MAFF UK - SURVEY FOR AFLATOXINS, OCHRATOXIN A, FUMONISINS AND ZEARALENONE IN RAW MAIZE

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Summary

A JFSSG survey for aflatoxins, ochratoxin A, fumonisins and zearalenone in 139 samples of raw maize has been completed. Samples were taken at UK ports or on entry to large maize mills. Eleven further samples were taken from some consignments following an industrial physical cleaning procedure, to determine the effect of such cleaning on contamination levels. The analytical limit of detection (LOD) for the mycotoxins analysed was 0.1 µg/kg for each aflatoxin (B_1, B_2, G_1 and G_2) and ochratoxin A, 10.0 µg/kg for each fumonisin (B_1, B_2 and B_3) and 5.0 microgram/kg for zearalenone.

Reassuringly, over 92 per cent of samples were below the levels of 4 and 2 µg/kg for total aflatoxins (B_1, B_2, G_1 and G_2) and aflatoxin B_1 respectively which are the limits set by EC legislation for cereals and cereal products intended for direct human consumption or use as an ingredient in foodstuffs. However, 1 sample was found to be highly contaminated at 29.1 microgram/kg total aflatoxins. The importer has been informed and has taken appropriate action. The maximum concentration of ochratoxin A found was 1.5 µg/kg. Fumonisins and zearalenone were detected in almost every sample with 48 per cent of samples containing more than 1000 µg/kg total fumonisins (B_1, B_2 and B_3) and 42 per cent of samples containing more than 100 µg/kg of zearalenone. Physical cleaning of raw maize reduced aflatoxin concentrations by about 40 per cent and total fumonisins by 32 per cent, however, the results for zearalenone were inconclusive. Imported raw maize undergoes cleaning and further processing before use for direct consumption or as an ingredient in foodstuffs and, therefore, the amounts of mycotoxins in raw maize are unlikely to reflect the concentrations in the finished products for human consumption.

Background

The aflatoxins, ochratoxin A, zearalenone and the fumonisins are naturally occurring mycotoxins produced by moulds. The aflatoxins are produced by certain mould species found in tropical and sub-tropical climates. Ochratoxin A, zearalenone and the fumonisins are produced by different moulds which generally favour climates with lower temperatures and humidity. Growth of the moulds and the production of mycotoxins is dependent upon a number of environmental factors such as temperature and humidity during growth, harvesting and subsequent storage of crops. Table 1 summarises the major fungal species responsible for the contamination of food by each of these mycotoxins, the foods that are susceptible and gives the latest expert committee opinion on possible effects on health.

To protect consumer safety, the Joint Food Safety and Standards Group (JFSSG) regularly carries out surveys of foodstuffs for mycotoxins. These surveys have identified foods at risk from contamination and have enabled estimates to be made of the exposure of UK consumers to mycotoxins. The surveillance programme has also identified contamination problems which have subsequently been addressed through improved production practices, supported by regulation and codes of practice. Further details of the food chemical surveillance programme can be found in the 1998 Annual Report on Food Chemical Surveillance.[1]

Regulation of mycotoxins

A European Commission Regulation (EC No. 1525/98)[2] on aflatoxins came into force on 1 January 1999. It offers even more consumer protection against aflatoxins than was provided by previous UK laws. The new Regulation sets limits for aflatoxin B_1 and total aflatoxins in groundnuts, nuts, dried fruit, cereals and a limit for aflatoxin M_1 in milk. Higher limits are provided for groundnuts, nuts and dried fruit intended for further processing before human consumption. No limits are set for cereals intended for further processing before human consumption, pending further information on processing to be provided by the industry. The industry was given until July 1999 to provide this information, although this deadline has now been extended to 1 July 2001 to allow time to provide sufficient technical data. In parallel, the EC adopted a Directive (98/53/EC)[3] prescribing sampling methods and performance criteria for methods of analysis for aflatoxins. Member States are required to implement this Directive by 31 December 2000.

