 100 g Test Portion, and TLC Analysis.

Evaluation Factors	Sample accept level=Guideline (μg/kg)				
(Avg. per 100 lots tested)	5	10	15	20	30
Total Lots Tested	100.00	100.00	100.00	100.00	100.00
Good Lots Tested	56.89	71.31	79.98	85.34	91.23
Bad Lots Tested	43.11	28.69	20.02	14.66	8.77
Avg μg/kg among all Lots Tested	14.38	14.38	14.38	14.38	14.38
Lots Accepted	71.83	78.74	82.95	85.89	89.78
Lots Rejected	28.17	21.26	17.05	14.11	10.22
Good Lots Accepted	53.17	65.92	74.06	79.47	86.08
Bad Lots Rejected	24.45	15.87	11.12	8.23	5.07
Correct Decisions	77.62	81.79	85.18	87.70	91.15
Good Lots Rejected	3.72	5.39	5.93	5.87	5.15
Bad Lots Accepted	18.66	12.82	8.90	6.42	3.70
Avg μg/kg Among Accepted lots	4.31	5.21	5.81	6.26	6.94
Avg μg/kg Among Rejected Lots	40.04	48.33	56.07	63.77	79.66

Table IV-4. Comparison of Five Sampling Plans for Raw Shelled Peanuts Using Five Different Sample Accept Levels, a 20 kg Sample, Hammer Mill, 100 g Test Portion, and TLC Analysis.

Evaluation Factors	Sample accept level=Guideline (μg/kg)				
(Avg. per 100 lots tested)	5	10	15	20	30
Total Lots Tested	100.00	100.00	100.00	100.00	100.00
Good Lots Tested	56.89	71.31	79.98	85.34	91.23
Bad Lots Tested	43.11	28.69	20.02	14.66	8.77
Avg μg/kg among all Lots Tested	14.38	14.38	14.38	14.38	14.38
Lots Accepted	64.97	75.05	81.08	85.10	90.05
Lots Rejected	35.03	24.93	18.92	14.90	9.95
Good Lots Accepted	52.52	65.78	74.38	80.14	87.10
Bad Lots Rejected	30.66	19.40	13.32	9.70	5.82
Correct Decisions	83.18	85.18	87.70	89.84	92.92
Good Lots Rejected	4.37	5.53	5.60	5.20	4.13
Bad Lots Accepted	12.45	9.29	6.70	4.96	2.95
Avg μg/kg Among Accepted Lots	3.02	4.17	4.98	5.60	6.51
Avg μg/kg Among Rejected Lots	35.44	45.10	54.67	64.52	85.58

PRACTICAL ISSUES ASSOCIATED WITH THE APPLICATION OF SAMPLING AND SAMPLE PREPARATION PROCEDURES

Introduction

A strategy for the regulatory control of aflatoxin in groundnut and corn commodities requires the collection and analysis of appropriate samples which are representative of carefully defined, readily identifiable lots.

The sequence of events required to satisfy these objectives may be expressed as follows:

(a) the identification of sampling points (i.e. those locations where the samples will be collected);

(b) the identification of lots which are representative of the consignment under study;

(c) the collection of a sample(s) which is representative of the lot(s);

(d) the subdivision of the sample(s) to afford a test portion which is representative of the original sample and, consequently, representative of the original lots/consignment.

A number of issues will effect the methods utilized in the collection of representative samples. Briefly, these will include the nature and location of the sampling points, the size and nature (i.e. in bulk, in sacks or other containers) of the consignment, the ease of sample collection and sample preparation and, importantly, the resources available at that particular import/export location.

Table 1. Commodities and Typical Trade Lot Sizes

Commodity	Typical Consignment Sizes	Typical Lot Sizes
Groundnuts In-shell and kernels	Large: 5,000 - 20,000 tonne Medium: 25 - 5,000 tonne Small: Less than 25 tonne	Bulk (Raw): 500 tonne Sacks (Raw Shelled): 25 tonne Sacks (Raw Unshelled): 35 tonne Equivalent to consignment: < 25 tonne
Processed Groundnuts eg. Peanut Butter	50 tonne maximum	20 tonne
Corn Raw Shelled	Large: 25,000-100,000 tonne Medium: 500-25,000 tonne Small: Less than 500 tonne	Large: 1500 tonne Medium: 500 tonne Small: Equivalent to consignment; < 500 tonne

RECOMMENDATIONS

Sample Collection

Wherever possible, it is most appropriate (and convenient) to collect the sample when the selected lots are mobile. The estimation of the true mean aflatoxin content of a stack of sacks, for example, will be facilitated when representative samples are collected during the construction or dismantling of the stack. Similarly, sampling of large shipments of groundnuts and corn can best be performed during the loading/unloading operation. In this situation, it is recommended that representative samples be collected from representative lots (as illustrated in Table 1) from, for example, ships holds, conveyer belts, dockside weighing towers, trucks or barges.

