Diet Study (TDS) – Mycotoxin Analysis

Final report

FS102081

February 2017

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1. Executive Summary

A total diet study (TDS) is representative of the whole diet. A TDS is different from many surveys in that foods are prepared for consumption (rather than being analysed as sold) before being pooled into groups before analysis. For this TDS, samples for 138 categories of foods established by the Food Standards Agency (FSA) were purchased from 24 local authorities (a total of 3312 samples). The categories were classified under twenty eight food groups and seventeen of these were analysed in the mycotoxin study. These included Bread, Miscellaneous Cereals, Offal, Oils and Fats, Eggs, Sugars and Preserves, Potatoes, Other Vegetables, Fresh Fruit, Fruit Products, Non-alcoholic Beverages, Milk, Dairy Products, Nuts, Alcoholic Drinks, Snacks and Sandwiches.

The samples were analysed for a range of mycotoxins; aflatoxins, ochratoxin A, fumonisins, patulin, zearalenone, trichothecenes, sterigmatocystin, ergot alkaloids, citrinin, cyclopiazonic acid and moniliformin. The 17 food group samples were also analysed for mycotoxins to compare with mathematical calculations carried out on the results for the food categories to check the homogeneity of the food groups.

The most frequently detected toxins were deoxynivalenol and ergot alkaloids which were detected in all bread samples and sandwiches as well as other cereal products. None of the samples exceeded any maximum permitted limit. Very few residues of any of the other mycotoxins analysed were found in the samples tested, most results were below the limits of quantification which were as low as technically achievable, and typically in the sub or low µg/kg range.

This is the first UK TDS study for mycotoxins. These results show very little incidence of mycotoxins in UK food samples, with very few results above the low limits of quantification. The data can be used for future intake calculations to calculate background exposure to various mycotoxins from the whole diet and also to compare exposures to those calculated by other sources.

2. Contents

3. Glossary

EMAN	European Mycotoxin Awareness Network
FB_1	Fumonisin B_1
FB_2	Fumonisin B_2
FB_3	Fumonisin B_3
Fera	Fera Science Ltd.
FSA	Food Standards Agency (UK)
HILIC	Hydrophobic Interaction Liquid Chromatography
HPLC	High Performance Liquid Chromatography
IAC	Immunoaffinity Column
LC-MS/MS	Liquid Chromatography tandem Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
MRM	Multiple Reaction Monitoring
m/z	Mass to charge ratio
PBS	Phosphate Buffered Saline
QC	Quality Control
RSD	Relative Standard Deviation
RSD_r	Repeatability
s : n	Signal to noise ratio
SOP	Standard Operating Procedure
TDS	Total Diet Study
UPLC-MS/MS	Ultra Performance Liquid Chromatography tandem Mass Spectrometry

4. Introduction

The key principle of a total diet study (TDS) is that it is representative of the whole diet. A TDS is different from many surveys in that foods are prepared for consumption (rather than being analysed as sold) before being pooled into groups before analyses. For this TDS, samples for each of the 138 categories of foods established by the FSA were purchased from 24 local authorities (a total of 3312 samples). Categories were grouped under twenty eight food groups so that commodities known to be susceptible to contamination (e.g. offal, fish) are kept separate, as are foods which are consumed in large quantities (e.g. bread, potatoes, milk). The relative proportion of foodstuffs in categories within a group (i.e. the amount of food in each category making up the pooled group sample) reflected its importance in the average UK household diet. This is based on three previous years of food purchase data from the Family Food Survey (previously the National Food Survey). The data from the family food survey is purchase data and not consumption data.

A TDS on the levels of metals and other elements has been carried out for the FSA (Fera FD Report 15/06). The samples collected for this main study were also used to measure the levels of mycotoxins in order to:

- calculate background exposure to various mycotoxins from the whole diet
- compare exposures to those calculated by other sources

The 28 food groups in the general TDS are Bread, Miscellaneous Cereals, Carcase Meat, Offal, Meat Products, Poultry, Fish, Oils and Fats, Eggs, Sugars and Preserves, Green Vegetables, Potatoes, Other Vegetables, Canned or Jarred Vegetables, Fresh Fruit, Fruit Products, Non-alcoholic Beverages, Milk, Dairy Products, Nuts, Alcoholic Drinks, Meat Substitutes, Snacks, Desserts Sandwiches, Condiments, Tap Water and Bottled water.

In the mycotoxin TDS, 17 of the 28 food groups which are known to have mycotoxin contamination were studied. These were Bread, Miscellaneous Cereals, Offal, Oils and Fats, Eggs, Sugars and Preserves, Potatoes, Other Vegetables, Fresh Fruit, Fruit Products, Non-alcoholic Beverages, Milk, Dairy Products, Nuts, Alcoholic Drinks, Snacks and Sandwiches.

5. Aims and Objectives

5.1. Scope

Samples prepared for the TDS on metals and other elements were used for this study. However not all mycotoxins were tested for in every food group and category.

The food groups included in the mycotoxin study are listed below:

Group 1 - Bread

Group 2 - Miscellaneous Cereals

Group 4 - Offal

Group 8 - Oils and Fats

Group 9 - Eggs

Group 10 - Sugars and Preserves

Group 12 - Potatoes

Group 13 - Other Vegetables

Group 15 - Fresh Fruit

Group 16 - Fruit Products

Group 17 - Beverages (With Mineral Water)

Group 18 - Milk

Group 19 - Dairy Products

Group 20 - Nuts

Group 21 - Alcoholic drinks

Group 23 - Snacks

Group 25 - Sandwiches

The categories that comprised each food group are shown in Table 1.

For consistency with previous total diet studies, the appropriate quantities of the homogenate from the 138 categories were combined into the respective food groups for analyses.

One of the problems with pooling the categories into food groups straight away is that contaminants are often diluted to the extent that they cannot be detected by the analytical method. As well as analysing the individual category samples, the food

groups were also analysed to compare with mathematical calculations carried out on the results for the 138 food categories to check the homogeneity of the food groups tested.

Table 1. Food groups and categories

Group		Category	
1	Bread	1	White sliced bread
		2	White unsliced bread
		3	Brown bread
		4	Wholemeal and granary bread
		5	Other bread
		NA	Group sample
2	Miscellaneous cereals	6	Flour
		7	Buns, cakes and pastries
		8	Savoury biscuits
		9	Sweet biscuits
		10	Chocolate biscuits
		11	Breakfast cereals
		12	Rice
		13	Other cereal products
		14	Pasta
		15	Pizza
		NA	Group sample
4	Offals	19	Lambs liver
		20	Pigs liver
		21	Other liver
		22	Kidney
		23	Other offals (excluding kidney and liver)
		NA	Group sample
8	Oils and fats	50	Vegetable oils
9	Eggs	53	Eggs
		54	Egg products
		NA	Group sample
10	Sugars and preserves	60	Chocolate confectionery
		61	Sugar confectionery
12	Potatoes	69	Fresh potatoes
		70	Potato products
		NA	Group sample
13	Other vegetables	71	Onions,leeks
		72	Carrots
		73	Turnips, swedes
		74	Other fresh vegetables
		75	Mushrooms
		76	Tomatoes
		77	Cucumbers
		78	Dried pulses
		79	Herbs, spices

Group		Category	
15	Fresh fruit	90	Oranges
		91	Other citrus fruits
		92	Apples
		93	Pears
		94	Stone fruit
		95	Bananas
		96	Grapes
		97	Other fresh fruit
		NA	Group sample
16	Fruit products	98	Canned peaches, pears, pineapples
		99	Other canned or frozen fruit
		100	Dried fruit
		101	Fruit juices and vegetable juices
		NA	Group sample
17	Non- alcoholic Beverages (with bottled water)	102	Tea
		103	Takeaway Tea
		104	Instant coffee
		105	Ground coffee
		106	Takeaway coffee
		107	Branded food drinks
		108	Cocoa, drinking chocolate
		113	Alternatives to milk
18	Milk	114	Whole (full fat) milk (cows)
		115	Skimmed/Semi skimmed milks (cows)
		NA	Group sample
19	Dairy products	116	Condensed milk or Evaporated Milk
		117	Instant milk
		118	Natural cheese
		119	Processed cheese
		120	Butter
		121	Ice-cream
		122	Yoghurt
		123	Other milk products
		124	Cream
		125	Canned milk puddings
		NA	Group sample
20	Nuts	126	Ground nuts including peanut butter
		127	Tree nuts
		NA	Group sample
21	Alcoholic drinks	128	Beer
		129	Cider
		130	Wine
		131	Alcopops and cocktails
23	Snacks	135	Other snacks (not potato based)
25	Sandwiches	138	Sandwiches
		NA	Group sample

The intention was that this approach would:

- provide specific data on mycotoxin concentrations at the food category level and food group level, rather than just at the food group level
- allow the FSA to refine exposure assessments for each food category
- allow the FSA to carry out exposure assessments for high consumers of specific foods

- help identify any ‘hotspots’ in particular food categories.

The samples were analysed for a range of mycotoxins; aflatoxins (B_1, B_2, G_1, G_2 and M_1), ochratoxin A, fumonisins (B_1, B_2 and B_3), patulin, zearalenone, trichothecenes (deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, nivalenol, fusarenon-X, diacetoxyscirpenol, neosolaniol, HT-2 toxin and T-2 toxin), sterigmatocystin, ergot alkaloids (ergocornine, ergocorninine, ergocristine, ergocristinine, ergocryptine, ergocryptinine, ergometrine, ergometrinine, ergosine, ergosinine, ergotamine and ergotaminine), citrinin, cyclopiazonic acid and moniliformin.

5.2. Sampling

Sampling was conducted by HallMark, contracted by the FSA. Samples were purchased throughout the UK and prepared according to a protocol agreed with the FSA. The protocol was intended to be suitable for metals/element analysis and was not designed for mycotoxin analysis.

5.3. Analysis

The samples were extracted using solvent and the cleaned-up extracts were analysed using the method that would allow the maximum sensitivity (lowest limit of quantification). In many cases, state of the art LC-MS/MS was used due to the high sensitivity and selectivity of the instrumentation, however HPLC-fluorescence was also used for some analyses where method performance was already well established or better sensitivity could be achieved.

In total 615 separate mycotoxin determinations were requested, and 634 results are reported.

5.4. Method Development and Validation

Many methods used were UKAS accredited. Where required, improved extraction and instrumental methods were developed. Validation samples were analysed to establish method repeatability and recovery but full single laboratory validation was not undertaken as there was insufficient time to do this for all the methods within the time constraints of this project.

5.5. Quality Control

Fully established UKAS accredited methods were used for this study where they were available. Blank samples and spikes were included. In some cases, the scope of these accredited methods did not cover all the matrices included in this study. In the cases where non-accredited methods were used quality control (QC) was increased by overspiking all test samples or by the inclusion of an isotopically labelled internal standard. For accredited methods, samples were batched into similar products with recovery adjustment being made using recovery determined for

the product type that was the closest match to the sample, or the group sample recovery was used to adjust for all the category samples that made up the group.

5.6. Reporting of Results

Results have been assessed and the recovery value calculated for individual samples, or group or close equivalent sample type. Each sample has been assessed and an individual limit of quantification (LOQ) calculated. This takes into account the apparent recovery from a spiked sample, therefore samples with a lower recovery will have a higher apparent LOQ than might be suggested by measuring signal to noise (s : n) from solvent calibration standards. This is a 'truer' representation of what could be measured in each sample type. All results are reported corrected for recovery and are in µg/kg.

6. Materials and Methods

6.1. Sampling

Sampling was conducted by another contractor appointed by the FSA. Details of the sampling plan and delivery schedule are in the report of the metals analysis of TDS samples. Samples were delivered to Fera in batches.

Samples were stored under suitable dry conditions, frozen if necessary, before being prepared and homogenised.

6.2. Sample Preparation

Sample preparation was carried out according to a previously agreed protocol for metals analysis (Metals TDS report FD Report 15/06). All samples were stored at -18°C after preparation until analysis.

6.3. Chemicals and Reagents

For extraction, acetonitrile (HPLC grade) (Sigma-Aldrich, Gillingham, UK) and water (18.2 MΩ/cm Purelab Ultra laboratory purification system) (Elga, Marlow, UK) were used. Methanol, acetonitrile, ammonium formate, formic acid, 99 % (UPLC/MS grade) (Biosolve, Dieuze, France via Greyhound, Birkenhead, UK) were used for eluent preparation for LC-MS/MS analysis.

To compensate for matrix effects during LC-MS/MS analysis, carbon-13 (^{13}C) isotopically labelled internal standards were used. The internal standards:

U-[$^{13}C_{18}$]-Sterigmatocystin in acetonitrile

U-[$^{13}C_{13}$]-Citrinin in acetonitrile

U-[$^{13}C_{20}$]-Cyclopiazonic acid in acetonitrile

U-[$^{13}C_{15}$]-Deoxynivalenol in acetonitrile

U-[$^{13}C_{7}$]-Patulin in acetonitrile

were purchased from Romerlabs, but were sourced from Biopure (Tulln, Austria). The analytical reference standards of sterigmatocystin, ochratoxin A, patulin, aflatoxins B_1, B_2, G_1, G_2 and M_1 and zearalenone were purchased as solid (dry film, Sigma Chemicals, ≥ 98 % purity). The analytical reference standards deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, nivalenol, fusarenon-X, diacetoxyscirpenol, neosolaniol, T-2 toxin, HT-2 toxin, citrinin, cyclopiazonic acid, moniliformin, fumonisin B_1, B_2 and B_3 were purchased as solutions in acetonitrile from Romerlabs, but were sourced from Biopure (Tulln, Austria). Ergot alkaloids (6 ine plus inine forms) were produced at IFA, Tulln and were gifted to Fera; further ergot alkaloids standards were purchased via Romerlabs from Biopure (Tulln, Austria).

Immunoaffinity columns for ochratoxin A, aflatoxins, zearalenone and fumonisins and Mycosep clean-up columns for trichothecenes were purchased from R-Biopharm (Rhone). Prototype citrinin immunoaffinity columns were a gift from R-Biopharm (Rhone). Dispersive SPE material (Bondesil PSA, 40 µm) was from Varian. Syringe filters (0.22 µm nylon, 13 mm) were from Anachem.

6.4. Extraction and Clean-Up

Targeted methods were used for each toxin or group of toxins however, where possible, common extraction and clean-up strategies were employed. In all cases the sample was extracted with organic solvent and water. This was followed by either a filtration or centrifugation step. The extract was diluted and cleaned-up or, for some LC-MS/MS methods, diluted and analysed directly (‘dilute and shoot’). Isotopically labelled (carbon-13) standards were used to internally standardise and adjust for matrix effects for LC-MS/MS methods where they were available.

UKAS accredited procedures for Quality Assurance, calibration and calculation of results were used.

Where LC-MS/MS was used the accredited SOP for LC-MS analysis was followed. This stipulates criteria for ion ratio and retention time acceptance following the criteria outlined in SANCO/12571/2013 (Pesticides criteria document).

Further details of the methods used are given below.

6.5. LC-MS/MS analysis

For all LC-MS/MS methods the following conditions and equipment were used:

Chromatograph: Waters Acquity UPLC

Autosampler: Waters Acquity UPLC

Weak needle wash:	80:20 (v/v) water : methanol
Strong needle wash:	20:40:40 (v/v/v) water : methanol : acetonitrile
Mass spectrometer:	Waters Xevo TQ-S triple quadrupole
Desolvation temperature:	500^{o}C
Desolvation gas flow rate:	1000 L/h
Nebuliser gas flow:	7 bar
Source temperature:	150 C
Cone gas flow rate:	100 L/h
Resolution:	Unit mass

6.5.1. UPLC Gradient “Curve”

In the tables for each method below a gradient “curve” is given. This is the gradient profile defined by the instrument software and are defined as follows.

Curve 6 is a linear change from one condition to the next.

Curve 1 is a step change in conditions at the start of the time segment.

Curve 5 is a variable rate of change from one condition to the next, with the rate of change slightly more rapid at the start of the time segment than at the end.

6.5.2. Data Analysis and Quantification

MassLynx software (Waters) was used for data-evaluation. Peak assignment and integration were manually verified by the operator. Quantification was based on multi-level calibration using solvent standards. Where available a ^{13}C-labelled internal standard was used to internally standardise the method, and inherently correct analytical results.

7. UKAS Accredited Methods

UKAS accredited protocols for the extraction and clean-up of samples for aflatoxins, ochratoxin A, fumonisins, patulin, zearalenone and trichothecenes were used, following the extraction procedure validated for the matrix under test, or for a similar matrix. The methods for ergot alkaloids and sterigmatocystin are also being submitted for UKAS accreditation under Extension of Scope.

7.1. Aflatoxins / Ochratoxin A

7.1.1. Extraction

Samples were extracted with a mixture of organic solvent and water, or sodium hydrogen bicarbonate solution depending on the matrix, using protocols from in-house methods FSG 251, FSG 252 and FSG 261. Following dilution with phosphate buffered saline (PBS) extracts were cleaned up by immunoaffinity column, using either Afla/Ochra columns where both analytes were required, or Aflaprep, or Ochraprep columns where only one analyte was requested. Most samples were spiked at 5 µg/kg each aflatoxin and ochratoxin A, alcoholic beverages were spiked at 2 µg/kg. Where the method was not accredited for the matrix, each sample was overspiked.

Samples in Group 19 Dairy products were analysed for aflatoxin M_1, using in-house method FSG 300, and were cleaned up with Aflaprep columns. Milk samples and samples 116 and 117 (condensed milk and instant milk) were analysed using in house method FSG 253.

7.1.2. HPLC Analysis

Cleaned up extracts were analysed by reversed phase HPLC with fluorescence detection, either using a method for aflatoxins B_1, B_2, G_1 and G_2, or ochratoxin A, or a multi method capable of detecting all compounds. Post-column derivatisation using a KOBRA cell was used to derivatise aflatoxin B_1 and G_1. The HPLC pump to deliver mobile phase was set at 1.0 mL/min, a Gilson automatic sample processor and injector (or equivalent) and a Fluorescence detector with programmable functions (Jasco FP1520 or similar) were used. Calibration standards in the range equivalent to 1 to 5 µg/kg for each toxin were used.

The HPLC column was a Spherisorb ODS1-Excel (25 cm x 4.6 mm i.d.), 250 Å pore size, 5 µm spherical particles (or equivalent).

Mobile Phase A: Acetonitrile : methanol : 0.1 % orthophosphoric acid solution (24 : 24 : 52, v/v/v).

Mobile Phase B: Acetonitrile : methanol : 0.1 % orthophosphoric acid solution (14.4 : 54.4 : 31.2, v/v/v).

The gradient profile used for joint aflatoxin/ochratoxin A analysis is given in Table 2.

Table 2. Gradient profile for joint aflatoxin / ochratoxin A method.

Time	Mobile phase A	Mobile phase B
0 to 17 minutes	100 %	0 %
19 to 29 minutes	0 %	100 %
31 to 40 minutes	100 %	0 %

Aflatoxin M_1 samples were analysed by HPLC with fluorescence detection. The HPLC column was a Spherisorb ODS2-Excel (25 cm x 4.6 mm i.d.), 5 µm particle size, 250 Å pore size (or equivalent) with guard column (C_{18}, 2.5 cm x 4.6 mm i.d.). Mobile phase of water : methanol : acetonitrile (60 : 10 : 30, v/v/v) was pumped at 1 mL/min. Calibration standards in the range equivalent to 0.02 to 0.1 µg/kg were used.

