

Aflatoxins in Your Food - and their Effect on Your Health

Aflatoxin Introduction

Aflatoxin is a potent human carcinogen. It is a naturally occurring toxic metabolite produced by certain fungi (*Aspergillus flavus*), a mold found on food products such as corn and peanuts, peanut butter. It acts as a potent liver carcinogen in rodents (and, presumably, humans). They are probably the best known and most intensively researched mycotoxins in the world. Aflatoxins have been associated with various diseases, such as [aflatoxicosis](#), in livestock, domestic animals and humans throughout the world.

The occurrence of aflatoxins is influenced by the weather, (temperature, and humidity - warm & wet is worst!); so the extent of contamination will vary with geographic location, agricultural and agronomic practices, and the susceptibility of the peanuts (etc.) to fungus before they are harvested, and during storage, and/or processing periods. Aflatoxins have received greater attention than any other mycotoxins because they clearly have a potent [carcinogenic effect](#) in laboratory rats and their acute poisonous effects in humans.

In the 1960 more than 100,000 young turkeys on poultry farms in England died in the course of a few months from an apparently new disease that was termed "**Turkey X disease**". It was soon found that the difficulty was not limited to turkeys. Ducklings and young pheasants were also affected and heavy mortality was experienced.

A careful survey of the early outbreaks showed that they were all associated with feeds, namely Brazilian peanut meal. An intensive investigation of the suspect peanut meal was undertaken and it was quickly found that this peanut meal was highly toxic to poultry and ducklings with symptoms typical of Turkey X disease.

Speculations made during 1960 regarding the nature of the toxin suggested that it might be of fungal origin. In fact, the toxin-producing fungus was identified as [Aspergillus flavus](#) (1961) and the toxin was given the name **Aflatoxin** by virtue of its origin (A.flavis--> Afla).

This discovery has led to a growing awareness of the potential hazards of these substances as contaminants of food and feed causing illness and even death in humans and other mammals.

US FDA Regulations

Even though absolute safety can never be achieved , many countries have attempted to limit exposure to aflatoxins by imposing [regulatory limits](#) on commodities intended for use as food and feed .

U.S. FDA Action Levels for Aflatoxins	
Commodity	Level (in ng/g)
All products, except milk, designated for humans	20
Milk	0.5
Corn for immature animals and dairy cattle	20
Corn for breeding beef cattle, swine and mature poultry	100
Corn for finishing swine	200
Corn for finishing beef cattle	300
Cottonseed meal (as a feed ingredient)	300
All feedstuff other than corn	20

More importantly, research supported by our institute has provided clear evidence that aflatoxin ingestion through contaminated foodstuffs is one of the major etiological factors in human hepatocellular carcinoma (HCC) in China and sub-Saharan Africa. In certain regions of these countries, at least 250,000 deaths from HCC occur annually.

Scientific Background

Aflatoxin is a naturally occurring mycotoxin produced by two types of mold: *Aspergillus flavus* and *Aspergillus parasiticus*. *Aspergillus flavus* is common and widespread in nature and is most often found when certain grains are grown under stressful conditions such as drought. The mold occurs in soil, decaying vegetation, hay, and grains undergoing microbiological deterioration and invades all types of organic substrates whenever and wherever the conditions are favorable for its growth. Favorable conditions include high moisture content and high temperature. At least 13 different types of aflatoxin are produced in nature with aflatoxin B1 considered as the most toxic. While the presence of *Aspergillus flavus* does not always indicate harmful levels of aflatoxin it does mean that the potential for aflatoxin production is present.

Evidence that aflatoxin ingestion through contaminated foodstuffs is one of the major etiological factors in human hepatocellular carcinoma (HCC) in China and sub-Saharan Africa. In certain regions of these countries, at least 250,000 deaths from HCC occur annually. A natural progression from the etiological studies discussed is the introduction of preventive strategies to reduce the risk of liver cancer by exposure to aflatoxin. Investigators funded by NIEHS (Groopman, P01ES06052, P30ES03819) initiated a Phase II Oltipraz chemoprevention trial in China to define a dose and schedule of Oltipraz for reducing levels of validated aflatoxin biomarkers and to characterize dose-limiting toxicities. Oltipraz reduces the biologically effective dose of aflatoxin by inhibiting its activation by P450s and by inducing detoxification pathways. Preliminary results show a small but significant reduction of aflatoxin-albumin adducts at the highest dose level. Another intervention using chlorophyllin is also planned. Chlorophyllin is a water soluble derivative of chlorophyll that forms molecular complexes with aflatoxin and thereby reduces its bioavailability. Modulation of blood and urine aflatoxin biomarkers will be studied.

