FINAL REPORT

STERIGMATOCYSTIN IN CEREAL PRODUCTS

FS 102055

NOVEMBER 2015

Irene Leon, Joanna Stratton, Julie Clough, Monika Sehnalova and Susan MacDonald

Fera Science Ltd., York (UK)

This report has been produced by Fera under contracts placed by the UK Food Standards Agency and by the European Food Safety Authority. The views expressed herein are not necessarily those of these two funding bodies. Fera warrants that all reasonable skill and care has been used in preparing this report. Notwithstanding this warranty, Fera shall not be under any liability for loss of profit, business, revenues or any special indirect or consequential damage of any nature whatsoever or loss of anticipated saving or for any increased costs sustained by the client or his or her servants or agents arising in any way whether directly or indirectly as a result of reliance on this report or of any error or defect in this report.

1 Executive Summary

The mycotoxin sterigmatocystin, is a genotoxic carcinogen which can occur in grains and grain-based products. The European Food Safety Authority (EFSA) issued a call for proposals to address a lack of suitable data for risk assessment of the occurrence of sterigmatocystin, concentrating on grains and grain-based products for human consumption in Europe. Five organisations partnered to answer the call: RIKILT – Wageningen UR, (Netherlands), Università Cattolica del Sacro Cuore (UCSC, Italy), Food and Environment Research Agency (Fera, now Fera Science Ltd., UK), Benaki Phytopathological Institute (BPI, Greece) and Netherlands Food and Consumer Product Safety Authority (VWA).

This report details work by Fera on samples obtained in the UK, the results of which were fed into the wider EFSA survey.

Highly sensitive analytical methods based on liquid-chromatography with tandem mass spectrometry (LC-MS/MS) were used for the determination of sterigmatocystin in cereal grain and cereal products. The methods were validated to 0.5 µg/kg in each of the matrices and met the requirements as laid down in Commission Regulation (EC) No 401/2006 as amended. The comparability of the results generated by the partner laboratories was demonstrated and method performance was adequate throughout the analysis of the survey samples. The average recoveries and repeatabilities all met the requirements showing that the method used was fit-for-purpose for quantitative analysis of sterigmatocystin in cereals based samples at a limit of quantification (LOQ) of 0.5 µg/kg. This was used as a standardised reporting limit by all partners for the whole EFSA survey. The limit of detection (LOD) achieved by Fera was 0.1 µg/kg, with a working LOQ of 0.2 µg/kg (equivalent to the lowest calibration standard). Therefore Fera was able to measure samples with very low levels of sterigmatocystin.

Samples were collected from a variety of sources such as during storage, production and retail (including internet shopping). Samples were analysed by the partnership laboratories and reported to EFSA. A total of 1259 samples were analysed in the EFSA study. These were 1142 samples categorised as ‘all cereals’, 53 samples of beer and 64 samples of nuts.

In total 277 cereal samples, comprising 93 samples of unprocessed cereal grains and 184 cereal products were collected and analysed in the UK by Fera in multiple analytical batches. In addition as part of the wider EFSA survey, Fera collected nine beer and fourteen nut samples in the UK. These samples were sent to partner laboratories for analysis.

Sterigmatocystin was found in 12 UK samples at levels >0.5 µg/kg; this represents 4% of the UK cereal samples analysed and was in line with the results of the wider EFSA survey of 1142 cereal samples.

Another 32 of the 277 samples (12%) had sterigmatocystin in the range (0.1 µg/kg to 0.5 µg/kg). This gives a total of 44 samples where sterigmatocystin was detected representing 16% of all samples analysed in the UK. This is a slightly higher rate of incidence than the EFSA study as a whole which reported that sterigmatocystin was identified in 11% of the samples, however Fera had a lower limit of detection than

some partners. Sterigmatocystin was present in UK samples at levels above 0.1 µg/kg in 14% of unprocessed grains and 17% of cereal products. This compares with occurrence of 13% and 10% for these two categories, respectively, for the wider survey.

Rice and oats grains were most likely to contain sterigmatocystin and consequently processed products containing these ingredients were more likely to contain sterigmatocystin.

In the EFSA study sterigmatocystin levels reported in samples with residues were mainly in the range between 0.5 to 5 µg/kg.

One rice sample reported to EFSA by the partnership had a level of sterigmatocystin of 5.5 µg/kg (rice crop, Greece). Rice samples analysed by Fera were all processed samples from a variety of origins. Fera results for rice were in the range 0.22 µg/kg up to 1.00 µg/kg for a brown rice of unknown origin.

For oats, 33 µg/kg was the highest level of sterigmatocystin reported to EFSA by the partnership. This was a sample of oat grain. The highest concentrations of sterigmatocystin found in UK oat samples analysed at Fera were 0.97 µg/kg for an unprocessed cereal grain and 1.41 µg/kg in an oat based breakfast cereal.

The highest level of sterigmatocystin found in the Fera survey of samples collected in the UK was in a sample of wholemeal rye crisp bread at 3.65 µg/kg.

In the EFSA survey 53 samples of beer, 28 samples of peanuts and 36 samples of hazelnuts were also analysed. All partners collected beer and nut samples but due to the limited number of samples involved analysis was conducted at only 2 partner laboratories, BPI (nuts) and UCSC (beer). Results showed that no beer and nut samples contained sterigmatocystin.

This survey captures a first look on the occurrence of sterigmatocystin in food products grown and consumed in the EU. The number of samples for each product is too low for an in-depth assessment of any relationship between occurrence and country of origin, year of harvest, organic, non-organic or other variables. Mycotoxin formation depends on various factors including climatic conditions, moisture content at storage, and product treatment. This study reports results from approximately one year of sampling (2013-2014). Further analyses of samples from different crop years would be necessary to obtain information on seasonal variations.

Since rice flour and to a lesser extent oats are important ingredients in cereal-based infant food, further study of products containing rice or oats as a major ingredient could be considered to provide additional information on these products.

All results have been submitted to EFSA data collection unit and uploaded to the DCF database.

2 Contents

Tables

Figures

Appendices

3 Glossary

BPI	Benaki Phytopathological Institute (Greece)
EFSA	European Food Safety Authority
EU	European Union
Fera	Food and Environment Research Agency (UK)
FSA	Food Standards Agency (UK)
LC-MS/MS	Liquid Chromatography tandem Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantification
MU	Measurement Uncertainty
m/z	Mass to charge ratio
RIKILT	RIKILT Wageningen UR
RSD	Relative Standard Deviation
RSD_r	Repeatability
S/N	Signal to noise ratio
UCSC	Università Cattolica del Sacro Cuore (Italy)
UPLC-MS/MS	Ultra Performance Liquid Chromatography tandem Mass Spectrometry

4 Introduction

Mycotoxins are toxic compounds produced by fungi which frequently contaminate cereal crops. They commonly enter the food chain through contaminated food and feed crops, mainly cereals. They can occur in a wide range of foods, often with no visible signs of mould spoilage to the food. They have a wide range of chemical properties and toxicities to humans and food-producing animals.

The mycotoxin sterigmatocystin is a genotoxic carcinogen which can occur in grains and grain-based products, green coffee beans, beer, spices, nuts, cheese and feed.

The European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM Panel) issued a call for proposals [1] to address a lack of suitable data for risk assessment on the occurrence of sterigmatocystin. The objective of the call was to obtain representative occurrence data for sterigmatocystin in food samples with special focus on grains and grain-based products for human consumption from different geographic regions in Europe. This information would then be available to the CONTAM Panel for future exposure assessment for sterigmatocystin.

Five organisations partnered to answer the call: RIKILT – Wageningen UR, (Netherlands), Università Cattolica del Sacro Cuore (UCSC, Italy), Food and Environment Research Agency (Fera, UK), Benaki Phytopathological Institute (BPI, Greece) and Netherlands Food and Consumer Product Safety Authority (VWA). The group was led by RIKILT. Four laboratories analysed cereal samples: RIKILT, UCSC, Fera and BPI. Beer and nuts were analysed by UCSC and BPI respectively.

