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Survey of mycotoxins in stored grain from the 1999 harvest in the U.K.

by

A J Prickett, S MacDonald and K B Wildey

Central Science Laboratory, Sand Hutton, York YO41 1LZ

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CONTENTS

Abstract 2

Introduction 3

Methods 4

Sites visited 4

Sampling 4

Mycotoxin analyses 5

Samples of grain collected 7

Analytical results 8

Ochratoxin A 8

Deoxynivalenol 10

Discussion 10

Acknowledgements 12

Figure 1. Distribution of sample collection sites in England and Scotland. 13

Table 1. Regional UK cereal production (wheat, barley and oats) and the number of grain samples collected per region. 14

Table 2. Types of site from which samples were collected. 15

Table 3. Storekeeper's view of the intended use of the grain which was sampled. 15

Table 4. Levels of ochratoxin A found in each type of grain. 16

Table 5. Levels of deoxynivalenol (DON) found in each type of grain. 16

Table 6. Levels of ochratoxin A found for each intended use of grain specified by the storekeeper. 17

Table 7. Levels of deoxynivalenol (DON) found for each intended use of grain specified by the storekeeper. 17

Table 8. Levels of ochratoxin A found in each UK Region. 18

Table 9. Levels of ochratoxin A found in relation to the moisture contents of the grain samples. 19

Appendix I. Results of analyses for ochratoxin A (OA) and deoxynivalenol (DON) for each of the 320 samples, expressed as μg/kg. 20

Appendix II. Results of further trichocecene analyses. 22

Abstract

A survey of stored grain was carried out to determine the levels of two mycotoxins, deoxynivalenol (DON) and ochratoxin A. The first of these substances is produced by naturally-occurring fungi or moulds which attack the growing crop, such as Fusarium ear blight. The second is produced by moulds growing on the grain during storage. Both present a risk to human and animal health. The European Commission is currently considering proposals for legislative limits for both of these contaminants but more information is needed on their normal levels in grain. Consequently the Home-Grown Cereals Authority commissioned a survey of UK grain in store from the 1999 harvest to see how the levels compared with the proposed limits. Samples of grain were collected across the UK from farms, central stores, mills, maltings and ports from the beginning of February to the beginning of April 2000. A total of 320 samples was collected, consisting of 201 wheat, 106 barley and 13 oats, which is approximately proportional to the hectares of each grown in the UK. A small number of organic samples was also collected.

For DON the limits under consideration include 500µg/kg for retail products such as breakfast cereal, bread, pasta, etc. and 750µg/kg for grain or any grain product (such as flour) used as a raw material by industry (1µg/kg is 1 part per 1,000,000,000). Analyses of the samples showed that none exceeded 750µg/kg of DON but one exceeded 500µg/kg with a level of 600µg/kg. The proposed limit for ochratoxin A is 5µg/kg, in line with its much greater toxicity relative to DON. The great majority of samples (97%), including all of the organic samples, had levels of ochratoxin A which were below the proposed limits. Measurable levels were present in 16% of samples but only 3% were above the limit. However two samples had significantly higher levels of more than 100µg/kg. Finally, a limited study was made of the range of trichocenes found in the higher contaminated DON samples. The results indicated that DON was the major trichothecene present.

These results represent a snap-shot in time, in terms of both the length of time since harvest and the year of the survey, which provide a base-line for comparison with the proposed limits and with any future surveys.

Introduction

Mycotoxins are toxic chemicals which are produced by naturally-occurring moulds on a variety of foodstuffs. They can contaminate food as a result of fungal infection in crops in the field or during storage. These substances are a risk to both human and animal health. The only mycotoxins for which European Legislation is currently in place are the aflatoxins. The EC is working currently towards harmonisation of the permitted levels of two other mycotoxins - ochratoxin A (OA) and deoxynivalenol (DON), both of which may be found in grain. DON is produced by *Fusarium* species of moulds which attack the growing crop, causing, for example, Fusarium ear blight. DON is a member of a group of structurally related compounds called the trichothecenes. These are classified according to their basic structure, with Type A (e.g. T2 toxin and HT2 toxin) and Type B (including DON, 3-Acetyl DON, 15-Acetyl DON and nivalenol) being the most commonly reported to occur naturally in cereals. The trichothecenes have been found to inhibit protein synthesis and to have immunosuppressive and haemorrhaging effects, with toxicity characterised by feed refusal and weight loss, and increased susceptibility to infectious diseases . For DON the limits under consideration (which may be applied as a total trichothecene level) include 500µg/kg for retail products such as breakfast cereal, bread, pasta, etc. and 750µg/kg for grain and any grain products (such as flour) used as a raw material by industry. OA is produced by moulds which occur on stored grain, such as species of *Penicillium* (in the UK) and *Aspergillus* (the latter also produces aflatoxins). The proposed regulatory limits for ochratoxin A are 5µg/kg for cereals and 3µg/kg for cereal products.

To determine how these proposed limits relate to levels which occur in stored grain in the UK, a survey of grain from the 1999 harvest was commissioned by the HGCA (see Appendix II). DON is the trichothecene most frequently detected and in the largest concentration, the other trichothecenes rarely occurring without DON being present. For this reason samples were initially analysed for DON only, by a quantitative HPLC method. Samples subsequently found to exceed a level of 150 µg/kg were subsequently analysed by GC-MS for eight trichothecenes (including DON) to determine the extent to which these other compounds occurred. This report presents the levels of ochratoxin A and deoxynivalenol found. The results of the additional GC-MS analyses are given in Appendix II, with a short description of the method and discussion of the findings.

Methods

Sites visited

The aim was to collect samples which were representative of cereal production in the UK. This entailed matching the required number of samples to the hectares of cereal grown in each of the UK Regions and keeping the ratio of wheat, barley and oats proportional to their production. The type of sites included farm grain stores, central stores, mills, maltsters and ports. Unfortunately the source of random addresses which was to be used for this survey was unexpectedly unavailable. However, an alternative source of addresses was identified and letters were sent explaining the purpose of the survey and asking for permission to visit and collect samples. Almost half of the recipients who responded did not have any grain left in store. Sites were visited between the beginning of February and the beginning of April 2000.

Wherever possible, information which might be relevant to the results was collected at each site. This included the type of site, variety of grain, moisture content, type of storage, tonnage on site, size of sampled lot, intended use of the grain, harvest location, whether it was organic, pesticide use and a brief description of its storage history.