7.1.3. HPLC Detection

For aflatoxin / ochratoxin A the programmable fluorescence detector was set to the following parameters:

Excitation = 364 nm, Emission = 440 nm from 0 to 18 min

Excitation = 333 nm, Emission = 477 nm from 18 min onwards

For aflatoxin M_1 the fluorescence detector was set at:

Excitation = 364 nm, Emission = 434 nm.

7.2. Zearalenone

7.2.1. Extraction

Analysis was carried out following in-house method FSG 258. Sample (12.5 g) was weighed into a plastic beaker, and extraction solution of acetonitrile : water (75 : 25, v/v) added. Samples were homogenised for 3 minutes with an Ultra Turrax set at high speed, then filtered through Whatman No. 4 or Whatman 113V (fluted filter). An aliquot (12 mL) of filtrate was diluted with 88 mL of PBS in a conical flask. The diluted filtrate was transferred to a pre-labelled tube for automated immunoaffinity column clean-up using a ZONprep IAC.

For oil, 2.0 g oil was weighed into a centrifuge tube and 2 mL hexane and 20 mL methanol/ammonium carbonate solution added. This was vortex mixed then shaken for 15 minutes. The extracts were centrifuged at 4000 rpm, 4 °C for 10 minutes and then 10 mL of the upper methanol-water layer removed and adjusted to pH 6 to ≤ 7.5. An aliquot (5 mL) of the extract was evaporated to dryness under nitrogen, and redissolved in 1 mL HPLC mobile phase. The extract was analysed by HPLC.

Samples were spiked at a level equivalent to 50 µg/kg. For accredited matrices samples were grouped and a representative sample spiked, for non-accredited matrices each sample was overspiked.

7.2.2. HPLC Analysis

Clean-up and analysis were carried out automatically by a Gilson ASPEC system (except for oils). The HPLC column was a Spherisorb ODS2-Excel (25 cm x 4.6 mm i.d.), 5 µm particle size, 250 Å pore size or equivalent, with a mobile phase of water : acetonitrile (45 : 50 v/v) pumped at 1 mL/min. Detection was by fluorescence with excitation and emission wavelengths of 275 and 450 nm. Calibration range was equivalent to 2.5 to 50 µg/kg.

7.3. Fumonisins

7.3.1. Extraction

Samples were extracted following in-house method FSG 264. Sample (12.5 g) was weighed into a beaker. Extraction solvent (62.5 mL, water : acetonitrile : methanol, 50 : 25 : 25, v/v/v) was added and the sample homogenised using an Ultra Turrax blender for approximately 5 minutes. The sample was filtered through 113V filter paper, then an aliquot (10 mL) transferred into a flask containing 40 mL of PBS. This was mixed then filtered through microfibre filter paper.

For oil, 12.5 g was measured into a separating funnel and shaken with 62 mL of extraction solvent (0.1 M o-phosphoric acid solution : acetonitrile : methanol, 50 : 25 : 25, v/v/v). The bottom, aqueous, layer was drained into a flask, 10 mL was diluted with 40 mL PBS and filtered through microfibre filter paper.

Samples were cleaned-up by immunoaffinity column. Fumonisins were eluted with 1.5 mL of methanol followed by 1.5 mL of water. An aliquot was filtered through a 0.22 µm syringe filter and transfer to a vial for LC-MS/MS analysis. Spiking level was at 250/171/125 µg/kg FB_1/FB_2/FB_3 and 100/50/50 µg/kg FB_1/FB_2/FB_3 for the two batches.

7.3.2. LC-MS/MS Analysis

Cleaned-up extracts were analysed by UPLC-MS/MS. Fumonisins B_1, B_2 and B_3 were included in the method. The column was a Waters Acquity HSS T3 1.8 µm (100 x 2.1 mm) at a column temperature of 40 ^oC. The injection volume was 2 µL. The following UPLC gradient was used:

Mobile phase A: 0.1 % formic acid in water

Mobile phase B: 0.1 % formic acid in 1:1 (v/v) methanol : acetonitrile

The gradient profile used is given in Table 3. MRM transitions are given in Table 4.

Table 3. Gradient profile for Fumonisin analysis.

Time / min	% B	Flow rate / mL/min	Curve
0	15	0.4	-
4.5	55	0.4	6
5.5	99	0.4	6
5.6	99	0.6	6
7.0	99	0.6	6
7.1	15	0.4	6
9.0	15	0.4	6

Table 4. MRM Transitions for Fumonisin analysis.

Compound	Parent	Fragment	Cone Voltage / V	Collision Energy / eV
Fumonisin B_1	722.4	334.2	40	37
		352.2		34
Fumonisin B_2	706.4	336.2	40	34
		318.2		37
Fumonisin B_3	706.4	336.2	40	34
		354.2		32

MassLynx software (Waters) was used for data-evaluation. Peak assignment and integration were manually verified by the operator. Quantification was based on multi-level calibration using solvent standards of fumonisins. Concentrations corresponding to 40/20/20 to 500/250/250 µg/kg FB_1/FB_2/FB_3 in the sample were used in one batch and 5/2.5/2.5 to 250/125/125 µg/kg used in the second.

7.4. Trichothecenes

The trichothecenes included in the study were deoxynivalenol, 3-acetyl-deoxynivalenol, 15-acetyldeoxynivalenol, nivalenol, fusarenon-X, diacetoxyscirpenol, neosolaniol, HT-2 toxin and T-2 toxin.

7.4.1. Extraction

10 g of sample was weighed into a beaker, and 50 mL of extraction solvent (acetonitrile : water, 84 : 16, v/v) was added. This was homogenised using an Ultra Turrax blender for approximately 5 minutes. Extracts were filtered through 113V filter paper. An aliquot (6-7 mL) of the filtered extract was transferred into a glass tube supplied with the charcoal/alumina column. The clean-up column was pushed through the extract at a rate of about 1 mL per 30 seconds until approximately 3-4 mL of cleaned up extract has collected above the bed of the clean-up column. 4 mL of cleaned-up filtrate was transferred into a vial. The cleaned-up filtrate was

evaporated to dryness under nitrogen then re-dissolved in methanol : water (20 : 80, v/v) and filtered through a syringe filter prior to LC-MS/MS analysis.

Selected samples were re-analysed to confirm initial results, at which time a ^{13}C-labelled internal standard for deoxynivalenol was included to account for matrix effects.

7.4.2. LC-MS/MS analysis

Samples were analysed by UPLC-MS/MS. The column was a Restek Raptor Biphenyl 2.7 µm (100 x 2.1 mm) maintained at 40 °C. The injection volume was 5 µL. The following UPLC gradient was used:

Mobile phase A: 1 mM ammonium formate in water

Mobile phase B: 1:1 (v/v) methanol : acetonitrile

The gradient profile used is given in Table 5. Nine compounds were included in the method and the MRM Transitions used are given in Table 6.

MassLynx software (Waters) was used for data-evaluation. Peak assignment and integration were manually verified by the operator. Quantification was based on multi-level calibration using solvent standards of trichothecenes (concentrations corresponding to 5, 10, 25, 50, 100 and 200 µg/kg in the sample, internal standard at 50 µg/kg). Samples were spiked at 100 µg/kg each trichothecene.

Table 5. Gradient profile used for trichothecene analysis.

Time / min	% B	Flow rate / mL/min	Curve
0	5	0.3	-
0.5	5	0.3	6
3.5	30	0.3	5
8.5	70	0.3	6
9.0	99	0.3	1
11.5	99	0.5	1
13.0	5	0.4	1
13.5	5	0.3	6

Table 6. MRM Transitions used for trichothecene analysis.

Compound	Parent	Fragment	Cone Voltage / V	Collision Energy / eV
Deoxynivalenol	297.1	249.1	20	10
		231.1		12
$^{13}C_{15}$-Deoxynivalenol	312.1	263.1	20	10
		245.1		12
Nivalenol	313.1	175.0	15	19
		125.0		12
3- and 15-Acetyl-Deoxynivalenol	339.1	231.0	30	12
		203.0		16
Fusarenon X	355.1	247.1	20	12
		229.0		15
Diacetoxyscirpenol	384.2	307.2	20	10
		247.1		13
Neosolaniol	400.2	305.1	20	11
		215.1		18
HT-2 toxin	442.4	263.1	20	11
		215.1		12
T-2 toxin	484.3	305.1	20	13
		215.1		18

7.5. Ergot Alkaloids

The ergot alkaloids included in the study were ergocornine, ergocorninine, ergocristine, ergocristinine, ergocryptine, ergocryptinine, ergometrine, ergometrinine, ergosine, ergosinine, ergotamine and ergotaminine.

7.5.1. Extraction

The method used for ergot alkaloids has been validated and published (Krska et al, 2008). In-house method FSG 601 was used. Samples (5 g) were extracted with a mixture of acetonitrilie : ammonium carbonate solution (84:16, v/v) on an orbital shaker for 30 minutes. After shaking the sample extracts were filtered. 1 mL of sample was removed, and transferred into a 4 mL amber glass vial containing 50 mg of Varian Bondesil solid phase material. Samples were vortex mixed then an aliquot filtered through a 13 mm PTFE 0.22 µm filter into a 2 mL amber vial and transferred to a 200 µL vial ready for UPLC-MS/MS.

7.5.2. LC-MS/MS Analysis

Samples were analysed using a modified version of the published LC-MS/MS method. The method was capable of detecting all 6 major ergot alkaloids and their -inine epimers and used alkaline mobile phase conditions to prevent unwanted epimerisation. The column was a Waters Acquity BEH C_{18} 1.7 µm (100 x 2.1 mm) at 40 ^{o}C. The injection volume was 2 µL. The mobile phase and gradient profile were as follows:

Mobile phase A: 200 mg/L ammonium carbonate in water

Mobile phase B: Acetonitrile

The gradient profile used is given in Table 7.

Twelve compounds were included in the method and the MRM transitions used are given in Table 8.

MassLynx software (Waters) was used for data-evaluation. Peak assignment and integration were manually verified by the operator. Quantification was based on multi-level calibration using solvent standards of ergot alkaloids (concentrations corresponding to 1 to 200 µg/kg equivalent in the sample, samples were spiked at 50 µg/kg).

Table 7. Gradient profile used for ergot alkaloid analysis.

Time / min	% B	Flow rate / mL/min	Curve
0	5	0.5	-
1.5	45	0.5	6
3.5	50	0.5	6
6.0	70	0.5	6
9.0	99	0.5	1
12.0	5	0.5	1

Table 8. MRM transitions used for ergot alkaloid analysis.

Compound	Parent	Fragment	Cone Voltage / V	Collision Energy / eV
Ergometrine	326.2	223.1	30	24
		208.1		27
Ergometrinine	326.2	208.1	30	27
		223.1		24
Ergosine	548.3	223.1	30	30
		208.1		40
Ergosinine	548.3	223.1	30	30
		208.1		40
Ergocornine	562.3	268.1	30	23
		223.1		35
Ergocorninine	562.3	544.3	30	14
		223.1		35
		277.1		25
Ergocryptine	576.3	268.1	30	24
		223.1		35
Ergocryptinine	576.3	558.3	30	14
		223.1		35
		305.2		28
Ergotamine	582.3	223.1	30	32

Compound	Parent	Fragment	Cone Voltage / V	Collision Energy / eV
		208.1		40
Ergotaminine	582.3	564.3	30	14
		223.1		32
		277.1		25
Ergocristine	610.3	223.1	30	35
		208.1		45
Ergocristinine	610.3	592.3	30	13
		223.1		35
		305.2		26

7.6. Patulin

7.6.1. Extraction

The extraction procedure in the UKAS accredited in-house method FSG 254 was used with some minor modifications. The method is only accredited for apple juice, other fruit juices and apple puree. Sample (10 g) was weighed, and extracted with ethyl acetate. The ethyl acetate was washed with aqueous sodium hydrogen carbonate solution which was discarded. Acetic acid was added to the ethyl acetate and this was evaporated to dryness and extracts redissolved in water adjusted to pH 4 by addition of acetic acid.

For fruit and vegetable samples the method for apple puree was followed. A portion of sample was weighed, mixed with water and incubated with pectinase enzyme. After centrifugation the supernatant was extracted with ethyl acetate as above. ^{13}C-labelled patulin internal standard was added to all samples prior to extraction.

7.6.2. LC-MS/MS Analysis

Samples were analysed by UPLC-MS/MS. The column was a Waters Acquity HSS T3 1.8 µm (100 x 2.1 mm), maintained at 40 ^{o}C, with mobile-phase gradient of 1 mM ammonium formate in water and methanol : acetonitrile (50:50 v/v) from 99:1 (0 min) to 70:30 in 3 min, then to 1:99 in 3 min, and back to 99:1 by 8 min. The injection volume was 5 µL.

For patulin, using $[M+H]^+$ (m/z 153) as precursor ion, two transitions were measured: fragment m/z 109 (35 V) [quantification ion], and m/z 81 (35 V). For the isotopic label ($^{13}C_7$), 160 m/z was used as precursor ion with m/z 115 (35 V) as product ion.

MassLynx software (Waters) was used for data-evaluation. Peak assignment and integration were manually verified by the operator. Quantification was based on multi-level calibration using solvent standards of patulin (concentrations corresponding to 2, 5, 10, 25, 40 and 50 µg/kg in the sample, internal standard at 25 µg/kg). Since the internal standard was added to the sample before extraction, it

corrected for both recovery and matrix effects. Hence in this case, for positive samples, the concentration found was inherently corrected for the recovery.

7.7. Sterigmatocystin

7.7.1. Extraction

To 5 g dry milled homogenate 20 mL of acetonitrile : water (80 : 20 v/v) was added and the sample extracted using an orbital shaker for 2 hours. After centrifugation, 500 µL of the clear supernatant was transferred into a vial and diluted with 500 µL acetonitrile : water 20 : 80 v/v. The extract was filtered using a 0.22 µm syringe filter into an autosampler vial and analysed by LC-MS/MS.

Isotopically labelled sterigmatocystin in acetonitrile was added (equivalent to 1.5 µg/kg) was added to the sample prior to extraction.

7.7.2. LC-MS/MS Analysis

Sample extract (2 µL) was injected into an UPLC-MS/MS system. Separation was performed on a Waters Acquity HSS T3 1.8 µm (100 x 2.1 mm) maintained at 40 ^{o}C, with a mobile phase gradient of 1 mM ammonium formate in water and methanol : acetonitrile (50 : 50, v/v) from 95:5 (0.2 min) to 5 : 95 in 3 min, then isocratic for 3.6 min. The flow rate was 0.4 mL/min.

For sterigmatocystin, using $[M+H]^+$ (m/z 325) as precursor ion, two transitions were measured: m/z 310 (23 eV) [quantification ion], and m/z 281 (35 eV). For the isotopic label ($^{13}C_{18}$), m/z 343 was used as precursor ion with m/z 297 (36 eV) as product ion.

MassLynx software (Waters) was used for data-evaluation. Peak assignment and integration were manually verified by the operator. Quantification was based on multi-level calibration using solvent standards of sterigmatocystin (concentrations corresponding to 0.20, 0.50, 1.5, 2.5, 5.0 and 10 µg/kg in the sample, internal standard at 1.5 µg/kg). Responses in extracts and standards were normalized to the internal standard. Since the internal standard was added to the sample before extraction, it corrected for both recovery and matrix effects. Hence in this case, for positive samples, the concentration found was inherently corrected for the recovery.

7.8. Citrinin

7.8.1. Extraction

Samples were extracted with methanol and water, and after filtration the extract was diluted with water adjusted to pH 7.4 and cleaned up by immunoaffinity column. 5 g sample was weighed and ^{13}C-citrinin internal standard added at a level equivalent to 25 µg/kg. 25 mL extraction solvent methanol : water (75 : 25, v/v) was added and samples were mixed by vortex mixing then placed on a shaker for 30 minutes. After extraction samples were filtered through Whatman 113V equivalent filter paper and 2 mL of filtered extract was added to 18 mL PBS. This was mixed well then filtered

through GFA filter paper. Extracts were cleaned up using immunoaffinity columns (RBiopharm Rhone columns). Samples were eluted in 2 mL methanol, then this was evaporated to dryness under nitrogen and redissolved in 1 mL methanol : water (1 : 1, v/v). The sample was transferred to a vial for LC-MS/MS analysis.

7.8.2. LC-MS/MS Analysis

Samples were analysed by LC-MS/MS using acidic mobile phase conditions. The ^{13}C-citrinin standard was used to internally standardise the method and adjust for matrix effects. The column used was a Waters Acquity HSS T3 1.8 µm (100 x 2.1 mm), at 40 ^{o}C. The injection volume was 5 µL. The following UPLC gradient profile was used:

Mobile phase A: 0.1 % formic acid in water

Mobile phase B: 0.1 % formic acid in 1 : 1 (v/v) methanol : acetonitrile

The gradient profile used in given in Table 9 and the MRM transitions used are in Table 10.

Table 9. Gradient profile used for citrinin analysis.

Time / min	% B	Flow rate / mL/min	Curve
0	15	0.4	-
4.5	55	0.4	6
5.5	99	0.4	6
5.6	99	0.6	6
7.0	99	0.6	6
7.1	15	0.4	6
9.0	15	0.4	6

Table 10. MRM Transitions for citrinin analysis.

Compound	Parent	Fragment	Cone Voltage / V	Collision Energy / eV
Citrinin	251.1	233.1	25	16
	251.1	205.1		26
	251.1	91.0		40
$^{13}C_{13}$-Citrinin	264.1	246.1	25	16

MassLynx software (Waters) was used for data-evaluation. Peak assignment and integration were manually verified by the operator. Quantification was based on multi-level calibration using solvent standards of citrinin (concentrations

corresponding to 1, 2.5, 5, 10, 25, and 50 µg/kg in the sample, internal standard at 25 µg/kg).

7.9. Cyclopiazonic acid and Moniliformin

7.9.1. Extraction

Samples were extracted using a common extraction solvent of acetonitrile : water : acetic acid (79 : 20 : 1, v/v/v). 20 mL of extraction solvent was added to 5 g sample and samples were shaken for 2 hours. Carbon-13 labelled cyclopiazonic acid was added to all samples before extraction at 25 µg/kg. After extraction samples were centrifuged, diluted with an equal volume of acetonitrile : water : acetic acid (20 : 79 : 1), then filtered prior to analysis by LC-MS/MS using separate methods for each analyte.

7.9.2. Cyclopiazonic Acid – LC-MS/MS Analysis

Samples were analysed by LC-MS/MS using an alkaline mobile phase. Carbon-13 labelled cyclopiazonic acid was used to internally standardise the method and compensate for matrix effects. The column used was a Waters Acquity BEH C_{18} 1.7 µm (100 x 2.1 mm), at 40 ^{o}C. The injection volume was 3 µL. The following UPLC gradient profile was used:

Mobile phase A: 200 mg/L ammonium carbonate

Mobile phase B: 1:1 (v/v) methanol : acetonitrile

The gradient profile used is given in Table 11 and the MRM transitions used are given in Table 12.