This report details work by Fera on samples obtained in the UK. The report for the wider EFSA project is available[2].

The work at Fera was funded by EFSA with co-funding from the Food Standards Agency (FSA).

5 Aims and Objectives

5.1 Scope

This work was part of an extensive European survey on sterigmatocystin in food tendered by EFSA to gain an insight into the occurrence of sterigmatocystin especially in cereals and cereal-based products as consumed in the EU.

This report details the UK contribution of results generated by Fera to the data set submitted to EFSA.

5.2 Sampling

EFSA requirements were for two categories of samples: cereal grains (raw agricultural commodities) and processed cereal products. At least 400 samples of grains including wheat, barley, rye, oats and rice, and at least 500 samples of grain-based products for human consumption including flour, bread and rolls, pasta, cereal flakes and muesli were required. In addition as an extra benefit to the project EFSA requested the collection and analysis of at least fifty samples of beer and fifty samples of nuts across all the partnership organisations; Fera collected some beer and nut samples in the UK and passed them to partner laboratories for analysis. Table 1 gives the breakdown of sample types requested and the minimum target number required for the overall project as well as the minimum target numbers for Fera.

Objective sampling was carried out, with emphasis placed on wheat which is consumed widely in the European Union (EU). Minimum sample numbers were set for other cereals types (rye, maize, rice, barley and oats) to represent regional consumption and to represent dietary groups (coeliac). The sampling plan involved different countries and regions including Eastern Europe to represent the whole of the EU.

The choice of product categories and numbers within each category were specified by EFSA and was based on intake estimations from EFSA consumption data. For the five minor grains the number of samples in the sampling plan was set at a fixed minimum to ensure a statistically valid number of samples were included and to avoid results for commodities such as oats being based on a very limited number of samples. Processed cereal products were divided into two subgroups: food ingredients that are typically further processed (e.g. baking or cooking) and products that are consumed as such without further processing.

A target of approximately 5% of organic produce was to be included. Fera obtained a total of 277 cereal based samples and 9 beer and 14 nut samples that were sent to partners.

Table 1 summarises the target sampling to be carried out for the whole project and the target proportion of samples to be obtained by Fera. The minimum numbers listed were those suggested in the original EFSA tender document. The target numbers were agreed at the initial EFSA kick off meeting. The actual number of

samples analysed during the EFSA study and obtained and analysed by Fera is also given in Table 1.

	Target Number		Samples analysed	
Sample description	**Total EFSA**	**Fera**	**Total EFSA**	**Fera**
Grains (bulk): min. 400*	**410**	**100**	**429**	**93**
wheat (soft)	160	40	169	5
wheat (hard/durum)	50	10	52	33
rye	40	10	35	3
maize	40	10	33	7
rice	40	10	28	-
barley	40	10	59	26
oats	40	10	21	19
spelt	-	-	2	-
Cereal products: min. 500*	**500**	**100**	**713**	**184**
Cereal products, processed grains	**200**	40	**329**	**92**
grain milling products	60	12	125	44
rice	40	8	89	26
pasta	100	20	115	22
Cereal products consumed as such	**300**	**60**	**384**	**92**
bread/rolls	150	30	143	31
breakfast cereals (incl. muesli)	50	10	97	37
fine bakery ware	60	12	90	12
cereal-based infant food	40	8	54	12
Nuts: min. 50*	**56**	12	**64**	**14**
peanuts	28	6	28	5
hazelnuts	28	6	36	9
Beer: min. 50*	**50**	8	53	9

*Minimum number of samples requested in EFSA tender document

Table 1: Target Sampling Plan and number of samples analysed

5.3 LC-MS/MS Analysis

The samples were extracted using solvent and the cleaned-up extracts were analysed for sterigmatocystin using state of the art LC-MS/MS, due to the high sensitivity and selectivity of the instrumentation no sample clean-up was used.

5.4 Validation

A validation exercise was performed by the four laboratories carrying out cereal analysis to demonstrate the fitness-for-purpose of the analysis methods used for the survey.

5.5 Interlaboratory Comparison

No reference material with naturally contaminated sterigmatocystin or proficiency test materials were available at the time of the study. Therefore the comparability of results between the four laboratories performing the analyses was verified by exchange of solvent standards used for spiking and calibration, and by analysis of a sample containing sterigmatocystin.

5.6 Quality Control

It was agreed at the start of the project, that as a minimum all partner laboratories would include one reagent blank and a minimum of two fortified samples with each batch of samples, for each matrix group (cereal grains and cereal products).

5.7 Reporting of Results

Results were to be submitted to follow EFSA Guidance on standard sample description and submitted via the Evidence Management Unit's (DATA) call for continuous collection of chemical contaminants occurrence data in food and feed with standard sample description details specified in the most recent EFSA Guidance.

6 Materials and Methods

6.1 Sampling

In the majority of the cases, sampling was done according to the guidelines for the official control of foodstuffs as described in Commission Regulation (EC) No 401/2006. Detailed sampling information was collected.

Samples were taken at various points in the food chain including primary production, storage, import activities, processing plants (mills), wholesale, and retail sale. Samples were supplied by cereal trade bodies, food industry collaborators and obtained by laboratory staff.

Upon receipt in the analytical laboratory, samples were given an unique code. Samples were stored under suitable dry conditions until homogenised. Packed products were stored at ambient conditions unless the label specified otherwise and processed before the 'best before' date.

6.2 Sample Preparation

Dry samples were homogenised by milling (0.5 or 1 mm) using a centrifugal mill (Retsch). For certain products (e.g. fruit-containing breakfast cereals) solid carbon dioxide was added during the milling process. In the case of some retail products, (breakfast cereals containing pieces of fruit and chocolate, some breads, fine bakery goods and ready to use noodles) a wet slurry preparation method was used. Slurry choice and water ratio was dependent on the sample type and was carried out to ensure the final sample was adequately ground and homogenised. In all cases the full sample provided to the laboratory was prepared. Sub portions of homogenates were stored at <-18°C.

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, carbon-13 (^{13}C) isotopically labelled sterigmatocystin was used as internal standard throughout. The analytical reference standard of sterigmatocystin was purchased as solid (dry film, Sigma Chemicals, ≥98% purity). The internal standard U-[$^{13}C_{18}$]-Sterigmatocystin in acetonitrile was purchased from Romerlabs, but was sourced from Biopure (Tulln, Austria).

6.4 Extraction and Clean Up

To 5 gram dry milled homogenate, 15 µL of isotopically labelled sterigmatocystin in acetonitrile was added (equivalent to 1.5 µg/kg). The sample was extracted with 20 mL of acetonitrile/water (80/20 v/v) using an orbital shaker for 2 hours. For

slurried samples the amount of sample used and proportion of acetonitrile/water in the extraction solvent were adjusted depending on the amount of water used in the slurry preparation. 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 cooled overnight in a refrigerator and then filtered using a 0.22 µm nylon syringe filter into an autosampler vial. The amount of matrix equivalent in the final extract was 0.125 g sample/mL.

6.5 LC-MS/MS Analysis

2 µL of the extract was injected into an UPLC-MS/MS system. The system consisted of a Waters Acquity LC-system (degasser, pumps, autosampler, column oven) and a TQ-S triple quadrupole MS/MS system from Waters. Separation was performed on a HSS T3 column (1.8 µm particle size, 100 x 2.1 mm, Waters) maintained at 40°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.

LC-MS/MS measurement was performed using positive electrospray ionisation applying the following conditions: capillary voltage, 3 kV; desolvation temperature, 500°C; desolvation gas flow rate, 1000 L/h; nebuliser gas flow, 7 bar; source temperature, 150°C; cone gas flow rate, 100 L/h; cone voltage, 40 V. For sterigmatocystin, using $[M+H]^+$ (m/z 325) as precursor ion, two transitions were measured: m/z 310 (23 V) [quantification ion], and m/z 281 (35 V). For the isotopic label ($^{13}C_{18}$), 343 m/z was used as precursor ion with m/z 297 (36 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 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.