The above EC Regulation and Directive were implemented in the UK in June this year. The Contaminants in Food (Amendment) Regulations 1999[4] make provision for their enforcement and revoke the previous UK legislation, *The Aflatoxins in Nuts, Nut Products, Dried Figs and Dried Fig Products Regulations 1992*.[5] The new legislation indicates that products intended for human consumption or as an ingredient in foodstuffs must comply with limits of 2 μg/kg aflatoxin B_1 and 4 μg/kg total aflatoxins. A separate limit of 0.05 μg/kg aflatoxin M_1 is applicable in milk and milk products. Separate Regulations also set maximum limits for aflatoxin B_1 in animal feedingstuffs.[6,7] Aflatoxin B_1 in animal feed is metabolised by ruminants such as cows, sheep and goats, leaving the metabolic product aflatoxin M_1 in the milk.

Possible EC legislative limits are currently being considered for aflatoxins in certain spices and for ochratoxin A in a variety of foods. Other mycotoxins such as fumonisins, deoxynivalenol and zearalenone may be the subject of future EC regulation.

The current survey

This survey aimed to check the compliance of raw maize intended for further processing with anticipated new EC regulations on aflatoxins in such products which, as discussed above, were due to come into force in July 1999. However, the deadline for setting a limit in raw cereals intended for further processing has now been extended until 1 July 2001.

The opportunity was taken to analyse the same samples of maize for ochratoxin A, fumonisins and zearalenone as the acquisition of maize samples is costly. The data obtained from this survey will be used to inform:

- negotiations on EC legislation setting limits for aflatoxins in cereals for further processing;
- discussions on possible EC legislation on ochratoxin A;
- on the extent and prevalence of aflatoxins, ochratoxin A, fumonisins and zearalenone contamination in raw maize for use in assessing consumer exposure;
- all interested parties, including consumers and the industry.

Previous surveys

During the period 1991-6, the annual amount of raw maize used in the UK has remained constant at about 1.6 million tonnes, of which about 1.35 million tonnes is used for human and industrial use and 0.25 million tonnes for animal feed. Imported raw maize currently comes principally from France and Argentina. Maize grown in the UK is used either as 'corn on the cob' or for animal feed, usually as maize silage.

In the UK, surveys have been carried out to determine the incidence and concentrations of mycotoxins in a range of products including cereals and animal feeds. [8, 9, 10, 11] The most recent JFSSG survey of UK cereals was for ochratoxin A and included 300 samples (barley, oats, rye and wheat). [12] Ochratoxin A was detected in 21 per cent of samples (limit of detection 0.1 μg/kg). Only five samples of barley and 3 samples of wheat contained ochratoxin A above 5 μg/kg, the maximum limit for ochratoxin A in cereals currently proposed by the Codex Alimentarius.

A survey of ethnic food [13] involved mycotoxin analysis of samples purchased from specialist shops. Thirty two cereal and cereal product samples were found to contain total aflatoxins at levels between 0.1 and 3 μg/kg, ochratoxin A levels between 0.2 and 0.9 μg/kg, fumonisin levels between 26 and 218 μg/kg and zearalenone levels between 5 and 41 μg/kg. In another survey on fumonisins in maize-based foods [14] the mycotoxin was detected in 26 per cent (76/291) of all samples, at concentrations ranging from 10-2124 μg/kg.

Raw maize undergoes further processing including cleaning, milling, fractionation and processing to produce a range of corn products a few of which are available for direct human consumption. The survey investigated whether the initial cleaning stage effectively removed any mycotoxins in the raw material. The products of dry milling of maize include grits, meal and flour. For example, flaking grits are used for the manufacture of the breakfast cereal 'cornflakes'. Coarse grits and medium grits are used in the manufacture of cereal products and snack foods. Fine grits are used in brewing.

In summary, the contamination of maize by aflatoxins and fumonisins, and other cereals by ochratoxin A is well documented in the scientific literature. Also zearalenone has been frequently found as a contaminant of cereals.

Methodology

Sampling Strategy

In total 139 consignments of raw maize were sampled over a 9 month period (Summer 1998 to Spring 1999) on arrival at three ports (Manchester, Mersey and Hull and Goole) by the Port Health Authorities (PHAs) or on entry to three large maize mills. Eleven of these consignments were re-sampled after undergoing an initial cleaning procedure to remove dust, husks, small broken particles and foreign matter.