For unprocessed material, each sample should be composed of at least one hundred incremental samples, taken in a representative manner (using a systematic random sampling method), from locations throughout the lot.

For processed material, a representative sample may be composed of a large number of combined retail packs (e.g. jars of peanut butter) which have been collected, by systematic random sampling, from the production line; the lot, in this case, may be composed of the output of a single shift or of a consignment for a specific customer.

Ideally, especially when dealing with large consignments, automatic sampling equipment should be utilized. If, however, such equipment is either unavailable or inappropriate, sampling probes/triers of a design appropriate to the commodity should be used.

For large lots, the initial collection of very large portions of material, of at least 30-40 kg, may be unavoidable. In such a situation, it is imperative that the final sample is produced in as random a manner as possible by utilising riffle dividers.

Sample preparation

As already described, it is imperative that the preparation of the final test portion retains the representative nature of the original sample. This is achieved by withdrawing a representative subsample from the collected sample which, in turn, is used to produce a representative test portion.

A flow chart describing some of the methods available for the sample preparation of groundnuts is shown in Figure 1. The method of choice will be determined by the type of sample preparation equipment available.

Ideally, the complete sample should be simultaneously comminuted and subdivided using a subsampling mill (Method 3, Figure 1). If this approach is untenable, the complete sample should be comminuted and subdivided using a static rotary riffle divider (Methods 4a and 5a). (The technique of coneing and quartering, for the production of subsamples, should only be utilised as a last resort).

If a riffle divider is not available, an alternative strategy involves the thorough mixing of the comminuted sample (as a dry powder, aqueous slurry or paste) followed by the withdrawal of appropriate subsamples (Methods 4b, 1 and 6 respectively).

Methods 2 and 5 are time-consuming but suited to those laboratories with limited sample preparation equipment.

In Method 2, for example, the total sample is comminuted with water, using a suitable blender, as a series of separate operations. Equivalent portions of the resultant slurries are then combined to produce a single slurry which, in turn, affords the test portion(s).

In Method 5, the total sample is comminuted dry, as a succession of small portions, using a small mill/coffee grinder. The comminuted portions are then combined to produce a single ground sample which, in turn, will afford a representative subsample by mixing or riffle division.

Method 2 is probably best suited to those laboratories with very limited sample preparation equipment (i.e. an approximately 4 litre capacity blender, only) but with adequate labour resources. However, a suitable method for the disposal of significant quantities of aqueous slurry will have to be established by laboratories using this approach.

For groundnuts, it is recommended that the subsamples are converted into homogenous, stable slurries by blending with an appropriate volume of water. The ratio of subsample:water is typically 1:1.25, 1:2 and 1:1.5 for corn, groundnut kernels and peanut butter, respectively. However, the ratio which produces a stable slurry will vary, for example, with the commodity variety and country of origin. Replicate aliquots (of at least 100 g) of slurry are withdrawn, the selected extraction solvent added and the chosen analytical procedure implemented.

Good Laboratory Practice (including participation in check sample programmes) in combination with accepted quality assurance procedures should be implemented throughout the execution of sampling/sampling methods.

Recommendations for Further Action

Regional training courses should be implemented in sampling, sample preparation and analytical methodology to maximize precision and accuracy.

Regional reference laboratories should be established to facilitate the optimisation of sampling, sample preparation and analytical efficiency, by the organisation of collaborative studies, workshops etc.

Further studies should be performed on:

(a) the relative efficiencies of automatic and manual sampling methods;

(b) the comparison of the precision of sample preparation methods;

(c) the distribution of aflatoxin in finished corn and groundnut commodities involved in international trade;

(d) the efficiency of the developed sampling and sample preparation methods as applied to other mycotoxins in corn and corn products.