6.6 Validation

Six cereal products from different food categories (pasta, breakfast cereals/muesli, bread, biscuit, pastry/cake and cereal-based infant food) were used for this purpose. The validation was performed by spiking each of the six types of cereal grains to be included in the survey in duplicate at two levels (0.5 µg/kg and 5 µg/kg). A similar approach was followed for the cereal products. All matrices included in the validation were also analysed without spiking, as well as a reagent blank.

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

Validation criteria included average recovery. The recommended value for average recovery was 50-120% at the 0.5 µg/kg level and 70-110% at the 5 µg/kg level (derived from Commission Regulation (EC) No 401/2006 for aflatoxin B_1). To measure selectivity, the response for sterigmatocystin in the non-fortified samples should not exceed 30% of the response at the lowest fortification level.

For precision, the recommended repeatability (RSD_r) was based on Horwitz and should not exceed 33% at the 0.5 µg/kg level and 23% at the 5 µg/kg level (derived from Commission Regulation (EC) No 401/2006 for aflatoxin B_1).

The Limit of Detection (LOD) is defined here as the level corresponding to a signal-to-noise ratio (S/N) of three. Noise is 'peak-to-peak noise' as manually determined from extracted ion chromatograms of the 0.5 µg/kg fortifications. The response should be taken for the transition with the lowest S/N (i.e. the qualifier ion).

In this study, the Limit of Quantification (LOQ) is defined as the lowest level for which it has been demonstrated that the criteria for average recovery and repeatability are met, i.e. where validation data were obtained. The target LOQ for this study was 0.5 µg/kg.

Identification of sterigmatocystin was based on retention time and ion ratio of coinciding peaks for at least two diagnostic transitions in the correct abundance ratio. Retention time was set within 0.05 minutes of its isotopic internal standard. The ion ratio of the two diagnostic ions (least abundant/most abundant) of sterigmatocystin in the samples should be consistent with that obtained during validation and not deviate more than ±30%.

6.7 Interlaboratory Comparison

Solvent standards containing sterigmatocystin at 50-100 ng/mL in acetonitrile were exchanged between the four laboratories. Each laboratory diluted the solutions supplied to achieve a concentration within the calibration range. The concentration was then determined and compared with the theoretical one. Standards were injected in at least triplicate and the differences between the absolute average responses of each standard were calculated.

Naturally contaminated rice (feed material) was prepared by RIKILT but there was insufficient material to perform a full homogeneity study. Portions of the dry milled sample material were sent to the partner laboratories and analysed.

6.8 Quality Control

With each batch of survey samples, one or more spiked samples were included to assess method performance over time and different commodities. This was typically a random sample representative of the commodities being analysed in the batch. The fortification level for these samples was set at 1.5µg/kg. In addition the naturally incurred rice sample supplied by RIKILT was included in every batch. These data were also used to establish the long-term within-laboratory reproducibility and for estimation of measurement uncertainty.

6.9 Reporting of Results

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

Under the EFSA reporting format there were three possible types of analysis result:

‘Numerical Value’: interpreted as samples in which sterigmatocystin was found at levels equal to or higher than the lowest validated level (≥0.5 µg/kg).

‘Value below the lower limit of the working range’, interpreted as samples in which sterigmatocystin was identified but below the lowest validated level (<0.5 µg/kg).

‘Non Detected Value (<LOD)’, interpreted as samples in which no sterigmatocystin was identified.

Values below the lower limit of the working range (<0.5 µg/kg) are included but as these values are above the LOD of 0.1 µg/kg but below the LOQ of 0.5 µg/kg they should be regarded as indicative. Tabulated values for this data are shown in brackets.

7 Results

7.1 Sampling

A total of 277 UK-sourced samples were analysed for the presence of sterigmatocystin. There were 93 samples of cereal grains and 184 cereal products. 8% of samples were from organic production.

To contribute to the overall EFSA project objectives, Fera collected nine beers and fourteen samples of nuts in the UK. This was slightly in excess of the sampling plan but made up for sampling shortfalls elsewhere in the partnership. These samples were passed on for analysis to partner laboratories (USCS and BPI).

7.2 LC-MS/MS Analysis

The retention times within the validation sequences were very consistent. Deviations of the absolute retention time of sterigmatocystin relative to the reference value (average of solvent standards injected in the same sequence) were less than 0.05 min. Deviations of individual ion ratios relative to the reference were typically 10%, with maximum deviations up to 18% for some of the 0.5 µg/kg spikes.

During LC-MS/MS analysis transition m/z 325 → m/z 310 was slightly lower in abundance than m/z 325 → 281 but in many cases it provided a better signal-to-noise ratio in the samples and therefore it was used as default for quantification.

Examples of extracted ion chromatograms for calibration standards at a level corresponding to approximately half the lowest validation level, blank samples, and samples spiked at 0.5 µg/kg are shown in Figures 1 to 3.

Example chromatograms for sterigmatocystin obtained during validation (barley). (Left: m/z 325 → m/z 310, right: transition m/z 325 → 281).

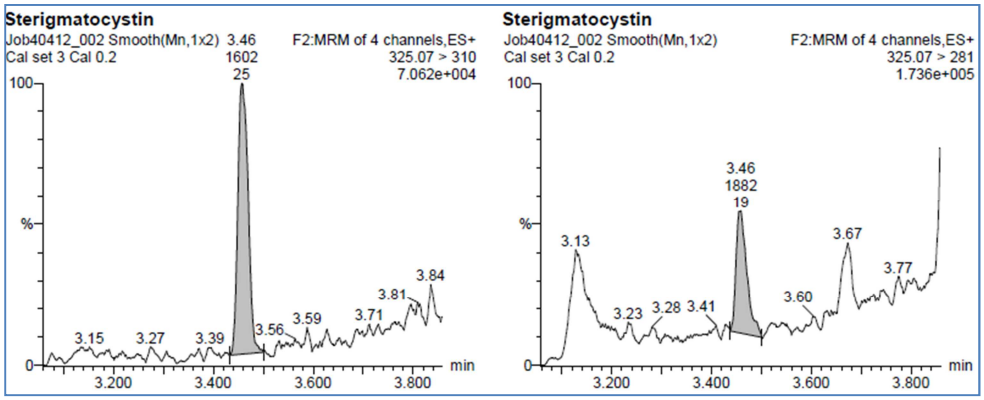

Figure 1: Calibration standard in solvent 0.025 ng/mL
(corresponding to 0.20 µg/kg in cereal)

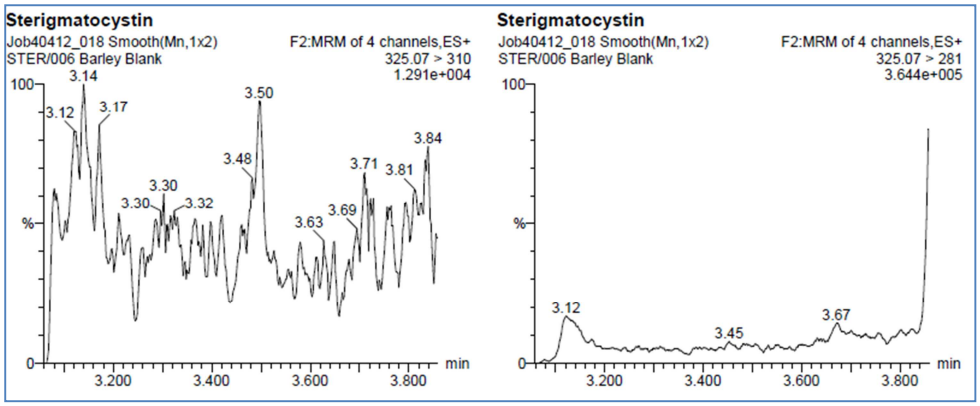

Figure 2: Barley (non-fortified).