Sampling

The uneven distribution of ochratoxin A contamination in a bulk means that care must be taken to obtain a sample of grain which is as representative as possible of the whole bulk. The method used was based on that suggested in Commission Directive 98/53/EC (Annex I) for sampling grain for aflatoxins, in which 100 small samples are taken to produce one aggregate sample. The grain was sampled using purpose-built five-compartment grain spears which were adjusted so that each compartment could hold 30g of wheat. By sampling at 20 locations in a grid pattern across the surface of the bulk, at depths of 0.2 to 1.7m, 100 sub-samples of 30g each were obtained, giving an aggregate sample of 3kg. Since the spears were set for 30g of wheat per compartment, extra sampling was necessary with barley and oats to obtain the required 3kg.

The need to sample by spear across the surface of the grain, as well as safety considerations, meant that the method of storage imposed restrictions as to whether grain could be sampled. Generally, silos and sealed bins could not be sampled so almost all samples came from floor

stores and open bins. However, where only silos were present but it was possible to sample safely from lorries as they delivered grain, then this was done.

The samples were sealed in polythene bags and put into padded bags to prevent any condensation due to changes in temperature. The samples were stored cool and returned to the laboratory where they were held at 4°C until analysed.

Before the grain was ground for analysis, the moisture content was determined using a Protimeter Grainmaster calibrated for wheat at 15% m.c.

Mycotoxin Analyses

1. Extraction.

The 3kg sample was finely ground and fully mixed. A sub-sample (20 ± 0.1g) was weighed into an extraction flask (250ml round bottom flask). The extraction solvent (acetonitrile : water (84:16, v/v), 100ml) was added. The flask was stoppered and shaken by hand to mix then placed on shaker for 120 minutes to extract. The resulting extract was filtered through Whatman No.4 or Whatman 113V filter paper.

2a. Sample clean-up for deoxynivalenol (DON).

A portion of filtrate was transferred to a clean glass tube. A Mycosep trichothecene clean-up column was used to clean-up the sample. The rubber flange end of the column was pushed into the glass tube until sufficient cleaned up extract had passed through the column to fill the plastic tube. In total 10 ml of extract was cleaned-up for analysis. The extract was evaporated to dryness under nitrogen in a heating block set at 50^{o}C. The dried residue was redissolved with 1ml distilled water.

2b. Manual immunoaffinity column clean-up for deoxynivalenol (DON).

The aqueous sample extract was loaded onto a DONtest immunoaffinity column using a pasteur pipette. The extract was passed completely through the column at a rate of about 1 drop/second. The column was washed with 5ml distilled water and then dried completely by pushing air through the column. The column was eluted with methanol (1 ml) at very slow speed. and the eluate collected in a 4ml amber vial. The eluate was evaporated to dryness in a heating block at 50^{o}C under nitrogen. The dried residue was redissolved with 500µl of mobile phase. This was transferred into a 800µl amber crimp top vial ready for HPLC analysis.

2c. Sample clean-up for ochratoxin A.

Phosphate buffered saline (PBS, 55ml) was measured into a conical flask. An aliquot of filtered extract (5 ml) was added. The flask was stoppered and shaken well to mix. The diluted extract was transferred to a plastic sample tube and placed on a Gilson ASPEC for automated immunoaffinity column clean-up.

2d. Immunoaffinity column clean-up for ochratoxin A.

The column was conditioned with PBS (20ml). Sample (50ml) was added at a rate of no more than 3 ml/minute. The column was washed with PBS (10 ml), then eluted with 2% acetic acid in methanol (2ml). An aliquot of eluate was diluted with water before HPLC analysis.

3a. HPLC conditions for deoxynivalenol (DON).

Autosampler : Gilson 231 autosampler

Column : Phenomenex Prodigy ODS3, 150 x 4.6mm i.d.

Flow rate : 1 ml/minute

Mobile phase: HPLC water : methanol, (85:15, v/v).

Wash solution: Methanol : water, (50 :50, v/v).

Mobile phase programme : Mobile phase 0-15 minutes, wash 15-25 minutes, mobile phase 25-35 minutes.

Injection volume : 300 µl

Detection : UV at 220nm

3b. HPLC conditions for ochratoxin A.

Autosampler : Gilson ASPEC,

Column : Spherisorb ODS2-Excel, 250 x 4.6 mm i.d.

Mobile phase : acetonitrile : water : acetic acid, (99 : 99 : 2, v/v/v)

Flow rate : 1 ml/minute

Injection volume : 6 - 800 µl.

Detection : Fluorescence Excitation : 333nm, Emission : 477 nm.

To enhance sensitivity a post column reaction system consisting of a Valco dead volume t-piece, and reaction coil tubing 1.5m x 0.010' was used.

Post column reaction solution **:** 1.1M NH_3

Flow rate : 0.3 ml/minute

Detection : Fluorescence Excitation : 390 nm, Emission : 477 nm

4. Spiked samples

Each analytical batch contained at least one spiked sample to check analytical recovery. Samples were spiked with solutions of both ochratoxin A and deoxynivalenol. The solvent was allowed to evaporate before the samples were extracted and analysed using the method described above. All analytical recovery values obtained during the survey were within the acceptable range.

The lower limits of detection were 0.1µg/kg for ochratoxin A and 20µg/kg for DON. However the measurable limit for OA was 0.3µg/kg and this reporting level is used in this report. Results below these values are recorded as zero in Appendix I. Spike recovery checks for ochratoxin A were done at a level of 5µg/kg and those for DON were at 500µg/kg. All results in Appendix I are corrected for analytical recovery with the exception of the few samples containing OA levels greater than 10µg/kg, which have not been corrected as they obviously exceeded the level of the spike. The levels reported for these samples should be read as "greater than" the stated value.

Samples of grain collected

A total of 320 grain samples was collected - 201 wheat, 106 barley and 13 oats. On a country basis, 269 samples were collected in England, 45 in Scotland and 6 in Northern Ireland. Attempts to obtain a proportionate 5 samples from Wales were unsuccessful: after much effort four sites with grain were identified but two could not be sampled because the grain was in closed bins and upon visiting the remaining two it was found that the actual harvest sites were just inside England. The distribution of collection sites in England and Scotland is shown in Fig.1. Northern Ireland could not be represented on this map but, of the six samples, three came from Co. Londonderry and three from Co. Down. The correspondence between the target numbers of samples required for full proportionality (calculated from June 1999 census data for each Region) and the actual numbers collected, is shown in Table 1. Overall the agreement is good but the difficulty of finding the right grain in the right place at the time of year of the survey meant that some compromises were necessary. This is reflected in the slight undersampling of barley which occurred in the Eastern and West Midlands regions of England and the consequential oversampling in northern England. In addition, in some areas where it was difficult to find stored grain, some samples were collected on the basis of local knowledge rather than random addresses. According to MAFF statistics, grain stocks on farm as a percentage of total production had fallen to 52% for wheat and 42% for barley by

December 1999. On the basis of the figures for March 1999, the stocks remaining at the end of March 2000 would have fallen to approximately 25% for wheat and 9% for barley.