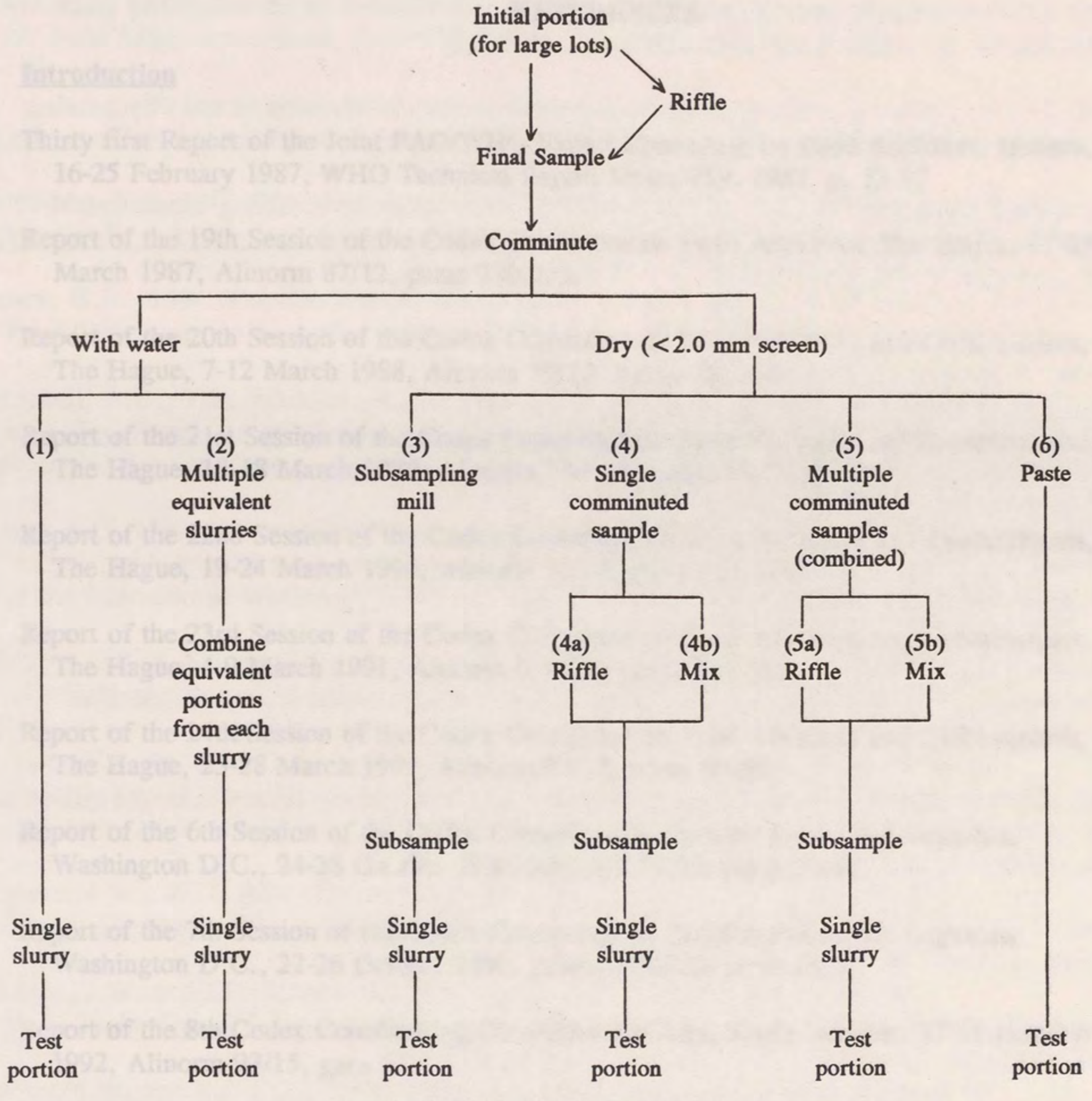


Figure 1. SAMPLE PREPARATION OF GROUNDNUTS

(c) the distribution of aflatoxin in finished corn and groundnut commodities involved in international trade.

(d) the efficiency of the developed sampling and sample preparation methods as applied to other mycotoxins in corn and corn products.

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ANNEX I

FAO TECHNICAL CONSULTATION ON
SAMPLING PLANS FOR AFLATOXIN ANALYSIS IN PEANUTS AND CORN

Programme

Monday 3 May

09:00 - 09:30	Opening Session
	Welcome addresses
	- by Mr. John R. Lupien, Director, Food Policy and Nutrition Division, FAO
	- by Dr. Marina Miraglia, Senior Researcher, Food Laboratory, Istituto Superiore di Sanità
	Explanation of administrative arrangements
09:30 - 10:00	Coffee break
10:00 - 10:15	Organizational arrangements
	- Election of Chairman and Co-chairman
	- Working groups
10:15 - 10:30	Summary of Codex work related to the subject of the Consultation
10:30 - 12:30	General discussion on the objectives of the Consultation
12:30 - 14:00	Lunch
14:00 - 17:00	Presentation and discussion of the following papers:
	- Sampling for Aflatoxin - Literature review (Janet Springer)
	- Sampling of Foods and Feeds for Aflatoxin (Ray Coker)
	- Sampling of Grains for Aflatoxin (Jeremy Wu)

Tuesday 4 May

09:00 - 12:00	Presentation and discussion of the following papers:
	- Theoretical Models that Describe the Distribution of Sample Aflatoxin Test Results when Sampling Bulk Lot of Peanuts and Corn for Aflatoxin (Tom Whitaker)
	- Guidelines for the Development of Sampling Plans for Aflatoxin (Mike Read)