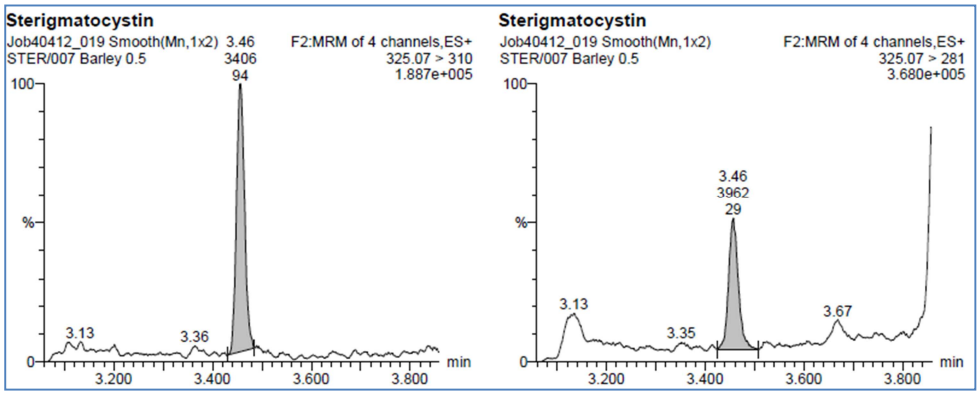

Figure 3: Barley (fortified at 0.5 µg/kg).

7.3 Validation

Quantitative analysis was validated at ≤0.5 µg/kg. This is below the current recommended limit of quantification (LOQ) of 1.5 µg/kg (EFSA, 2013).

The method for the determination of sterigmatocystin in the different cereals and processed cereal products was validated at 0.5 µg/kg and 5 µg/kg by each of the analytical laboratories. Fera was able to achieve a lower limit of detection than some of the partner laboratories. An analytical standard equivalent to 0.2 µg/kg was included in all batches and allowed reliable detection of sterigmatocystin down to a level equivalent to 0.1 µg/kg in samples. For the purpose of this wider study, as it was lowest level used in the validation and to ensure consistency between the partner laboratories 0.5 µg/kg was accepted as the LOQ for all partners.

An oat sample was analysed as a blank matrix by Fera in the initial validation and revealed under confirmatory analysis that the sample was positive for sterigmatocystin. For this reason another oat sample was taken and the analysis repeated.

Table 2 gives the recoveries for sterigmatocystin spiked in duplicate into six cereal grain matrices fortified at two different levels.

Product Group 1: Cereal Grains		
Matrix	**Spike (µg/kg)**	**Recovery (%)**
wheat	0.5	109
wheat	0.5	114
wheat	5	105
wheat	5	97
barley	0.5	111
barley	0.5	107
barley	5	102
barley	5	88
oats	0.5	74
oats	0.5	79
oats	5	71
oats	5	70
maize	0.5	114
maize	0.5	110
maize	5	107
maize	5	105
rye	0.5	122
rye	0.5	109
rye	5	97
rye	5	113
rice	0.5	109
rice	0.5	124
rice	5	101
rice	5	100
Average	0.5	107
RSD (%)	0.5	14
Average	5	96
RSD (%)	5	14

Table 2: Recoveries obtained for sterigmatocystin in cereals.

Table 3 gives the recoveries for sterigmatocystin spiked in duplicate into six cereal product matrices fortified at two different levels.

Product Group 2: Processed Cereal Products		
Matrix	**Spike (µg/kg)**	**Recovery (%)**
pasta (spaghetti)	0.5	110
pasta (spaghetti)	0.5	119
pasta (spaghetti)	5	94
pasta (spaghetti)	5	103
breakfast cereal	0.5	119
breakfast cereal	0.5	111
breakfast cereal	5	100
breakfast cereal	5	104
bread	0.5	115
bread	0.5	87
bread	5	112
bread	5	78
biscuit	0.5	94
biscuit	0.5	88
biscuit	5	93
biscuit	5	100
cake/pastry	0.5	109
cake/pastry	0.5	104
cake/pastry	5	101
cake/pastry	5	107
infant food	0.5	103
infant food	0.5	104
infant food	5	98
infant food	5	96
Average	0.5	105
RSD (%)	0.5	10
Average	5	99
RSD (%)	5	9

Table 3: Recoveries obtained for sterigmatocystin in processed cereal products.

7.4 Interlaboratory Comparison

The solvent standards exchanged between laboratories were analysed by RIKILT, Fera, UCSC and BPI and results differed by 9% or less.

The naturally contaminated rice sample prepared by RIKILT contained approximately 2 µg/kg and Table 4 gives the individual analysis results for the different replicates. The results were in good agreement with each other, indicating that the four laboratories produced comparable and consistent results.

Additionally, Fera analysed this incurred rice sample as a quality control sample with every analysis batch.

	Measured concentration in µg/kg			
Replicate	RIKILT	UCSC	Fera	BPI
1	2.05	1.9	1.81	2.16
2	2.39	2.1	1.64	2.31
3	2.24	2.2		2.16
4	2.01			2.02
5	1.85			
Average	2.11	2.07	1.73	2.16
RSD (%)	10	7.4	7.0	5.4

Table 4: Analysis results for a naturally contaminated rice sample analysed by four laboratories.

7.5 Quality Control

As part of the batch quality control, one or more samples fortified at 1.5 µg/kg were included with each batch of samples analysed. Fera also included the incurred rice sample with every batch.

Most of the individual recoveries were within 70-110%. In some cases higher recoveries were obtained, however, the average recoveries were always within the recommended range.

The average recovery and the reproducibility (RSD_R) obtained for each of the fortified food commodities (cereal grains, processed cereal products) are summarised in Table 5.

	Number	Average (%)	RSD_R[(a)] (%)	Exp. MU[(b)] (%)
Fera				
Cereal grains	21	99	14	
Cereal products	18	101	14	
Cereal grains/products	39	100	14	
Incurred rice (feed)	9	2.07[(c)]	20	40

(a): relative standard deviation under reproducibility conditions;
(b): expanded relative measurement uncertainty
(c): average concentration in incurred quality control sample in µg/kg

Table 5: Average recovery and reproducibility of quality control samples.

The RSD_R averaged 14% for all sample types which was well within the recommended value from Commission Regulation (EC) No 401/2006 for aflatoxin B_1 (Horwitz equation at 1.5 µg/kg = 43%).

7.6 Results

Linearity requirements in the range equivalent to 0.2-10 µg/kg were met.

Sterigmatocystin was not identified in blank samples.

Matrix effects were compensated for through the use of the isotopically labelled internal standard and so were not evaluated in detail. In general, matrix effects were not very pronounced which may in part be due to dilution used during extraction.

The results obtained for the survey samples were only considered valid when the linearity and recoveries obtained for a particular batch complied with the criteria outlined in Section 6.6 and when in a known (reagent) blank no sterigmatocystin was identified.

Sterigmatocystin was found in 12 samples at levels above the LOQ of 0.5 µg/kg. Of these 5 were unprocessed grains and 7 were processed cereal products (Table 6).

LOD (µg/kg)	0.1
LOQ (µg/kg)	0.5

Sample	Product		Sterigmatocystin (µg/kg)
S14-050048	Rice, brown	Processed	0.56
S14-042620	Oat flakes	Processed	0.60
S14-042609	Oats, grain	Unprocessed	0.63
S14-010945	Rice, long-grain	Processed	0.64
S14-042614	Oats, grain	Unprocessed	0.67
S14-011012	Infant cereal	Processed	0.75
S14-042607	Oats, grain	Unprocessed	0.97
S14-048782	Rice, brown	Processed	1.00
S14-052405	Wheat grain	Unprocessed	1.26
S14-051243	Corn grain	Unprocessed	1.32
S14-010988	Oat porridge	Processed	1.41
S14-048744	Crisp bread, rye wholemeal	Processed	3.65

Table 6: Sterigmatocystin levels in food (>0.5 µg/kg)

Sterigmatocystin was found in 32 samples at levels above the LOD (0.1 µg/kg) but below the LOQ of 0.5 µg/kg (Table 7). Of these 8 samples were unprocessed grains and 24 were processed cereal products.