The 139 samples included 97 from France, 37 from Argentina, 3 from Spain, 1 from Hungary and 1 from Germany. This corresponds well with UK import figures which show that raw maize comes principally from France (1.0 to 1.3 million tonnes) and Argentina (0.2 to 0.3 million tonnes) with much smaller amounts from other countries.

Maize from France arrives in vessels usually holding 2,000 to 6,000 tonnes. On the other hand, Argentinian maize arrives in larger consignments of 20,000 to 30,000 tonnes and the amount imported annually can be supplied by as few as 10 consignments. It is estimated that the samples taken as part of this survey were drawn from almost half of the consignments of maize imported into the UK during the nine month period.

Sampling at the ports
Samples for analysis consisted of aggregates of incremental samples taken randomly throughout the consignments of raw maize. At Manchester, health and safety considerations ruled out sampling on board ship, particularly during unloading, because safe access to sample in a systematic manner was considered impractical. After unloading the maize was transferred to silos until required, at which time it was discharged by conveyer direct to a mill. Twenty incremental samples each of 0.5 kg were taken from the conveyer during a 2 hour period and bulked. At Merseyside and Hull and Goole it was feasible to sample individual holds and 100 incremental samples of 300 grams were obtained from holds during discharge. The 30 kg bulk sample was thoroughly mixed and a 10 kg sub-sample sent to the laboratory for analysis.

Sampling at maize mills
Samples were either taken from lorries, normally carrying approximately 25 tonne loads, or from a moving belt as the consignment was being discharged from the ship directly to silos at the mill. At one mill, 0.5 kg incremental samples were taken from 20 consecutive lorries using an automatic sampling probe. The incremental samples were bulked to give a 10 kg laboratory sample representing 500 tonnes. At a second mill 30 incremental 'tailgate' samples of 1.5 kg were taken from every third lorry arriving at the mill. An incremental sample of 0.5 kg was taken from each of the 30 samples and the incremental samples bulked to give a 15 kg laboratory sample. At the third mill 20 grab samples of 1.5 kg were taken from a moving belt on discharge from a holding silo over a 5 day period. An incremental sample of 0.5 kg was taken from each of the 20 samples and bulked to give a 10 kg laboratory sample.

Methods of Analysis

One hundred and fifty samples of raw maize were received directly from PHAs or from maize millers, 11 of which were obtained after the original maize had undergone an industrial physical cleaning process. The analysis was carried out by RHM Technology Ltd, High Wycombe. Samples ranged in weight from 10 to 15 kg depending on the method of sampling and were ground, homogenised, sub-sampled and extracted using an appropriate solvent.

Limit of detection and limit of quantification
The limit of detection (LOD) of a method was defined as the minimum detectable level of an analyte under the assay conditions during a particular assay. The limit of quantification (LOQ) was defined as the minimum level of analyte that could reliably be quantified. Both the LOD and LOQ are dependent on the sensitivity for the analyte and the base-line noise at the time of analysis, in this survey the LOD was defined as 3 times the electronic baseline noise and the LOQ as 6 times baseline noise. The LODs and LOQs for each mycotoxin are given in Table 2.

Extraction, clean-up and determination of mycotoxins in maize
The methods used for aflatoxins, ochratoxin A and zearalenone were modifications of the method of Howell *et al.* [15] The method used for fumonisins was a modification of the method of Shephard *et al.* [16] All RHM Technology Ltd analytical methods are UKAS accredited.
Mycotoxin analysis involved a chloroform and water extraction; the exact protocol used depended on which mycotoxin was being analysed. The extract was filtered and diluted before being passed through an appropriate immunoaffinity column under gravity. The mycotoxin was eluted under

gravity. The eluate was reduced to dryness, a volume of mobile phase added and the extract was transferred to a vial.
Determination of each mycotoxin was carried out using High Performance Liquid Chromatography with fluorescence detection. The excitation and emission wavelengths of the detector used depended on the mycotoxin being analysed. For each mycotoxin chromatography was performed using a suitable column with an appropriate mobile phase.