Where does it occur?

In Raw Agricultural Products :

Aflatoxins often occur in crops in the field prior to harvest. After harvest contamination can occur if crop drying is delayed and during storage of the crop if the [crop](#) is kept too moist. Insect or rodent infestations facilitate mold invasion of some stored commodities.

Aflatoxins are detected occasionally in milk, cheese, corn, peanuts, cottonseed, nuts, almonds, figs, spices, and a variety of other foods and feeds. Milk, eggs, and meat products are sometimes contaminated because of the animal consumption of aflatoxin-contaminated feed. However, **the commodities with the highest risk of aflatoxin contamination are corn, peanuts, and cottonseed**

In Processed Foods :

Corn is probably the commodity of greatest worldwide concern, because it is grown in climates that are likely to have perennial contamination with aflatoxins and corn is the staple food of many countries. However, procedures used in the processing of corn help to reduce contamination of the resulting food product. This is because although aflatoxins are stable to moderately stable in most food processes, they are unstable in processes such as those used in making tortillas that employ alkaline conditions or oxidizing steps. Aflatoxin-contaminated corn and cottonseed meal in dairy rations have resulted in aflatoxin M1 contaminated milk and milk products, including non-fat dry milk, cheese, and yogurt.

Aflatoxins and Human Health

Humans are exposed to aflatoxins by consuming foods contaminated with products of fungal growth . Such exposure is difficult to avoid because fungal growth in foods is not easy to prevent . Even though heavily contaminated food supplies are not permitted in the market place in developed countries, concern still remains for the possible adverse effects resulting from long-term exposure to low levels of aflatoxins in the food supply .

Evidence of acute aflatoxicosis in humans has been reported from many parts of the world , namely the Third World Countries, like Taiwan, Ouganda, India, and many others . The syndrome is characterized by vomiting, abdominal pain, pulmonary edema, convulsions, coma, and death with cerebral edema and fatty involvement of the liver , kidneys , and heart.

Conditions increasing the likelihood of acute aflatoxicosis in humans include limited availability of food, environmental conditions that favor fungal development in crops and commodities, and lack of regulatory systems for aflatoxin monitoring and control.

Because aflatoxins, especially aflatoxin B1, are potent carcinogens in some animals, there is interest in the effects of long-term exposure to low levels of these important mycotoxins on humans . **In 1988, the IARC placed aflatoxin B1 on the list of human carcinogens.** This is supported by a number of epidemiological studies done in Asia and Africa that have demonstrated a **positive association between dietary aflatoxins and Liver Cell Cancer (LCC)** . Additionally , the expression of aflatoxin-related diseases in humans may be influenced by factors such as age, sex, nutritional status, and/or concurrent exposure to other causative agents such as viral hepatitis (HBV) or parasite infestation.

Recommendations

So what can you do to avoid aflatoxins?

1. Don't keep grains and nuts (particularly, corn, peanuts and cottonseed) for long periods (more than a few months) before eating them.
2. Store them in a dry (low humidity) cool environment - a freezer is excellent!
3. Buy from known, reputable sources - where you know it is fresh and has been handled properly.

Sampling, sample handling and preparation in grains and cereals

by Traisat Hongsuwong,

This paper is presented in the Training Course on Mycotoxin Prevention and Control in Field of Sampling, Sample Handling and Preparation in Grains/Cereals. It is a collection from many ideas of selected literatures and is aimed to share some knowledge to improve your work.

SAMPLING

By the result of some sort of a test of a portion of the material with its quality criterion to judge whether each article is non-defective or defective, or with an acceptability criterion to judge whether a lot is acceptable or not, the portion of the material in a sample used to judge the whole material, improper sampling will lead to inappropriate grading even with correct testing.

In general, sampling is conducted in such a way that the sample represents the population, but in the same case a sample is taken from an especially good or bad section. Without understanding the sampling method of the test sample, one can not evaluate correctly about the quality of the material being inspected.