Table 11. Gradient profile used for cyclopiazonic acid analysis.

Time / min	% B	Flow rate / mL/min	Curve
0	10	0.4	-
4.0	50	0.4	6
4.5	99	0.4	6
5.0	99	0.6	6
7.0	99	0.6	6
8.9	10	0.6	1
9.0	10	0.4	6

Table 12. MRM Transitions used for cyclopiazonic acid analysis.

Compound	Parent	Fragment	Cone Voltage / V	Collision Energy / eV
Cyclopiazonic acid	337.2	196	30	22
		182		18
$^{13}C_{20}$-Cyclopiazonic acid	357.2	210	30	22

MassLynx software (Waters) was used for data-evaluation. Peak assignment and integration were manually verified by the operator. Quantification was based on multi-level calibration using solvent standards of cyclopiazonic acid (concentrations corresponding to 1, 2.5, 5, 10, 25, and 50 µg/kg in the sample, internal standard at 25 µg/kg).

7.9.3. Moniliformin– LC-MS/MS Analysis

Samples were analysed by LC-MS/MS using HILIC chromatography. The column used was a SeQuant ZIC-HILIC 5 µm 200 Å (150 x 2.1 mm), with a column filter (Phenomenex KrudKatcher 0.5 µm). The column temperature was 40 °C and the injection volume was 5 µL. The following gradient profile was used:

Mobile phase A: 50 mM ammonium formate in water

Mobile phase B: Acetonitrile

The gradient profile used is given in Table 13. It was only possible to determine one transition for moniliformin, the MRM transition information is given in Table 14.

Table 13. Gradient profile for moniliformin analysis.

Time / min	% B	Flow rate / mL/min	Curve
0	95	0.2	-
5.0	50	0.2	6
8.0	50	0.2	6
8.1	95	0.2	6
23.0	95	0.2	6

Table 14. MRM Transition for moniliformin analysis.

Compound	Parent	Fragment	Cone Voltage / V	Collision Energy / eV
Moniliformin	97.0	41.0	35	12

MassLynx software (Waters) was used for data-evaluation. Peak assignment and integration were manually verified by the operator. Quantification was based on multi-level calibration using solvent standards of moniliformin (concentrations corresponding to 1, 2.5, 5, 10, 25, and 50 µg/kg in the sample, samples were overspiked with moniliformin at a level of 25 µg/kg prior to extraction).

7.10. Validation Analyses

Six spiked samples were used to establish recovery and repeatability for citrinin, cyclopiazonic acid and moniliformin. The validation was performed by spiking six replicates of a cereal sample at 25 µg/kg. Samples used in the validation were also analysed without spiking, and where internal standard was used, a double blank with no internal standard was also analysed.

The linearity of the LC-MS/MS measurement was established through calibration standards in solvent, covering the relevant concentration range. From these initial in-house validations, the linearity, recovery, repeatability, selectivity, LOQ and limit of detection (LOD) were derived. In addition, the stability of retention time and ion ratios in solvent standards and extracts were determined.

The LOD is defined here as the level corresponding to a signal-to-noise ratio (s : n) of three.

Identification of analytes was based on retention time and ion ratio of coinciding peaks for at least two diagnostic transitions in the correct abundance ratio. The LC-MS SOP states that Retention Time should be within 2.5 % of the mean of the standards. The ion ratio of the two diagnostic ions (least abundant/most abundant) in the samples should be consistent with that obtained during validation and not deviate more than ± 20 %.

7.11. Quality Control

With each batch of survey samples, one or more spiked samples were included to assess method performance and recovery for different commodities. In many analyses every sample was spiked with the compound of interest and/or the isotopically labelled internal standard where it was used. For some analyses samples were grouped into similar types and spikes were made into one sample chosen to be representative of the group.

7.12. Reporting of Results

Where labelled internal standard was added to the sample before extraction recovery correction was inherent to the procedure.

For samples spiked with parent compound the recovery for each sample or group of samples was calculated and used to correct the results.

In the majority of cases the LOQ was set at level equivalent to the bottom calibrations standard (as stated in the LC-MS/MS SOP), as long as the s : n was good enough (>10 : 1). Some LOQs could be lower based on s : n. LOD was determined by s : n but was also influenced by carry-over and background peaks. Limits of quantification were calculated from signal to noise, and were adjusted for the recovery measured. Results were expressed as a numerical value corrected for recovery where the residue was above the LOQ. Where a residue was seen below the LOQ but above the LOD the result was reported as less than LOQ (<LOQ) and the value found and the LOQ given. Where no residue was detected the result was reported as <LOD, and the calculated (corrected) LOD given.

8. Results

8.1. Existing Mycotoxins Methods

Various procedures were already established in-house for the extraction of different mycotoxins from samples. In many cases it was possible to apply these methods without deviation from the SOP, however the diverse range of sample matrices resulted in the need for adaptations to the extraction procedures.

A generic extraction procedure has also been established for a broad range of mycotoxins in a multi-mycotoxin LC-MS/MS suite. This involves extraction by shaking in 20:79:1 water : acetonitrile : acetic acid followed by centrifugation, dilution in an equal volume of 79:20:1 water : acetonitrile : acetic acid and filtration of the supernatant. This method was also used or adapted for some of the analytes in this study.

Using the multi-mycotoxin method, mycotoxins extracts are analysed using an LC-MS/MS method which is split into two parts, one with neutral pH mobile phase, which is used for the majority of the analytes in the suite, and one with acidic pH mobile phase which is required to improve the peak shape or response of selected analytes. For some analytes these two multi-mycotoxin methods were adapted in order to maximise response and/or throughput. For some analytes it was necessary to develop a new LC-MS/MS method from scratch. The method used has been described fully in the previous section. Modifications and the reason they were made are given in the results section below for each analyte.

Fully tabulated results are given in Appendix 1. Tables of individual results are given below. Aflatoxins, ochratoxin A, zearalenone, fumonisins, trichothecenes and patulin

methods are already UKAS accredited, although not for all the matrices included in this study. Methods performed well and met QC criteria for plate count, linear regression, and in most cases recovery. Due to the variability in sample types extra spiked samples were included, and in many cases an overspike was carried out for each category sample.

LC-MS/MS analysis is also accredited to UKAS as the accredited protocol for data review, ion ratio for confirmation, and retention time requirements were met in the majority of cases, and where they were not this is indicated in the results tables. In each case the data has been analysed manually and assessed to allow as low an LOD as possible to be calculated for these samples. This is not done routinely in the accredited method to make the method more time efficient but it was thought to be necessary in this case to achieve as low an LOQ as possible. In many cases the range of the calibration series has been changed so the lowest calibration standard is lower than used for normal practice to allow lower LOQs to be reported. The QC data is summarised in Table 15 and Table 16, this gives the range, mean and median of recovery, the LOD where it was possible to calculate it, and LOQ. For those analytes where only LOQ was reported the reason for this is given.

Table 15. Summarised Recovery and LOD/LOQ data.

Analyte	3-Acetyl-Deoxynivalenol	15-Acetyl-Deoxynivalenol	Deoxynivalenol	Diacetoxyscirpenol	Fusarenon_X	HT2_Toxin	Neosolaniol	Nivalenol	T2_Toxin
Recovery Range	14-82 %	9-92 %	3-106 %	22-122 %	12-91 %	19-100 %	21-109 %	15-59 %	13-140 %
Mean Recovery	55%	44%	41%	84%	51%	75%	80%	34%	90%
Median Recovery	57%	42%	37%	86%	52%	76%	83%	34%	99%
LOD Range / µg/kg	1.10-7.62	5.45-55.94	1.39-33.25	0.08-0.46	0.70-2.29	1.00-5.39	0.46-2.34	8.51-45.26	0.10-0.78
LOQ Range / µg/kg	6.08-36.70	5.45-55.94	4.73-166.67	4.11-22.83	5.52-40.35	4.98-26.94	4.57-23.40	8.51-33.54	3.58-38.94

Analyte	Fumonisin B1	Fumonisin B2	Fumonisin B3	Zearalenone	Sterigmatocystin	Patulin	Cyclopiazonic Acid
Recovery Range	13-83 %	14-92 %	16-96 %	26-102 %	37-133 %	15-115 %	74-150 %
Mean Recovery	72%	78%	88%	64%	79%	51%	107%
Median Recovery	75%	84%	90%	70%	78%	53%	107%
LOD Range / µg/kg	3.87-37.05	3.24-36.84	3.12-31.50	0.29-1.15	0.15-0.54	1.74-13.58	0.50
LOQ Range / µg/kg	6.49-296.43	5.41-147.38	5.20-125.99	2.46-9.56	0.15-0.54	4.35-33.95	0.67-1.35

Table 16. Summarised Recovery and LOQ data.

Analyte	Ergocornine	Ergocorninine	Ergocristine	Ergocristinine	Ergocryptine	Ergocryptinine	Ergometrine	Ergometrinine	Ergosine	Ergosinine	Ergotamine	Ergotaminine
Recovery Range	78-103 %	70-111 %	77-116 %	78-129 %	77-104 %	78-117 %	80-114 %	82-116 %	82-138 %	76-106 %	80-112 %	77-120 %
Mean Recovery	97%	101%	108%	115%	98%	108%	97%	93%	93%	98%	102%	110%
Median Recovery	100%	111%	112%	113%	99%	111%	93%	95%	92%	99%	106%	111%
LOQ Range / µg/kg	0.25-1.14	0.25-0.90	0.25-1.02	0.25-0.88	0.25-1.05	0.25-0.90	0.91-1.26	0.55-1.0	0.25-0.85	0.25-0.85	0.25-0.89	0.25-1.00
Why LOQ?	Background response	Background response	Background response	Background response	Background response	Background response	Background response	Background response	Background response	Background response	Background response	Background response

Analyte	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2	Ochratoxin A	Aflatoxin M1	Citrinin	Moniliformin
Recovery Range	9-116 %	12-158 %	8-103 %	10-116 %	32-100 %	43-85 %	46-93 %	3-76 %
Mean Recovery	78%	93%	70%	67%	78%	57%	78%	20%
Median Recovery	82%	96%	81%	80%	82%	60%	79%	8%
LOQ Range / µg/kg	0.17-2.26	0.14-1.60	0.19-2.41	0.17-1.97	0.20-0.56	0.02-0.05	1.07-2.16	1.31-39.50
Why LOQ?	Usual method of reporting	Usual method of reporting	Usual method of reporting	Usual method of reporting	Usual method of reporting	Usual method of reporting	Peaks in several samples	Peaks in most samples

8.2. Validation of New Methods

The sterigmatocystin method was validated as part of another project (FSA/EFSA sterigmatocystin survey).

The methods for citrinin, cyclopiazonic acid and moniliformin were each validated by the analysis of replicate spiked samples (n = 6) once the optimum sample preparation and analysis conditions had been developed. Results for the validation of these methods are given in the results section for each of the analytes in the sections below.

8.3. Aflatoxins

Aflatoxins were analysed using the established accredited method, although the method has not been fully validated for all the matrices tested in this study. The results for aflatoxins are summarised in Table 17 and Table 18. Aflatoxin B_1, B_2, G_1 and G_2 were not detected in any sample. Limits of quantification calculated ranged from 0.13 µg/kg for aflatoxin B_2 in dried fruit to 2.41 µg/kg for aflatoxin G1 in sugar confectionary. The majority of the calculated limits of quantification were at or around 0.2 to 0.3 µg/kg. The higher limits of quantification were a result of lower recovery values.

All dairy and egg products were analysed for aflatoxin M_1 only, as this is the metabolite of aflatoxin B_1 that would be present in these products if the animals had been exposed to significant aflatoxin concentrations in their diets. None of the samples analysed for aflatoxin M_1 contained residues above the calculated LOQs that ranged from 0.02 to 0.05 µg/kg. Offal samples were analysed for aflatoxins B_1, B_2, G_1 and G_2 as these are the analytes these products are routinely test for in the UK for the Statutory Veterinary Residues Monitoring programme under EC Directive 96/23 - Measures to monitor certain substances and residues thereof in live animals and animal products.

8.4. Ochratoxin A

Ochratoxin A was analysed using the established accredited method, although the method has not been fully validated for all the matrices tested in this study. The results for ochratoxin A are summarised in Table 17 and Table 18. Five samples contained residues of ochratoxin A. The highest level found was 5.6 µg/kg in fruit & vegetable juices, although this result was corrected for a low recovery (32 %). The second highest value was 1.65 µg/kg in the dried fruit samples (100 % recovery). The other samples that contained residues were herbs and spices (0.63 µg/kg), brown bread (0.53 µg/kg) and granary bread (0.45 µg/kg). All levels measured were below the maximum permitted levels according to Regulation (EC) 1881/2006 (and amendments).

Table 17. Aflatoxins and Ochratoxin A Results – Part 1.

Group		Category		LIMS Number		Ochratoxin / Aflatoxin Concentration / µg/kg					Aflatoxin M1 Concentration / µg/kg
						Concentrations are corrected for recovery. ' QC cereal sample recovery value. " Sweet biscuits recovery value. "' Breakfast cereals recovery value. "" Rice recovery value. """ Group sample recovery value. ^ Liver validation spikes recovery value. ^^ Only one result because the category and group samples are identical.					Concentrations are corrected for recovery. ' Group sample recovery value. " Whole milk sample recovery value.
						Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2	Ochratoxin A	
1	Bread	1	White sliced bread	S14-042856	Result	< LOQ (< 0.24)	< LOQ (< 0.18)	< LOQ (< 0.19)	< LOQ (< 0.25)	< LOQ (< 0.22)	
					Recovery	82% '	112% '	103% '	80% '	91% '	
		2	White unsliced bread	S14-042857	Result	< LOQ (< 0.24)	< LOQ (< 0.18)	< LOQ (< 0.19)	< LOQ (< 0.25)	< LOQ (< 0.22)	
					Recovery	82% '	112% '	103% '	80% '	91% '	
		3	Brown bread	S14-042858	Result	< LOQ (< 0.24)	< LOQ (< 0.18)	< LOQ (< 0.19)	< LOQ (< 0.25)	**0.53**	
					Recovery	82% '	112% '	103% '	80% '	91% '	
		4	Wholemeal and granary bread	S14-042859	Result	< LOQ (< 0.24)	< LOQ (< 0.18)	< LOQ (< 0.19)	< LOQ (< 0.25)	**0.45**	
					Recovery	82% '	112% '	103% '	80% '	91% '	
		5	Other bread	S14-042860	Result	< LOQ (< 0.24)	< LOQ (< 0.18)	< LOQ (< 0.19)	< LOQ (< 0.25)	< LOQ (< 0.22)	
					Recovery	82% '	112% '	103% '	80% '	91% '	
		NA	Group sample	S14-042828	Result	< LOQ (< 0.24)	< LOQ (< 0.18)	< LOQ (< 0.19)	< LOQ (< 0.25)	< LOQ (< 0.22)	
					Recovery	82% '	112% '	103% '	80% '	91% '	
2	Miscellaneous cereals	6	Flour	S14-042861	Result	< LOQ (< 0.22)	< LOQ (< 0.21)	< LOQ (< 0.24)	< LOQ (< 0.26)	< LOQ (< 0.24)	
					Recovery	93%	96%	85%	77%	85%	
		7	Buns, cakes and pastries	S14-042862	Result	< LOQ (< 0.20)	< LOQ (< 0.21)	< LOQ (< 0.25)	< LOQ (< 0.30)	< LOQ (< 0.23)	
					Recovery	99%	94%	79%	67%	87%	
		8	Savoury biscuits	S14-042863	Result	< LOQ (< 0.18)	< LOQ (< 0.17)	< LOQ (< 0.20)	< LOQ (< 0.22)	< LOQ (< 0.26)	
					Recovery	113% "	121% "	101% "	89% "	76% "	
		9	Sweet biscuits	S14-042864	Result	< LOQ (< 0.18)	< LOQ (< 0.17)	< LOQ (< 0.20)	< LOQ (< 0.22)	< LOQ (< 0.26)	
					Recovery	113%	121%	101%	89%	76%	
		10	Chocolate biscuits	S14-042865	Result	< LOQ (< 0.18)	< LOQ (< 0.17)	< LOQ (< 0.20)	< LOQ (< 0.22)	< LOQ (< 0.26)	
					Recovery	113% "	121% "	101% "	89% "	76% "	
		11	Breakfast cereals	S14-042866	Result	< LOQ (< 0.20)	< LOQ (< 0.20)	< LOQ (< 0.33)	< LOQ (< 0.33)	< LOQ (< 0.21)	
					Recovery	102%	98%	60%	60%	96%	
		12	Rice	S14-042867	Result	< LOQ (< 0.20)	< LOQ (< 0.21)	< LOQ (< 0.23)	< LOQ (< 0.24)	< LOQ (< 0.30)	
					Recovery	99%	94%	88%	84%	67%	
		13	Other cereal products	S14-042868	Result	< LOQ (< 0.20)	< LOQ (< 0.20)	< LOQ (< 0.33)	< LOQ (< 0.33)	< LOQ (< 0.21)	
					Recovery	102% "'	98% "'	60% "'	60% "'	96% "'	
		14	Pasta	S14-042869	Result	< LOQ (< 0.20)	< LOQ (< 0.21)	< LOQ (< 0.23)	< LOQ (< 0.24)	< LOQ (< 0.30)	
					Recovery	99% """	94% """	88% """	84% """	67% """	
		15	Pizza	S14-042870	Result	< LOQ (< 0.25)	< LOQ (< 0.25)	< LOQ (< 0.31)	< LOQ (< 0.36)	< LOQ (< 0.20)	
					Recovery	80% """	81% """	64% """	55% """	99% """	
		NA	Group sample	S14-042829	Result	< LOQ (< 0.25)	< LOQ (< 0.25)	< LOQ (< 0.31)	< LOQ (< 0.36)	< LOQ (< 0.20)	
					Recovery	80%	81%	64%	55%	99%	
4	Offals	19	Lambs liver	S14-042874	Result	< LOQ (< 0.20)	< LOQ (< 0.21)	< LOQ (< 0.23)	< LOQ (< 0.24)	< LOQ (< 0.30)	
					Recovery	98% ^	96% ^	86% ^	82% ^	66% ^	
		20	Pigs liver	S14-042875	Result	< LOQ (< 0.20)	< LOQ (< 0.21)	< LOQ (< 0.23)	< LOQ (< 0.24)	< LOQ (< 0.30)	
					Recovery	98% ^	96% ^	86% ^	82% ^	66% ^	
		21	Other liver	S14-042876	Result	< LOQ (< 0.20)	< LOQ (< 0.21)	< LOQ (< 0.23)	< LOQ (< 0.24)	< LOQ (< 0.30)	
					Recovery	98% ^	96% ^	86% ^	82% ^	66% ^	
		22	Kidney	S14-042877	Result	< LOQ (< 0.20)	< LOQ (< 0.21)	< LOQ (< 0.23)	< LOQ (< 0.24)	< LOQ (< 0.30)	
					Recovery	98% ^	96% ^	86% ^	82% ^	66% ^	
		23	Other offals (excluding kidney and liver)	S14-042878	Result	< LOQ (< 0.20)	< LOQ (< 0.21)	< LOQ (< 0.23)	< LOQ (< 0.24)	< LOQ (< 0.30)	
					Recovery	98% ^	96% ^	86% ^	82% ^	66% ^	
		NA	Group sample	S14-042831	Result	< LOQ (< 0.20)	< LOQ (< 0.21)	< LOQ (< 0.23)	< LOQ (< 0.24)	< LOQ (< 0.30)	
					Recovery	98% ^	96% ^	86% ^	82% ^	66% ^	
8	Oils and fats	50	Vegetable oils	S14-042905	Result	< LOQ (< 0.29)	< LOQ (< 0.19)	< LOQ (< 0.40)	< LOQ (< 0.21)	< LOQ (< 0.24)	
					Recovery	68%	103%	50%	96%	82%	
9	Eggs	53	Eggs	S14-042908	Result						< LOQ (< 0.04)
					Recovery						47% '
		54	Egg products	S14-042909	Result						< LOQ (< 0.04)
					Recovery						47% '
		NA	Group sample	S14-042836	Result						< LOQ (< 0.04)
					Recovery						47%
10	Sugars and preserves	60	Chocolate confectionery	S14-042915	Result	< LOQ (< 2.26)	< LOQ (< 1.23)	< LOQ (< 1.15)	< LOQ (< 0.75)	< LOQ (< 0.41)	
					Recovery	9%	16%	17%	27%	49%	
		61	Sugar confectionery	S14-042916	Result	< LOQ (< 2.07)	< LOQ (< 1.60)	< LOQ (< 2.41)	< LOQ (< 1.97)	< LOQ (< 0.56)	
					Recovery	10%	12%	8%	10%	36%	
13	Other vegetables	78	Dried pulses	S14-042933	Result	< LOQ (< 0.20)	< LOQ (< 0.21)	< LOQ (< 0.23)	< LOQ (< 0.24)	< LOQ (< 0.30)	
					Recovery	99% """	94% """	88% """	84% """	67% """	
		79	Herbs, spices	S14-042934	Result	< LOQ (< 0.20)	< LOQ (< 0.15)	< LOQ (< 0.27)	< LOQ (< 0.20)	**0.63**	
					Recovery	102%	134%	73%	98%	84%	
16	Fruit products	100	Dried fruit	S14-042952	Result	< LOQ (< 0.17)	< LOQ (< 0.13)	< LOQ (< 0.24)	< LOQ (< 0.17)	**1.65**	
					Recovery	116%	158%	83%	116%	100%	
		101	Fruit juices and vegetable juices	S14-042953	Result	< LOQ (< 0.19)	< LOQ (< 0.14)	< LOQ (< 0.26)	< LOQ (< 0.19)	**5.62**	
					Recovery	106%	148%	78%	104%	32%	