Results

The analytical results for aflatoxins, ochratoxin A, fumonisins and zearalenone are given in Tables 3-9.
Recoveries obtained for each analytical method fell within the acceptable range throughout the study (70-110 per cent) and all results are corrected for recovery. On-going control of the methods was monitored using in-house naturally contaminated reference material. The relative standard deviation of the recoveries for the individual mycotoxins throughout the study was aflatoxins (6.1 per cent), ochratoxin A (5 per cent), fumonisins (4.7 per cent) and zearalenone (3.2 per cent).
Uncertainty for the aflatoxin and ochratoxin A methods has been estimated using RHM Technology's performance in the FAPAS proficiency-testing scheme. A 'best estimate' of the method measurement reliability was calculated for the following three average aflatoxins levels:
(1) 20 μg/kg plus or minus 2.3.
(2) 50 μg/kg plus or minus 4.3.
(3) 150 μg/kg plus or minus 9.6.
A 'best estimate' of the method measurement reliability was calculated for the following three average ochratoxin A levels:
(4) 0.4 μg/kg plus or minus 0.4.
(5) 3.5 μg/kg plus or minus 0.5.
(6) 10 μg/kg plus or minus 0.9.
When further laboratory data are available, an assessment of method measurement reliability for the other mycotoxins will be possible.

Total aflatoxins and aflatoxin B_1
Results for total aflatoxins and aflatoxin B_1 are given in Tables 3 and 4. The majority of the French maize samples, 95 out of 97 (98 per cent) contained low (less than 4.0 μg/kg) or undetectable levels of total aflatoxins compared with 33 out of 37 (89 per cent) for the Argentinian maize samples. Two samples of French maize, 3 samples of Argentinian maize and a sample of Spanish maize were slightly above the total aflatoxins level of 4 μg/kg where EC legislation currently applies for direct human consumption. One sample of Argentinian maize was found to be heavily contaminated with total aflatoxins at 29.1 μg/kg.
Aflatoxin B_1 was between 50 per cent and 90 per cent of the total aflatoxins, with 10 samples (7 per cent) in excess of 2 μg/kg, the level where EC legislation currently applies for direct human consumption. Not surprisingly, the Argentinian maize sample most heavily contaminated with total aflatoxins also contained the highest level of aflatoxin B_1 at 16.4 μg/kg.

Ochratoxin A
Results for ochratoxin A are given in Table 5. Ochratoxin A was not detectable in the majority of

samples, 125 out of 139 (90 per cent). Only 1 sample of Argentinian maize contained ochratoxin A, at a low level, while 10 samples (10 per cent) of French maize contained detectable levels of ochratoxin A. The maximum ochratoxin A level found was 1.5 microgram/kg which was in a single sample of maize from Hungary. This sample also contained low levels of fumonisins and zearalenone but no aflatoxins were detected.

Fumonisins
The results are given in Tables 6 and 7. All but 5 samples of French and other European produced maize contained detectable amounts of total fumonisins up to a maximum level of 2123 µg/kg total fumonisins. However, nearly all Argentinian maize exceeded 1000 µg/kg for both total fumonisins and fumonisin B_1, with a maximum concentration of 5007 microgram/kg total fumonisins comprising 3406 µg/kg of fumonisin B_1, 1268 microgram/kg of fumonisin B_2, 333 µg/kg of fumonisin B_3. Fumonisin B_1 was usually present in highest concentrations with fumonisin B_2 and fumonisin B_3 contributing approximately 30 per cent and 10 per cent respectively of the total fumonisins. The widespread occurrence and the relative proportions of each fumonisin detected in this survey are similar to those reported elsewhere. [17]

Zearalenone
Table 8 gives the results obtained for zearalenone in this survey. This mycotoxin was frequently detected in raw maize with 135 (97 per cent) samples being contaminated. Only 3 per cent of the samples contained less than 4 µg/kg of zearalenone and 42 per cent exceeded 100 µg/kg.