Uniform sampling

In this method, a sample is taken so as to represent the average of the whole population. Samples are taken in a small quantity from each section of the population. In this case, the total amount of the sampling method of the test sample, one cannot of it is used for testing. Sampling in this case has to be evenly reduced. The reduction procedure is called dividing, which is performed by quartering, dividing or the use of divider.

Selective sampling

When the products are disposed according to the lowest quality, sampling is made from sections with particularly poor quality. For example, to judge baking condition of bread through determination of moisture a sample is taken from the central part of the bread.

Random sampling

This method is applied in cases of the several samples are taken from a product to be uniform and when they do not have the same quality. In this sampling, individual samples, an amount of sampling, and in some case, sampling period are not fixed before sampling. Strictly random sampling is rather difficult, and so the subjects of sampling are chosen by the use of dice, lottery, or random table. The random sampling can prevent unfair action of inspect.

SAMPLING, SAMPLE HANDLING IN GRAIN AND CEREALS (ISO)

Correct sampling is an operation that requires most careful attention. Emphasis cannot therefore be too strongly laid on the necessity of obtaining a properly representative sample of grain. Careless or inaccurate sampling could lead to misunderstanding and unwarranted financial adjustments.

Samples shall be fully representative of the lots from which they are taken. Therefore, as the composition of the lot is seldom uniform, a sufficient number of increments shall be taken and carefully mixed, thus giving a bulk sample from which are obtained, by successive divisions, the laboratory samples.

Apparatus

Apparatus is required as follows, and many types and variations of apparatus are available.

Method of taking samples from carried in bulk.

When sampling takes place while the product is in motion, increments shall be taken at time intervals dependent on the rate of flow.

When bulk grain is sampled in the hold during discharge, increments shall be taken from as many places as possible, excluding the run, and at intervals determined by the rate of discharge.

Method of taking samples from cereals carried in bags.

The increments shall be taken from different parts of bag example top, middle and bottom, by means of a sack-type spear from the number of bags specified in the table below.

If sampling takes place from weight hoppers, increments shall be taken by means of cylindrical samplers, shovels, or mechanical samplers in accordance with the practice of the port.

The procedure for silos or warehouses is necessarily dependent on local conditions.

If sampling takes place from laden wagons or lorries, the increments shall be taken throughout the whole depth of the layer, by means of a cylindrical sampler and at the following points.

Table 1. Number of bags to be sampled.

in consignment	Number of bags to be sampled.
Up to 10	Each bag
10 to 100	10, taken at random.
More than 100	Square root (approximately) of total number, taken according to a suitable sampling scheme.

Table 2. Sampling scheme for consignments of more than 100 bags.

N = Number of bags in consignment; n = Number of bags in group.

N	n	N	n	N	n
101 - 121	11	1,601-1,681	41	4,901-5,041	71
122 - 144	12	1,682-1,764	42	5,024-5,184	72
145 - 169	13	1,765-1,849	43	5,185-5,329	73
170 - 196	14	1,850-1,936	44	5,330-5,476	74
197 - 225	15	1,937-2,025	45	5,477-5,625	75
226 - 256	16	2,026-2,116	46	5,626-5,766	76
257 - 289	17	2,117-2,209	47	5,777-5,929	77
290 - 324	18	2,210-2,304	48	5,930-6,084	78
325 - 361	19	2,305-2,401	49	6,085-6,241	79
326 - 400	20	2,402-2,500	50	6,242-6,400	80
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
1,226-1,296	36	4,226-4,356	66	9,026-9,216	96
1,297-1,369	37	4,357-4,489	67	9,217-9,409	97
1,370-1,444	38	4,490-4,624	68	9,410-9,604	98
1,445-1,521	39	4,625-4,761	69	9,605-9,801	99
1,522-1,600	40	4,762-4,900	70	9,802-10,000	100

For consignments larger than 10,000 bags, n equals the square root Of N. rounded upwards.

Samples

- Laboratory Samples

The bulk sample shall be divided to obtain the required number of laboratory samples by use of the apparatus mentioned as follow. The number of laboratory samples to be taken for analysis and arbitration shall be specified in the contract or otherwise agreed between the buyer and the seller.

- Size of samples

Samples of the sizes given in Table 3 are usually suitable for all grains. Larger or smaller laboratory samples may be required in some cases, according to the tests to be carried out.