Table 18. Aflatoxins and Ochratoxin A Results – Part 2.

Group		Category		LIMS Number		Ochratoxin / Aflatoxin Concentration / µg/kg					Aflatoxin M1 Concentration / µg/kg
						Concentrations are corrected for recovery. ' QC cereal sample recovery value. "					Concentrations are corrected for recovery. '
						Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2	Ochratoxin A	
17	Non- alcoholic Beverages (with bottled water)	102	Tea	S14-042954	Result	< LOQ (< 0.46)	< LOQ (< 0.40)	< LOQ (< 0.35)	< LOQ (< 1.30)	< LOQ (< 0.24)	
					Recovery	43%	50%	57%	15%	84%	
		103	Takeaway Tea	S14-042955	Result	< LOQ (< 0.46)	< LOQ (< 0.40)	< LOQ (< 0.35)	< LOQ (< 1.30)	< LOQ (< 0.24)	
					Recovery	43% ^^	50% ^^	57% ^^	15% ^^	84% ^^	
		104	Instant coffee	S14-042956	Result	< LOQ (< 0.61)	< LOQ (< 0.45)			< LOQ (< 0.23)	
					Recovery	33%	44%			88%	
		105	Ground coffee	S14-042957	Result	< LOQ (< 0.61)	< LOQ (< 0.45)			< LOQ (< 0.23)	
					Recovery	33% ^^^	44% ^^^			88% ^^^	
		106	Takeaway coffee	S14-042958	Result	< LOQ (< 0.61)	< LOQ (< 0.45)			< LOQ (< 0.23)	
					Recovery	33% ^^^	44% ^^^			88% ^^^	
		107	Branded food drinks	S14-042959	Result	< LOQ (< 0.42)	< LOQ (< 0.32)	< LOQ (< 0.44)	< LOQ (< 0.35)	< LOQ (< 0.22)	
					Recovery	48%	63%	45%	56%	90%	
		108	Cocoa, drinking chocolate	S14-042960	Result	< LOQ (< 0.75)	< LOQ (< 0.52)	< LOQ (< 0.77)	< LOQ (< 0.53)	< LOQ (< 0.25)	
					Recovery	27%	39%	26%	38%	81%	
		113	Alternatives to milk	S14-042963	Result	< LOQ (< 0.31)	< LOQ (< 0.25)	< LOQ (< 0.41)	< LOQ (< 0.33)	< LOQ (< 0.22)	
					Recovery	64%	80%	49%	60%	92%	
18	Milk	114	Whole (full fat) milk (cows)	S14-042964	Result						< LOQ (< 0.02)
					Recovery						60%
		115	Skimmed/Semi skimmed milks (cows)	S14-042965	Result						< LOQ (< 0.02)
					Recovery						60% '
		NA	Group sample	S14-042845	Result						< LOQ (< 0.02)
					Recovery						60% '
19	Dairy products	116	Condensed milk or Evaporated	S14-042966	Result						< LOQ (< 0.02)
					Recovery						60% '
		117	Instant milk	S14-042967	Result						< LOQ (< 0.02)
					Recovery						60% '
		118	Natural cheese	S14-042968	Result						< LOQ (< 0.03)
					Recovery						72%
		119	Processed cheese	S14-042969	Result						< LOQ (< 0.05)
					Recovery						43% '
		120	Butter	S14-042970	Result						< LOQ (< 0.02)
					Recovery						85%
		121	Ice-cream	S14-042971	Result						< LOQ (< 0.05)
					Recovery						43% '
		122	Yoghurt	S14-042972	Result						< LOQ (< 0.05)
					Recovery						43% '
		123	Other milk products	S14-042973	Result						< LOQ (< 0.03)
					Recovery						79%
		124	Cream	S14-042974	Result						< LOQ (< 0.05)
					Recovery						43% '
		125	Canned milk puddings	S14-042975	Result						< LOQ (< 0.03)
					Recovery						73%
		NA	Group sample	S14-042846	Result						< LOQ (< 0.05)
					Recovery						43%
20	Nuts	126	Ground nuts including peanut butter	S14-042976	Result	< LOQ (< 0.26)	< LOQ (< 0.26)	< LOQ (< 0.55)	< LOQ (< 0.71)	< LOQ (< 0.30)	
					Recovery	78% """	76% """	36% """	28% """	68% """	
		127	Tree nuts	S14-042977	Result	< LOQ (< 0.26)	< LOQ (< 0.26)	< LOQ (< 0.55)	< LOQ (< 0.71)	< LOQ (< 0.30)	
					Recovery	78% """	76% """	36% """	28% """	68% """	
		NA	Group sample	S14-042847	Result	< LOQ (< 0.26)	< LOQ (< 0.26)	< LOQ (< 0.55)	< LOQ (< 0.71)	< LOQ (< 0.30)	
					Recovery	78%	76%	36%	28%	68%	
21	Alcoholic drinks	128	Beer	S14-042978	Result	< LOQ (< 0.58)	< LOQ (< 0.26)	< LOQ (< 0.91)	< LOQ (< 0.66)	< LOQ (< 0.29)	
					Recovery	35%	77%	22%	30%	69%	
		129	Cider	S14-042979	Result	< LOQ (< 0.26)	< LOQ (< 0.14)	< LOQ (< 0.43)	< LOQ (< 0.32)	< LOQ (< 0.30)	
					Recovery	76%	146%	46%	62%	67%	
		130	Wine	S14-042980	Result	< LOQ (< 0.28)	< LOQ (< 0.15)	< LOQ (< 1.22)	< LOQ (< 0.79)	< LOQ (< 0.27)	
					Recovery	71%	137%	16%	25%	73%	
		131	Alcopops and cocktails	S14-042981	Result	< LOQ (< 0.32)	< LOQ (< 0.15)	< LOQ (< 0.41)	< LOQ (< 0.30)	< LOQ (< 0.29)	
					Recovery	63%	132%	49%	67%	69%	
23	Snacks	135	Other snacks (not potato based)	S14-042985	Result	< LOQ (< 0.24)	< LOQ (< 0.18)	< LOQ (< 0.19)	< LOQ (< 0.25)	< LOQ (< 0.22)	
					Recovery	82% '	112% '	103% '	80% '	91% '	
25	Sandwiches	138	Sandwiches	S14-042988	Result						
					Recovery	< LOQ (< 0.24) ^^	< LOQ (< 0.18) ^	LOQ (< 0.19) ^	< LOQ (< 0.25) ^^	< LOQ (< 0.22) ^	
		NA	Group sample	S14-042852	Result						
					Recovery	82% '	112% '	103% '	80% '	91% '	

Grey shading indicates that no analysis was requested.

8.5. Zearalenone

Zearalenone was analysed using the established accredited method, although the method has not been fully validated for all the matrices tested in this study. Full zearalenone results are given in Table 19. Five samples contained residues above the LOD but below the LOQ, levels ranged from 0.57 to 1.92 µg/kg, although these values are not quantitative. The pizza sample contained a level of 16.5 µg/kg corrected for a recovery for 47 %, this was the highest level measured in all the samples. All levels measured were well below the maximum permitted levels in legislation.

8.6. Fumonisins B_1, B_2 and B_3

The fumonisins were analysed using the established accredited acidic multi-mycotoxin method. Full results are given in Table 20.

Fumonisin B_1 was detected in the sample of herbs and spices at a level of 5.53 µg/kg, below the LOQ of 7.15 µg/kg. Fumonisins were not detected in any other samples above the limit of detection (LOD). Limits of detection for Fumonisin B_1 ranged from 3.87 µg/kg for beers and cider to 7.95 µg/kg for flour, and chocolate biscuits had a much higher LOD of 37.1 µg/kg. For Fumonisin B_2 LODs were in the range 3.4 to 8.9 µg/kg apart from chocolate biscuits where it was 36.8 µg/kg. For fumonisin B_3 LODs were in the range 3.1 to 6.5 µg/kg, with a LOD in chocolate biscuits of 31.5 µg/kg.

Table 19. Zearalenone Results.

Group		Category		LIMS Number		Zearalenone Concentration / µg/kg
						Concentrations are corrected for recovery. ' Brown bread sample recovery value. "Fresh potatoes sample recovery value. '"Dried plses sample recovery value. Beer sample recovery value. '""Sandwiches sample recovery value. ^ Wholemeal bread sample recovery value. ^^ Sweet biscuits smple recovery value. ^^^ Rice sample recovery value.
1	Bread	1	White sliced bread	S14-042856	Result	< LOD (< 0.34)
					Recovery	87% ^
		2	White unsliced bread	S14-042857	Result	< LOD (< 0.42)
					Recovery	72% '
		3	Brown bread	S14-042858	Result	< LOD (< 0.42)
					Recovery	72%
		4	Wholemeal and granary bread	S14-042859	Result	< LOD (< 0.34)
					Recovery	87%
		5	Other bread	S14-042860	Result	< LOD (< 0.42)
					Recovery	72% '
		NA	Group sample	S14-042828	Result	< LOD (< 0.71)
					Recovery	42%
2	Miscellaneous cereals	6	Flour	S14-042861	Result	< LOD (< 0.34)
					Recovery	87% ^
		7	Buns, cakes and pastries	S14-042862	Result	< LOD (< 0.29)
					Recovery	102% ^^
		8	Savoury biscuits	S14-042863	Result	< LOD (< 0.29)
					Recovery	102% ^^
		9	Sweet biscuits	S14-042864	Result	0.57 < LOQ (< 2.46)
					Recovery	102%
		10	Chocolate biscuits	S14-042865	Result	0.85 < LOQ (< 2.46)
					Recovery	102% ^^
		11	Breakfast cereals	S14-042866	Result	< LOD (< 0.34)
					Recovery	87% ^
		12	Rice	S14-042867	Result	< LOD (< 0.41)
					Recovery	73%
		13	Other cereal products	S14-042868	Result	< LOD (< 0.29)
					Recovery	102% ^^
		14	Pasta	S14-042869	Result	< LOD (< 0.41)
					Recovery	73% ^^^
		15	Pizza	S14-042870	Result	**16.45**
					Recovery	47%
		NA	Group sample	S14-042829	Result	< LOD (< 0.70)
					Recovery	43%
12	Potatoes	69	Fresh potatoes	S14-042924	Result	< LOD (< 0.78)
					Recovery	38%
		70	Potato products	S14-042925	Result	1.92 < LOQ (< 6.54)
					Recovery	38% "
		NA	Group sample	S14-042839	Result	< LOD (< 0.66)
					Recovery	45%
13	Other vegetables	78	Dried pulses	S14-042933	Result	1.33 < LOQ (< 6.95)
					Recovery	36%
		79	Herbs, spices	S14-042934	Result	< LOD (< 0.83)
					Recovery	36% "'
17	Non- alcoholic Beverages (with bottled water)	107	Branded food drinks	S14-042959	Result	< LOD (< 0.60)
					Recovery	50%
		108	Cocoa, drinking chocolate	S14-042960	Result	< LOD (< 1.15)
					Recovery	26%
		113	Alternatives to milk	S14-042963	Result	< LOD (< 0.53)
					Recovery	57%
21	Alcoholic drinks	128	Beer	S14-042978	Result	< LOD (< 0.43)
					Recovery	70%
		129	Cider	S14-042979	Result	< LOD (< 0.43)
					Recovery	70% ""
23	Snacks	135	Other snacks (not potato based)	S14-042985	Result	1.09 < LOQ (< 6.73)
					Recovery	37%
25	Sandwiches	138	Sandwiches	S14-042988	Result	< LOD (< 0.81)
					Recovery	37%
		NA	Group sample	S14-042852	Result	< LOD (< 0.81)
					Recovery	37% '""

Table 20. Fumonisin Results.

Group		Category		LIMS Number		Fumonisin Concentration / µg/kg		
						Concentrations are corrected for recovery. ' Group sample recovery value. " Wine sample recovery value.		
						Fumonisin B1	Fumonisin B2	Fumonisin B3
1	Bread	1	White sliced bread	S14-042856	Result	< LOD (< 6.69)	< LOD (< 7.32)	< LOD (< 5.64)
					Recovery	75% '	68% '	89% '
		2	White unsliced bread	S14-042857	Result	< LOD (< 6.69)	< LOD (< 7.32)	< LOD (< 5.64)
					Recovery	75% '	68% '	89% '
		3	Brown bread	S14-042858	Result	< LOD (< 6.69)	< LOD (< 7.32)	< LOD (< 5.64)
					Recovery	75% '	68% '	89% '
		4	Wholemeal and granary bread	S14-042859	Result	< LOD (< 6.69)	< LOD (< 7.32)	< LOD (< 5.64)
					Recovery	75% '	68% '	89% '
		5	Other bread	S14-042860	Result	< LOD (< 6.69)	< LOD (< 7.32)	< LOD (< 5.64)
					Recovery	75% '	68% '	89% '
		NA	Group sample	S14-042828	Result	< LOD (< 6.69)	< LOD (< 7.32)	< LOD (< 5.64)
					Recovery	75%	68%	89%
2	Miscellaneous cereals	6	Flour	S14-042861	Result	< LOD (< 7.95)	< LOD (< 8.79)	< LOD (< 6.48)
					Recovery	63%	57%	77%
		7	Buns, cakes and pastries	S14-042862	Result	< LOD (< 6.57)	< LOD (< 5.53)	< LOD (< 5.28)
					Recovery	76% '	90% '	95% '
		8	Savoury biscuits	S14-042863	Result	< LOD (< 6.57)	< LOD (< 5.53)	< LOD (< 5.28)
					Recovery	76% '	90% '	95% '
		9	Sweet biscuits	S14-042864	Result	< LOD (< 6.57)	< LOD (< 5.53)	< LOD (< 5.28)
					Recovery	76% '	90% '	95% '
		10	Chocolate biscuits	S14-042865	Result	< LOD (< 37.05)	< LOD (< 36.84)	< LOD (< 31.50)
					Recovery	13%	14%	16%
		11	Breakfast cereals	S14-042866	Result	< LOD (< 6.57)	< LOD (< 5.53)	< LOD (< 5.28)
					Recovery	76% '	90% '	95% '
		12	Rice	S14-042867	Result	< LOD (< 6.57)	< LOD (< 5.53)	< LOD (< 5.28)
					Recovery	76% '	90% '	95% '
		13	Other cereal products	S14-042868	Result	< LOD (< 6.57)	< LOD (< 5.53)	< LOD (< 5.28)
					Recovery	76% '	90% '	95% '
		14	Pasta	S14-042869	Result	< LOD (< 6.82)	< LOD (< 7.06)	< LOD (< 5.83)
					Recovery	73%	71%	86%
		15	Pizza	S14-042870	Result	< LOD (< 6.03)	< LOD (< 6.83)	< LOD (< 5.46)
					Recovery	83%	73%	92%
		NA	Group sample	S14-042829	Result	< LOD (< 3.94)	< LOD (< 3.32)	< LOD (< 3.17)
					Recovery	76%	90%	95%
12	Potatoes	69	Fresh potatoes	S14-042924	Result	< LOD (< 4.02)	< LOD (< 3.24)	< LOD (< 3.12)
					Recovery	75% '	92% '	96% '
		70	Potato products	S14-042925	Result	< LOD (< 4.02)	< LOD (< 3.24)	< LOD (< 3.12)
					Recovery	75% '	92% '	96% '
		NA	Group sample	S14-042839	Result	< LOD (< 4.02)	< LOD (< 3.24)	< LOD (< 3.12)
					Recovery	75%	92%	96%
13	Other vegetables	78	Dried pulses	S14-042933	Result	< LOD (< 4.26)	< LOD (< 4.28)	< LOD (< 3.78)
					Recovery	70%	70%	79%
		79	Herbs, spices	S14-042934	Result	5.53 < LOQ (< 7.15)	< LOD (< 4.09)	< LOD (< 4.05)
					Recovery	70%	73%	74%
17	Non- alcoholic Beverages (with bottled water)	107	Branded food drinks	S14-042959	Result	< LOD (< 4.10)	< LOD (< 3.49)	< LOD (< 3.27)
					Recovery	73%	86%	92%
		113	Alternatives to milk	S14-042963	Result	< LOD (< 4.27)	< LOD (< 3.70)	< LOD (< 3.38)
					Recovery	70%	81%	89%
21	Alcoholic drinks	128	Beer	S14-042978	Result	< LOD (< 3.87)	< LOD (< 3.38)	< LOD (< 3.14)
					Recovery	77% "	89% "	95% "
		129	Cider	S14-042979	Result	< LOD (< 3.87)	< LOD (< 3.38)	< LOD (< 3.14)
					Recovery	77% "	89% "	95% "
		130	Wine	S14-042980	Result	< LOD (< 3.87)	< LOD (< 3.38)	< LOD (< 3.14)
					Recovery	77%	89%	95%
23	Snacks	135	Other snacks (not potato based)	S14-042985	Result	< LOD (< 6.84)	< LOD (< 7.52)	< LOD (< 5.79)
					Recovery	73%	66%	86%
25	Sandwiches	138	Sandwiches	S14-042988	Result	< LOD (< 4.09)	< LOD (< 3.56)	< LOD (< 3.39)
					Recovery	73% '	84% '	89% '
		NA	Group sample	S14-042852	Result	< LOD (< 4.09)	< LOD (< 3.56)	< LOD (< 3.39)
					Recovery	73%	84%	89%

8.7. Trichothecenes

Samples were analysed by UPLC-MS/MS using a method that allowed the separation of nine trichothecenes - deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, nivalenol, fusarenon-X, diacetoxyscirpenol, neosolaniol, T-2 toxin, HT-2 toxin. The existing neutral mobile phase multi-mycotoxin LC-MS method did not give separation of the isomers 3- and 15-acetyldeoxynivalenol and this could not be achieved by changing the gradient profile so a new column type, the Restek Raptor Biphenyl 2.7 µm (100 x 2.1 mm), was selected to replace the Waters Acquity HSS T3 1.8 µm (100 x 2.1 mm). The mobile phases were not changed but the gradient was re-optimised to give separation of these two isomers. The injection volume was increased because the aqueous content of the sample extracts was higher than in the usual multi-mycotoxin method so it was possible to inject more without deterioration of the peak shapes of early-eluting compounds.