Mycotoxin levels following cleaning
The first stage in the industrial processing of maize is a cleaning step which is carried out to remove dust, husks, small broken particles and foreign matter. The results of the effect on cleaning are shown in table 9. No comparison was possible for ochratoxin A but 3 samples contained sufficient aflatoxin for a comparison to be made. The results are very variable but indicate that cleaning does reduce aflatoxin contamination. However, the number of samples was limited and further studies will be necessary to produce sufficient data for the EC to consider when setting limits for unprocessed maize. The highly contaminated sample with total aflatoxins at 29.1 µg/kg was found to contain 7.1 microgram/kg after cleaning, a reduction of 76 per cent, but a second sample taken from the same consignment contaminated at 23.3 µg/kg showed only a 6 per cent reduction in contamination after cleaning.
Zearalenone and fumonisins were present in all samples used for the cleaning studies. For total fumonisins a mean 32 per cent reduction occurred in cleaned samples, although this was less than 2 per cent for zearalenone. The percentage reduction of zearalenone was affected by 3 samples for which an apparent increase in zearalenone occurred although no obvious explanation for this can be offered as amounts of fumonisin in these samples declined as expected.

Interpretation

This survey reports the concentrations of aflatoxins, ochratoxin A, fumonisins and zearalenone found in samples of imported raw maize over the period Summer 1998 to Spring 1999. However, care should be exercised in comparing maize from different sources. Little information was available about treatment of grain before shipment and it has been suggested that some maize undergoes a preliminary screen or selection before export. Lower concentrations in samples from some sources may partly reflect more rigorous selection and testing prior to export in response to the quality requirements of the buyer.

Total aflatoxin and aflatoxin B_1

Of the 139 raw maize samples, 132 (95 per cent) were below 4 µg/kg for total aflatoxins in cereals. Aflatoxin B_1 levels in 129 out of 139 samples (93 per cent) were below 2.0 µg/kg. Aflatoxin B_1 often comprises the major component produced when aflatoxin contamination occurs (commonly between 50 per cent and 90 per cent of the total aflatoxins).

One shipment was found to be highly contaminated at 29.1 µg/kg total aflatoxins (16.4 µg/kg aflatoxin B_1), which is substantially in excess of the possible future EC limit for cereals. A further sample was taken from this consignment and was found to contain 23.3 µg/kg total aflatoxins. The importer has been informed and has taken appropriate action.

Aflatoxin contamination in maize grown in Europe was generally low. However, previously published data indicate that maize from South America, Southern USA, Philippines and Thailand are occasionally contaminated with high levels of aflatoxins. For example, in one study levels of aflatoxin B_1 found in Argentinian maize were between 5 and 560 µg/kg and the toxin was detected in 20 per cent of the samples (454/2271). [18] The findings of the current survey compare favourably with contamination levels reported elsewhere.

Ochratoxin A

Discussion about possible future EC legislation for mycotoxins, other than aflatoxins, is currently centred on ochratoxin A and, although no limit has been agreed, a maximum permitted level of 5 microgram/kg is being considered for raw cereals. All maize samples in this survey were found to contain substantially less than this level.

Fumonisins

There are currently a number of toxicological studies and surveys in progress aimed at assessing the significance of the fumonisins for human health. The results of a study carried out under the National Toxicological Programme in the USA indicated that fumonisin B_1 was carcinogenic in male rats and female mice. However, there is little evidence at present to suggest that fumonisins pose a significant risk to the UK consumer.

All but 5 samples of French maize contained detectable amounts of fumonisins. The maximum level found was 2123 µg/kg total fumonisins. However, usually fumonisin B_1 was present in highest concentrations with approximately 30 per cent and 10 per cent of the total fumonisin concentration present as fumonisin B_2 and fumonisin B_3 respectively. The relative proportions and widespread occurrence are consistent with previous reports. In common with the other mycotoxins studied the relationship between concentrations in raw maize and consumer products is unclear, although surveys of maize based consumer foods in the UK have shown levels to be generally low.

The maximum concentration of fumonisin found was 5007 µg/kg total fumonisins, comprising 3406 microgram/kg fumonisin B_1, 1268 µg/kg fumonisin B_2 and 333 µg/kg fumonisin B_3. However, nearly all Argentinian maize exceeded 1000 µg/kg of total fumonisins and fumonisin B_1 while French and other European maize tended to contain lower concentrations. This difference may be due to climatic or seasonal differences and/or production practices.