Table 3. Sizes of samples

LOT	Increment	Bulk sample	Laboratory sample
Up to	1 Kg.	100 Kg.	5 Kg.
500 tons.	(max.)		

MAIZE - SAMPLING. (OCS)

The Office of Commodity Standards, Department of Foreign Trade, Ministry of Commerce empowered by the Export Standards Act B.E. 2503 (1960) amended by the Export Standards Act (No.2), B.E. 2522 (1979), is responsible for the control of products to be exported as follows:

- To specify the standardized products
- To prepare export commodity standards
- To control exporters, surveyors and inspectors
- To provide inspection service
- To issue certificates for commodity standards on quality, volume, weight, and origin of products
- To prevent and suppress deception of commodities to be exported, and
- To collect statistics concerning manufacture, market needs, price level and value of exports and publicize such information to concerned persons so that they can use the information for production and export targets.

At the time being, twelve products have already been standardized. According to the 6th National Economic and Social Development Plan (1987/1991), seven additional products will also be standardized.

Standardized commodities:

- Jute and Kenaf
- Maize (corn)
- Castor seed
- Kapok
- Salt
- Teak Conversions
- Sorghums
- Thai silverware
- Tapioca products
- Thai silk and silk products

- Green bean
- Fish meal

(6) "Greatly spoiled seeds" mean seeds which, the whole part, are rotten, mouldy, containing no starch, sprout.

(7) "Seeds destroyed by weevils" mean seeds which are bitten or bore by weevils or other insects.

(8) "Broken seeds" mean sound seeds which are broken into pieces and each piece less than a half of natural sound seed, but not immature seeds, spoiled seeds, or weevilled seeds.

(9) "Foreign material" means all matter other than maize.

Clause 2. The standards of maize shall be classified into two grades as follows:

(1) Grade 1 maize.

(2) Grade 2 maize.

Clause 3. The standard specifications for each grade of maize shall be as follows:

A. Grade 1 maize shall be sound seeds, but the following tolerances are allowed:

(1) Seeds of other colours, not exceeding 1.0 per cent by weight.

(2) Partially spoiled seeds together with greatly spoiled seeds, not exceeding 4.0 per cent by weight but greatly spoiled seeds, not exceeding 1.5 per cent by weight.

(3) Seeds destroyed by weevils, not exceeding 2.0 per cent by weight.

(4) Broken seeds together with immature seeds, not exceeding 2.0 per cent by weight.

(5) Foreign material, not exceeding 1.5 per cent by weight, but have no oil seeds or poisonous matter.

(6) Moisture content on the average, not exceeding 14.5 per cent by weight and there shall be no part having moisture content in excess of 15.0 per cent by weight.

B. Grade 2 maize shall be sound seeds, but the following tolerances are allowed:

Standards for Maize

Clause 1. Definitions

(1) "Maize" means seeds of *Zea mays* which are split from the cob.

(2) "Sound seeds" mean seeds which are not immature, spoiled, destroyed by weevils, broken, or seeds of other colours.

(3) "Seeds of other colours" mean seeds which are not of the colours as agreed upon.

(4) "Immature seeds" mean seeds which are not fully developed.

(5) "Partially spoiled seeds" mean seeds which, any part, are rotten, mouldy or containing no starch.

Clause 4. In case of disputes or contentious problems concerning to clause 3, the latest sample provided by the Office of Commodity Standards shall be taken as the basis of determination.

Clause 5. In case of selling maize by sample, which has been approved by the Office of Commodity Standards, the standard of such maize shall not be inferior to the sample or condition agreed by buyers.

Clause 6. In case of maize exported in gunny bags, those gunny bags shall be new ones, which are the same type, size and weight as the gunny bags used for packing rice (Heavy Cee); they shall be in good condition, suitable for export, not torn, not leaked and free from bad odor. The mouth of the bags shall be tightly sewn across and reverse, with double jute twine, each way not less than 8 stitches for the bags with width not exceeding 60 cm. and not less than 11 stitches for the bags with width exceeding 60 cm., but not over 86 cm. Nevertheless, except the buyer has made an agreement with the exporter concerning the type, size and weight of the gunny bags including the sewing of the mouth of the gunny bags which differs from the preceded mention and the exporter has declared such agreement in details in the application form for standard certificate.

