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#### **Food Additives and Contaminants**



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### Fusarium mycotoxin content of UK organic and conventional barley

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#### Abstract

Each year (2002-2005), approximately 100 samples of barley from fields of known agronomy were analysed for 10 trichothecenes by GC/MS including deoxynivalenol (DON), nivalenol, 3-acetylDON, 15-acetylDON, fusarenone X, T-2 toxin (T2), HT-2 toxin (HT2), diacetoxyscirpenol, neosolaniol and T-2 triol. Samples were also analysed for moniliformin and zearalenone by HPLC. Of the 10 trichothecenes analysed from 446 harvest samples of barley only two, diacetoxyscirpenol and neosolaniol were not detected. The concentration of type A trichothecenes were similar to those that occurred in wheat over the same period whilst those of type B trichothecenes were markedly lower. Deoxynivalenol was the most frequently detected *Fusarium* mycotoxin, present above the limit of quantification (10  $\mu$ g kg<sup>-1</sup>) in 57% of samples, and was usually present at the highest concentration. A single sample (0.2%) exceeded the legal limit for DON in unprocessed barley over the fouryear period. Moniliformin and zearalenone were both rarely detected (2% of samples greater than 10  $\mu$ g kg<sup>-1</sup> for both toxins) with maximum concentrations of 45 and 44  $\mu$ g  $kg^{-1}$  respectively. Year and region had a significant effect on DON and HT2+T2 but there was no significant difference in the concentration of these mycotoxins between organic and conventional samples. Overall, the risk of UK barley exceeding the newly introduced legal limits for Fusarium mycotoxins in cereals intended for human consumption is very low, but the percentage of samples above these limits will fluctuate between years.

**Keywords:** trichothecenes, deoxynivalenol, nivalenol, HT-2 toxin, T-2 toxin, zearalenone, moniliformin, organic, conventional

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#### Introduction

All cereal crops are prone to *Fusarium* head blight and resulting contamination of harvested grains by *Fusarium* mycotoxins. Of the cereal species durum wheat, followed by soft wheat are usually the most susceptible. Barley, rye and oats are considered less susceptible (Langevin et al. 2004) although there is considerable variation between geographic regions. In eastern Canada, the DON concentration of barley was lower than in wheat (Campbell et al. 2002), whereas in Minnesota during the epidemics of 1993 and 1994 the average DON content of Barley was higher than that for wheat (Jones and Mirocha 1999). A study in Norway found higher DON concentrations on oats, then barley and least on wheat samples (Langseth and Elen 1996). There are limited data on occurrence of *Fusarium* mycotoxins in UK cereals prior to 2001. A previous survey conducted in 1999 found highest amounts of DON on wheat, with lower levels on barley and oats (MacDonald et al. 2004).

There are less data as to the relative concentration of other *Fusarium* mycotoxins in wheat, barley and oats. For HT2 and T2, highest levels were detected on oats, then barley and lowest in wheat samples in Norway (Langseth and Rundberget 1999). Moniliformin has been detected in cereal samples from Nordic countries. In Norway, highest levels were observed on wheat, with similar, lower amounts on barley and oats (Uhlig *et al.* 2004).

The European Commission (EC) has set legislative limits for the *Fusarium* mycotoxins including the trichothecene, DON and ZON in cereal grains and cerealbased products intended for human consumption (Anon. 2006a). A combined limit for the trichothecenes HT2 and T2 will be introduced in the near future. The European Commission also set guideline limits in 2006 for *Fusarium* mycotoxins in animal feed (Anon. 2006b). The presence of relationships between mycotoxin concentrations are important as they allow assumptions for one mycotoxin based on the concentration of another. For example, legislative limits have not been set for NIV as it is considered by the EC to be a co-contaminant of DON and as such can be controlled by controlling DON (Anon. 2006a). Relationships between mycotoxins also provide evidence of the relationships that may exist between the *Fusarium* species that produce these mycotoxins.

Several studies have compared the mycotoxin content of wheat and wheat products in organic and conventional samples. These studies have either identified no differences (Champeil et al. 2004, Jestoi et al. 2004) or found slightly less DON in organic samples (Schollenberger et al. 2002, Schollenberger et al. 2005). No studies have been conducted on barley, although one study in Belgium compared levels of DON in organic and conventional beers and found no significant differences (Anselme 2006).