Elsewhere, even higher levels of fumonisin contamination have been reported. For example, the results of a survey carried out in China indicated that 56 per cent of samples (134/240) were found to contain fumonisin B_1 at levels ranging from 50-34868 μg/kg. [19] Germany reported detection of fumonisins in 27 per cent of samples (86/317) and levels ranged from 6-7132 μg/kg. [20]

Zearalenone
There are no current or proposed EC limits for zearalenone, although it is recognised as a frequent contaminant of maize and maize products. This survey confirms that zearalenone is frequently associated with raw maize, with levels ranging from less than 4 microgram/kg to greater than 100 μg/kg with a maximum of 584 microgram/kg. In comparison with other studies worldwide this UK survey shows low levels of contamination. The results compare favourably with a Canadian survey which found zearalenone in 69 per cent of samples (87/126) with levels from 5-647 μg/kg. [21] In Argentina zearalenone was detected in 30 per cent of cases with levels from 5-2000 μg/kg. [18]
A JFSSG survey of ethnic diets in 1994 [13] showed that processed foods, including maize based products, contained zearalenone at significantly lower levels than those found in the current survey of raw maize. This indicates that processing of raw maize may reduce zearalenone concentrations in the resulting consumer products.

Mycotoxin levels following cleaning
One objective of this survey was to examine the effect of the initial cleaning process on concentrations of aflatoxins and the other mycotoxins in the cleaned product. Industry has been invited by the EC to provide data for raw and processed cereals by July 2001 to determine whether sorting or other physical techniques could be used to identify and remove heavily contaminated samples. A 2-tier system might then be adopted for separate maximum permissible levels in cereals for direct human consumption and for further processing. Similar data might be required to support future legislation for ochratoxin A in cereals.
The survey found that aflatoxins and ochratoxin A do not often occur in imported maize. It was therefore impossible to be sure that samples selected for examination before and after cleaning would initially contain these mycotoxins. Because of this difficulty, it was decided that the last 11 consecutive samples of Argentinian maize would be examined before and after cleaning. As the last sample contained a high amount of aflatoxins (29.1 μg/kg), a further sample was included additional to the planned survey. The subsequent sample was found to contain 23.3 μg/kg total aflatoxins.
Results on the effects of cleaning are shown in Table 9. No comparison was possible for ochratoxin A because of the low levels of contamination found, but 3 samples contained sufficient aflatoxin for comparison. For these samples the mean reduction for total aflatoxins was 41 per cent, although the small sample number should be borne in mind when considering this figure.
Zearalenone and fumonisins were present in all 11 samples. For total fumonisins a mean 32 per cent reduction occurred in cleaned samples, whereas only 2 per cent reduction was recorded for zearalenone. The average reduction for zearalenone was low because 3 samples had an apparent increase. No obvious explanation for this can be offered, particularly as the amounts of fumonisin in these samples were reduced as expected. It is clear that a simple cleaning of raw maize may significantly reduce aflatoxin and fumonisin concentrations and other studies suggest that further processing is likely to lead to further significant losses. The effect of processing on mycotoxin levels needs to be studied further to provide sufficient data to support any future separate regulatory limits.

Conclusions

Most raw maize entering the UK during the period of this survey had no or low levels of aflatoxins. However, 7 samples contained greater than 4 microgram/kg total aflatoxins and 10 greater than 2 µg/kg aflatoxin B_1. One sample was very high at 29 µg/kg total aflatoxins and the importer has been contacted.

The incidence and levels of ochratoxin A were low with only 2 samples exceeding a concentration of 1 microgram/kg and a maximum of 1.5 µg/kg. This level is below the possible EC regulatory limits being discussed.

Fusarium mycotoxins were present in nearly all samples examined. Total fumonisin concentrations were greater than 1000 µg/kg in 35 per cent of the samples while concentrations of zearalenone exceeded 100 µg/kg in over 40 per cent of samples.