In case of maize exported in bulk, but due to the necessity to use gunny bags for packing some portion of maize in order to prevent the movement of maize in the hatch of the outgoing vessel, those gunny bags may be used ones, but they shall be strong, durable and in good condition, not torn, not leaked and free from bad odor. The mouth of the bags shall be tightly sewn, in order to prevent the maize moving or leak from the gunny bags in the loading time.

(1) Seeds of other colours, not exceeding 3.0 per cent by weight.

(2) Partially spoiled seeds together with greatly spoiled seeds, not exceeding 6.0 per cent by weight but greatly spoiled seeds, not exceeding 2.0 per cent by weight.

(3) Seeds destroyed by weevils, not exceeding 3.0 per cent by weight.

(4) Broken seeds together with immature seeds, not exceeding 3.0 per cent by weight.

(5) Foreign material, not exceeding 2.0 per cent by weight, but have no oil seeds or poisonous matter.

(6) Moisture content not exceeding 15.5 per cent by weight.

A. Pre-Loading Samples

(1) In case of maize in bags, the increments shall be taken by random at least 2 sides of the pile (upper and other sides) and the number of bags no less than 5% of bags in the pile.

(2) In case of maize in bulk pile, the increment shall be taken by random and throughout no less than 0.5 meter depth of the layer, and 2 meters of neighboring points, overall the pile, each point at least 0.5 Kg. of sample by means of a cylindrical sampler.

(3) In case of maize in a storage silo, the increments shall be taken no less than 1 Kg. each of 1 meter depth from upper layer until 3/4 of maize height, by means of pneumatic probe sampler or sampling takes place by means of maize in circulate motion, increments shall be taken no less than 0.5 Kg. each 1 M.ton. maize circulation, until 2 % or more of maize which stored in the silo.

(4) When the bulk maize is sampled in the hold during storage in silos or warehouses, increments shall be taken at least 0.5 Kg. lorries, or unit. In case of bag, it shall be taken at least 0.5 Kg. per truck or wagon or unit and no less than 5% of bag in each unit.

(5) The bulk sample of each pile, bin or silo shall be formed by combining the increments and mixing to obtain uniformity and sub-dividing to obtain about 3 Kg., and six samples to be taken for analysis and arbitration.

B. Loading samples

(1) The maize, which is to be dispatched, have to be certified as to the quality by means of preloading samples to be based on standards or agreement.

Sampling Procedure (Official Inspection) for maize.

There are two kinds of inspection samples. The first is called a "pre - loading" sample and is taken to be representative of maize in a storage bin, silo, godown (in bag or in bulk). The second is a "loading" sample and is collected as maize is loaded, onto a barge, lighter or ship for export.

(2) Physical quality inspection and packaging checks are to be performed, and samples taken from every bag of maize by means of sampling spear or 0.5 Kg. from each wagon, lorry, unit or time intervals dependent on the rate of flow.

(3) During inspection, each 100 M.tons. loaded, take at least 0.5 Kg. sample by random from pile or silo for moisture testing and each of two for discrimination testing.

When the quality of maize tested inferior to the standards or agreements, the inspection shall be temporary stopped to take away the inferior part. Then, samples are taken by random from another part for moisture testing and discrimination testing, if the quality is to be accepted, the maize shall be loaded again.

In case the inferior maize is not due to moisture content, it can be mixed with another that its quality has to be accepted by means of pre-loading samples, and it can be loaded again. If the quality is to be accepted, samples are taken and tested as in the first paragraph.

SAMPLING, SAMPLE PREPARATION, AND SAMPLING PLANS FOR MYCOTOXIN ANALYSIS IN U.S.A.

It is now well established that aflatoxin (mycotoxin) tends to be distributed very heterogeneously

The official first action method for corn specified by the Association of Official Analytical chemists (AOAC) does not designate sample size, but it requires that the entire sample of shelled corn be ground to pass a No.14 sieve, and that a 1-2 Kg. sub-sample of this material be ground to pass a No. 20 sieve. A 50 Kg. sub-sample of the finely ground material is then analysed by the CB. method. Whitaker, Dickens and Monroe developed the following equations for variance (error) terms related to this test procedure:

$$V = S + C + F + Q$$

V = Total variance (total error)

S = Error in sampling = 3.9539 P/Ws

C = Error in sub-sampling the coarse ground material = 0.1196 P/Wc

F = Error in sub-sampling the fine ground material = 0.0125 P/Wf

within a batch of maize. Traditional means of sampling and sample preparation of agricultural crops and foodstuffs are generally not adequate for mycotoxin analyses.