LOD (µg/kg)	0.1
LOQ (µg/kg)	0.5

Sample	Product		Sterigmatocystin (µg/kg)
S14-010991	Wheat semolina, Durum	Processed	(0.13)
S14-011054	Oat flakes	Processed	(0.13)
S14-052345	Wheat flour, white	Processed	(0.16)
S14-043556	Barley grain, whole	Unprocessed	(0.18)
S14-043555	Barley grain, whole	Unprocessed	(0.19)
S14-050879	Wheat germ bread	Processed	(0.21)
S14-048776	Rice	Processed	(0.22)
S14-050051	Rice, brown	Processed	(0.22)
S14-050049	Rice, long-grain	Processed	(0.23)
S14-023272	Mixed breakfast cereals	Processed	(0.24)
S14-010947	Cornmeal	Processed	(0.25)
S14-050034	Pasta (Raw)	Processed	(0.25)
S14-050055	Pasta, wheat, without eggs	Processed	(0.25)
S14-050064	Rye milling products	Processed	(0.26)
S14-023269	Breakfast cereals (bran base)	Processed	(0.28)
S14-042608	Oats, grain	Unprocessed	(0.28)
S14-042601	Oats, grain	Unprocessed	(0.30)
S14-023270	Oat bran	Processed	(0.31)
S14-050052	Pasta, wheat, without eggs	Processed	(0.33)
S14-042610	Oats, grain	Unprocessed	(0.33)
S14-048761	Rice flour	Processed	(0.34)
S14-050847	Wheat rolls, white	Processed	(0.35)
S14-010990	Oat porridge	Processed	(0.39)
S14-052956	Wheat bread, white	Processed	(0.41)
S14-043575	Oats, grain	Unprocessed	(0.44)
S14-010935	Rice, brown	Processed	(0.45)
S14-048792	Rice, brown	Processed	(0.45)
S14-011057	Crisp bread, rye wholemeal	Processed	(0.47)
S14-048743	Crisp bread, rye wholemeal	Processed	(0.47)
S14-010957	Breakfast cereals	Processed	(0.47)
S14-042600	Oats, grain	Unprocessed	(0.48)
S14-043576	Oats, grain	Unprocessed	(0.49)

( ) Level above 0.1 µg/kg LOD but below 0.5 µg/kg LOQ

Table 7: Sterigmatocystin levels in food (0.1 µg/kg to 0.5 µg/kg)

Fully tabulated results are given in Appendices 1 and 2.

8 Discussion

8.1 Occurrence of Sterigmatocystin in UK Collected Samples

In this survey 277 samples were collected in the UK and analysed in multiple analytical batches.

Sterigmatocystin was detected in 12 samples (>0.5 µg/kg); this represents 4% of the UK samples analysed. The highest level was in a processed cereal sample of wholemeal rye crisp bread (3.65 µg/kg).

Another 32 samples (12%) had levels of sterigmatocystin detected at a value below the lower limit of the working range (0.1 µg/kg to 0.5 µg/kg).

In total, sterigmatocystin was found above 0.1 µg/kg in 44 samples.

Sterigmatocystin was not detected in 233 samples (84%).

All results were submitted to EFSA in the SSD format, with the other data from the project, and have been accepted by the data collection unit.

8.2 Cereal Grains

93 samples of unprocessed cereal grains were received and analysed. Sterigmatocystin was detected in 13 samples including barley, oat, wheat and maize but not in the limited number of rye samples (n=3).

Of these, 5 samples contained sterigmatocystin above 0.5 µg/kg; 3 oat grain samples (0.63 to 0.97 µg/kg), 1 wheat grain (1.26 µg/kg) and a corn grain at (1.32 µg/kg).

The other 8 samples contained levels of sterigmatocystin at a value below the lower limit of the working range (0.1 µg/kg to 0.5 µg/kg). Two samples of barley (0.18 µg/kg and 0.19 µg/kg) and six samples of oat grain (0.28 µg/kg to 0.49 µg/kg) contained sterigmatocystin.

8.3 Cereal Products

184 cereal products samples were collected as i) 92 samples of processed grains and ii) 92 samples of products consumed as such. In total, sterigmatocystin was not detected in 153 samples.

7 samples contained sterigmatocystin above 0.5 µg/kg. The highest level was in a sample of wholemeal rye crisp bread (3.65 µg/kg) and this was the highest level found in all samples of all types analysed by Fera. The other 6 samples were rice (3 samples) or contained oats (3 samples) and had sterigmatocystin in the range 0.56 µg/kg to 1.41 µg/kg. Below the lower limit of the working range (0.1 µg/kg to 0.5 µg/kg), 24 samples contained sterigmatocystin from 0.13 µg/kg to 0.47 µg/kg.

The 92 cereal products classified as ‘processed grains’ consisted of 44 grain milling products (such as flour), 26 rice samples and 22 samples of pasta (including noodles).

Whilst grain milling products did not contain sterigmatocystin above 0.5 µg/kg, 5 samples did give results in the range 0.13 µg/kg to 0.31 µg/kg across maize, oat, wheat and rye.

No samples of pasta (including noodles) had a level of sterigmatocystin above 0.5 µg/kg but 3 samples showed occurrence in the range of 0.25 µg/kg to 0.33 µg/kg.

The 92 cereal products classified as ‘consumed as such’, comprised four broad categories of samples. These were bread and rolls (13 samples), breakfast cereals (37 samples), fine bakery wares (30 samples) and cereal based infant food (12 samples).

Bread and rolls did not contain sterigmatocystin above 0.5 µg/kg; 3 samples had levels between 0.21 µg/kg to 0.41 µg/kg.

Oat based breakfast cereals showed a prevalence for the presence of sterigmatocystin. Two breakfast cereals had sterigmatocystin at 0.6 µg/kg (formed oat flakes) and 1.41 µg/kg (rolled porridge oats). A further 5 breakfast cereals had levels of sterigmatocystin between 0.13 µg/kg to 0.47 µg/kg. Four of these were oat based or contained oats with the highest of these containing nearly 25% oat flour. The other breakfast cereal showing presence of sterigmatocystin was a bran flake sample at 0.28 µg/kg.

The highest level of sterigmatocystin found in all the samples collected in the UK and analysed by Fera was found in wholemeal rye crisp bread (3.65 µg/kg). Two other rye based crisp breads had a lower level at 0.47 µg/kg. Other fine bakery wares such as biscuits, crackers and oatcakes did not contain detectable sterigmatocystin.

8.4 Rice

Within the cereal products (processed grains) category, 27 samples of rice were analysed with 18 samples not showing the presence of sterigmatocystin. 6 samples contained sterigmatocystin in the range (0.22 µg/kg to 0.45 µg/kg) and 3 rice samples were in the range (0.56 µg/kg to 1.00 µg/kg).

8.5 Cereal Based Infant Foods

Included in the range of cereal products and considered separately here, there were 12 samples of infant foods. Eight of these require reconstitution (usually with milk) before consumption (six porridge, one infant rice and one infant cereal). The analytical results are for the samples as received i.e. with no reconstitution. The other four samples were ready meals or biscuits. Of the 12 samples, only 1 (a porridge sample) contained sterigmatocystin, at 0.75 µg/kg. Amongst other ingredients, that porridge sample contained wheat, oats, rice, millet, barley, maize and rye.

8.6 Comparison of Fera Results to EFSA Survey CP/EFSA/CONTAM/2013/02

Sterigmatocystin occurred above 0.1 µg/kg in 44 of the 277 samples and represents 16% of the samples collected in the UK. The wider EFSA survey that these UK analysed results fed into found sterigmatocystin in 124 of 1259 (10%) of the total number of samples.