Full trichothecene results are given in Table 21. Nine trichothecenes were included in the analysis. 3-Acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol, and nivalenol were not detected in any sample above the LOD. In addition neosolaniol was not detected in any sample requested above the LOD, however a few additional analyses (not originally requested by the FSA) have been included and neosolaniol was found in the vegetable oils sample between LOD and LOQ at level of 0.73 µg/kg.

T-2 and HT-2 toxin were also detected in a number of samples above LOD but below LOQ. Most samples with detectable residues were cereal products, however they were both detected in vegetable oils and cider, and T-2 toxin was seen in the potatoes group sample and in dried pulses.

Deoxynivalenol was detected in all cereal products, snack and sandwiches at levels from 11.2 to 166 µg/kg. It was also seen at concentrations between LOD and LOQ in herbs and spices, vegetable oil and beer. The recovery of some of the initial analysis was low, as while there are regulatory limits for DON in cereal products the accepted standardised method has only been validated for flours rather than processed products, e.g. bread. To confirm the initial results the analysis was repeated using ^{13}C-deoxynivalenol internal standard to control the measurement. This second analysis confirmed the presence of deoxynivalenol in all the samples tested, and in most cases the concentration found was in good agreement. The apparent recovery values reported for the second analysis also appear low, but in fact the result reported was inherently corrected by the presence of the internal standard, so these recovery values were not used for correction. It is reassuring that the reported values between the two analyses are in good agreement. The largest variation was for the sample of ‘other bread’ where the initial result was 166 µg/kg but the repeat analysis

found 79 µg/kg. This is around 50 % difference which is close to the measurement uncertainty for this measurement at 166 µg/kg of 39 % (64 µg/kg). [1]

[1] The difference could also in some part be due to the sample preparation process as the samples were prepared initially for metals analysis not mycotoxins, and may not have been homogenous for mycotoxins as the sample contained lumps and pieces of crust and seeds were visible. It has been shown that to produce a homogenous sample for mycotoxin analysis samples should be slurried with water or milled to a very small particle size (< 500 µm sieve size) followed by mixing [Spanjer et al, 2006]. This was not done for these samples as they were initially intended for metals analysis, the slurry method would have made some samples incompatible with the analytical methods used, and ultimately this additional preparation would have added a considerable resource burden and cost to the whole project that it was not possible to meet. For the majority of samples the homogenisation was sufficient, but for a small number, such as in this case it may have resulted in less than homogenous test portions.

Table 21. Trichothecene Results

Group		Category		LIMS Number		Trichothecene Concentration / µg/kg Concentrations are corrected for recovery. ^ Identity of the residue fails to confirm by ion ratio. Concentrations for Deoxynivalenol repeat results are inherently corrected for recovery using an isotope-labelled internal standard. Recovery values in table are for this internal standard.									
						3-Acetyl-Deoxynivalenol	15-Acetyl-Deoxynivalenol	Deoxynivalenol		Diacetoxyscirpenol	Fusarenon_X	HT2_Toxin	Neosolaniol	Nivalenol	T2_Toxin
								Original Result	Repeat						
1	Bread	1	White sliced bread	S14-042856	Result	< LOD (< 1.50)	< LOD (< 11.69)	**37.02**	**60.13**	< LOD (< 0.12)	< LOD (< 0.87)	< LOD (< 1.41)	< LOD (< 0.54)	< LOD (< 17.35)	0.14 < LOQ (< 5.46)
					Recovery	60%	43%	61%	13%	85%	58%	71%	92%	29%	92%
		2	White unsliced bread	S14-042857	Result	< LOD (< 2.47)	< LOD (< 7.34)	**72.43**	**64.15**	< LOD (< 0.09)	< LOD (< 1.51)	< LOD (< 1.04)	< LOD (< 0.58)	< LOD (< 12.33)	< LOD (< 0.45)
					Recovery	81%	68%	37%	17%	111%	66%	96%	87%	41%	112%
		3	Brown bread	S14-042858	Result	< LOD (< 3.78)	< LOD (< 15.68)	**105.5**	**90.23**	< LOD (< 0.11)	< LOD (< 2.13)	1.39 < LOQ (< 6.54)	< LOD (< 0.64)	< LOD (< 17.69)	< LOD (< 0.44)
					Recovery	53%	32%	23%	13%	94%	47%	76%	78%	28%	114%
		4	Wholemeal and granary bread	S14-042859	Result	< LOD (< 1.59)	< LOD (< 14.39)	**48.05**	**106.8**	< LOD (< 0.10)	< LOD (< 0.96)	2.66 < LOQ (< 6.85)	< LOD (< 0.59)	< LOD (< 36.37)	0.21 < LOQ (< 4.82)
					Recovery	57%	35%	60%	15%	98%	52%	73%	84%	27%	104%
		5	Other bread	S14-042860	Result	< LOD (< 4.34)	< LOD (< 12.16)	**166.4**	**78.94**	< LOD (< 0.15)	< LOD (< 2.29)	< LOD (< 1.76)	< LOD (< 0.95)	< LOD (< 23.47)	< LOD (< 0.72)
					Recovery	46%	41%	17%	17%	65%	44%	57%	52%	21%	70%
		NA	Group sample	S14-042828	Result	< LOD (< 3.58)	< LOD (< 8.45)	**77.42**	**76.73**	< LOD (< 0.09)	< LOD (< 1.57)	< LOD (< 1.13)	< LOD (< 0.62)	< LOD (< 13.56)	< LOD (< 0.46)
					Recovery	56%	59%	32%	16%	108%	64%	89%	81%	37%	109%
2	Miscellaneous cereals	6	Flour	S14-042861	Result	< LOD (< 1.10)	< LOD (< 6.58)	**27.24**		< LOD (< 0.08)	< LOD (< 0.55)	1.21 < LOQ (< 5.14)	< LOD (< 0.49)	< LOD (< 8.51)	0.15 < LOQ (< 3.58)
					Recovery	82%	76%	106%		121%	91%	97%	103%	59%	140%
		7	Buns, cakes and pastries	S14-042862	Result	< LOD (< 1.38)	< LOD (< 11.41)	**10.72**		< LOD (< 0.11)	< LOD (< 1.05)	< LOD (< 1.24)	< LOD (< 0.52)	< LOD (< 17.33)	< LOD (< 0.10)
					Recovery	65%	44%	66%		89%	48%	81%	96%	29%	100%
		8	Savoury biscuits	S14-042863	Result	< LOD (< 2.93)	< LOD (< 23.06)	**153.2**	**90.76**	< LOD (< 0.23)	< LOD (< 1.85)	< LOD (< 2.37)	< LOD (< 1.18)	< LOD (< 33.54)	< LOD (< 0.23)
					Recovery	31%	22%	18%	12%	43%	27%	42%	43%	15%	43%
		9	Sweet biscuits	S14-042864	Result	< LOD (< 1.51)	< LOD (< 9.60)	**23.75**		< LOD (< 0.11)	< LOD (< 0.92)	1.5 < LOQ (< 5.72)	< LOD (< 0.53)	< LOD (< 20.73)	0.64 < LOQ (< 4.67)
					Recovery	60%	52%	57%		87%	55%	87%	95%	24%	107%
		10	Chocolate biscuits	S14-042865	Result	< LOD (< 1.54)	< LOD (< 9.56)	**17.32**		< LOD (< 0.11)	< LOD (< 1.71)	< LOD (< 1.10)	< LOD (< 0.51)	< LOD (< 16.60)	0.17 < LOQ (< 4.64)
					Recovery	58%	52%	56%		94%	29%	91%	98%	30%	108%
		11	Breakfast cereals	S14-042866	Result	< LOD (< 1.70)	< LOD (< 12.67)	**34.79**		< LOD (< 0.12)	< LOD (< 1.11)	3.03 ^ < LOQ (< 6.51)	< LOD (< 0.53)	< LOD (< 17.85)	0.85 < LOQ (< 5.03)
					Recovery	53%	39%	55%		85%	45%	77%	94%	28%	99%
		12	Rice	S14-042867	Result	< LOD (< 1.36)	< LOD (< 11.48)	1.4 < LOQ (< 6.37)		< LOD (< 0.12)	< LOD (< 0.80)	< LOD (< 1.32)	< LOD (< 0.51)	< LOD (< 10.99)	< LOD (< 0.10)
					Recovery	66%	44%	79%		86%	62%	76%	98%	46%	99%
		13	Other cereal products	S14-042868	Result	< LOD (< 1.17)	< LOD (< 9.04)	**18.61**		< LOD (< 0.10)	< LOD (< 0.70)	< LOD (< 1.00)	< LOD (< 0.46)	< LOD (< 10.30)	< LOD (< 0.08)
					Recovery	77%	55%	104%		98%	71%	100%	109%	49%	133%
		14	Pasta	S14-042869	Result	< LOD (< 1.59)	< LOD (< 13.72)	**11.18**		0.28 < LOQ (< 6.46)	< LOD (< 0.91)	1.7 < LOQ (< 7.22)	< LOD (< 0.54)	< LOD (< 11.59)	0.93 < LOQ (< 8.17)
					Recovery	56%	36%	71%		77%	55%	69%	92%	43%	61%
		15	Pizza	S14-042870	Result	< LOD (< 1.36)	< LOD (< 13.64)	**23.50**		0.08 < LOQ (< 4.11)	< LOD (< 0.95)	1.17 < LOQ (< 5.23)	< LOD (< 0.52)	< LOD (< 13.34)	0.56 < LOQ (< 3.98)
					Recovery	66%	37%	61%		122%	52%	96%	97%	37%	126%
		NA	Group sample	S14-042829	Result	< LOD (< 2.65)	< LOD (< 7.38)	**37.97**	**44.55**	< LOD (< 0.09)	< LOD (< 1.65)	1.24 ^ < LOQ (< 6.19)	< LOD (< 0.53)	< LOD (< 14.57)	< LOD (< 0.41)
					Recovery	75%	68%	33%	16%	108%	61%	81%	95%	34%	121%
8	Oils and fats	50	Vegetable oils	S14-042905	Result	1.42 < LOQ (<	< LOD (< 9.21)	2.07 < LOQ (< 8.36)		0.66 < LOQ (< 6.53)	1.02 < LOQ (< 7.73)	1.45 < LOQ (< 6.28)	0.73 < LOQ (< 4.98)	< LOD (< 8.98)	1.32 < LOQ (< 6.18)
					Recovery	66%	54%	60%		77%	65%	80%	100%	56%	81%
12	Potatoes	69	Fresh potatoes	S14-042924	Result	< LOD (< 3.12)	< LOD (< 12.00)	< LOD (< 2.60)		< LOD (< 0.15)	< LOD (< 1.84)	< LOD (< 1.42)	< LOD (< 0.82)	< LOD (< 32.45)	< LOD (< 0.16)
					Recovery	29%	42%	38%		69%	27%	70%	61%	31%	63%
		70	Potato products	S14-042925	Result	< LOD (< 1.44)	< LOD (< 16.23)	< LOD (< 1.86)		< LOD (< 0.12)	< LOD (< 0.97)	< LOD (< 1.17)	< LOD (< 0.64)	< LOD (< 14.58)	< LOD (< 0.12)
					Recovery	62%	31%	54%		86%	52%	86%	78%	34%	86%
		NA	Group sample	S14-042839	Result	< LOD (< 2.86)	< LOD (< 12.06)	< LOD (< 2.31)		0.23 < LOQ (< 6.55)	< LOD (< 1.56)	< LOD (< 1.32)	< LOD (< 0.80)	< LOD (< 45.26)	0.27 < LOQ (< 6.05)
					Recovery	31%	41%	43%		76%	32%	76%	63%	22%	83%
13	Other Vegetables	78	Dried pulses	S14-042933	Result	< LOD (< 1.39)	< LOD (< 10.54)	< LOD (< 1.39)		0.25 < LOQ (< 6.94)	< LOD (< 0.97)	< LOD (< 1.43)	< LOD (< 0.64)	< LOD (< 12.31)	0.49 < LOQ (< 9.30)
					Recovery	65%	47%	72%		72%	52%	70%	78%	41%	54%
		79	Herbs, spices	S14-042934	Result	< LOD (< 6.61)	< LOD (< 55.94)	6.85 ^ < LOQ (< 33.25)	**16.51**	< LOD (< 0.46)	15.36 ^ < LOQ (< 40.35)	< LOD (< 5.39)	< LOD (< 2.34)		< LOD (< 0.78)
					Recovery	14%	9%	15%	3%	22%	12%	19%	21%		13%
17	Non- alcoholic Beverages (with bottled water)	107	Branded food drinks	S14-042959	Result	< LOD (< 1.95)	< LOD (< 20.36)	**11.14**		< LOD (< 0.17)	< LOD (< 1.19)	< LOD (< 1.46)	< LOD (< 0.77)	< LOD (< 14.22)	< LOD (< 0.16)
					Recovery	46%	25%	58%		57%	42%	68%	65%	35%	63%
		113	Alternatives to milk	S14-042963	Result	< LOD (< 2.84)	< LOD (< 5.45)	< LOD (< 3.10)		< LOD (< 0.11)	< LOD (< 1.42)	< LOD (< 1.11)	< LOD (< 0.62)	< LOD (< 11.75)	< LOD (< 0.55)
					Recovery	70%	92%	32%		91%	70%	90%	81%	43%	91%
21	Alcoholic drinks	128	Beer	S14-042978	Result	< LOD (< 3.20)	< LOD (< 28.58)	3.39 < LOQ (< 11.05)		< LOD (< 0.23)	< LOD (< 1.30)	< LOD (< 1.64)	< LOD (< 0.88)	< LOD (< 21.84)	< LOD (< 0.20)
					Recovery	28%	17%	45%		43%	38%	61%	57%	23%	49%
		129	Cider	S14-042979	Result	< LOD (< 1.92)	< LOD (< 23.40)	< LOD (< 1.90)		< LOD (< 0.18)	< LOD (< 0.84)	1.99 < LOQ (< 9.97)	< LOD (< 0.60)	< LOD (< 15.02)	0.89 < LOQ (< 12.63)
					Recovery	47%	21%	53%		57%	60%	50%	83%	33%	40%
23	Snacks	135	Other snacks (not potato based)	S14-042985	Result	< LOD (< 2.50)	< LOD (< 16.25)	**35.53**		< LOD (< 0.14)	< LOD (< 1.16)	< LOD (< 1.18)	< LOD (< 0.72)	< LOD (< 24.08)	0.09 < LOQ (< 4.47)
					Recovery	36%	31%	50%		72%	43%	84%	70%	21%	112%
25	Sandwiches	138	Sandwiches	S14-042988	Result	< LOD (< 3.55)	< LOD (< 9.60)	**41.65**	**60.82**	< LOD (< 0.09)	< LOD (< 1.80)	< LOD (< 1.47)	< LOD (< 0.60)	< LOD (< 12.49)	< LOD (< 0.43)
					Recovery	56%	52%	30%	16%	109%	56%	68%	84%	40%	116%
		NA	Group sample	S14-042852	Result	< LOD (< 3.58)	< LOD (< 12.60)	**42.26**	**48.06**	< LOD (< 0.09)	< LOD (< 1.88)	< LOD (< 1.53)	< LOD (< 0.68)	< LOD (< 13.00)	< LOD (< 0.46)
					Recovery	56%	40%	31%	14%	106%	53%	65%	73%	38%	108%

8.8. Ergot alkaloids

An established HPLC method for the analysis of ergots was transferred onto a UPLC system. The original method used a Phenomenex Gemini C_{18} 5 µm (150 x 2.1 mm) HPLC column. No UPLC column with this phase was available therefore a Waters Acquity BEH C_{18} 1.7 µm (100 x 2.1 mm) was selected because the BEH substrate is stable in high pH mobile phases. The same mobile phases as the original method were used and the gradient profile was re-optimised for the UPLC column. The method run time was shortened from 21 min to 12 min as a result of the transfer to UPLC.

Results for ergot alkaloid analysis are given in Table 22. All bread samples contained some or all of the 12 ergot alkaloids included in the analytical method. Levels found ranged from < 1 µg/kg to 7.51 µg/kg for individual ergot alkaloids in the samples. Wholemeal and granary bread contained a total of 34 µg/kg alkaloids. Ergot alkaloids were also detected in sandwiches at a similar level to bread samples, and at lower levels in other cereal products such as flour, breakfast cereals, biscuits and pizza as well as the group sample for these products. The alkaloids were not detected in branded food drinks, beer, cider or alternatives to milk.

8.9. Patulin

Patulin was analysed using conditions very similar to the multi-mycotoxin method; the same column and mobile phases were used and the gradient was kept the same until the patulin peak was eluted but was then rapidly increased to flush the column before re-equilibration. The injection volume was also increased because sample extracts were submitted in aqueous solvent therefore more could be injected without deterioration of the peak shape of this early-eluting compound. Carbon-13 labelled patulin internal standard was used to calculate recovery and to compensate for matrix effects.

Patulin results are presented in Table 23. Patulin was not detected in any sample. Individual LODs calculated for the samples analysed ranged from 1.7 µg/kg for the mushroom sample to 13.6 µg/kg for the group cereal sample. All samples were overspiked with ^{13}C-patulin. Apparent patulin recovery values appeared low however the analysis was fully controlled by the use of the internal standard.

Table 22. Ergot Alkaloids Results.