The number of "organic" samples collected was 9, which was the target number calculated from an estimated 86,400 hectares of organic cereal out of a UK total of 3,109,025 hectares of wheat, barley and oats. The 9 samples consisted of five wheat, 3 oats and one barley.

The range of premises visited and the number of samples from each is given in Table 2. Overall, 222 samples came from sites which were primarily farms, 68 from sites which were primarily central stores and 30 from mills, maltsters and ports. The intended use of the grain (in the storekeeper's view) is given in Table 3. The main single intended uses of the grain were feed (195 samples), flour (61) and malting (34). Where the intended use was said to be malting, a further small sample was collected. These samples were subsequently examined by a professional representative from the Maltsters Association of Great Britain (MAGB) to check whether the grain was acceptable for malting.

Analytical results

Analytical recovery of mycotoxins from spiked grain

Analytical recovery of ochratoxin A from grain spiked at 5µg/kg ranged from 64% to 93% with an average of 75% for 33 spikes. The range for the recovery of DON from grain spiked at 500µg/kg was from 60% to 90% with an average of 72% for 26 spikes.

Ochratoxin A

The level of ochratoxin A was below the measurable detection limit in 84% of samples (Table 4) and below the proposed 5µg/kg limit in 97%. The remaining 3% (11 samples - 6 wheat and 5 barley) which were above 5µg/kg ranged from 5.2µg/kg to a maximum of 231µg/kg in a sample of wheat.

Categorising the samples by the intended use of the grain, as specified by the storekeeper, showed that 10 of the 11 samples which exceeded the 5µg/kg level were from grain intended for use as feed (Table 6). The other sample was barley said to be intended for malting. However this was one of two samples rejected by the professional maltster (who had no knowledge of the results of the mycotoxin analyses) as being unacceptable for malting

because of fungal infection. Thus none of the samples which were found acceptable for malting exceeded the proposed 5µg/kg limit and only three were above the measurable detection level of 0.3µg/kg. Similarly none of the samples of wheat intended for flour exceeded the limit and 89% of them were below the detectable limit. Ochratoxin A was below the measurable detection limit in all of the 9 organic samples.

The levels of ochratoxin A in each Region are given in Table 8. The 5 samples of barley which had more than 5µg/kg (ranging from 8.5 to 117µg/kg) were from northern areas - three from the north-east of Scotland, one from the east of Northern Ireland and one from Yorkshire & Humberside. There was no commonality in the type of site from which these samples were collected or in the variety of barley. The sampled lot sizes varied from 10 to 50 tonnes and the moisture contents from 15.5 to 18.6%. The 6 wheat samples which exceeded the proposed limit (ranging from 5.2 to 231µg/kg) came from the south-east of Scotland and the east, south-east and west-midlands of England. In all but one case they were from stores on arable farms. The measured moisture contents were from 14.1 to 15.8%, the varieties differed and the lot sizes ranged from 25 to 500 tonnes.

In Table 9 the ochratoxin A levels are tabulated against the moisture contents of the grain, as determined from the samples prior to analysis. The distributions of moisture contents for wheat and barley were very similar: the wheat samples ranged from 11.5 to 17.7% m.c. with a mean of 14.6% whilst barley ranged from 11.9 to 18.6% with a mean of 14.7%. Oats differed with 12 of the 13 samples ranging from 11.1 to 13.5% and the remaining sample had a moisture content of 19.3%. In the 51 samples of grain where ochratoxin A was detected, there was no significant correlation between moisture content and the level of ochratoxin A (Pearson correlation coefficient = 0.266, probability = 0.06). The relationship between moisture content and the presence or absence of ochratoxin A was tested by grouping the samples into two categories - those of 14.5% m.c. or less (162 samples) and those of more than 14.5% (156 samples). Ochratoxin A was detected in 13% of the lower m.c. group and 19% of the higher group but these percentages were not statistically different (probability = 0.17, chi-squared test with Yates correction). However, the level of ochratoxin A was above 5µg/kg in 5.8% of the higher m.c. group but only 1.2% of the lower group and this difference was statistically significant (probability = 0.03, Fisher's exact test).

Deoxynivalenol

DON was detected in 88% of samples but the majority (83%) contained less than 100µg/kg. Only one sample exceeded 500µg/kg, with a level of 600µg/kg, and none exceeded 750µg/kg (Table 5.). However another 6 samples fell between 250 and 499µg/kg. Of these 7 samples, three were intended for feed, one for malting and three for "other uses" (see Table 7). The maximum value for the 9 organic samples was 118µg/kg.

Discussion

That the great majority of samples (97%) were shown to contain less than the proposed limit of 5µg/kg ochratoxin A, and that 84% were below the measurable detection limit of 0.3µg/kg, may be taken as a good indicator of acceptable storage conditions at the great majority of sites. Cool and dry conditions limit the development of both moulds and insect pests. However, the levels in some of the remaining 3% are of concern. Ochratoxin A has been classified as a genotoxic carcinogen by the UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment, which recommended that ochratoxin levels should be reduced to the lowest level technologically achievable. The intended use of the grain was as specified by the storekeeper at the time that the samples were collected and obviously this may not always be the same as its actual use, since this will depend upon various factors such as quality tests. This was well demonstrated by the samples of barley intended for malting which were rejected by MAGB as being of unsuitable quality for malting. However the samples of grain with the higher levels of ochratoxin A were intended for use as animal feed and this contaminant has been shown to have an adverse effect upon the health and productivity of stock. Whether this grain would have been mixed with other grain, thereby lowering the levels of ochratoxin A, and whether it was to be offered to feed mills or as straights to farms, was information that was not collected (and may not have been known) during the survey.

The conclusions that can be drawn from this survey with regard to the relationship between ochratoxin A and the moisture content of the grain are limited. Although such a relationship might be expected (because the fungi producing the mycotoxin require a relatively high moisture content to grow), the results did not demonstrate a relationship between moisture content and either the level of ochratoxin A or the prevalence of samples containing this mycotoxin. They did however indicate a relationship between the prevalence of samples with high levels of ochratoxin A and moisture content. Nevertheless, these comparisons were necessarily limited to using the moisture content of the grain as measured just prior to analysis, which may or may not reflect the history of the moisture content of that grain. This

may explain why, in this study, no direct relationship was found between the level of ochratoxin A and moisture content.