In the EFSA survey, beer and nuts represented 117 samples and none of these samples were found to be contaminated with sterigmatocystin. Disregarding these beer and nut samples from the EFSA dataset leaves a pool of 1142 cereal results to more directly compare with 277 UK cereal results. Sterigmatocystin occurred in approximately 11% (i.e. 124/1142) of the EFSA set compared with 16% (44/277) for the UK-sourced samples.

93 UK samples of unprocessed cereal grains were analysed. Sterigmatocystin was detected above 0.1 µg/kg in 13 samples (14%) and 5 of those 13 positive samples had sterigmatocystin above 0.5 µg/kg. This is similar to the 13% rate of detection in the wider EFSA survey.

184 cereal products samples were collected in the UK and analysed. Sterigmatocystin was detected in a total of 31 samples (17%) above 0.1 µg/kg, and 7 of those 31 positive samples contained sterigmatocystin above 0.5 µg/kg.

Fera contributed just over 25% of the samples for cereal products in the wider EFSA survey. These Fera samples contributed nearly 45% of all samples containing sterigmatocystin to the EFSA results and this is reflected in the higher incidence rate of sterigmatocystin in UK sourced samples compared to the EFSA survey as a whole. This, however, is more a reflection of the lower limit of detection that Fera achieved than a wider problem of higher incidence of sterigmatocystin in the UK. Two of the partners could not quantify levels below 0.25 µg/kg so any samples below this would have been reported as not detected.

Overall, sterigmatocystin occurred most often in cereal products in Fera samples. Occurrence of sterigmatocystin in UK-sourced cereal grains was comparable to the wider EFSA survey. Whilst for the wider survey the levels in cereal products were typically lower than in the grains, Fera samples had similar levels across the two groups.

Fera and the wider EFSA survey results for cereals (i.e. nuts and beers excluded) are compared in Table 8.

Total	Fera Samples	EFSA Survey
Number of cereal samples	277	1142
Number detected (>0.1µg/kg)	44	124
% detected (>0.1µg/kg)	16%	11%
Cereal Grains		
Number of samples	93	429
Number detected (>0.1µg/kg)	13	55
% detected (>0.1µg/kg) of Total Samples	14%	13%
Cereal Products		
Number of samples	184	713
Number detected (>0.1µg/kg)	31	69
% detected (>0.1µg/kg) of Total Samples	17%	10%

Table 8: Comparison of Occurrence of Sterigmatocystin for Fera and EFSA

More than 50% of the contaminated samples contained levels between LOD and 0.5 µg/kg for the EFSA survey and other samples were all in the range 0.5 to 6 µg/kg with one exception (33 µg/kg in oats). The highest level of sterigmatocystin in Fera samples was in a sample of processed cereal wholemeal rye crisp bread (3.65 µg/kg).

The rice samples from Fera were all processed samples with a variety of origins with 4 European samples and 11 from outside the EU (mainly from India, Pakistan and Thailand). The limited information on some packaging and produce of more than one country accounted for 11 samples. Fera results for rice were in the range 0.22 to 1.00 µg/kg (brown rice from unknown origin). In the overall EFSA survey virtually all unprocessed rice samples (all from Europe) contained detectable sterigmatocystin.

The highest amount of sterigmatocystin found in an oat sample analysed at Fera was in an oat based breakfast cereal at 1.41 µg/kg and 0.97 µg/kg in an unprocessed cereal grain. By comparison 33 µg/kg was reported in a sample of oat grain from the Netherlands in the EFSA survey.

9 Statistics

9.1 Measurement Uncertainty (MU)

For Fera, the measurement uncertainty was estimated by the reproducibility of the measurement of the incurred rice sample that was analysed with every batch. This also covered the contribution from sample inhomogeneity. The expanded measurement uncertainty was the combined variability multiplied by a coverage factor (k) of 2, and was 40%.

10 Conclusions

Highly sensitive LC-MS/MS-based methods for the determination of sterigmatocystin in various food matrices were successfully validated to the level of 0.5 µg/kg in four laboratories, this was below the 1.5 µg/kg required by EFSA to facilitate risk assessment.

The average recoveries and repeatabilities all met the requirements showing that the method used by Fera was fit-for-purpose for quantitative analysis of sterigmatocystin in cereal based samples down to 0.5 µg/kg.

In this survey 277 cereal samples were collected in the UK and analysed in multiple analytical batches. In total, sterigmatocystin was detected in 12 samples (>0.5 µg/kg); this represents 4% of samples analysed and was in line with the wider EFSA study of 1142 cereal samples (disregarding beer and nuts).

Another 32 samples (12%) contained levels of sterigmatocystin in the range (0.1 µg/kg to 0.5 µg/kg). This gives a total of 44 samples where sterigmatocystin was detected representing 16% of all samples analysed in the UK. This is a slightly higher rate of incidence than the EFSA study as a whole which reported that sterigmatocystin was identified in 11% of the cereal samples in this concentration range. Sterigmatocystin was present in UK samples at levels above 0.1 µg/kg in 14% of unprocessed grains and 17% of cereal products.

There were 93 samples of unprocessed cereal grains and 184 cereal products so this study based on UK samples had fewer unprocessed grains than cereal products. Sterigmatocystin was present at levels above 0.5 µg/kg in 5% of unprocessed grains and 4% of cereal products. This broadly agrees with incidence in the wider EFSA study that these results fed into and which had many more samples.

The highest level of sterigmatocystin found in this survey was in a sample of wholemeal rye crisp bread at 3.65 µg/kg. In comparison, the wider EFSA survey saw levels mainly between 0.5 to 5 µg/kg with one rice sample at 5.5 µg/kg and an oat grain sample at a high level of 33 µg/kg.

Rice and oats grains were most likely to contain sterigmatocystin and consequently some products containing these ingredients also contained sterigmatocystin.

This survey captures a first look on the occurrence of sterigmatocystin in food products grown and consumed in the EU. For the UK samples and the wider EFSA project, the number of samples for each product is too low for an in-depth assessment of the level of sterigmatocystin for any relationship between country of origin, year of harvest, organic, non-organic or other variables.

All results have been submitted to EFSA data collection unit and have been uploaded to the database.

11 Publications

Output from this project is in the form of the report to EFSA:

Hans G.J. Mol, Amedeo Pietri, Susan J. MacDonald, Christos Anagnostopoulos, Martien Spanjer (2015) CP/EFSA/CONTAM/2013/02 'Survey on sterigmatocystin in food'.

This can be found at:

http://www.efsa.europa.eu/en/supporting/pub/774e.htm

12 Recommendations for Further Work

Mycotoxin formation depends on various factors including climatic conditions, moisture content at storage, and product treatment. This study reports results from approximately one year of sampling (2013-2014). Further analyses of samples from different crop years would be necessary to obtain information on seasonal variations.

Since rice flour, and to a lesser extent oats, are important ingredients in cereal-based infant food, further study of products containing rice or oats as a major ingredient could be considered to provide additional information on these products. As oat samples showed higher occurrence of sterigmatocystin, analysis of UK grown oat crops before and after processing would provide additional information on the occurrence of sterigmatocystin.

13 References

[1] http://www.efsa.europa.eu/en/contam201302/docs/gpefsacontam201302guide.pdf

[2] Hans G.J. Mol, Amedeo Pietri, Susan J. MacDonald, Christos Anagnostopoulos, Martien Spanjer (2015) CP/EFSA/CONTAM/2013/02 'Survey on sterigmatocystin in food'.

14 Acknowledgements

Fera would like to thank the following for their kind assistance in supply of samples to support this project:

Derek Croucher, Morning Foods Ltd, Cheshire, UK.

Member companies of the Maltsters' Association of Great Britain.

Maizecor Ltd, Hull, UK.

NABIM: National Association of British and Irish Flour Millers, UK.

Hovis, Premier Foods, High Wycombe, Buckinghamshire, UK.