Initial cleaning of raw Argentinian maize showed some significant reduction of aflatoxins and fumonisins although the number of comparisons possible for aflatoxins was small. The effects of cleaning on zearalenone were less conclusive as levels increased in 3 samples, although the reasons for this were unclear.

More information on the fate of aflatoxins and other mycotoxins during processing is required so that the daily intake of mycotoxins from maize products can be estimated and can be related to the levels in raw maize. These data are required to support any future discussion relating to EC legislation for mycotoxins in raw cereals and cereal-based foods.

Summary of units

kilogram (kg) : one thousand grams
microgram : one millionth of a gram
µg : one millionth of a gram
nanogram (ng) : one thousand millionth of a gram (or one thousandth of a microgram)

Table 1: Summary of mycotoxins covered by this survey

Mycotoxin	Predominant Fungal Species	Foodstuffs susceptible	Assessment of the effects on health by expert committees *
Aflatoxins are a group of closely related compounds (B_1, B_2, G_1, G_2)	*Aspergillus flavus* and *Aspergillus parasiticus*	Cereals, Nuts, Spices, Dried Figs.	All aflatoxins are acutely and chronically toxic in animal studies. They are recognised animal and human carcinogens, with aflatoxin B_1 being the most potent (COT). Recommendation to reduce levels to lowest technologically achievable (FAC).
Ochratoxin A	*Aspergillus ochraceus* And *Penicillium verrucosum*	Stored cereals, Beer, Cocoa Coffee, Dried Fruit, Pulses, Grape Juice, Wine.	Ochratoxin A has been linked with kidney damage in pigs and in humans has been associated with Balkan endemic nephropathy. Appears to be an animal carcinogen and is regarded as a genotoxic carcinogen (COT). Recommendation to reduce levels to lowest technologically achievable (FAC).
Fumonisins are a group of closely related compounds (B_1, B_2, B_3)	*Fusarium moniliforme*	Maize	Fumonisins cause leukoencephalomalacia which affects the central nervous system of horses, carcinogenic in rats and mice. The potential carcinogenic effects of fumonisins were considered (COC) . No firm conclusions were made on the evidence available in July 1993.

Zearalenone	Various *Fusarium* Species	Cereals primarily Maize, Wheat, Barley	Tests have shown that zearalenone is a weak genotoxin and has oestrogenic properties, but no evidence to indicate that it is mutagenic or teratogenic (COM).

* In the UK, the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT), the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) and the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) assess the significance of mycotoxins for human health. These committees then make recommendations to the Food Advisory Committee (FAC) who will endorse these recommendations as appropriate. Further details of the advice given by these committees, along with other International expert scientific opinions are given in Annex I.

Table 2: Analytical parameters for mycotoxin analysis

	Concentration, microgram/kg	
Mycotoxin	Limit of detection	Limit of quantification
Aflatoxins B_1	0.1	0.2
Aflatoxins B_2	0.1	0.2
Aflatoxins, G_1	0.1	0.2
Aflatoxins G_2	0.1	0.2
Total Aflatoxins	0.4	0.8
Ochratoxin A	0.1	0.2
Zearalenone	5	10
Fumonisin B_1	10	20
Fumonisin B_2	10	20
Fumonisin B_3	10	20
Total Fumonisins	30	60

Table 3: Total aflatoxins in raw maize, microgram/kg

		Number of samples in the range Total aflatoxins				
Sample origin	No. of samples	Less Than 0.4	0.4-1.0	1.1-4.0	More Than 4.0	Maximum
France	97	82	10	3	2	6.0
Argentina	37	25	4	4	4	29.1
Other European	5	2	0	2	1	4.9
All	139	109	14	9	7	29.1

Table 4: Aflatoxin B_1 in raw maize, microgram/kg

		Number of samples in the range Aflatoxin B_1				
Sample origin	No. of samples	Less Than 0.1	0.1-1.0	1.1-2.0	More Than 2.0	Maximum
France	97	65	27	2	3	5.8
Argentina	37	21	8	3	5	16.4
Other European	5	2	1	0	2	3.7
All	139	88	36	5	10	16.4