The levels of deoxynivalenol, which are dictated by growing conditions rather than storage conditions, were all acceptable in terms of expected legislative limits but there was a single sample which was close to the 750μg/kg limit. Although much of the country experienced above average rainfall in 1999 and the occurrence of Fusarium ear blight on winter wheat was higher than average (MAFF data), the species of *Fusarium* involved were not overly dominated by those which are regarded are mycotoxin-producing species. In the previous year (1998) ear blight had been twice as high as in 1999 but very few predominantly mycotoxin-producing species were present. One should therefore consider that 1999 was neither a particulary serious year for tricothecene production, nor an especially 'clean' one.

The data obtained in this survey represent a snap-shot in time, both in relation to the time of harvest and the year. The grain that was sampled came from approximately the one-third of wheat and one-quarter of the barley that was still in store from the last harvest. Whether this grain was fully representative of the harvest is questionable. It could be argued that lower quality grain may have been moved on more quickly before further deterioration occurred, leaving relatively higher quality grain, or conversely lower quality was still in store because it could not be moved on. Whatever the scenario, the timing of the survey was such that possible deterioration in storage and the consequential contamination with ochratoxin A was perhaps near to its maximum. In the case of deoxynivalenol, since there were relatively low levels of mycotoxin-producing *Fusarium* in the field, the levels of DON should probably be seen as representing a relatively "good" year.

Nevertheless, the samples collected gave a good representation of the UK grain present in store at the time of the survey, in terms of geographical distribution, type of grain and in the various types of site storing grain. The data obtained give a reliable base-line against which other data can be judged.

Acknowledgements

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Figure 1. Distribution of sample collection sites in England and Scotland

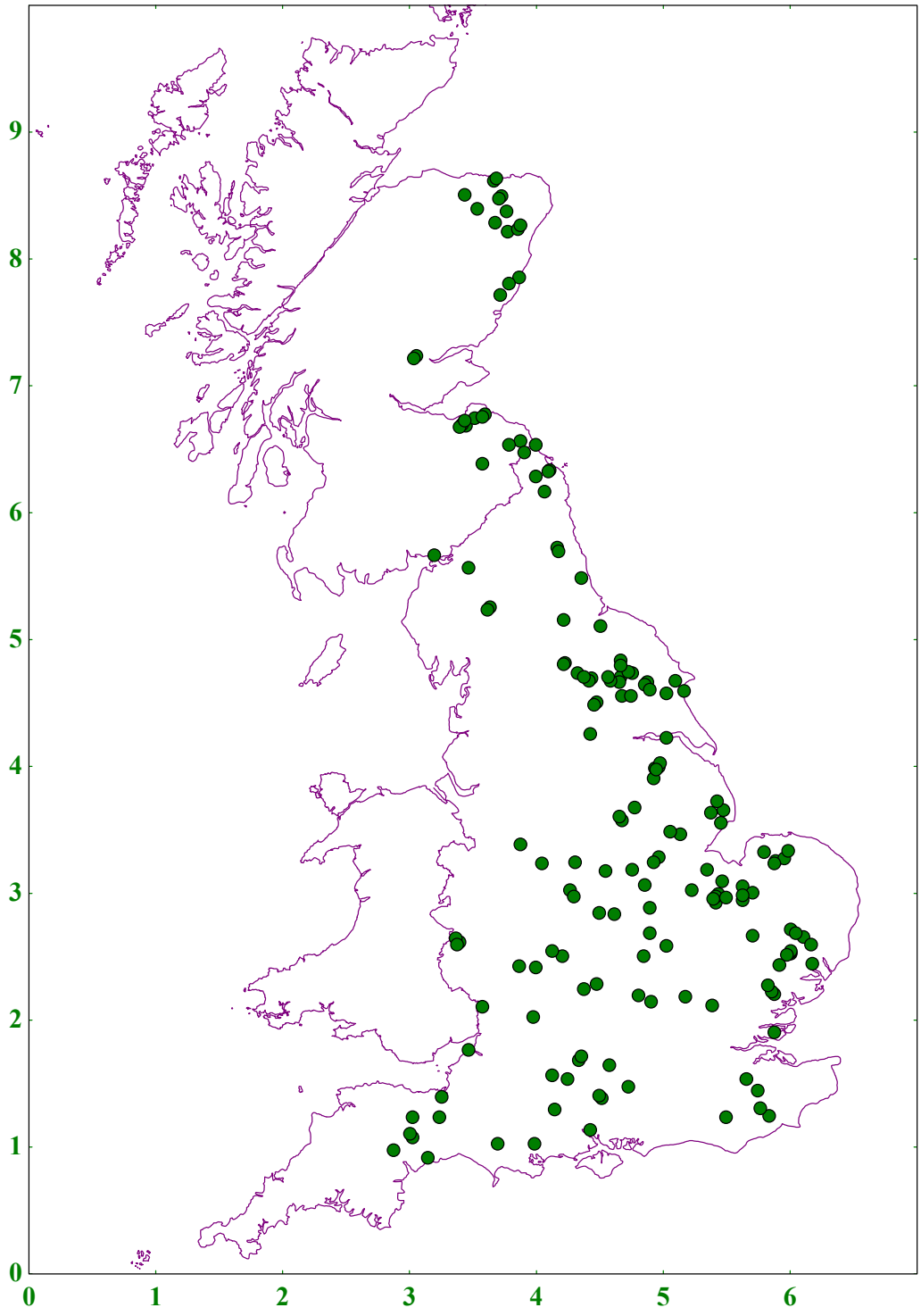

Table 1. Regional UK cereal production (wheat, barley and oats) and the number of grain samples collected per region