Thanks also to Fera staff, Irene Leon, Julie Clough, Monika Sehnalova and Joanna Stratton for sample preparation and analyses.

Appendix 1: Sterigmatocystin in Unprocessed Cereals

LOD (µg/kg)	0.1
LOQ (µg/kg)	0.5

Sample	Product	Sterigmatocystin (µg/kg)
S14-050851	Wheat grain, soft	--
S14-050852	Wheat grain, soft	--
S14-050853	Wheat grain, soft	--
S14-050854	Wheat grain, soft	--
S14-050855	Wheat grain, soft	--
AS14-051244-003	Wheat grain	--
AS14-051245-003	Wheat grain	--
AS14-051246-003	Wheat grain	--
AS14-051247-003	Wheat grain	--
S14-052357	Wheat grain	--
S14-052358	Wheat grain	--
S14-052359	Wheat grain	--
S14-052360	Wheat grain	--
S14-052361	Wheat grain	--
S14-052363	Wheat grain	--
S14-052364	Wheat grain	--
S14-052366	Wheat grain	--
S14-052367	Wheat grain	--
S14-052371	Wheat grain	--
S14-052372	Wheat grain	--
S14-052373	Wheat grain	--
S14-052374	Wheat grain	--
S14-052377	Wheat grain	--
S14-052379	Wheat grain	--
S14-052401	Wheat grain	--
S14-052402	Wheat grain	--
S14-052403	Wheat grain	--
S14-052404	Wheat grain	--
S14-052405	Wheat grain	1.26
S14-052408	Wheat grain	--
S14-052409	Wheat grain	--
S14-052410	Wheat grain	--
S14-052411	Wheat grain	--
S14-052412	Wheat grain	--
S14-052415	Wheat grain	--
S14-052416	Wheat grain	--
S14-052417	Wheat grain	--

-- Level below 0.1 µg/kg LOD

( ) Level above 0.1 µg/kg LOD but below 0.5 µg/kg LOQ

Sterigmatocystin in Unprocessed Cereals (continued)

LOD (µg/kg)	0.1
LOQ (µg/kg)	0.5

Sample	Product	Sterigmatocystin (µg/kg)
S14-050056	Rye grain	--
S14-050062	Rye grain	--
AS14-051281-003	Rye grain	--
S14-051238	Corn grain	--
S14-051239	Corn grain	--
S14-051240	Corn grain	--
S14-051241	Corn grain	--
S14-051242	Corn grain	--
S14-051243	Corn grain	1.32
AS14-051279-003	Corn grain	--
S14-043549	Barley grain, whole	--
S14-043550	Barley grain, whole	--
S14-043551	Barley grain, whole	--
S14-043552	Barley grain, whole	--
S14-043553	Barley grain, whole	--
S14-043554	Barley grain, whole	--
S14-043555	Barley grain, whole	(0.19)
S14-043556	Barley grain, whole	(0.18)
S14-043557	Barley grain, whole	--
S14-043558	Barley grain, whole	--
S14-043559	Barley grain, whole	--
S14-043560	Barley grain, whole	--
S14-043561	Barley grain, whole	--
S14-043562	Barley grain, whole	--
S14-043563	Barley grain, whole	--
S14-043564	Barley grain, whole	--
S14-043565	Barley grain, whole	--
S14-043566	Barley grain, whole	--
S14-043567	Barley grain, whole	--
S14-043568	Barley grain, whole	--
S14-043569	Barley grain, whole	--
S14-043570	Barley grain, whole	--
S14-043571	Barley grain, whole	--
S14-043572	Barley grain, whole	--
S14-043573	Barley grain, whole	--
S14-043574	Barley grain, whole	--
S14-043575	Oats, grain	(0.44)
S14-043576	Oats, grain	(0.49)
S14-042605	Oats, grain	--

-- Level below 0.1 µg/kg LOD
( ) Level above 0.1 µg/kg LOD but below 0.5 µg/kg LOQ

Sterigmatocystin in Unprocessed Cereals (continued)

LOD (µg/kg)	0.1
LOQ (µg/kg)	0.5

Sample	Product	Sterigmatocystin (µg/kg)
S14-042600	Oats, grain	(0.48)
S14-042601	Oats, grain	(0.30)
S14-042602	Oats, grain	--
S14-042603	Oats, grain	--
S14-042604	Oats, grain	--
S14-042606	Oats, grain	--
S14-042607	Oats, grain	0.97
S14-042608	Oats, grain	(0.28)
S14-042609	Oats, grain	0.63
S14-042610	Oats, grain	(0.33)
S14-042611	Oats, grain	--
S14-042612	Oats, grain	--
S14-042613	Oats, grain	--
S14-042614	Oats, grain	0.67
AS14-051276-003	Oats, grain	--
AS14-051277-003	Oats, grain	--
AS14-051278-003	Oats, grain	--

-- Level below 0.1 µg/kg LOD

( ) Level above 0.1 µg/kg LOD but below 0.5 µg/kg LOQ

Appendix 2: Sterigmatocystin in Processed Cereal Products

Grain Milling Products

LOD (µg/kg)	0.1
LOQ (µg/kg)	0.5

Sample	Product	Sterigmatocystin (µg/kg)
S14-048738	Barley, pearled	--
S14-048740	Corn grain	--
S14-048741	Corn grain	--
S14-010947	Cornmeal	(0.25)
S14-048771	Oat bran	--
S14-023270	Oat bran	(0.31)
S14-023266	Oat milling products	--
S14-010946	Wheat semolina, Durum	--
S14-010991	Wheat semolina, Durum	(0.13)
S14-010948	Wheat semolina, Durum	--
S14-050059	Rye flour, light	--
S14-048758	Rye flour, wholemeal	--
S14-050063	Rye flour, wholemeal	--
S14-050058	Rye milling products	--
S14-050064	Rye milling products	(0.26)
S14-010949	Wheat flour, brown	--
S14-048763	Wheat flour, brown	--
S14-011002	Wheat flour, white	--
S14-010939	Wheat flour, white	--
S14-010952	Wheat flour, white	--
S14-048757	Wheat flour, white	--
S14-048760	Wheat flour, white	--
S14-052345	Wheat flour, white	(0.16)
S14-052347	Wheat flour, white	--
S14-052362	Wheat flour, white	--
S14-052365	Wheat flour, white	--
S14-052369	Wheat flour, white	--
S14-052375	Wheat flour, white	--
S14-052376	Wheat flour, white	--
S14-052378	Wheat flour, white	--
S14-052399	Wheat flour, white	--
S14-052400	Wheat flour, white	--
S14-052407	Wheat flour, white	--
S14-052413	Wheat flour, white	--
S14-052414	Wheat flour, white	--
S14-010941	Wheat flour, wholemeal	--

-- Level below 0.1 µg/kg LOD

( ) Level above 0.1 µg/kg LOD but below 0.5 µg/kg LOQ

Grain Milling Products (continued)

LOD (µg/kg)	0.1
LOQ (µg/kg)	0.5

Sample	Product	Sterigmatocystin (µg/kg)
S14-048738	Barley, pearled	--
S14-048740	Corn grain	--
S14-048741	Corn grain	--
S14-010947	Cornmeal	(0.25)
S14-048771	Oat bran	--
S14-023270	Oat bran	(0.31)
S14-023266	Oat milling products	--
S14-010946	Wheat semolina, Durum	--