Associated errors and error reduction

In a study with corn, the total error was broken down into four components: sampling error, coarse subsampling, fine sub-sampling, and analytical error.

Q = Error due to quantification = 0.0699 P²/Nq

Ws = mass of sample in kg.

WC = mass of coarse sub-sample in kg.

Wf = mass of fine sub-sample in kg.

Nq = the number of times the aflatoxin in the solvent extract is quantified on a separate TLC.

P = Aflatoxin concentration (ug/kg) in the lot.

These studies draw attention to the fact that the sampling error is usually the largest contributor to the total error, so improved sampling can make the greatest contribution toward the accuracy of analytical results from which acceptance or rejection decisions are made.

Some methods to increase the precision of aflatoxin tests are to increase sample size, to increase the size of the sub-sample used for aflatoxin analysis, and to increase the number of analyses.

Different costs are associated with each method and careful study is required to determine the testing program that will provide the most precision for a given cost. The optimum balance in sample size, degree of comminution, sub-sample size, and number of analyses will vary according to the cost of the sample to be comminuted, the cost of sample and subsampling, the cost of analysis, and other factors. In general, the costs of properly designed aflatoxin testing programs will increase as precision increases.

Sampling Procedure

Samples may be taken from crops growing in the field, during handling, storage, and at other points in the production. Marketing samples can best be obtained by the use of automatic continuous samplers in situations where such equipment can be used, such as manufacturing process streams of materials. When this is not possible, e.g., when a bulk lot is in a bin, truck, box car or similar container, probe samples should be taken by means of probes which can reach to the bottom of the container. When the lot is bagged, samples are best taken from the bags while they are being gridded or emptied into containers. These samples may consist of portions taken by scoop or by hand, "grabs" and composited in a collection container. After the bags are closed the job becomes more difficult, but samples can be removed by means of small triers (probes). For lots comprising a relatively small number of bags it is best to sample each bag. As the number of bags in a lot becomes large, a good practice is to remove material from one-fourth of the bags.

Since the recognition of the aflatoxin problem, it has generally been the practice to require at least 1 kg. samples; and the U.S. Food and Drug Administration has advocated a minimum of a 15 lb. (6.8 kg.) sample. The size of the lot under investigation usually does not affect the random variability associated with sampling if the sample size is small compared to the size of the lot. A properly drawn 48 lb. sample is as representative of a 100,000 lb. lot of raw shelled peanuts as it is for a 40,000 lb. lot. Over the years, the size of the sample for the control of aflatoxin in peanuts in the United States has risen from 12 lbs. (5.4 kg.), to 24 lbs. (10.9 kg.), to 48 lbs. (21.8 kg.), to the current 144 lb. sample (three 48 lb. samples). This increase in size evolved as more reliable test results were required by the manufacturer. Increasing sample size has the advantage of simultaneously reducing the number of good lots rejected and the number of bad lots accepted by a testing program.

Usually the amount of sample material removed from the lot is more than is required, so it is necessary to thoroughly mix this material before removing the required amount of sample. After mixing, the sample can be sub-divided to the required size by use of mechanical dividers or by applying the "quartering" technique.

Sample Preparation

Assuming that a representative lot sample can be obtained, the next step in the process is to prepare the sample for analysis. In general, this will involve mixing and blending of the material, coarse grinding to reduce the particle size so the material will pass a standard # 14 mesh screen, mixing to obtain a portion for further grinding to produce a flowable material which can be sub-divided to the specified size of analytical sample.

INSPECTION SCHEMES FOR SHELLED PEANUTS WITH REGARD TO AFLATOXIN IN THE NETHERLANDS

In the Netherlands, a provisional Code of Practice for the peanut wholesalers and processors has been laid down. The provisional Code deals with inspection practices with regard to aflatoxin for lots of shelled peanuts prior to processing and/or selling to retailers, restaurants, etc. The Code should be used by wholesalers and processors.