UK region	UK production (% of hectares) from June 1999 census data				Target number of samples for full proportionality				Actual number of samples collected			
	Wheat	Barley	Oats	Total	Wheat	Barley	Oats	Total	Wheat	Barley	Oats	Total
North Eastern	2.04	1.41	0.13	3.58	6.5	4.5	0.4	11.4	16	11	1	28
North Western	0.75	1.41	0.08	2.25	2.4	4.5	0.3	7.2	1	4	1	6
Yorks & Humber	7.50	3.91	0.13	11.55	24.0	12.5	0.4	36.9	27	16	3	46
East Midlands	11.62	3.14	0.22	14.98	37.2	10.1	0.7	47.9	34	13	1	48
West Midlands	4.82	2.39	0.41	7.61	15.4	7.6	1.3	24.4	16	1	0	17
Eastern	15.74	5.57	0.14	21.46	50.4	17.8	0.5	68.7	50	10	0	60
South Eastern	7.81	3.01	0.48	11.30	25.0	9.6	1.5	36.2	22	10	1	33
South Western	5.81	4.01	0.44	10.26	18.6	12.8	1.4	32.8	18	12	1	31
Total England	56.10	24.85	2.04	82.98	179.5	79.5	6.5	265.5	184	77	8	269
North Western	0.09	0.91	0.13	1.14	0.3	2.9	0.4	3.6	0	0	0	0
North Eastern	0.40	3.98	0.22	4.59	1.3	12.7	0.7	14.7	4	14	2	20
South Eastern	2.04	4.75	0.28	7.07	6.5	15.2	0.9	22.6	11	11	0	22
South Western	0.10	1.21	0.08	1.40	0.3	3.9	0.3	4.5	0	1	2	3
Total Scotland	2.64	10.85	0.71	14.20	8.4	34.7	2.3	45.4	15	26	4	45
Wales	0.41	0.95	0.11	1.47	1.3	3.0	0.3	4.7	0	0	0	0
Northern Ireland	0.11	1.15	0.09	1.34	0.3	3.7	0.3	4.3	2	3	1	6
Total UK	59.25	37.80	2.94	100.00	189.6	121.0	9.4	320.0	201	106	13	320

Table 2. Types of site from which samples were collected.

Type of site	Number of samples Wheat	Barley	Oats	Total
Arable farm	84	26	0	110
Arable farm and feed mill	1	2	0	3
Mixed farm	60	29	9	98
Mixed farm and feed mill	2	9	0	11
Central co-operative	20	13	0	33
Central co-operative and feed mill	1	2	1	4
Central commercial	14	9	1	24
Central commercial and feed mill	0	1	1	2
Central commercial and flour mill	0	0	1	1
Central commercial and maltster	0	4	0	4
Feed mill	10	4	0	14
Flour mill	5	0	0	5
Maltster	2	6	0	8
Port	2	1	0	3
Total samples	201	106	13	320

Table 3. Storekeeper's view of the intended use of the grain which was sampled.

Intended use of grain	Number of samples Wheat	Barley	Oats	Total
Feed	117	68	10	195
Flour	61	0	0	61
Flour and feed	4	0	1	5
Malting	4	30	0	34
Distilling	2	0	0	2
Porridge or flake	0	0	2	2
Seed	1	4	0	5
Export	0	2	0	2
Export and feed	2	1	0	3
Unknown	10	1	0	11
Total samples	201	106	13	320

Table 4. Levels of ochratoxin A found in each type of grain

Grain	Number of samples tested	Number of samples in each range (µg/kg) of ochratoxin A									Maximum value (µg/kg)
		Not detected*	0.3-0.9	1.0-2.4	2.5-4.9	5.0-9.9	10.0-19.9	20.0-49.9	50.0-99.9	100 or more	
Wheat	201	169	8	11	7	1	3	0	1	1	231.0
Barley	106	86	9	4	2	1	2	1	0	1	117.0
Oats	13	13	0	0	0	0	0	0	0	0	0
Total	320	268	17	15	9	2	5	1	1	2	
	% =	**83.8**	**5.3**	**4.7**	**2.8**	**0.6**	**1.6**	**0.3**	**0.3**	**0.6**	

*Not detected = below the measurable detection limit of 0.3µg/kg.

Table 5. Levels of deoxynivalenol (DON) found in each type of grain

Grain	Number of samples tested	Number of samples in each range (µg/kg) of DON							Maximum value (µg/kg)
		Not detected*	20-49	50-99	100-249	250-499	500-749	750 or more	
Wheat	201	3	45	101	47	4	1	0	600
Barley	106	31	48	24	1	2	0	0	370
Oats	13	13	6	3	1	0	0	0	108
Total	320	37	99	128	49	6	1	0	
	% =	**11.6**	**30.9**	**40.0**	**15.3**	**1.9**	**0.3**	**0**	

*Not detected = below the measurable detection limit of 20µg/kg.

Table 6. Levels of ochratoxin A found for each intended use of grain specified by the storekeeper.

Intended use of grain	No. of samples tested	Number of samples in each range (µg/kg) of ochratoxin A									Maximum value (µg/kg)
		Not detected	0.3-0.9	1.0-2.4	2.5-4.9	5.0-9.9	10.0-19.9	20.0-49.9	50.0-99.9	100 or more	
Feed - wheat	117	95	5	5	6	1	3	0	1	1	231
Feed - barley	68	54	6	4	0	1	1	1	0	1	117
Feed - oats	10	10	0	0	0	0	0	0	0	0	0
Flour - wheat	61	54	2	4	1	0	0	0	0	0	2.8
Malting - barley	30	26	2	0	1	0	1**	0	0	0	13.8
Malting - wheat	4	4	0	0	0	0	0	0	0	0	0
Other*	30	25	2	2	1	0	0	0	0	0	3.9
Total	320	268	17	15	9	2	5	1	1	2	
of which Organic	9	9	0	0	0	0	0	0	0	0	0

*The category "other" includes samples where multiple uses were intended and those where the intended use was not ascertained.
**This sample was judged by a professional maltster to be of unacceptable quality for malting.

Table 7. Levels of deoxynivalenol (DON) found for each intended use of grain specified by the storekeeper.

Intended use of grain	No. of samples tested	Number of samples in each range (µg/kg) of DON							Maximum value (µg/kg)
		Not detected	20-49	50-99	100-249	250-499	500-749	750 or more	
Feed - wheat	117	2	18	61	33	2	1	0	600
Feed - barley	68	20	34	13	1	0	0	0	126
Feed - oats	10	3	4	2	1	0	0	0	108
Flour - wheat	61	1	22	30	8	0	0	0	234
Malting - barley	30	7	13	9	0	1	0	0	311
Malting - wheat	4	0	1	1	2	0	0	0	110
Other	30	4	7	12	4	3	0	0	370
Total	320	37	99	128	49	6	1	0	
of which Organic	9	1	5	2	1	0	0	0	118

Table 8. Levels of ochratoxin A found in each UK Region.