Rice

Sample	Product	Sterigmatocystin (µg/kg)
S14-048774	Rice	--
S14-048775	Rice	--
S14-048776	Rice	(0.22)
S14-048778	Rice	--
S14-048784	Rice	--
S14-048785	Rice	--
S14-048789	Rice	--
S14-050050	Rice	--
S14-048761	Rice flour	(0.34)
S14-010935	Rice, brown	(0.45)
S14-048779	Rice, brown	--
S14-048780	Rice, brown	--
S14-048782	Rice, brown	1.00
S14-048792	Rice, brown	(0.45)
S14-050051	Rice, brown	(0.22)
S14-050048	Rice, brown	0.56
S14-010945	Rice, long-grain	0.64
S14-048773	Rice, long-grain	--
S14-048781	Rice, long-grain	--
S14-048783	Rice, long-grain	--
S14-048787	Rice, long-grain	--
S14-050049	Rice, long-grain	(0.23)
S14-010921	Rice, long-grain	--
S14-010978	Rice, popped with sugar	--
S14-048777	Rice, red	--
S14-048788	Rice, white	--
S14-048742	Noodle, rice	--

-- Level below 0.1 µg/kg LOD

( ) Level above 0.1 µg/kg LOD but below 0.5 µg/kg LOQ

Pasta

LOD (µg/kg)	0.1
LOQ (µg/kg)	0.5

Sample	Product	Sterigmatocystin (µg/kg)
S14-050034	Pasta (Raw)	(0.25)
S14-050035	Pasta (Raw)	--
S14-048756	Pasta, gluten free	--
S14-048751	Pasta, mixed cereal flour	--
S14-048754	Pasta, mixed cereal flour	--
S14-050054	Pasta, mixed cereal flour	--
S14-048752	Pasta, mixed cereal flour	--
S14-048749	Pasta, wheat flour, without eggs	--
S14-050053	Pasta, wheat flour, without eggs	--
S14-050052	Pasta, wheat flour, without eggs	(0.33)
S14-050055	Pasta, wheat flour, without eggs	(0.25)
S14-052942	Pasta, wheat flour, without eggs	--
S14-052943	Pasta, wheat flour, without eggs	--
S14-052944	Pasta, wheat flour, without eggs	--
S14-048755	Pasta, wheat flour, without eggs	--
S14-048750	Pasta, wheat wholemeal, without eggs	--
S14-048753	Noodle, wheat flour, without eggs	--
S14-050033	Noodle, wheat flour, without eggs	--
S14-050036	Noodle, wheat flour, without eggs	--
S14-050037	Noodle, wheat flour, without eggs	--
S14-050038	Noodle, wheat flour, without eggs	--

Bread/ Rolls

Sample	Product	Sterigmatocystin (µg/kg)
S14-050846	Wheat rolls, brown	--
S14-052932	Wheat rolls, brown	--
S14-052951	Wheat rolls, brown	--
S14-050847	Wheat rolls, white	(0.35)
S14-050881	Wheat rolls, white	--
S14-052954	Wheat rolls, with oil seeds	--
S14-050878	Wheat bread, brown	--
S14-052933	Wheat bread, brown	--
S14-050877	Wheat bread, white	--
S14-052945	Wheat bread, white	--
S14-052956	Wheat bread, white	(0.41)
S14-050879	Wheat germ bread	(0.21)
S14-052934	Wheat germ bread	--

-- Level below 0.1 µg/kg LOD

( ) Level above 0.1 µg/kg LOD but below 0.5 µg/kg LOQ

Sterigmatocystin in Processed Cereal Products (continued)

Breakfast Cereals (including muesli)

LOD (µg/kg)	0.1
LOQ (µg/kg)	0.5

Sample	Product	Sterigmatocystin (µg/kg)
S14-010968	Breakfast cereals	--
S14-010957	Breakfast cereals	(0.47)
S14-01100	Breakfast cereals	--
S14-010956	Breakfast cereals	--
S14-011055	Breakfast cereals	--
S14-023269	Breakfast cereals	(0.28)
S14-010962	Corn flakes	--
S14-023272	Mixed breakfast cereals	(0.24)
S14-010974	Mixed cereal flakes	--
S14-011054	Oat flakes	(0.13)
S14-042619	Oat flakes	--
S14-042620	Oat flakes	0.60
S14-023273	Muesli	--
S14-023274	Muesli	--
S14-010981	Muesli with fruits and nuts	--
S14-011056	Muesli with fruits and nuts	--
S14-042615	Muesli with fruits and nuts	--
S14-042616	Muesli with fruits and nuts	--
S14-042621	Muesli with fruits and nuts	--
S14-042622	Muesli with fruits and nuts	--
S14-010980	Oat porridge	--
S14-011998	Oat porridge	--
S14-010988	Oat porridge	1.41
S14-010990	Oat porridge	(0.39)
S14-010931	Oat porridge	--
S14-011060	Oat porridge	--
S14-011058	Oat porridge	--
S14-048766	Oat porridge	--
S14-048767	Oat porridge	--
S14-048768	Oat porridge	--
S14-048769	Oat porridge	--
S14-048772	Oat porridge	--
S14-048765	Oat porridge	--
S14-048770	Porridge	--
S14-010986	Wheat flakes	--
S14-023268	Wheat flakes	--
S14-023265	Hazelnuts (Corylus avellana)	--

-- Level below 0.1 µg/kg LOD

( ) Level above 0.1 µg/kg LOD but below 0.5 µg/kg LOQ

Sterigmatocystin in Processed Cereal Products (continued)

Fine Bakery Ware

LOD (µg/kg)	0.1
LOQ (µg/kg)	0.5

Sample	Product	Sterigmatocystin (µg/kg)
S14-052955	Biscuits, salty	--
S14-010937	Biscuits, salty, with cheese	--
S14-052940	Biscuits, salty, with cheese	--
S14-052948	Biscuits, sweet, plain	--
S14-052941	Biscuits, sweet, plain	--
S14-052958	Biscuits, sweet, plain	--
S14-052947	Butter biscuits	--
S14-010922	Crisp bread, rye wholemeal	--
S14-011057	Crisp bread, rye wholemeal	(0.47)
S14-048743	Crisp bread, rye wholemeal	(0.47)
S14-048744	Crisp bread, rye wholemeal	3.65
S14-050057	Crisp bread, rye wholemeal	--
S14-052953	Crisp bread, rye wholemeal	--
S14-010950	Crisp bread, rye wholemeal	--
S14-052968	Crisp bread, wheat, wholemeal	--
S14-010936	Fine bakery wares	--
S14-050880	Fine bakery wares	--
S14-050882	Fine bakery wares	--
S14-052952	Matzo	--
S14-052957	Breadcrumbs	--
S14-050883	Scone	--
S14-052938	Unleavened bread, crisp bread and rusk	--
S14-052935	Unleavened bread, crisp bread and rusk	--
S14-052936	Unleavened bread, crisp bread and rusk	--
S14-052937	Unleavened bread, crisp bread and rusk	--
S14-052939	Unleavened bread, crisp bread and rusk	--
S14-052946	Unleavened bread, crisp bread and rusk	--
S14-052949	Unleavened bread, crisp bread and rusk	--
S14-052959	Unleavened bread, crisp bread and rusk	--
S14-052950	Waffles	--

-- Level below 0.1 µg/kg LOD
( ) Level above 0.1 µg/kg LOD but below 0.5 µg/kg LOQ

Sterigmatocystin in Processed Cereal Products (continued)

Cereal Based Infant Food

LOD (µg/kg)	0.1
LOQ (µg/kg)	0.5

Sample	Product	Sterigmatocystin (µg/kg)
S14-011070	Ready-to-eat meal for children, cereal-based	--
S14-011051	Ready-to-eat meal for children, cereal-based	--
S14-011052	Biscuits, rusks and cookies for children	--
S14-011052	Biscuits, rusks and cookies for children	--
S14-011023	Simple cereals *	--
S14-011010	Simple cereals *	--
S14-011012	Simple cereals *	0.75
S14-011019	Simple cereals *	--
S14-011018	Simple cereals *	--
S14-011020	Simple cereals *	--
S14-011013	Simple cereals *	--
S14-011024	Simple cereals *	--

* Simple cereals which are or have to be reconstituted with milk or other appropriate nutritious liquids

-- Level below 0.1 µg/kg LOD
( ) Level above 0.1 µg/kg LOD but below 0.5 µg/kg LOQ