The aims of the present study were: i) to identify the distribution of *Fusarium* mycotoxins in UK barley, ii) to identify relationships between the concentrations of *Fusarium* mycotoxins detected, iii) to identify how *Fusarium* mycotoxin distributions vary across seasons and regions and iv) to identify if there were differences in the *Fusarium* mycotoxin concentrations of organic and conventional barley in the UK.

#### Material and methods

Sample collection

Each year, from 2002-2005, about one hundred barley grain samples and related agronomic data were collected by agronomists and growers. Samples were collected at harvest from fields of known agronomy as detailed previously (Edwards In press a). Near equivalent numbers of samples were collected from six geographic regions of the UK (Table I). Of a total of 446 samples collected, approximately 25% of samples were collected from organic growers (Table II).

On receipt of samples they were processed as described previously (Edwards In press a).

#### (INSERT Table 1 and 2 here)

#### Mycotoxin analysis

All mycotoxin analysis was conducted by RHM Technology (High Wycombe). Ten trichothcenes (deoxynivalenol (DON), nivalenol (NIV), 3-acetylDON, 15-acetylDON, fusarenone X, T-2 toxin (T2), HT-2 toxin (HT2), diacetoxyscirpenol (DAS), neosolaniol and T-2 triol) were quantified by GC/MS and zearalenone and moniliformin were quantified by HPLC using previously published methods (Sharman et al. 1991, Patel 1996, Scudamore et al. 2007). The limit of quantification (LoQ) was determined as six times the baseline noise and calculated to be 10  $\mu$ g kg<sup>-1</sup> for trichothecenes and moniliformin and 3  $\mu$ g kg<sup>-1</sup> for zearalenone.

All methods used were UKAS accredited with acceptable recovery ranges of 70-110%. For this study the calculation of the measurement uncertainty was carried out using in-house data, performance in international collaborative trials and the Food Analysis Performance Assessment Scheme (www.fapas.co.uk/fapas.cfm) thus incorporating repeatability and reproducibility data. Only in house data was used for moniliformin as there have been no FAPAS or external collaborative trials for this mycotoxin. The expanded measurement of uncertainty was calculated using a standard coverage factor of two, equivalent to a confidence of approximately 95% that the actual level of the mycotoxin being measured lies within the quoted range. The expanded measurement of uncertainty was calculated to be  $\pm 25$ , 18 and 21% for trichothecenes, zearalenone and moniliformin respectively.

#### Statistical analysis

Summary statistics for each mycotoxin were calculated using Excel (Microsoft Office 2003). Statistics calculated included the percentage greater than 10  $\mu$ g kg<sup>-1</sup> (LoQ for trichothecenes), the mean, median, 90th percentile, 95th percentile and the maximum concentration for each mycotoxin detected. For the calculation of the mean, samples below the LoQ were assigned a value of (LoQ)/6, ie 1.667 for trichothecenes. This allows data to be directly compared to recently compiled *Fusarium* mycotoxin occurrence data from EU member states (Anon. 2003)

For regression analysis samples below the LoQ were removed from the dataset and concentrations  $\log_{10}$  transformed to normalise the variance. For ANOVA samples below the LoQ were assigned a value of (LoQ)/2, ie 5 µg kg<sup>-1</sup> for trichothecenes;  $\log_{10}$  transformed to stabilise the variance and analysed using Genstat (version 10, Lawes Agricultural Trust). For mycotoxins with a low number of detectable samples, the mycotoxin distribution was analysed by analysis of deviance of incidence data and

ANOVA of positive samples. For analysis of deviance samples below the LoQ were allocated a value of zero, those above the LoQ were allocated a value of one and analysed using a Bernoulli distribution with Genstat.

#### **Results and discussion**

#### Mycotoxin concentration

In the first two years moniliformin was only detected above 10  $\mu$ g kg<sup>-1</sup> in 2% of samples (n=239); the maximum was 45  $\mu$ g kg<sup>-1</sup>. Consequently analysis of moniliformin was not performed in the final two years. In the same period, zearalenone was rarely detected and when detected was only found at low concentrations, consequently analysis for zearalenone was reduced to 50 samples per year. Over four years, 339 samples were analysed for zearalenone. Ten percent of samples were above the LoQ of 3  $\mu$ g kg<sup>-1</sup>, 2% of samples exceeded 10  $\mu$ g kg<sup>-1</sup> and the maximum detected was 44  $\mu$ g kg<sup>-1</sup>. The legal limit for zearalenone in unprocessed barley intended for human consumption is 100  $\mu$ g kg<sup>-1</sup> (Anon. 2006a).