Provisional Inspection Scheme

Classification of peanuts in the lot (average)	Number and weight of subsamples per lot 1	Acceptance criterion (microgram Aflatoxine B1 per kg)
60 nuts per ounce or more	4 x 5 kg	In each subsample 3 ppb or less
less than 60 nuts per ounce	4 x 10 kg	Ditto.

Before sampling, the lot should be divided into four equal parts. From each part a sub-sample is taken. The sub-sample should be made up of small equal samples which are taken out of each 250 kg of the part of the lot.

Classification of the peanuts in a lot (average)	Probability of acceptance (%) when the average aflatoxine B1 content of the lot is (microgram per kg):							
	Aflatoxine							
	1	2	3	5	10	15	20	30
60/oz or more	71	29	15	7	4	3	3	2.5
less than 60/oz	74	29	14	6	3	2	2	2

Probability of Acceptance

When the above described scheme is applied, the probability of acceptance calculated according to the method and on the basis of the distribution of Aflatoxine B. in peanuts as described by J. Walbel in his article "Stichprobengrosse für die Bestimmung von Aflatoxin in Erdnüssen", in Deutschen Lebensmittel-Rundschau (vol. 73, nr. 11, November 1977, page 353 t/m 357), is as follows.

In this case it is assumed that a sorted and cleaned lot has a degree of contamination of 1 peanut/15.000 peanuts. Besides, it is assumed that the average weight per peanut is respectively 0.35 g (classification 60/oz or more) and 0.65 g (classification less than 60/oz).

Desirable Inspection Scheme

The inspection scheme described above is agreed upon only for the time being.

Regarding the intake of aflatoxin, the stand of the State Supervisory Agency for Public Health in the Netherlands is as follows:

1. All measures which are feasible should be taken to avoid the contamination of foods with aflatoxine.
2. In case of an accidental "one-off" intake of aflatoxine (for instance when products with contaminated whole or broken peanuts are consumed), the intake should in no case be more than ± 50 microgram aflatoxine B1.
3. In case of an accidental "sub-chronic" intake of aflatoxine (for instance when products with contaminated milled peanuts like peanut butter are consumed) the intake should in no case be more than ± 0.5 microgram Aflatoxine B1 per day over a short period.

Condition 2 implies in fact that lots of peanuts which contain contaminated peanuts with ± 50 microgram Aflatoxine B1 or more should always be rejected when inspected. This means that an inspection scheme should be used which offers a probability of acceptance of 0 for lots with nuts containing 50 microgram Aflatoxine B1 or more. Using the same method of calculation and the same assumptions as referred to above, this implies that in case of gradings of 60/oz or more the probability of acceptance of a lot with on average Aflatoxine B. content of 10 micrograms per kg should be 0 ($\leq 0.1\%$) and in case of gradings of less than 60/oz the probability of acceptance should be 0 ($\leq 0.1\%$) when the lot contains on average 5 microgram per kg.

Condition 3 implies that since in the Netherlands a high individual consumption of peanuts from products like peanut butter is about 35 g per person per day, the probability of acceptance of a lot of peanuts which is intended for milled peanut products should be 0 ($\leq 0.1\%$) when the average Aflatoxine B. content of the lot is 15 microgram per kg (for all classifications).

The provisional inspection scheme which is now used for some months by the wholesalers and processors may after some time be adapted so that they conform more with the conditions described above.

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SAMPLING, SAMPLE HANDLING AND PREPARATION IN TAIWAN. (R.O.C.)

The Bureau of Commodity Inspection and Quarantine, Ministry of Economic Affairs is responsible for the control of products (such as corn) to be imported by means of CNS. (Chinese Standards)

Sampling Procedure

1. The products (corn) are to be rejected if the quantity of aflatoxin is greater than 50 ppb.
2. Pneumatic-Sampler or Probe-A-Vac. are apparatus for taking sample from corn carried in bulk from hatch of the vessel.
3. The increments shall be taken every meter throughout the whole depth of the layer, with 4-6 sampling points taken at random.

Size of sample (Laboratory sample)

The bulk sample shall be formed by combining the increments at least 66 Kg., and mixing them well and sub-dividing to obtain three of 21 Kgs. samples.

Sample Preparation

The 21 kgs. sample will mixing, blending and coarse grinding (about 1 mm.), then mixing to obtain uniformity and sub-dividing to obtain about 2 Kgs. And further grinding to reduce the particle size so the material will pass a standard # 20 Mesh screen for aflatoxin analysis.