Region	No. of samples tested	Number of samples in each range (μg/kg) of ochratoxin A								Maximum value (μg/kg)
		Not detected	0.3-0.9	1.0-2.4	2.5-4.9	5.0-9.9	10.0-19.9	20.0-49.9	50.0-99.9	100 or more	
England											
North Eastern	28	23	3	2	0	0	0	0	0	0	1.7
North Western	6	6	0	0	0	0	0	0	0	0	0
Yorks & Humber	46	40	1	2	2	0	0	0	0	1	117.0
East Midlands	48	44	1	1	2	0	0	0	0	0	3.7
West Midlands	17	12	1	2	1	0	0	0	0	1	231.0
Eastern	60	52	1	2	3	0	1	0	1	0	50.0
South Eastern	33	26	2	3	0	0	2	0	0	0	19.9
South Western	31	27	2	1	1	0	0	0	0	0	3.9
Scotland											
North Eastern	20	12	3	2	0	1	2	0	0	0	15.6
Southern	25	21	3	0	0	1	0	0	0	0	5.2
Northen Ireland											
East & West	6	5	0	0	0	0	0	1	0	0	24.5
Total	320	268	17	15	9	2	5	1	1	2	231.0

Table 9. Levels of ochratoxin A found in relation to the moisture contents of the grain samples.

Moisture content range (%)	Number of samples tested*	Number of samples in each range (μg/kg) of ochratoxin A								
		Not detected	0.3-0.9	1.0-2.4	2.5-4.9	5.0-9.9	10.0-19.9	20.0-49.9	50.0-99.9	100 or more
Wheat										
11.1-12.0	1	1	-	-	-	-	-	-	-	-
12.1-13.0	7	7	-	-	-	-	-	-	-	-
13.1-14.0	55	48	2	3	2	-	-	-	-	-
14.1-15.0	81	68	4	3	2	-	2	-	1	1
15.1-16.0	43	34	1	3	3	1	1	-	-	-
16.1-17.0	9	9	-	-	-	-	-	-	-	-
17.1-18.0	4	1	1	2	-	-	-	-	-	-
Barley										
11.1-12.0	2	1	1	-	-	-	-	-	-	-
12.1-13.0	9	6	2	-	1	-	-	-	-	-
13.1-14.0	18	18	-	-	-	-	-	-	-	-
14.1-15.0	36	31	4	-	1	-	-	-	-	-
15.1-16.0	27	22	1	1	-	1	1	1	-	-
16.1-17.0	7	5	1	1	-	-	-	-	-	-
17.1-18.0	5	3	-	1	-	-	1	-	-	-
18.1-19.0	1	0	-	-	-	-	-	-	-	1
Oats										
11.1-12.0	3	3	-	-	-	-	-	-	-	-
12.1-13.0	6	6	-	-	-	-	-	-	-	-
13.1-14.0	3	3	-	-	-	-	-	-	-	-
19.1-20.0	1	1	-	-	-	-	-	-	-	-

*The moisture contents of one wheat sample and one barley sample were not determined.

Appendix I. Results of analyses for ochratoxin A (OA) and deoxynivalenol (DON) for each of the 320 samples, expressed as μg/kg.

Sample	OA	DON	Sample	OA	DON	Sample	OA	DON
Wheat001	20	148	Wheat108	0	44	Barley014	0	54
Wheat002	0	358	Wheat109	0	39	Barley015	1.1	0
Wheat003	0	53	Wheat110	0.4	50	Barley016	0	0
Wheat004	0	54	Wheat111	0	107	Barley017	0	0
Wheat005	0	82	Wheat112	0	120	Barley018	0	311
Wheat006	0	39	Wheat113	0	56	Barley019	0	30
Wheat007	0	68	Wheat114	1.1	101	Barley020	0	370
Wheat008	0.3	62	Wheat115	0	191	Barley021	0	55
Wheat009	0	118	Wheat116	0	47	Barley022	0	62
Wheat010	0	112	Wheat117	0	37	Barley023	0	27
Wheat011	0	31	Wheat118	0	97	Barley024	0	0
Wheat012	0	95	Wheat119	0	65	Barley025	0	50
Wheat013	15	154	Wheat120	0	131	Barley026	0	31
Wheat014	1.1	82	Wheat121	0	75	Barley027	0	36
Wheat015	0	41	Wheat122	0	50	Barley028	0	27
Wheat016	0	63	Wheat123	50	225	Barley029	0	0
Wheat017	1.6	84	Wheat124	0	115	Barley030	0	44
Wheat018	0	103	Wheat125	0	70	Barley031	0	0
Wheat019	0	49	Wheat126	0	60	Barley032	0	126
Wheat020	0	78	Wheat127	0	103	Barley033	0	61
Wheat021	0.8	55	Wheat128	0	76	Barley034	0	0
Wheat022	0	40	Wheat129	0	46	Barley035	0	42
Wheat023	2.8	51	Wheat130	0	48	Barley036	3.9	87
Wheat024	0	59	Wheat131	0	48	Barley037	0	54
Wheat025	0	48	Wheat132	0	41	Barley038	0	31
Wheat026	0	57	Wheat133	0	59	Barley039	0	32
Wheat027	0	144	Wheat134	0	32	Barley040	0	51
Wheat028	0	36	Wheat135	0	66	Barley041	0	30
Wheat029	0	39	Wheat136	0	31	Barley042	0	38
Wheat030	0	170	Wheat137	0	98	Barley043	0	37
Wheat031	3.3	55	Wheat138	0	76	Barley044	0	39
Wheat032	1.5	41	Wheat139	0.7	94	Barley045	0	41
Wheat033	0	0	Wheat140	0	63	Barley046	0	59
Wheat034	0	46	Wheat141	0	132	Barley047	0	49
Wheat035	0	108	Wheat142	0	64	Barley048	0	54
Wheat036	0	73	Wheat143	0	59	Barley049	0	37
Wheat037	0	49	Wheat144	0	118	Barley050	0	37
Wheat038	0	98	Wheat145	0	84	Barley051	0	0
Wheat039	0	256	Wheat146	0	244	Barley052	0	0
Wheat040	0	63	Wheat147	0	87	Barley053	0	49
Wheat041	0	69	Wheat148	0	65	Barley054	0	0
Wheat042	0	50	Wheat149	0	88	Barley055	0	74
Wheat043	0	0	Wheat150	0	56	Barley056	0	44
Wheat044	0	27	Wheat151	0	60	Barley057	0	28
Wheat045	0	56	Wheat152	0	121	Barley058	0	33
Wheat046	0	53	Wheat153	0	39	Barley059	0	20
Wheat047	0	64	Wheat154	0	43	Barley060	0	0
Wheat048	0	60	Wheat155	0	158	Barley061	0	31
Wheat049	2.8	76	Wheat156	4.6	35	Barley062	0	28
Wheat050	0	59	Wheat157	0	110	Barley063	0.5	0
Wheat051	0	47	Wheat158	0	351	Barley064	0.3	24
Wheat052	0	53	Wheat159	0	131	Barley065	1.7	0
Wheat053	0	62	Wheat160	0	243	Barley066	1.3	39
Wheat054	0	37	Wheat161	11.0	115	Barley067	0	34