Of the ten trichothecenes analysed, eight were detected, of these DON, NIV, and HT2 were detected above 100  $\mu$ g kg<sup>-1</sup>. Table III shows the summary statistics for each trichothecene detected over the four year period. Combined values are provided for HT2 and T2 (HT2+T2) as these closely related mycotoxins have equivalent toxicity and it is likely European legal limits will be based on a combined concentration.

DON was detected in 57% of samples and one sample (0.2% of total)exceeded the legal limit for DON in unprocessed barley intended for human consumption (1250 µg kg<sup>-1</sup>) (Anon. 2006a). This sample, harvested in 2005, had a DON concentration of 1416  $\mu$ g kg<sup>-1</sup>. This was the only sample with detectable 3- and 15-acetyl DON as co-contaminants of DON. HT-2 and T-2 were detected in 36 and 12% of samples respectively; the concentrations were not high compared with UK oats (Edwards In press b). One point one percent of samples exceeded 100  $\mu$ g kg<sup>-1</sup> and the combined maximum concentration was 138  $\mu$ g kg<sup>-1</sup> HT2+T2. Nivalenol, although relatively common (detected in 25% of samples) was not found at high concentrations (maximum 157  $\mu$ g kg<sup>-1</sup>). Fusarenone X, an acetylated version of nivalenol, was detected at low concentrations in four samples in 2004. This trichothecene has not been reported in UK cereals previously, including the two recent studies of wheat and oats (Edwards In press a, In press b). T2 triol was detected just above the limit of quantification in a single sample as a co-contaminant of HT2 and T2, in the same year. Diacetoxyscirpenol and neosolaniol were not detected in any sample (LoO =  $10 \text{ µg kg}^{-1}$ ).

#### (Insert Table 3 here)

#### Regression analysis

The relationship between HT2 and T2 was analysed by regression analysis of logarithmic transformed values (Figure 1). The regression was highly significant (p < 0.001) and accounted for 37% of the variance. There were no other positive relationships between the concentrations of other commonly detected *Fusarium* 

mycotoxins detected in UK barley. Both NIV and DON showed signs of mutual exclusion with HT2 and to one another (Figures 2-4).

#### (Insert Figure 1-4 here)

#### Year, region and practice

The impact of practice on the DON concentration was analysed using ANOVA. Year and region were entered into the model first to account for temporal and spatial variation. There was a highly significant interaction (p=0.003) between year and region with higher concentrations been detected in the South, declining northwards (Figure 5). There was no significant difference in DON concentration between organic and conventional samples (p=0.263).

The low incidence of samples with HT2+T2 above the limit of quantification resulted in the residuals were not normally distributed, consequently the concentration of these mycotoxins could not be analysed by ANOVA. Incidence of HT2+T2 was analysed by analysis of deviance using a Bernoulli distribution. Concentration of HT2+T2 was then analysed for the subset of samples with concentrations above the limit of quantification using ANOVA. As for DON analysis, year and region were entered into the models first to account for temporal and spatial variation. For HT2+T2 incidence there were highly significant differences between years (p<0.001) and regions (p<0.001) but no significant interaction (p=0.316) (Figure 6A). There was no significant difference (p=0.362) in the HT2+T2 incidence of organic and conventional samples. ANOVA of HT2+T2 concentration in positive samples (n=159) indicated a significant interaction between year and region (p=0.004) (Figure 6B) but no significant difference between organic and conventional samples (p=0.839).

There were too few samples with detectable zearalenone to allow any statistical analysis.

#### General discussion

This research has clearly identified the extent to which UK organic and conventional barley contains *Fusarium* mycotoxins at harvest. Amounts of *Fusarium* mycotoxins in UK barley samples from 2002-2005 were generally low. In comparison to wheat, barley levels of all mycotoxins were much lower except for HT2 and T2, which were present at similar concentrations. Only a single barley sample (0.2% of total) exceeded the newly introduced limit of 1250  $\mu$ g kg<sup>-1</sup> DON in unprocessed cereals intended of human consumption.

Regression analysis showed a weak relationship between HT2 and T2. This is similar to the correlation reported for these related type A trichothecenes in wheat, which had a similar incidence and concentration range (Edwards In press a). Both wheat and barley had much weaker correlations between HT2 and T2 compared with oats which had a high incidence and wide concentration range of these mycotoxins (Edwards In press b). Scatter plots of HT2+T2 against DON and NIV and of DON against NIV were similar to those reported for wheat and oats. There was a consistent pattern of mutual exclusion at high concentrations. This would indicate that the species responsible for the production of DON, NIV and HT2+T2 either compete actively against one another, or prefer different environmental conditions.