Group		Category		LIMS Number		Ergot Concentration / µg/kg												
						Concentrations are corrected for recovery. ' Corrected using group sample recovery value. ^ Identity of the residue fails to confirm by ion ratio. ^^ Only one result because the category and group samples are identical.												
						Ergocornine	Ergocorninine	Ergocristine	Ergocristinine	Ergocryptine	Ergocryptinine	Ergometrine	Ergometrinine	Ergosine	Ergosinine	Ergotamine	Ergotaminine	Total Ergot Alkaloids
1	Bread	1	White sliced bread	S14-042856	Result	**0.81**	**0.77**	**2.70**	**2.11**	**1.37**	**1.02**	**1.31**	0.95 < LOQ (< 1.0)	**1.22**	**0.74**	**1.36**	**0.67**	**14.08**
					Recovery	100% '	96% '	112% '	112% '	99% '	111% '	114% '	91% '	87% '	95% '	104% '	111% '	
		2	White unsliced bread	S14-042857	Result	**0.71**	**0.63**	**1.70**	**1.22**	**0.91**	**0.63**	**1.07**	0.85 < LOQ (< 1.0)	**1.71**	**1.00**	**1.58**	**0.73**	**11.88**
					Recovery	100% '	96% '	112% '	112% '	99% '	111% '	114% '	91% '	87% '	95% '	104% '	111% '	
		3	Brown bread	S14-042858	Result	**1.46**	**1.26**	**5.36**	**3.98**	**2.35**	**1.53**	**2.01**	**1.19**	**2.13**	**1.41**	**3.01**	**1.61**	**27.29**
					Recovery	100% '	96% '	112% '	112% '	99% '	111% '	114% '	91% '	87% '	95% '	104% '	111% '	
		4	Wholemeal and granary bread	S14-042859	Result	**1.69**	**1.54**	**7.51**	**5.71**	**2.79**	**2.03**	**2.05**	**1.24**	**2.38**	**1.56**	**3.44**	**1.75**	**33.69**
					Recovery	100% '	96% '	112% '	112% '	99% '	111% '	114% '	91% '	87% '	95% '	104% '	111% '	
		5	Other bread	S14-042860	Result	**1.32**	**1.16**	**4.08**	**3.05**	**1.96**	**1.27**	**1.91**	**1.01**	**2.07**	**1.57**	**2.41**	**1.47**	**23.29**
					Recovery	100% '	96% '	112% '	112% '	99% '	111% '	114% '	91% '	87% '	95% '	104% '	111% '	
		NA	Group sample	S14-042828	Result	**1.05**	**0.91**	**3.63**	**2.77**	**1.54**	**1.05**	**1.51**	**1.00**	**1.67**	**1.17**	**1.99**	**1.14**	**19.43**
					Recovery	100%	96%	112%	112%	99%	111%	114%	91%	87%	95%	104%	111%	
2	Miscellaneous cereals	6	Flour	S14-042861	Result	**0.44**	**0.40**	**1.85**	**1.15**	**0.54**	**0.35**	0.96 < LOQ (< 1.0)	< 1.0	**1.16**	**0.61**	**1.19**	**0.55**	**8.25**
					Recovery	103% '	111% '	116% '	129% '	104% '	117% '	93% '	95% '	92% '	106% '	106% '	120% '	
		7	Buns, cakes and pastries	S14-042862	Result	0.22 < LOQ (< 0.25)	0.23 < LOQ (< 0.25)	**0.32**	**0.33**	0.22 < LOQ (< 0.25)	**0.26**	0.69 < LOQ (< 1.0)	< 1.0	**0.51**	**0.28**	**0.54**	0.24 < LOQ (< 0.25)	**2.23**
					Recovery	103% '	111% '	116% '	129% '	104% '	117% '	93% '	95% '	92% '	106% '	106% '	120% '	
		8	Savoury biscuits	S14-042863	Result	**0.32**	**0.55**	**1.09**	**1.98**	**0.49**	**0.72**	**1.01**	0.79 < LOQ (< 1.0)	**0.77**	**0.84**	**0.88**	**0.69**	**9.34**
					Recovery	103% '	111% '	116% '	129% '	104% '	117% '	93% '	95% '	92% '	106% '	106% '	120% '	
		9	Sweet biscuits	S14-042864	Result	**0.34**	**0.32**	**0.65**	**0.60**	**0.38**	**0.31**	0.80 < LOQ (< 1.0)	0.74 < LOQ (< 1.0)	**0.53**	**0.54**	**0.65**	**0.59**	**4.90**
					Recovery	103% '	111% '	116% '	129% '	104% '	117% '	93% '	95% '	92% '	106% '	106% '	120% '	
		10	Chocolate biscuits	S14-042865	Result	0.15 < LOQ (< 0.25)	**0.31**	**0.32**	**0.71**	0.14 < LOQ (< 0.25)	**0.33**	0.94 < LOQ (< 1.0)	0.79 < LOQ (< 1.0)	**0.38**	**0.35**	**0.36**	**0.32**	**3.07**
					Recovery	103% '	111% '	116% '	129% '	104% '	117% '	93% '	95% '	92% '	106% '	106% '	120% '	
		11	Breakfast cereals	S14-042866	Result	**0.45**	**0.63**	**0.53**	**0.63**	**0.47**	**0.53**	**1.16**	0.94 < LOQ (< 1.0)	**0.83**	**0.54**	**0.84**	**0.47**	**7.08**
					Recovery	103% '	111% '	116% '	129% '	104% '	117% '	93% '	95% '	92% '	106% '	106% '	120% '	
		12	Rice	S14-042867	Result	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 1.0	< 1.0	< 0.25	< 0.25	< 0.25	< 0.25	0.00
					Recovery	103% '	111% '	116% '	129% '	104% '	117% '	93% '	95% '	92% '	106% '	106% '	120% '	
		13	Other cereal products	S14-042868	Result	0.14 < LOQ (< 0.25)	0.14 < LOQ (< 0.25)	0.22 < LOQ (< 0.25)	0.24 < LOQ (< 0.25)	0.13 < LOQ (< 0.25)	0.16 < LOQ (< 0.25)	0.65 < LOQ (< 1.0)	< 1.0	**0.31**	0.20 < LOQ (< 0.25)	**0.34**	0.18 < LOQ (< 0.25)	**0.64**
					Recovery	103% '	111% '	116% '	129% '	104% '	117% '	93% '	95% '	92% '	106% '	106% '	120% '	
		14	Pasta	S14-042869	Result	0.08 < LOQ (< 0.25)	0.03 < LOQ (< 0.25)	0.17 < LOQ (< 0.25)	0.09 < LOQ (< 0.25)	0.08 < LOQ (< 0.25)	0.05 < LOQ (< 0.25)	0.60 < LOQ (< 1.0)	< 1.0	0.11 < LOQ (< 0.25)	0.05 < LOQ (< 0.25)	0.12 < LOQ (< 0.25)	0.04 < LOQ (< 0.25)	0.00
					Recovery	103% '	111% '	116% '	129% '	104% '	117% '	93% '	95% '	92% '	106% '	106% '	120% '	
		15	Pizza	S14-042870	Result	**0.47**	**0.37**	**1.07**	**0.78**	**0.59**	**0.37**	0.90 < LOQ (< 1.0)	0.72 < LOQ (< 1.0)	**0.97**	**0.68**	**1.04**	**0.62**	**6.94**
					Recovery	103% '	111% '	116% '	129% '	104% '	117% '	93% '	95% '	92% '	106% '	106% '	120% '	
		NA	Group sample	S14-042829	Result	**0.32**	**0.29**	**0.53**	**0.46**	**0.38**	**0.31**	0.78 < LOQ (< 1.0)	0.72 < LOQ (< 1.0)	**0.54**	**0.40**	**0.68**	**0.41**	**4.30**
					Recovery	103%	111%	116%	129%	104%	117%	93%	95%	92%	106%	106%	120%	
17	Non- alcoholic Beverages (with bottled water)	107	Branded food drinks	S14-042959	Result	< LOQ (< 0.63)	< LOQ (< 0.61)	< LOQ (< 0.63)	< LOQ (< 0.62)	< LOQ (< 0.63)	< LOQ (< 0.62)	< LOQ (< 1.21)	< LOQ (< 0.59)	< LOQ (< 0.56)	< LOQ (< 0.63)	< LOQ (< 0.59)	< LOQ (< 0.63)	0.00
					Recovery	80%	81%	79%	81%	79%	81%	83%	84%	89%	79%	84%	80%	
		113	Alternatives to milk	S14-042963	Result	< LOD (< 1.14)	< LOD (< 0.90)	< LOD (< 1.02)	< LOD (< 0.88)	< LOD (< 1.05)	< LOD (< 0.90)	< LOD (< 0.91)	< LOD (< 0.86)	< LOD (< 0.85)	< LOD (< 1.07)	< LOD (< 0.89)	< LOD (< 1.00)	0.00
					Recovery	88%	111%	98%	113%	95%	111%	110%	116%	117%	93%	112%	100%	
21	Alcoholic drinks	128	Beer	S14-042978	Result	< LOQ (< 0.64)	< LOQ (< 0.61)	< LOQ (< 0.64)	< LOQ (< 0.61)	< LOQ (< 0.64)	< LOQ (< 0.62)	< LOQ (< 1.26)	< LOQ (< 0.61)	< LOQ (< 0.59)	< LOQ (< 0.64)	< LOQ (< 0.61)	< LOQ (< 0.64)	0.00
					Recovery	78%	82%	78%	81%	78%	81%	80%	82%	84%	78%	82%	79%	
		129	Cider	S14-042979	Result	< LOQ (< 0.64)	< LOQ (< 0.64)	< LOQ (< 0.65)	< LOQ (< 0.64)	< LOQ (< 0.65)	< LOQ (< 0.64)	< LOQ (< 1.24)	< LOQ (< 0.61)	< LOQ (< 0.61)	< LOQ (< 0.65)	< LOQ (< 0.63)	< LOQ (< 0.65)	0.00
					Recovery	78%	79%	77%	78%	77%	79%	81%	83%	82%	76%	80%	77%	
23	Snacks	135	Other snacks (not potato based)	S14-042985	Result	< LOQ (< 0.62)	**0.52**	< LOQ (< 0.58)	**0.76**	< LOQ (< 0.56)	**0.51**	< LOQ (< 1.21)	< LOQ (< 0.55)	**1.12**	**0.74**	< LOQ (< 0.52)	< LOQ (< 0.50)	**3.65**
					Recovery	81%	108%	86%	110%	89%	105%	83%	91%	105%	96%	95%	100%	
25	Sandwiches	138	Sandwiches	S14-042988	Result	**0.75 ^^**	**0.92 ^^**	**1.80 ^^**	**2.05 ^^**	**1.08 ^^**	**1.06 ^^**	**1.26 ^^**	0.92 < LOQ (< 1.0) ^^	**1.39 ^^**	**0.95 ^^**	**1.36 ^^**	**0.84 ^^**	**13.46 ^^**
					Recovery													
		NA	Group sample	S14-042852	Result													
					Recovery	92%	70%	110%	101%	91%	78%	86%	87%	138%	99%	102%	99%	

Table 23. Patulin Results.

Group		Category		LIMS Number		Patulin
						Concentrations are inherently corrected for recovery using an isotope-labelled internal standard. Recovery values in table are for this internal standard.
1	Bread	NA	Group sample	S14-042828	Result	< LOD (< 10.73)
					Recovery	19%
2	Miscellaneous cereals	NA	Group sample	S14-042829	Result	< LOD (< 13.58)
					Recovery	15%
12	Potatoes	69	Fresh potatoes	S14-042924	Result	< LOD (< 10.04)
					Recovery	20%
		70	Potato products	S14-042925	Result	< LOD (< 8.16)
					Recovery	25% "
		NA	Group sample	S14-042839	Result	< LOD (< 9.60)
					Recovery	21%
13	Other vegetables	71	Onions,leeks	S14-042926	Result	< LOD (< 3.90)
					Recovery	51%
		72	Carrots	S14-042927	Result	< LOD (< 2.33)
					Recovery	86% "
		73	Turnips, swedes	S14-042928	Result	< LOD (< 3.56)
					Recovery	56% "
		74	Other fresh vegetables	S14-042929	Result	< LOD (< 2.91)
					Recovery	69% "
		75	Mushrooms	S14-042930	Result	< LOD (< 1.74)
					Recovery	115% "
		76	Tomatoes	S14-042931	Result	< LOD (< 3.01)
					Recovery	66% "
		77	Cucumbers	S14-042932	Result	< LOD (< 3.45)
					Recovery	58% "
		78	Dried pulses	S14-042933	Result	< LOD (< 2.77)
					Recovery	72% "
		79	Herbs, spices	S14-042934	Result	< LOD (< 8.38)
					Recovery	24% "
15	Fresh fruit	90	Oranges	S14-042942	Result	< LOD (< 2.65)
					Recovery	75% "
		91	Other citrus fruits	S14-042943	Result	< LOD (< 4.44)
					Recovery	45% "
		92	Apples	S14-042944	Result	< LOD (< 4.26)
					Recovery	47% "
		93	Pears	S14-042945	Result	< LOD (< 3.00)
					Recovery	67% "
		94	Stone fruit	S14-042946	Result	< LOD (< 2.71)
					Recovery	74% "
		95	Bananas	S14-042947	Result	< LOD (< 2.25)
					Recovery	89% "
		96	Grapes	S14-042948	Result	< LOD (< 2.76)
					Recovery	72% "
		97	Other fresh fruit	S14-042949	Result	< LOD (< 3.10)
					Recovery	65% "
		NA	Group sample	S14-042842	Result	< LOD (< 3.33)
					Recovery	60% "

Group		Category		LIMS Number		Patulin
						Concentrations are inherently corrected for recovery using an isotope-labelled internal standard. Recovery values in table are for this internal standard.
16	Fruit products	98	Canned peaches, pears, pineapples	S14-042950	Result	< LOD (< 2.59)
					Recovery	77% "
		99	Other canned or frozen fruit	S14-042951	Result	< LOD (< 3.09)
					Recovery	65% "
		100	Dried fruit	S14-042952	Result	< LOD (< 2.41)
					Recovery	83% "
		101	Fruit juices and vegetable juices	S14-042953	Result	< LOD (< 2.75)
					Recovery	73% "
		NA	Group sample	S14-042843	Result	< LOD (< 2.13)
					Recovery	94% "
17	Non- alcoholic Beverages (with bottled water)	102	Tea	S14-042954	Result	< LOD (< 6.38)
					Recovery	31% "
		103	Takeaway Tea	S14-042955	Result	< LOD (< 5.47)
					Recovery	37% "
		104	Instant coffee	S14-042956	Result	< LOD (< 6.91)
					Recovery	29% "
		105	Ground coffee	S14-042957	Result	< LOD (< 5.67)
					Recovery	35% "
		106	Takeaway coffee	S14-042958	Result	< LOD (< 8.40)
					Recovery	24% "
		107	Branded food drinks	S14-042959	Result	< LOD (< 8.10)
					Recovery	25% "
		108	Cocoa, drinking chocolate	S14-042960	Result	< LOD (< 8.49)
					Recovery	24% "
		113	Alternatives to milk	S14-042963	Result	< LOD (< 4.29)
					Recovery	47% "
20	Nuts	126	Ground nuts including peanut butter	S14-042976	Result	< LOD (< 6.53)
					Recovery	31%
		127	Tree nuts	S14-042977	Result	< LOD (< 10.52)
					Recovery	19%
		NA	Group sample	S14-042847	Result	< LOD (< 7.77)
					Recovery	26%
21	Alcoholic drinks	128	Beer	S14-042978	Result	< LOD (< 3.44)
					Recovery	58%
		129	Cider	S14-042979	Result	< LOD (< 5.67)
					Recovery	35%
		130	Wine	S14-042980	Result	< LOD (< 3.48)
					Recovery	57%
		131	Alcopops and cocktails	S14-042981	Result	< LOD (< 3.69)
					Recovery	54%
23	Snacks	135	Other snacks (not potato based)	S14-042985	Result	< LOD (< 6.08)
					Recovery	33%
25	Sandwiches	138	Sandwiches	S14-042988	Result	< LOD (< 4.13)
					Recovery	48%
		NA	Group sample	S14-042852	Result	< LOD (< 3.56)
					Recovery	56%

8.10. Sterigmatocystin

Sterigmatocystin was analysed using conditions very similar to the multi-mycotoxin method; the same column and mobile phases were used but the gradient was run in a shorter time. The injection volume was also increased because the effect on peak shape of early-eluting compounds was no longer a concern. An isotope-labelled internal standard was also added to the samples.

The sterigmatocystin results are given in Table 24. Three samples contained sterigmatocystin below the LOQ but above the LOD at levels from 0.46 to 2.17 µg/kg. These were chocolate biscuits (0.46 µg/kg), white unsliced bread (0.58 µg/kg) and herbs & spices (2.17 µg/kg). These 3 results are not quantitative as they are outside the reliable quantification range but the result is given for information as it is of value as it indicates a low level presence of this analyte. The LOQ for herbs & spices is higher due to matrix interferences seen in the chromatograms due to the large background seen in this sample. Even using the internal standard to adjust for matrix effects a higher LOQ was observed. This was a consistent pattern across all analyses of this category sample and highlights the difficulty in the analysis of these products. The LOQ is lower for the staple foods such as bread and is still acceptable for this category which forms a very minor part of the overall diet.

8.11. Citrinin

The anaylsis of citrinin using the multi-mycotoxin method was often unsuccessful due to the presence of interferences and also a high level of ion suppression in the MS source. It was decided that the LOD of citrinin would be improved by cleaning up extracts prior to analysis. This was achieved using new immunoaffinity columns that were a gift from R-Biopharm (Rhone).

Citrinin was analysed using the acidic multi-mycotoxin method. The injection volume was increased because the extracts had been subjected to clean-up and therefore there was less risk of simply injecting more interferences onto the column. An isotope-labelled internal standard was also added to the samples. The validation data is given in Table 25. The mean recovery for cereal spiked at 25 µg/kg was 111 % (after correction by ^{13}C internal standard). The relative standard deviation was only 4 %, showing the method was very repeatable. Further analyses would be required to complete a full formal single laboratory validation, however the data and the use of the ^{13}C internal standard ensure a high degree of confidence in the results found in this study.

All citrinin results are given in Table 24. All samples were below the LOQ, this ranged from 1.1 to 1.8 µg/kg, except for spices where it was slightly higher at 2.16 µg/kg. Recovery values were also very good, again the use of ^{13}C-citrinin internal standard helped control for matrix effects. The lowest recovery was seen for

herbs & spices, where it was 46 %, highlighting the difficulty of analysing these samples.

Table 24. Results for Sterigmatocystin, Citrinin, Cyclopiazonic acid and Moniliformin.