Table 5: Ochratoxin A in raw maize, microgram/kg

		Number of samples in the range				
Sample origin	No. of samples	Less Than 0.1	0.1-1.0	1.1-4.0	More Than 4.0	Maximum
France	97	86	10	1	0	1.4
Argentina	37	36	1	0	0	0.3
Other European	5	3	1	1	0	1.5
All	139	125	12	2	0	1.5

Table 6: Fumonisin B_1 in raw maize, microgram/kg

		Number of samples in the range					
Sample origin	No. of samples	Less Than 10	10-100	101-500	501-1000	1001-5000	Maximum
France	97	0	29	41	23	4	1557
Argentina	37	0			3	34	3406
Other European	5	0	1	1	2	1	1311
All	139	0	30	42	28	39	3406

Table 7: Total fumonisins in raw maize, microgram/kg

		Number of samples in the range						
Sample origin	No. of samples	Less Than 30	30-100	101-500	501-1000	1001-5000	More Than 5000	Maximum
France	97	5	14	39	30	9	0	2123
Argentina	37	0	0	0	0	36	1	5007
Other European	5	0	1	1	0	3	0	1829
All	139	5	15	40	30	48	1	5007

Table 8: Zearalenone in raw maize, microgram/kg

		Number of samples in the range

Sample origin	No. of samples	Less Than 4	4.0-20	21-100	101-500	More Than 500	Maximum
France	97	2	11	51	32	1	584
Argentina	37	0	0	11	26	0	357
Other European	5	2	2	1	0	0	34
All	139	4	13	63	58	1	584

References

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ANNEX I
Summary of International and UK Expert Committee Opinions on Mycotoxins

Aflatoxins
The COT concluded in the light of evidence of animal carcinogenicity and probable human carcinogenicity and that aflatoxins in food should be reduced to the lowest level that is technologically achievable. a Following subsequent advice from the FAC, a in 1992 UK regulations for aflatoxins were introduced. EC aflatoxin regulations came into force in January 1999.

Ochratoxin A
The COT concluded that ochratoxin A is a genotoxic carcinogen (1992) b and advised that levels in food should be reduced to the lowest level that is technologically achievable. Ochratoxin A has other potential toxicological effects (in particular, it has been implicated in kidney malfunction). The COT requested that surveillance of ochratoxin A in UK-produced cereals and animal feedingstuffs should continue with the aim of developing a plan to reduce ochratoxin A contamination to the lowest level technologically achievable.
In 1995, the Joint Expert Committee on Food Additives (JECFA) c set a Provisional Tolerable Weekly Intake of 0.1 microgram/kg bw (i.e. circa 14 ng/kg b.w./day) and in September 1998 the EC's Scientific Committee on Food (SCF) d issued its latest opinion on ochratoxin A. It stated that 'it would be prudent to reduce exposure to ochratoxin A as much as possible, ensuring that exposures are towards the lower end of the range of tolerable daily intakes of 1.2-14 ng/kg b.w./day which have been estimated by other bodies, e.g. below 5 ng/kg b.w./day'.

Fumonisins
The potential carcinogenic effects of fumonisins were discussed by the Committee on Carcinogenicity (COC) in July 1993, e but it was unable to reach any firm conclusions on the available evidence at that time. However it will review fumonisins again when the results of current carcinogenicity studies become available.
A two-year National Toxicology Programme (NTP) rodent study requested by the US Food and Drug Agency has found that fumonisin B 1 can cause cancer. The 1999 draft report of the NTP study found fumonisins B 1 in high doses caused cancer in the kidneys of male rats and in the

livers of female mice and will be published shortly.

Zearalenone
Zearalenone is a non-steroidal, oestrogenic mycotoxin. JECFA has recently established a provisional tolerable maximum daily intake (PMTDI) of 0.5 microgram/Kg b.w. for zearalenone. f The COM considered zearalenone in 1997 as one of a number of compounds, which had been identified by the COC as mouse-specific carcinogens. The COM agreed in 1997 g that zearalenone should be provisionally considered as a potential genotoxic carcinogen and will review its opinion shortly.

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