Modelling of DON concentration of barley samples against year, region and practice identified a highly significant interaction between year and region. There was the same trend from North to South, as seen for DON in wheat (Edwards In press a), with a lower concentration in the North of Britain. This distribution matches the distribution of *F. graminearum* on wheat in the UK (Anon. 2009).

There was no significant difference in the mycotoxin content of organic and conventional barley samples. Recent studies have identified significantly lower HT2 and T2 in UK wheat and oats (Edwards In press a, In press b). Why differences were not detected in barley, compared with wheat, which had similar incidence and concentrations may be due to weaker statistical strength of the test, as fewer barley samples (n=446) were analysed compared with wheat (n=1624); or differences in the agronomy of conventional and organic barley compared with wheat resulting in lower HT2+T2 in organic wheat but not organic barley.

The relative levels of *Fusarium* mycotoxins in UK wheat, barley and oats are different to those observed in other countries (Langseth and Elen 1996, Campbell et al. 2002). Differences between countries in the relative level of *Fusarium* mycotoxins between cereals hosts maybe due to genetic, meteorological or agronomical differences. Comparison of *Fusarium* head blight infection of different cereal hosts under controlled conditions has identified genetic differences (Langevin et al. 2004) and comparison of replicated field trials to survey data has shown differences also occur due to climatic and/or agronomic factors (Langseth and Elen 1996). High concentrations of HT2 and T2 were recently reported in French barley (Barrier-Guillot 2008). Why these should occur in France and not other countries is not known and should be determined to help maintain low HT2 and T2 in barley worldwide.

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Table I.	Sample distribution	by year	and region.
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Voor	Region					Total	
i ear	South	East	Midlands	North	Scotland	N.Ireland	Total
2002	27	14	20	17	14	19	111
2003	21	20	17	25	21	24	128
2004	17	17	24	23	14	15	110
2005	12	15	23	15	19	13	97
Total	77	66	84	80	68	71	446

Table II. Sample distribution by year and practice.

Voor		Practice			
I Cal	Organic	Conventional	- 10tal		
2002	36	75	111		
2003	35	93	128		
2004	23	87	110		
2005	14	83	97		
Total	108	338	446		

Table III. Concentrations of all trichothecene mycotoxins detected in UK barley in2002-2005 (446 samples).

		Mycotoxin concentration (µg kg <sup>-1</sup> )				
	%>10					
	µg kg⁻¹	Mean <sup>a</sup>	Median	90th%	95th%	Max
DON	57	19	11	35	50	1416
15AcDON	0.5	<10	<10	<10	<10	35
3AcDON	0.2	<10	<10	<10	<10	15
NIV	25	<10	<10	24	45	157
FUS-X	0.7	<10	<10	<10	<10	55
T2 triol	0.2	<10	<10	<10	<10	11
T2	12	<10	<10	11	17	39
HT2	36	10	<10	28	45	105
HT2+T2	36	<20	<20	37	64	138

<sup>a</sup>Based on a value of 1.667 for all samples below the limit of quantification (10  $\mu$ g kg<sup>-1</sup>).



Figure 1. Relationship between T2 and HT2 concentration for UK barley 2002-2005 (n=446). Regression fitted for all samples above LoQ.



Figure 2. Relationship between nivalenol and deoxynivalenol concentration for UK barley 2002-2005 (n=445; one outlier excluded with DON and NIV concentration 1416 and 47  $\mu$ g kg<sup>-1</sup> respectively).



Figure 3. Relationship between HT2+T2 and deoxynivalenol concentration for UK barley 2002-2005 (n=445; one outlier excluded with DON and HT2+T2 concentration 1416 and 56  $\mu$ g kg<sup>-1</sup> respectively).



Figure 4. Relationship between nivalenol and HT2+T2 concentration for UK barley 2002-2005 (n=446).



Figure 5. Predicted mean DON concentration for UK wheat from each region in each year. Bars represent 95% confidence limits for predictions.



Figure 6. Predicted HT2+T2 incidence (A) and mean concentration of positive samples (B) for UK barley from each region in each year. Bars represent 95% confidence limits for predictions.