Group		Category		LIMS Number		Sterigmatocystin Concentration / µg/kg	Citrinin	Cyclopiazonic Acid	Moniliformin
						Concentrations are inherently corrected for recovery using an isotope-labelled internal standard. Recovery values in table are for this internal standard. ^ Identity of the residue fails to confirm by ion ratio.	Concentrations are inherently corrected for recovery using an isotope-labelled internal standard. Recovery values in table are for this internal standard. ^ Only one result because the category and group samples are identical.	Concentrations are inherently corrected for recovery using an isotope-labelled internal standard. Recovery values in table are for this internal standard. ^ Only one result because the category and group samples are identical.	Concentrations are corrected for recovery. ^ Only one result because the category and group samples are identical.
1	Bread	1	White sliced bread	S14-042856	Result	< LOD (< 0.24)	< LOQ (< 1.27)	< LOD (< 0.50)	< LOQ (< 13.61)
					Recovery	83%	79%	115%	7%
		2	White unsliced bread	S14-042857	Result	0.58 < LOQ (< 0.79)	< LOQ (< 1.23)	< LOD (< 0.50)	< LOQ (< 13.34)
					Recovery	76%	81%	117%	7%
		3	Brown bread	S14-042858	Result	< LOD (< 0.28)	< LOQ (< 1.36)	0.79 < LOQ (< 1.00)	< LOQ (< 23.06)
					Recovery	71%	74%	107%	4%
		4	Wholemeal and granary bread	S14-042859	Result	< LOD (< 0.33)	< LOQ (< 1.32)	< LOD (< 0.50)	< LOQ (< 20.68)
					Recovery	61%	76%	113%	5%
		5	Other bread	S14-042860	Result	< LOD (< 0.28)	< LOQ (< 1.38)	< LOD (< 0.50)	< LOQ (< 18.93)
					Recovery	72%	72%	113%	5%
		NA	Group sample	S14-042828	Result	< LOD (< 0.29)	< LOQ (< 1.32)	< LOD (< 0.50)	< LOQ (< 17.13)
					Recovery	69%	76%	113%	6%
2	Miscellaneous cereals	6	Flour	S14-042861	Result	< LOD (< 0.27)	< LOQ (< 1.24)	< LOD (< 0.50)	< LOQ (< 15.24)
					Recovery	73%	81%	104%	7%
		7	Buns, cakes and pastries	S14-042862	Result	< LOD (< 0.22)	< LOQ (< 1.34)	< LOD (< 0.50)	< LOQ (< 13.23)
					Recovery	89%	74%	124%	8%
		8	Savoury biscuits	S14-042863	Result	< LOD (< 0.37)	< LOQ (< 1.36)	< LOD (< 0.50)	< LOQ (< 24.21)
					Recovery	54%	73%	109%	4%
		9	Sweet biscuits	S14-042864	Result	< LOD (< 0.22)	< LOQ (< 1.20)	< LOD (< 0.50)	< LOQ (< 19.84)
					Recovery	89%	83%	127%	5%
		10	Chocolate biscuits	S14-042865	Result	0.46 < LOQ (< 0.66)	< LOQ (< 1.18)	< LOD (< 0.50)	< LOQ (< 15.44)
					Recovery	92%	85%	129%	6%
		11	Breakfast cereals	S14-042866	Result	< LOD (< 0.21)	< LOQ (< 1.27)	< LOD (< 0.50)	< LOQ (< 9.25)
					Recovery	94%	79%	117%	11%
		12	Rice	S14-042867	Result	< LOD (< 0.54)	< LOQ (< 1.28)	< LOD (< 0.50)	< LOQ (< 3.97)
					Recovery	37%	78%	77%	25%
		13	Other cereal products	S14-042868	Result	< LOD (< 0.22)	< LOQ (< 1.29)	< LOD (< 0.50)	< LOQ (< 8.72)
					Recovery	89%	77%	105%	11%
		14	Pasta	S14-042869	Result	< LOD (< 0.29)	< LOQ (< 1.37)	< LOD (< 0.50)	< LOQ (< 5.09)
					Recovery	68%	73%	90%	20%
		15	Pizza	S14-042870	Result	< LOD (< 0.43)	< LOQ (< 1.51)	< LOD (< 0.50)	< LOQ (< 20.46)
					Recovery	47%	66%	103%	5%
		NA	Group sample	S14-042829	Result	< LOD (< 0.25)	< LOQ (< 1.36)	< LOD (< 0.50)	< LOQ (< 14.19)
					Recovery	80%	73%	107%	7%
13	Other vegetables	78	Dried pulses	S14-042933	Result	< LOD (< 0.27)	< LOQ (< 1.53)	< LOD (< 0.50)	< LOQ (< 3.95)
					Recovery	73%	66%	80%	25%
		79	Herbs, spices	S14-042934	Result	2.17 ^ < LOQ (< 5.00)	< LOQ (< 2.16)	0.89 < LOQ (< 1.00)	< LOQ (< 39.50)
					Recovery	44%	46%	74%	3%
16	Fruit products	100	Dried fruit	S14-042952	Result	< LOD (< 0.15)	< LOQ (< 1.27)	< LOD (< 0.50)	< LOD (< 25.00)
					Recovery	133%	79%	150%	7%
		101	Fruit juices and vegetable juices	S14-042953	Result	< LOD (< 0.25)	< LOQ (< 1.07)	< LOD (< 0.50)	< LOQ (< 3.74)
					Recovery	81%	93%	125%	27%
17	Non- alcoholic Beverages (with bottled water)	102	Tea	S14-042954	Result	< LOD (< 0.22)	< LOQ (< 1.21)	< LOD (< 0.50)	< LOQ (< 1.45)
					Recovery	91%	83%	105%	69%
		103	Takeaway Tea	S14-042955	Result	< LOD (< 0.26)	< LOQ (< 1.13)	< LOD (< 0.50)	< LOQ (< 1.31)
					Recovery	78%	88%	97%	76%
		104	Instant coffee	S14-042956	Result	< LOD (< 0.20)	< LOQ (< 1.16)	< LOD (< 0.50)	< LOQ (< 2.89)
					Recovery	100%	86%	105%	35%
		105	Ground coffee	S14-042957	Result	< LOD (< 0.26)	< LOQ (< 1.08)	< LOD (< 0.50)	< LOQ (< 2.21)
					Recovery	77%	92%	108%	45%
		106	Takeaway coffee	S14-042958	Result	< LOD (< 0.30)	< LOQ (< 1.14)	< LOD (< 0.50)	< LOQ (< 2.95)
					Recovery	66%	88%	100%	34%
		107	Branded food drinks	S14-042959	Result	< LOD (< 0.35)	< LOQ (< 1.16)	< LOD (< 0.50)	< LOQ (< 7.64)
					Recovery	57%	86%	112%	13%
		108	Cocoa, drinking chocolate	S14-042960	Result	< LOD (< 0.36)	< LOQ (< 1.30)	< LOD (< 0.50)	< LOQ (< 3.43)
					Recovery	56%	77%	120%	29%
		113	Alternatives to milk	S14-042963	Result	< LOD (< 0.44)	< LOQ (< 1.20)	< LOD (< 0.50)	< LOQ (< 6.84)
					Recovery	113%	83%	109%	15%
20	Nuts	126	Ground nuts including peanut butter	S14-042976	Result	< LOD (< 0.19)	< LOQ (< 1.60)	< LOD (< 0.50)	< LOQ (< 14.41)
					Recovery	105%	63%	103%	7%
		127	Tree nuts	S14-042977	Result	< LOD (< 0.20)	< LOQ (< 1.22)	< LOD (< 0.50)	< LOQ (< 12.98)
					Recovery	102%	82%	85%	8%
		NA	Group sample	S14-042847	Result	< LOD (< 0.20)	< LOQ (< 1.21)	< LOD (< 0.50)	< LOQ (< 15.08)
					Recovery	100%	83%	96%	7%
21	Alcoholic drinks	128	Beer	S14-042978	Result	< LOD (< 0.19)	< LOQ (< 1.08)	< LOD (< 0.50)	< LOQ (< 1.73)
					Recovery	104%	93%	107%	58%
		129	Cider	S14-042979	Result	< LOD (< 0.20)	< LOQ (< 1.11)	< LOD (< 0.50)	< LOQ (< 1.57)
					Recovery	99%	90%	101%	64%
		130	Wine	S14-042980	Result	< LOD (< 0.20)	< LOQ (< 1.25)	< LOD (< 0.50)	< LOQ (< 3.48)
					Recovery	99%	80%	108%	29%
		131	Alcopops and cocktails	S14-042981	Result	< LOD (< 0.19)	< LOQ (< 1.12)	< LOD (< 0.50)	< LOQ (< 2.91)
					Recovery	107%	89%	120%	69%
23	Snacks	135	Other snacks (not potato based)	S14-042985	Result	< LOD (< 0.37)	< LOQ (< 1.45)	**4.27**	< LOQ (< 24.18)
					Recovery	54%	69%	99%	4%
25	Sandwiches	138	Sandwiches	S14-042988	Result	< LOD (< 0.40)	< LOQ (< 1.81) ^	< LOD (< 0.50) ^	< LOQ (< 18.98) ^
					Recovery	50%			
		NA	Group sample	S14-042852	Result	< LOD (< 0.44)	55%	77%	5%
					Recovery	45%			

Table 25. Validation results for citrinin.

Sample	Citrinin	Citrinin	$^{13}C_{13}$-Citrinin
	Conc. / µg/kg	**Apparent Recovery** (after correction by internal standard)	**Recovery**
Blank 1	NA		NA
Blank 2	0.37		81%
Spike 1 (25 µg/kg)	28.18	111%	66%
Spike 2 (25 µg/kg)	29.37	116%	58%
Spike 3 (25 µg/kg)	27.22	107%	54%
Spike 4 (25 µg/kg)	27.55	109%	57%
Spike 5 (25 µg/kg)	29.51	117%	55%
Spike 6 (25 µg/kg)	27.14	107%	52%
Mean	**28.16**	**111%**	**60%**
SD	**1.06**		
RSD	**4%**		

8.12. Cyclopiazonic Acid

Cyclopiazonic acid is usually analysed using the acidic multi-mycotoxin method, but the peak shape was asymmetric which affected integration and sometimes also resulted in interferences co-eluting with the peak tail. The peak shape with the neutral multi-mycotoxin method deteriorated further. It was decided to analyse cyclopiazonic acid at high pH. To allow this, the column had to be changed from the Waters Acquity HSS T3 1.8 µm (100 x 2.1 mm) normally used to a Waters Acquity BEH C_{18} 1.7 µm (100 x 2.1 mm) which is stable in high pH mobile phase. A simple gradient profile was used which was made steeper after the elution of cyclopiazonic acid in order to shorten the run time. An isotope-labelled internal standard was also added to the samples.

The validation data is given in Table 26. The mean recovery for cereal spiked at 25 µg/kg was 89 % (after correction by ^{13}C internal standard). The relative standard deviation was only 4 %, showing the method was very repeatable. Further analyses would be required to complete a full formal single laboratory validation, however the data and the use of the ^{13}C internal standard ensure a high degree of confidence in the results found in this study.

Cyclopiazonic acid results are given in Table 24. Most samples in the study were < LOD, which was set at 0.5 µg/kg. Two samples contained levels below LOQ (1.0 µg/kg), but above LOD. These were brown bread at 0.79 µg/kg and herbs and spices at 0.89 µg/kg. One sample (other snacks, not potato) contained a residue at 4.27 µg/kg. An internal standard was used for this analysis and in all cases recovery for TDS samples was in the range of 74 to 129 %. There are no limits for cyclopiazonic acid in legislation and EFSA have not evaluated it to derive a TDI. An LD_{50} of 2.3 mg/kg was observed (EMAN) and the levels found here are clearly significantly below that level.

Table 26. Validation data for Cyclopiazonic acid.

Sample	Cyclopiazonic Acid	Cyclopiazonic Acid	$^{13}C_{20}$-Cyclopiazonic Acid
	Conc. / µg/kg	Apparent Recovery (after correction by internal standard)	Recovery
Blank 1	NA		NA
Blank 2	0.00		97%
Spike 1 (25 µg/kg)	23.01	92%	100%
Spike 2 (25 µg/kg)	21.63	87%	100%
Spike 3 (25 µg/kg)	20.90	84%	96%
Spike 4 (25 µg/kg)	23.10	92%	87%
Spike 5 (25 µg/kg)	22.57	90%	97%
Spike 6 (25 µg/kg)	21.76	87%	99%
Mean	**22.16**	**89%**	**97%**
SD	**0.87**		
RSD	**4%**		

8.13. Moniliformin

Moniliformin is a very small, charged analyte which is highly soluble in water and unretained when analysed using the established multi-mycotoxin method. It elutes in the solvent front when the mobile phase is 99 % aqueous, which hinders ionisation and desolvation in the source. There is also a high possibility of co-elution with polar interferences leading to interference and ion suppression, and inaccuracy in quantification and ultimately higher LOD and LOQ. In order to improve the analytical performance and increase sensitivity a HILIC column was used to retain moniliformin in a method based on a published paper (Scarpino et al, 2013). The gradient used in this present study was steeper than that in the published method to decrease the run time. The ammonium formate buffer concentration in mobile phase A was also reduced from 100 mM to 50 mM because this resulted in a better MS response without significant deterioration of peak shape or loss of retention (reducing the concentration further resulted in a broad, early-eluting peaks). As is often the case with HILIC, a long re-equilibration time was required to obtain a stable retention time. Significant improvements were made to chromatography analytical performance. Attempts to improve the extraction and clean-up were made. Commercially available clean-up columns for moniliformin are available but by consulting literature and the manufacturer it was apparent they are only applicable for use with raw cereal flour (mainly maize), and would not be suitable for the TDS samples. Attempts were also made to source an isotopically labelled internal standard; however there are none available for moniliformin.

Other published methods used extensive concentration and blow down steps. These were mainly intended to concentrate the extracts to improve sensitivity, however as this had been achieved through the chromatography improvements by using HILIC there was no need to carry out this additional step. Based on literature and previous experience it was decided the best option would be a ‘dilute and shoot’ approach

using the standard extraction for the multi-mycotoxin method. It was hoped this approach would lead to minimal losses as no drying step, where losses can occur, was included.

The validation data is given in Table 27. The mean recovery for cereal spiked at 25 µg/kg was 11 %. The relative standard deviation was 11 %. Therefore the method gives low but repeatable results. It is unclear why the recovery was so low, particularly for the validation samples, as the extraction solvent used was similar to others reported to give higher recovery. Previously published methods were for raw cereals, there have been no other studies of complex sample types such as those included in the TDS study.

Table 27. Validation data for moniliformin

Sample	Moniliformin	Moniliformin
	Conc. / µg/kg	Recovery
Blank 1	0.08	
Blank 2	0.08	
Spike 1 (25 µg/kg)	1.94	7%
Spike 2 (25 µg/kg)	1.86	7%
Spike 3 (25 µg/kg)	1.85	7%
Spike 4 (25 µg/kg)	2.38	9%
Spike 5 (25 µg/kg)	1.80	7%
Spike 6 (25 µg/kg)	1.90	7%
Mean	**1.96**	**8%**
SD	**0.21**	
RSD	**11%**	

Moniliformin was not detected in any sample, full results are given in Table 24. LOQs were calculated to be from 1.3 µg/kg for takeaway tea to 39.5 µg/kg for herbs and spices. For the dried fruit sample the LOD was calculated as there was a large peak that co-eluted with moniliformin that made it difficult to accurately estimate the LOQ. Recovery values for moniliformin were extremely low, the reason for this is not known as previous work had shown the extraction method to be suitable. As the molecule is so small there is only one MRM transition that can be used for analysis. This means it is not possible to confirm the identity of the analyte using normal (triple quad) MS, which would typically be done by looking for the presence of a second and third transition and comparing the ratio of these to authentic standards. The use of Time of Flight-MS (High Resolution) was investigated, as this would allow confirmation using accurate mass, but was found to be less sensitive than LC-MS/MS and so wasn't used for the study. It is possible that due to the improvements made to the chromatography these are 'true' results. Previous studies may have overestimated moniliformin as it was unretained, co-eluted with other

small molecules and without a confirmation transition it would not be possible to discriminate between these compounds. It could simply be those samples were less complex and suffered fewer losses during extraction. The sensitivity of the LC-MS/MS method and the fact that every sample was overspiked at 25 µg/kg meant that even with very low recovery reasonable LOQs could be determined, and if moniliformin had been present in the samples it would have been detected.

9. Discussion

9.1. Calculation of Mycotoxin Levels in Group vs Category Samples.

The relative proportion of sample in each category taken to prepare a group sample was used as the basis to calculate the expected amount of toxin present in the group samples. This was then compared to the analytical result determined for that sample where residues had been detected.

9.1.1. Trichothecenes

Some samples were analysed a second time as deoxynivalenol had been found but the recovery measured was very low. The second analysis used ^{13}C-deoxynivalenol as an internal standard to internally correct the data. For both analyses the results of the sum of the category samples and group samples were in excellent agreement, both within the analysis group and between groups. For the bread category samples there were differences between the first and second analyses of up a factor or two, one result was approximately half the original result, two were approximately double and 2 were in good agreement. The fact that the sum and group samples were in good agreement would suggest that the individual differences were due to variations in the samples due to preparation variances. For the sandwiches the category and group sample were the same, the second analysis as the category sample gave a slightly higher value, but the group results were the same (42.3 compared to 48.1 µg/kg).

All other results for deoxynivalenol were in good agreement. For the other trichothecenes where residues were found (Diacetoxyscirpenol, HT-2 and T-2 toxin) the levels were very low but were in agreement. The comparison data is presented in Table 28.

9.1.2. Ergot alkaloids

Comparison of category and group results for ergot alkaloids are presented in Table 29. In all cases the sum of the category samples is in good agreement with the result measured for the group sample. For the sandwiches, the category and the group sample were the same sample therefore only one result is reported. The maximum difference was 39 % which is within the expected variability of the analytical method.

9.1.3. Ochratoxin A

Comparison data for ochratoxin A, zearalenone, sterigmatocystin and cyclopiazonic acid is summarised in Table 30. For the bread samples, low levels (below 1 µg/kg) were found in two category samples. When the results of the other breads were summed the predicted result for the group sample was 0.13 µg/kg and a result of < 0.22 µg/kg was measured. The highest levels of ochratoxin A found were in dried fruit and fruit juices. Analyses of the other food categories in this group or the group sample itself were not requested so no direct comparison can be made. For the other groups (misc. cereals and sandwiches) results were in agreement, no ochratoxin A was found in any of the samples.

9.1.4. Zearalenone

Only 2 groups contained measurable zearalenone. For the potatoes group the result measured was in agreement with the calculated value from the category samples (< 0.66 µg/kg compared to 0.61 µg/kg). For misc. cereals the pizza sample contained ZON, at 16.45 µg/kg, and 2 other samples (sweet biscuits and chocolate biscuits) contained levels just below the LOQ. The calculated value for the group sample was 1.77 µg/kg and < 0.7 µg/kg was measured. These levels are very close or below the normal reporting limit (LOQ) of the method and the difference is within the expected variability of the method.

9.1.5. Sterigmatocystin and Cyclopiazonic Acid

Very low levels of sterigmatocystin and cyclopiazonic acid were found in a small number of samples. The calculated values for the group samples were below the method LODs and the analytical results agreed with this.