Wheat055	0	46	Wheat162	0	51	Barley068	0	54
Wheat056	0	46	Wheat163	231	49	Barley069	0.4	54
Wheat057	0	0	Wheat164	0	51	Barley070	0	38
Wheat058	0	86	Wheat165	1.5	60	Barley071	0	67
Wheat059	0.5	600	Wheat166	0	52	Barley072	0	74
Wheat060	0	65	Wheat167	0	61	Barley073	0	46
Wheat061	0	48	Wheat168	0.6	59	Barley074	0	54
Wheat062	0	76	Wheat169	0	59	Barley075	3.1	41
Wheat063	0	49	Wheat170	0	48	Barley076	0	49
Wheat064	0	62	Wheat171	2.0	30	Barley077	0	0
Wheat065	0	112	Wheat172	0	108	Barley078	0	42
Wheat066	1.0	49	Wheat173	3.6	96	Barley079	8.5	47
Wheat067	0.9	87	Wheat174	0	66	Barley080	0.3	0
Wheat068	0	82	Wheat175	0	98	Barley081	13.8	0
Wheat069	0	151	Wheat176	1.4	58	Barley082	0	0
Wheat070	0	189	Wheat177	0	81	Barley083	1.4	27
Wheat071	0	289	Wheat178	0	68	Barley084	0.3	32
Wheat072	0	51	Wheat179	0	110	Barley085	0	0
Wheat073	0	57	Wheat180	1.2	114	Barley086	0	32
Wheat074	0	94	Wheat181	1.9	97	Barley087	0	0
Wheat075	3.7	110	Wheat182	0	114	Barley088	0	29
Wheat076	0.3	89	Wheat183	3.2	129	Barley089	0	0
Wheat077	0	106	Wheat184	0	24	Barley090	15.6	29
Wheat078	0	83	Wheat185	0	69	Barley091	0	0
Wheat079	0	107	Wheat186	0	144	Barley092	0	33
Wheat080	0	187	Wheat187	0	112	Barley093	0.4	51
Wheat081	0	108	Wheat188	0	78	Barley094	0	69
Wheat082	0	114	Wheat189	0	157	Barley095	0.4	32
Wheat083	0	34	Wheat190	0	67	Barley096	0.7	0
Wheat084	0	194	Wheat191	0	116	Barley097	0	39
Wheat085	0	150	Wheat192	5.2	72	Barley098	0	51
Wheat086	0	56	Wheat193	0	61	Barley099	0	50
Wheat087	0	74	Wheat194	0	77	Barley100	0	78
Wheat088	0	49	Wheat195	0	82	Barley101	0	47
Wheat089	0	65	Wheat196	0	93	Barley102	0	43
Wheat090	0	91	Wheat197	0	72	Barley103	0	82
Wheat091	0	234	Wheat198	0	70	Barley104	24.5	49
Wheat092	0	54	Wheat199	0	85	Barley105	0	49
Wheat093	0	30	Wheat200	0	84	Barley106	0	54
Wheat094	0	42	Wheat201	0	147	Oats001	0	35
Wheat095	0	79	Barley001	0	0	Oats002	0	35
Wheat096	0	59	Barley002	0.5	0	Oats003	0	0
Wheat097	0	76	Barley003	0	0	Oats004	0	0
Wheat098	0	38	Barley004	0	35	Oats005	0	82
Wheat099	0	42	Barley005	0	0	Oats006	0	37
Wheat100	0	58	Barley006	0	51	Oats007	0	108
Wheat101	0	29	Barley007	0	38	Oats008	0	31
Wheat102	0	36	Barley008	0	0	Oats009	0	0
Wheat103	0	53	Barley009	117	0	Oats010	0	28
Wheat104	0	49	Barley010	0	0	Oats011	0	56
Wheat105	0	50	Barley011	0	0	Oats012	0	41
Wheat106	1.0	66	Barley012	0	0	Oats013	0	61
Wheat107	0	60	Barley013	0	37			

Note: A value of zero indicates that if the mycotoxin was present, it was below the measurable level of detection. For ochratoxin A this was 0.3µg/kg and for deoxynivalenol (DON) it was 20µg/kg. Levels of ochratoxin A above 10.0µg/kg have not been corrected for the percentage recovery and should be read as "greater than" the stated value.

Appendix. II. Results of GC-MS Confirmation of Trichothecene Levels

1. During the initial analysis of survey samples by HPLC a number of samples were found to contain deoxynivalenol (DON) at a level of 150 µg/kg or greater. It was decided it would be prudent to confirm these results by repeating the analysis using an alternative analytical technique. GC-MS was used for confirmation as it allows the simultaneous detection of not only deoxynivalenol, but also a range of other trichothecenes that may co-occur with dexoynivalenol, and which may be important from both toxicological and regulatory or control perspectives. The semi-quantitative method used allows the simultaneous detection of deoxynivalenol, nivalenol, 3-acetyl deoxynivalenol, 15-O-acetyl 4-deoxynivalenol, fusarenon X, diacetoxyscirpenol, T2 toxin and HT2 toxin.

Twenty one samples reported as containing 150 µg/kg or greater DON were re-analysed using GC-MS. The sample extracts that had been prepared for HPLC had been stored at +4°C. These were cleaned-up then analysed by GC-MS.

2 . Sample clean-up and analysis
Sample extract was passed through a Romer Mycosep column until 10 ml cleaned up filtrate had been collected. The filtrate was evaporated under nitrogen at 50°C. Derivatising reagent (50 µl Tri Sil TBT) was added and the samples were incubated at 80°C for 30 minutes. After cooling hexane (500µl) and phosphate buffer (1ml) were added to the derivatised sample extracts. The samples were vigorously mixed using a vortex mixer, and an aliquot of the hexane layer was transferred to an autosampler vial.

The samples were analysed using the following conditions :
Column : 19CB 50 m x 0.25 mm x 0.2 µm
Carrier gas : Helium at 1.2 ml/minute
Temp. programme : Initial temp. 50 °C for 1 minute, increase at 45°C/minute to 200°C, hold for 5 minutes, increase at 3°C / minute to 280°C, and hold for 10 minutes.
The instrument was used in selected ion monitoring mode, at least three ions were monitored for each analyte. The toxins analysed were :
deoxynivalenol, nivalenol, 3-acetyl deoxynivalenol, 15-acetyl deoxynivalenol, fusarenon X, diacetoxyscirpenol, HT2 toxin and T2 toxin.