Aflatoxin analysis

A high pressure liquid chromatographic (HPLC) method is designed for determining aflatoxins in corn.

Schematic of Corn Aflatoxin Testing Program.

[Schematic of Corn Aflatoxin Testing Program.](#)

SAMPLING, SAMPLE HANDLING AND PREPARATION FOR AFLATOXIN DETERMINATION IN MAIZE (OCS unofficial)

7.1 Method of taking samples from maize stacked in bulk.

7.1.1 1,000 metric tons presume to be a lot, and 200 up to 1,000 metric tons remains to be a lot.

7.1.2 Increment shall be taken from a single position in the 200 metric tons lot, and the remains less than 200 metric tons is an increment.

7.1.3 Increments shall be taken through at least half depth of the layer. The sub-sample shall be formed by combining five increments (no less than 5 kgs./increment) and mixing them well, and dividing to obtain 2 kgs.

7.1.4 The sub-sample shall be divided to obtain four of 2 kgs. Laboratory samples.

Eg. 2,400 metric tons maize stock in bulk.

Lot.	1,000	1,000	400	metric tons
Increments	5	5	5	
Laboratory Samples	4	4	4	

7.2 Method of taking samples from maize stocked in bags.

7.2.1 1,000 metric tons presumed to be a lot and 200 up to 1,000 metric tons remains to be a lot.

7.2.2 Increments shall be taken from bags of each lot from at least four sides of stock, and from bags at top side which is pulled off one fourth of bags height. The number of bags to be sampled shall be no less than 20% of stock, and sub-sample shall be no less than 16 kgs.

7.2.3 The sub-sample shall be formed by combining the increments and mixing then well, and divided to obtain four of 2 kgs. Laboratory samples.

	Eg. 2,400 metric tons maize stock in bags.			
Lot	1,000	1,000	400	metric tons
Increment	16	16	16	kgs.
Laboratory Samples	4	4	4	

7.2.4 100% sampling of the bags shall be takes place while the bags is in motion to stock in go-down, a sample per pile and divided to obtain four of 2 kgs. Laboratory samples.

7.3 Method of taking samples from maze stocked in silo.

7.3.1 Sub-sample shall be taken from 200 metric tons of maize, and 100 upto 200 metric tons remains is a sub-sample. The sub-sample has to be no less than 5 kgs., mixing them well and divided to obtain 2 kgs.

7.3.2 The sub-samples shall be taken from the top and bottom of the silo by the ratio of 2:3 if sampling from the top is not available, it shall be taken from the bottom only.

Eg. 1,500 metric tons of maize stocked in silo.

$$= \frac{1,500}{200} \times \frac{2}{5} = 3$$

Number of sub-sample taken from the bottom of silo.

$$= \frac{1,500}{200} \times \frac{3}{5} = 5$$

7.3.3 How to get sampling from the top.

The increment shall be taken from every meter until about 5 - 7 meters depth of the layer or no less than $i/4$ of maize height in silo, and the sub-sample shall be taken from different directions.

7.3.4 Bottom Sampling.

The increments shall be taken by recycle 10 % of silo contents.

10 % of 1,500 M.ton. = 150 tons

5 % sub-sample taken from = $\frac{150}{5} = 30$ tons lot.

Sample for 30 tons lot taken at random for 5 kgs. sample size, are mixed well and divided to obtain 2 kgs. sub-sample.

In case top sampling is not possible to take, the increments (bottom sampling) shall be taken by recycling 20% of silo contents, and similar sampling as above.

7.4 The bulk sample.

The bulk sample shall be formed by combining the increments, and mixing them well, and dividing to obtain four 2 kgs.-samples for analysis.

7.5 Schematic of Corn Aflatoxin Testing Program.

$$2 \text{ kgs. SAMPLE 1 A B } \frac{1A + AB}{2}$$

(Contract, accept. >Contract. << 150 % Run sample 2 > 150 % Contract, reject.

$$2 \text{ kgs. SAMPLE 2 A B } \frac{1A + 1B + 2A + 2B}{4}$$

<= Contract., accept. > Contract <= 150 % Run sample 3) 150 % contract, reject. 2 Kgs. Sample
3 A B

$$\frac{1A + 1B + 2A + 2B + 3A + 3B}{6}$$