Table 28. Comparison of Category and Group Results for Trichothecenes

Group		Category		LIMS Number	Proportion of category in group sample	Trichothecene Concentration / µg/kg Concentrations are corrected for recovery. Concentrations for Deoxynivalenol repeat results are inherently corrected for recovery				
						Deoxynivalenol		Diacetoxyscirpenol	HT2_Toxin	T2_Toxin
						Original Result	Repeat			
1	Bread	1	White sliced bread	S14-042856	39%	**37.02**	**60.13**	< LOD (< 0.12)	< LOD (< 1.41)	0.14 < LOQ (< 5.46)
		2	White unsliced bread	S14-042857	5%	**72.43**	**64.15**	< LOD (< 0.09)	< LOD (< 1.04)	< LOD (< 0.45)
		3	Brown bread	S14-042858	6%	**105.5**	**90.23**	< LOD (< 0.11)	1.39 < LOQ (< 6.54)	< LOD (< 0.44)
		4	Wholemeal and granary bread	S14-042859	21%	**48.05**	**106.8**	< LOD (< 0.10)	2.66 < LOQ (< 6.85)	0.21 < LOQ (< 4.82)
		5	Other bread	S14-042860	28%	**166.4**	**78.94**	< LOD (< 0.15)	< LOD (< 1.76)	< LOD (< 0.72)
		NA	Group sample	S14-042828		**77.42**	**76.73**	< LOD (< 0.09)	< LOD (< 1.13)	< LOD (< 0.46)
			Sum of category samples			**82.00**	**77.35**	NA	0.65	0.10
2	Miscellaneous cereals	6	Flour	S14-042861	8%	**27.24**		< LOD (< 0.08)	1.21 < LOQ (< 5.14)	0.15 < LOQ (< 3.58)
		7	Buns, cakes and pastries	S14-042862	19%	**10.72**		< LOD (< 0.11)	< LOD (< 1.24)	< LOD (< 0.10)
		8	Savoury biscuits	S14-042863	2%	**153.2**	**90.76**	< LOD (< 0.23)	< LOD (< 2.37)	< LOD (< 0.23)
		9	Sweet biscuits	S14-042864	10%	**23.75**		< LOD (< 0.11)	1.5 < LOQ (< 5.72)	0.64 < LOQ (< 4.67)
		10	Chocolate biscuits	S14-042865	6%	**17.32**		< LOD (< 0.11)	< LOD (< 1.10)	0.17 < LOQ (< 4.64)
		11	Breakfast cereals	S14-042866	17%	**34.79**		< LOD (< 0.12)	3.03 ^ < LOQ (< 6.51)	0.85 < LOQ (< 5.03)
		12	Rice	S14-042867	11%	1.4 < LOQ (< 6.37)		< LOD (< 0.12)	< LOD (< 1.32)	< LOD (< 0.10)
		13	Other cereal products	S14-042868	6%	**18.61**		< LOD (< 0.10)	< LOD (< 1.00)	< LOD (< 0.08)
		14	Pasta	S14-042869	11%	**11.18**		0.28 < LOQ (< 6.46)	1.7 < LOQ (< 7.22)	0.93 < LOQ (< 8.17)
		15	Pizza	S14-042870	10%	**23.50**		0.08 < LOQ (< 4.11)	1.17 < LOQ (< 5.23)	0.56 < LOQ (< 3.98)
		NA	Group sample	S14-042829		**37.97**	**44.55**	< LOD (< 0.09)	1.24 ^ < LOQ (< 6.19)	< LOD (< 0.41)
			Sum of category samples			**21.63**	NA	0.04	1.07	0.39
12	Potatoes	69	Fresh potatoes	S14-042924	68%	< LOD (< 2.60)		< LOD (< 0.15)	< LOD (< 1.42)	< LOD (< 0.16)
		70	Potato products	S14-042925	32%	< LOD (< 1.86)		< LOD (< 0.12)	< LOD (< 1.17)	< LOD (< 0.12)
		NA	Group sample	S14-042839		< LOD (< 2.31)		0.23 < LOQ (< 6.55)	< LOD (< 1.32)	0.27 < LOQ (< 6.05)
			Sum of category samples			NA		NA	NA	NA
25	Sandwiches	138	Sandwiches	S14-042988	100%	**41.65**	**60.82**	< LOD (< 0.09)	< LOD (< 1.47)	< LOD (< 0.43)
		NA	Group sample	S14-042852		**42.26**	**48.06**	< LOD (< 0.09)	< LOD (< 1.53)	< LOD (< 0.46)
			Sum of category samples			**41.65**	**60.82**	NA	NA	NA

Table 29. Comparison of Category and Group Results for Ergot Alkaloids

Group		Category		LIMS Number	Proportion of category in group sample	Ergot Concentration / µg/kg Concentrations are corrected for recovery. ^ Identity of the residue fails to confirm by ion ratio. ^^ Only one result because the category and group samples are identical.					
						Ergocornine	Ergocorninine	Ergocristine	Ergocristinine	Ergocryptine	Ergocryptinine
1	Bread	1	White sliced bread	S14-042856	39%	0.81	0.77	2.70	2.11	1.37	1.02
		2	White unsliced bread	S14-042857	5%	0.71	0.63	1.70	1.22	0.91	0.63
		3	Brown bread	S14-042858	6%	1.46	1.26	5.36	3.98	2.35	1.53
		4	Wholemeal and granary bread	S14-042859	21%	1.69	1.54	7.51	5.71	2.79	2.03
		5	Other bread	S14-042860	28%	1.32	1.16	4.08	3.05	1.96	1.27
		NA	Group sample	S14-042828		1.05	0.91	3.63	2.77	1.54	1.05
			Sum of category samples			1.17	1.06	4.22	3.21	1.87	1.31
2	Miscellaneous cereals	6	Flour	S14-042861	8%	0.44	0.40	1.85	1.15	0.54	0.35
		7	Buns, cakes and pastries	S14-042862	19%	0.22 < LOQ (< 0.25)	0.23 < LOQ (< 0.25)	0.32	0.33	0.22 < LOQ (< 0.25)	0.26
		8	Savoury biscuits	S14-042863	2%	0.32	0.55	1.09	1.98	0.49	0.72
		9	Sweet biscuits	S14-042864	10%	0.34	0.32	0.65	0.60	0.38	0.31
		10	Chocolate biscuits	S14-042865	6%	0.15 < LOQ (< 0.25)	0.31	0.32	0.71	0.14 < LOQ (< 0.25)	0.33
		11	Breakfast cereals	S14-042866	17%	0.45	0.63	0.53	0.63	0.47	0.53
		12	Rice	S14-042867	11%	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25	< 0.25
		13	Other cereal products	S14-042868	6%	0.14 < LOQ (< 0.25)	0.14 < LOQ (< 0.25)	0.22 < LOQ (< 0.25)	0.24 < LOQ (< 0.25)	0.13 < LOQ (< 0.25)	0.16 < LOQ (< 0.25)
		14	Pasta	S14-042869	11%	0.08 < LOQ (< 0.25)	0.03 < LOQ (< 0.25)	0.17 < LOQ (< 0.25)	0.09 < LOQ (< 0.25)	0.08 < LOQ (< 0.25)	0.05 < LOQ (< 0.25)
		15	Pizza	S14-042870	10%	0.47	0.37	1.07	0.78	0.59	0.37
		NA	Group sample	S14-042829		0.32	0.29	0.53	0.46	0.38	0.31
			Sum of category samples			0.20	0.24	0.51	0.48	0.23	0.27
25	Sandwiches	138	Sandwiches	S14-042988	100%	0.75 ^^	0.92 ^^	1.80 ^^	2.05 ^^	1.08 ^^	1.06 ^^
		NA	Group sample	S14-042852							
			Sum of category samples			0.75 ^^	0.92 ^^	1.80 ^^	2.05 ^^	1.08 ^^	1.06 ^^

Group		Category		LIMS Number	Proportion of category in group sample	Ergot Concentration / µg/kg Concentrations are corrected for recovery. ^ Identity of the residue fails to confirm by ion ratio. ^^ Only one result because the category and group samples are identical.						
						Ergometrine	Ergometrinine	Ergosine	Ergosinine	Ergotamine	Ergotaminine	Total Ergot Alkaloids
1	Bread	1	White sliced bread	S14-042856	39%	1.31	0.95 < LOQ (< 1.0)	1.22	0.74	1.36	0.67	14.08
		2	White unsliced bread	S14-042857	5%	1.07	0.85 < LOQ (< 1.0)	1.71	1.00	1.58	0.73	11.88
		3	Brown bread	S14-042858	6%	2.01	1.19	2.13	1.41	3.01	1.61	27.29
		4	Wholemeal and granary bread	S14-042859	21%	2.05	1.24	2.38	1.56	3.44	1.75	33.69
		5	Other bread	S14-042860	28%	1.91	1.01	2.07	1.57	2.41	1.47	23.29
		NA	Group sample	S14-042828		1.51	1.00	1.67	1.17	1.99	1.14	19.43
			Sum of category samples			1.67	0.62	1.78	1.20	2.21	1.18	21.52
2	Miscellaneous cereals	6	Flour	S14-042861	8%	0.96 < LOQ (< 1.0)	< 1.0	1.16	0.61	1.19	0.55	8.25
		7	Buns, cakes and pastries	S14-042862	19%	0.69 < LOQ (< 1.0)	< 1.0	0.51	0.28	0.54	0.24 < LOQ (< 0.25)	2.23
		8	Savoury biscuits	S14-042863	2%	1.01	0.79 < LOQ (< 1.0)	0.77	0.84	0.88	0.69	9.34
		9	Sweet biscuits	S14-042864	10%	0.80 < LOQ (< 1.0)	0.74 < LOQ (< 1.0)	0.53	0.54	0.65	0.59	4.90
		10	Chocolate biscuits	S14-042865	6%	0.94 < LOQ (< 1.0)	0.79 < LOQ (< 1.0)	0.38	0.35	0.36	0.32	3.07
		11	Breakfast cereals	S14-042866	17%	1.16	0.94 < LOQ (< 1.0)	0.83	0.54	0.84	0.47	7.08
		12	Rice	S14-042867	11%	< 1.0	< 1.0	< 0.25	< 0.25	< 0.25	< 0.25	0.00
		13	Other cereal products	S14-042868	6%	0.65 < LOQ (< 1.0)	< 1.0	0.31	0.20 < LOQ (< 0.25)	0.34	0.18 < LOQ (< 0.25)	0.64
		14	Pasta	S14-042869	11%	0.60 < LOQ (< 1.0)	< 1.0	0.11 < LOQ (< 0.25)	0.05 < LOQ (< 0.25)	0.12 < LOQ (< 0.25)	0.04 < LOQ (< 0.25)	0.00
		15	Pizza	S14-042870	10%	0.90 < LOQ (< 1.0)	0.72 < LOQ (< 1.0)	0.97	0.68	1.04	0.62	6.94
		NA	Group sample	S14-042829		0.78 < LOQ (< 1.0)	0.72 < LOQ (< 1.0)	0.54	0.40	0.68	0.41	4.30
			Sum of category samples			0.22	0.00	0.54	0.35	0.57	0.28	3.89
25	Sandwiches	138	Sandwiches	S14-042988	100%	1.26 ^^	0.92 < LOQ (< 1.0) ^^	1.39 ^^	0.95 ^^	1.36 ^^	0.84 ^^	13.46 ^^
		NA	Group sample	S14-042852								
			Sum of category samples			1.26	0.00	1.39	0.95	1.36	0.84	13.46

Table 30. Comparison of Category and Group Results for Ochratoxin A, Zearalenone, Sterigmatocystin, and Cyclopiazonic Acid

Group		Category		LIMS Number	Proportion of category in group sample	Ochratoxin A Concentration / µg/kg	Zearalenone Concentration / µg/kg	Sterigmatocystin Concentration / µg/kg	Cyclopiazonic Acid
						Concentrations are corrected for recovery. ^ Only one result because the category and group samples are identical.	Concentrations are corrected for recovery.	Concentrations are inherently corrected for recovery using an isotope-labelled internal standard.	Concentrations are inherently corrected for recovery using an isotope-labelled internal standard. ^ Only one result because the category and group samples are identical.
1	Bread	1	White sliced bread	S14-042856	39%	< LOQ (< 0.22)	< LOD (< 0.34)	< LOD (< 0.24)	< LOD (< 0.50)
		2	White unsliced bread	S14-042857	5%	< LOQ (< 0.22)	< LOD (< 0.42)	0.58 < LOQ (< 0.79)	< LOD (< 0.50)
		3	Brown bread	S14-042858	6%	**0.53**	< LOD (< 0.42)	< LOD (< 0.28)	0.79 < LOQ (< 1.00)
		4	Wholemeal and granary bread	S14-042859	21%	**0.45**	< LOD (< 0.34)	< LOD (< 0.33)	< LOD (< 0.50)
		5	Other bread	S14-042860	28%	< LOQ (< 0.22)	< LOD (< 0.42)	< LOD (< 0.28)	< LOD (< 0.50)
		NA	Group sample	S14-042828		< LOQ (< 0.22)	< LOD (< 0.71)	< LOD (< 0.29)	< LOD (< 0.50)
			Sum of category samples			0.13	NA	0.03	0.05
2	Miscellaneous cereals	6	Flour	S14-042861	8%	< LOQ (< 0.24)	< LOD (< 0.34)	< LOD (< 0.27)	< LOD (< 0.50)
		7	Buns, cakes and pastries	S14-042862	19%	< LOQ (< 0.23)	< LOD (< 0.29)	< LOD (< 0.22)	< LOD (< 0.50)
		8	Savoury biscuits	S14-042863	2%	< LOQ (< 0.26)	< LOD (< 0.29)	< LOD (< 0.37)	< LOD (< 0.50)
		9	Sweet biscuits	S14-042864	10%	< LOQ (< 0.26)	0.57 < LOQ (< 2.46)	< LOD (< 0.22)	< LOD (< 0.50)
		10	Chocolate biscuits	S14-042865	6%	< LOQ (< 0.26)	0.85 < LOQ (< 2.46)	0.46 < LOQ (< 0.66)	< LOD (< 0.50)
		11	Breakfast cereals	S14-042866	17%	< LOQ (< 0.21)	< LOD (< 0.34)	< LOD (< 0.21)	< LOD (< 0.50)
		12	Rice	S14-042867	11%	< LOQ (< 0.30)	< LOD (< 0.41)	< LOD (< 0.54)	< LOD (< 0.50)
		13	Other cereal products	S14-042868	6%	< LOQ (< 0.21)	< LOD (< 0.29)	< LOD (< 0.22)	< LOD (< 0.50)
		14	Pasta	S14-042869	11%	< LOQ (< 0.30)	< LOD (< 0.41)	< LOD (< 0.29)	< LOD (< 0.50)
		15	Pizza	S14-042870	10%	< LOQ (< 0.20)	**16.45**	< LOD (< 0.43)	< LOD (< 0.50)
		NA	Group sample	S14-042829		< LOQ (< 0.20)	< LOD (< 0.7)	< LOD (< 0.25)	< LOD (< 0.50)
			Sum of category samples			NA	**1.77**	0.03	NA
12	Potatoes	69	Fresh potatoes	S14-042924	68%		< LOD (< 0.78)		
		70	Potato products	S14-042925	32%		1.92 < LOQ (< 6.54)		
		NA	Group sample	S14-042839			< LOD (< 0.66)		
			Sum of category samples				0.61		
16	Fruit products	98	Canned peaches, pears, pineapples	S14-042950	3%				
		99	Other canned or frozen fruit	S14-042951	5%				
		100	Dried fruit	S14-042952	6%	**1.65**		< LOD (< 0.15)	< LOD (< 0.50)
		101	Fruit juices and vegetable juices	S14-042953	86%	**5.62**		< LOD (< 0.25)	< LOD (< 0.50)
		NA	Group sample	S14-042843					
			Sum of category samples			**4.91**			
25	Sandwiches	138	Sandwiches	S14-042988	100%	< LOQ (< 0.22) ^	< LOD (< 0.81)	< LOD (< 0.40)	< LOD (< 0.50) ^
		NA	Group sample	S14-042852		< LOQ (< 0.22) ^	< LOD (< 0.81)	< LOD (< 0.44)	< LOD (< 0.50) ^
			Sum of category samples			NA	NA	NA	NA

10. Conclusions

Highly sensitive LC-MS/MS-based methods for the determination of sterigmatocystin, ergot alkaloids, cyclopiazonic acid, citrinin and moniliformin in TDS samples were developed and limited validation data derived. The methods developed were then used to analyse the various TDS samples successfully.

UKAS accredited methods were used for the analysis of aflatoxins, ochratoxin A, zearalenone, patulin, fumonisins and trichothecenes. A combination of LC-MS/MS and HPLC fluorescence methods were used, chosen to give maximum sensitivity.

The most frequently detected toxins were deoxynivalenol and ergot alkaloids which were detected in all bread samples, as well as other cereal products. None of the samples exceeded any maximum permitted limit. There were very few residues of any of the other mycotoxins analysed found in the samples tested, most results were at or below the limit of quantification which were as low as technically achievable, and were typically in the sub or low µg/kg range.

Agreement between individual food category results and those of the composite group samples was on the whole very good.

This is the first UK TDS study for mycotoxins. The analysis of the individual category samples has provided additional information about these products for the first time. These results show very little incidence of mycotoxins in UK food samples, with very few results above the low limits of quantification. The data can be used for future intake calculations, to calculate background exposure to various mycotoxins from the whole diet and also to compare exposure to those calculated by other sources.

11. References

EC Directive 96/23 - Measures to monitor certain substances and residues thereof in live animals and animal products

European Mycotoxin Awareness Network (EMAN) Cyclopiazonic Acid Factsheet http://services.leatherheadfood.com/eman/FactSheet.aspx?ID=15

FD Report 15/06 Total Diet Study of metals and other elements in food, Report for the UK Food Standards Agency (FS102081)

FSG 251 Determination of Aflatoxins B_1, B_2, G_1 and G_2 using Automated Immunoaffinity Column Clean-up and HPLC

FSG 252 Determination of Ochratoxin A using Automated Immunoaffinity Column Clean-up and HPLC

FSG 253 Determination of aflatoxin M_1 in milk by immunoaffinity column clean-up and HPLC

FSG 254 Determination of Patulin using HPLC or LC-MS/MS

FSG 258 Determination of Zearalenone using Immunoaffinity Column Clean-up and HPLC

FSG 261 Simultaneous Determination of Ochratoxin A and Aflatoxins B_1, B_2, G_1 and G_2 using Automated Immunoaffinity Column Clean-up and HPLC

FSG 263 Determination of a range of Trichothecenes using Solid Phase Column Clean-up and LC-MS/MS Detection

FSG 264 Determination of Fumonisins B_1, B_2 and B_3 using Immunoaffinity Column Clean-up and Either HPLC with Pre-column Derivatisation and Fluorescence Detection or LCMS/MS

FSG 300 Analysis of Milk Products for Aflatoxin M_1

FSG 601 LC-MS/MS Method for the Determination of Ergot Alkaloids in Cereals

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12. Acknowledgements

Thanks to Fera staff, Irene Leon, Joanna Stratton, Sharron Anderson, Julie Clough, Monika Sehnalova, Stephen Chapman, Paul Hepworth, Sharon Jardine, Julie Christy, Ryan Setter and Daniel Philips for their contribution to this study.

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