3. Results

The results of the GC-MS analysis are shown in Table 10. The results of the GC-MS analysis should be regarded as semi-quantitative. These results have been corrected for analytical recovery values determined for each analytical batch.
21 samples were analysed. Of these 21, 20 were confirmed as containing deoxynivalenol levels in agreement with the initial HPLC results. The result for sample BO020 however was much lower than the initial HPLC result. To check this finding the sample was re-extracted and analysed in duplicate by GC-MS. The repeat analyses supported the first GC-MS analysis, that the sample contains deoxynivalenol, but at a lower level than initially reported (approximately 50µg/kg). To further investigate this the sample was extracted and analysed in duplicate by HPLC, and a level of 26 µg/kg was found. All other samples are confirmed as containing deoxynivalenol in the range previously reported.

Eighteen of the samples were found to contain nivalenol above the reporting limit of 50 µg/kg. For sixteen of these samples the nivalenol level was lower than the DON level, but for two samples the nivalenol level was higher than the DON level. This is an indication that different strains of mould were responsible for the contamination. Neither of the acetyl derivatives of DON i.e. 3-acetyl deoxynivalenol, & 15-O-acetyl 4-deoxynivalenol were detected above the reporting limit of 50 µg/kg, but this is not surprising as these tend only to occur at levels of 10-20 % of the DON level, and the maximum DON level found in this study was 600 µg/kg (by HPLC, approximately 700 µg/kg by GC-MS - sample W059).
None of the other trichothecenes (diacetoxyscirpenol, HT2 toxin and T2 toxin) were found above the reporting limit of 50 µg/kg. Again this is not surprising as their occurrence is thought to be limited

4. Discussion

The Scientific Committee on Food published their opinion on deoxynivalenol on 2 December 1999 (1). In it they concluded that there were no indications for carcinogenic and or mutagenic properties for DON, but based on No Observed Adverse Effects Levels (NOAEL) and applying an uncertainty factor they derived a temporary Tolerable Daily Intake (tTDI) of 1 µg/kg body weight. The TDI is temporary because it was noted that DON belongs to a group of trichothecenes with a common basic chemical structure, which share basic mechanisms of toxic action, and when these have been evaluated the Committee will consider the combined total exposure to trichothecenes and whether a group TDI should be assigned. Problems with the occurrence of DON in cereals and cereal products in The Netherlands have led to discussions within the European Union on the need for legislation for DON. The Netherlands have suggested action levels of 750 µg/kg for flour as delivered to bakeries and

500 µg/kg for retail products (2). The UK have suggested that any proposed limit or new studies undertaken should also include other mycotoxins associated with DON (3). This is also in agreement with the SCF statement that further information is required on the occurrence of all trichothecenes (1). At the most recent meeting (31 May 2000) of the Commission Working Group on Agricultural Contaminants action levels (not regulatory limits) were proposed by the Commission (4). These were 500 µg/kg for cereal products as consumed and other cereal products at retail, 750 µg/kg for flour used as raw material in food products and 750 µg/kg as a monitoring level for raw cereals. There was discussion on how these action levels could be interpreted, and the Commission asked Member States to consider the practicalities of advising on and using the limits. Therefore it is still very unclear how any possible action levels or limits might be enforced or monitored. The UK have also commissioned a survey of retail products to check DON and trichothecene levels which will be reported next year.

The HPLC results from this study, which should be considered to be the quantitative values show that none of the 320 UK cereals sampled in this study exceeded the proposed action level of 750 µg/kg DON. However if the action level is for total trichothecenes then the GC-MS results, although semi-quantitative in this instance, indicate that at least one sample (W059) is at or above the suggested level. Whilst this is re-assuring for the UK cereal industry it must be made clear that this survey only represents a snapshot in time and that different results may be found for different harvests grown under different climatic conditions.

The status of proposed action levels is still unclear, but it would seem most likely that levels would include all trichothecenes as they are similar in chemical structure and are as or more toxic than DON (5), and the SCF opinion will be taken into consideration when levels are set. If this happens it will have the effect of effectively making the level for DON lower, as the other toxins (most importantly nivalenol) will also have to be considered.

References

1. European Commission Scientific Committee on Food (1999) Opinion on Fusarium Toxins, Part 1 : Deoxynivalenol (DON) expressed on 2 December 1999. SCF/CS/CNTM/MYC/19 Final.

2. MAFF Joint Food Safety and Standards Group. Letter Dated 29 February 2000 - Mycotoxins - EC Permitted Levels.

3. MAFF Joint Food Safety and Standards Group. Letter Dated 13 December 1999 - Mycotoxins - EC Permitted Levels.

4. Food Standards Agency. Letter Dated 5 June 2000 - Mycotoxins EC Permitted Levels.

5. IARC (1993) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 56 Some Naturally Occurring Substances. pp397-485.

Table 10 : Results of GC-MS analyses
All results corrected for recovery. The values are semi-quantitative.

	Corrected (µg/kg)							
Sample	**DON**	**FUS X**	**NIV**	**15AcDON**	**3AcDON**	**DAS**	**HT2**	**T2**
B018	210	<50	267	<50	<50	<50	<50	<50
W030	185	<50	127	<50	<50	<50	<50	<50
W039	174	<50	104	<50	<50	<50	<50	<50
W069	172	<50	83	<50	<50	<50	<50	<50
W059	767	<50	130	<50	<50	<50	<50	<50
W155	132	<50	<50	<50	<50	<50	<50	<50
W158	282	<50	<50	<50	<50	<50	<50	<50
W189	133	<50	95	<50	<50	<50	<50	<50
B020	52	<50	<50	<50	<50	<50	<50	<50
W002	281	<50	104	<50	<50	<50	<50	<50
W013	130	<50	87	<50	<50	<50	<50	<50
W070	110	<50	150	<50	<50	<50	<50	<50
W071	223	<50	93	<50	<50	<50	<50	<50
W080	115	<50	103	<50	<50	<50	<50	<50
W084	152	<50	89	<50	<50	<50	<50	<50
W085	170	<50	93	<50	<50	<50	<50	<50
W091	187	<50	77	<50	<50	<50	<50	<50
W115	149	<50	96	<50	<50	<50	<50	<50
W123	160	<50	87	<50	<50	<50	<50	<50
W146	182	<50	96	<50	<50	<50	<50	<50
W160	188	<50	104	<50	<50	<50	<50	<50
B020-1	53	<50	<50	<50	<50	<50	<50	<50
B020-2	50	<50	<50	<50	<50	<50	<50	<50
Mean Recovery (%) (n=4)	86	64	71	76	70	75	107	93