- Recommended Draft Sampling Plans for Aflatoxin Analysis to be Applied in Export and Import Control of Major Aflatoxin Susceptible Peanut and Corn commodities (Peter Defize)
- Review of Sampling Methodologies in Latin America and the Caribbean (Silvia Resnik)
- Basic Information on Sampling Peanuts and Sampling Plan for Analysis of Aflatoxins in Grains (Homero Fonseca)
- General Figures of Aflatoxin Contaminated Imported from 60 Countries in the Wold into Japan since 1972 (Kageaki Aibara)
- Some Data on Imports and Exports of Important Foodstuffs and Feed in stuffs (Jean-Marc Fremy)
- Occurrence of Aflatoxin in Groundnut Products and Corn in Senegal (Amadou Kane)

Oral Reports (Bill Tennant, Julie Adams, Jerry Cotter)

12:30 - 14:00	Lunch
14:00 - 14:30	Constitution of Working Groups
14:30 - 17:00	Working Groups in Session

<u>Wednesday 5 May</u>

09:00 - 12:30	Working Groups in Session (continued
12:00 - 14:00	Lunch
14:00 - 17:00	Preparation of Reports by Working Groups

<u>Thursday 6 May</u>

09:00 - 12:30	Presentation and discussion of Reports of the Working Groups
12:30 - 14:00	Lunch
15:00 - 16:00	Adoption of the Report of the Consultation
16:00 - 16:30	Closing of the Consultation

ANNEX II

FAO TECHNICAL CONSULTATION
ON SAMPLING PLANS
FOR AFLATOXIN ANALYSIS IN PEANUTS AND CORN

Rome, 3-6 May 1993

List of Participants

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ANNEX III

TASKS AND COMPOSITION OF THE WORKING GROUPS

WG 1:

1. To specify the type of commodities and their characteristics as they move through international trade (i.e. intermediate products such as corn, raw peanuts, corn meal, peanut cake, other processed products, etc.).

2. To specify possible levels of contamination of the above products as they move in international trade.

3. To identify potential effect of transportation/shipment conditions on aflatoxin level in above commodities during transit.

4. To prepare a report on the results of the Group's deliberations and recommendations.

Dr. D. Park (Chairman), Dr. J-M. Fremy (Co-chairman), Mrs. J. Adams, Dr. K. Aibara, Dr. J. Cotter, Dr. A. Kane and Dr. M. Miraglia.

WG 2:

1. To examine the scientific basis of sampling plans and procedures for aflatoxin in peanut and corn products, and to establish mathematical model(s) of the distribution of aflatoxin in the commodities on which the sampling plan is to be based.

2. To establish guidelines for he development of sampling plans, i.e. the required operating characteristics of the sampling plan (specified acceptance and rejection probabilities for commodity lots containing a certain level of aflatoxin).

3. To include recommendations for sampling plans to be used in export and import control, and predict percentage of commodity rejected when proposed sampling plans are applied.

4. To prepare a report on the results of the Group's deliberations and recommendations.

Dr. T. Whitaker (Chairman), Dr. M. Read (Co-chairman), Mr. P. Defize, Dr. A. Rainosek, Dr. J. Sastry, Ms. J. Springer and Dr. J. Wu.

WG 3:

1. To evaluate the effect and environment of sample collection and sample preparation procedures on the overall results of the analysis; specification should include representative samples, representative sub-samples and appropriate preparation of homogeneous composites.

2. To advise on the impact of different lot sizes or sampling methodology.

3. To advise on sampling and sample preparation equipment including automated devices to be used in different circumstances.

4. To prepare a report on the results of the Group's deliberations and recommendations.

Dr. R. Coker (Chairman), Dr. H. Fonseca (Co-chairman), Dr. G. Gomez, Dr. S. Resnik and Dr. B. Tennant.

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Availability: October 1993

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This document is the report of the FAO Technical Consultation on Sampling Plans for Aflatoxin Analysis in Peanuts and Corn, held in Rome, 3-6 May 1993. The consultation was convened at the recommendation of the Codex Committee on Food Additives and Contaminants to provide member countries with guidance on sampling procedures of aflatoxin-contaminated peanut and corn commodities. The report emphasizes the importance of aflatoxin contamination and its effects on international trade. It also recognizes the crucial importance of sample size on the acceptance or rejection of an aflatoxin-contaminated lot.

A sampling plan for peanuts and corn is developed and evaluated, based on two sample sizes, thin-layer chromatography (as analytical method) and five guideline levels of 5, 10, 15, 20 and 30 μg/kg, which reflect the levels most commonly found in national food legislations. Practical issues associated with the application of sampling and sample preparation procedures are also discussed.

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