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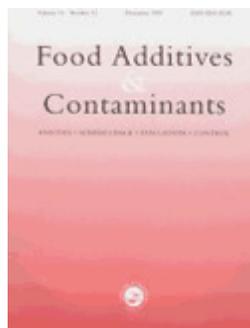
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Fusarium mycotoxin content of UK organic and conventional oats

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Abstract

Each year (2002-2005), approximately 100 samples of oats 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 ten trichothecenes analysed from 458 harvest samples of oat only three, 15-acetylDON, fusarenone X and diacetoxyscirpenol were not detected. Moniliformin and zearalenone were absent or rarely detected, respectively. HT2 and T2 were the most frequently detected fusarium mycotoxins, present above the limit of quantification ($10 \mu\text{g kg}^{-1}$) in 92 and 84% of samples respectively, and were usually present at the highest concentrations. The combined mean and median for HT2 and T2 (HT2 + T2) was 570 and $213 \mu\text{g kg}^{-1}$ respectively. There were good correlations between concentrations of HT2 and all other type A trichothecenes detected (T2, T2 triol and neosolaniol). Year and region had a significant effect on HT2 + T2 concentration. There was also a highly significant difference between HT2 + T2 content in organic and conventional samples with the predicted mean for organic samples five times lower than that of conventional samples. This is the largest difference reported for any mycotoxin content of organic and conventional cereals. No samples exceeded the legal limits for DON and zearalenone for oats intended for human consumption. Legislative limits for HT2 and T2 are currently under consideration by the European Commission. The levels detected in oats could have a serious consequence for the UK oat processing industry dependent on the limits set for unprocessed oats intended for human consumption.

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

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Fusarium head blight (FHB) of cereals may be caused by several fungal pathogens. Of these fungi, many produce a range of mycotoxins. The vast majority of research conducted on FHB is concerned with wheat as this is the most economically important small grain cereal world-wide and is the most susceptible to FHB and mycotoxin contamination in many countries. Few studies have compared the FHB severity or mycotoxin contamination of wheat, barley and oats either from replicated field experiments or observational studies. In eastern Canada, observational data showed the highest DON content was found on wheat, then barley and lowest amounts on oats from 1991 to 1998 (Campbell *et al.* 2002). The percentage of samples exceeding 1000 $\mu\text{g kg}^{-1}$ DON was 31, 22 and 1.4% for wheat, barley and oats respectively. This data were matched in a study of ear blight susceptibility of cereal species in inoculated glasshouse experiments (Langevin *et al.* 2004). In the epidemic years of 1993 and 1994 in Minnesota commercial cereal samples were analysed for DON. Average DON concentrations in wheat, barley and oat samples were 8.3, 10.4, and 1.4 mg kg^{-1} respectively (Jones and Mirocha 1999). In Norway, a large scale study over 6 years identified that highest DON concentrations occurred in oat samples, then wheat, and barley had the lowest DON average concentrations (Langseth and Elen 1996). The observed variation in contamination levels between cereals was not observed in experimental field trials indicating that the observed differences were not solely due to inherent differences in resistance but also due to differences in agronomy. There are less data as to the relative concentration of other fusarium mycotoxins in wheat, barley and oats. The type A trichothecenes, HT2 and T2 are potent inhibitors of protein synthesis and were implicated in alimentary toxic aleukia (ATA), which was responsible for many deaths in Russia in the 1940s due to the consumption of overwintered grain (Desjardins

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3 2006). The distribution of these mycotoxins is largely limited to Europe, although
4 this may, in part, be due to the lack of analysis in other regions of the world. Until
5 recently, the concentrations of HT2 and T2 detected in cereal samples were relatively
6 low with higher levels detected on oats, then barley and lowest in wheat samples in
7 Norway (Langseth and Rundberget 1999). Recently, higher concentrations of HT2
8 and T2 have been detected in oats in Nordic countries and the UK (Pettersson 2007,
9 Scudamore et al. 2007). Moniliformin has been detected in cereal samples from
10 Nordic countries. In Norway, highest levels were observed on wheat, with similar,
11 lower amounts on barley and oats (Uhlig *et al.* 2004). There are limited data on
12 occurrence of fusarium mycotoxins in UK cereals prior to 2001. A previous survey
13 conducted in 1999 found highest amounts of DON on wheat, with lower levels on
14 barley and oats (MacDonald et al. 2004). Other trichothecenes were not analysed
15 within this study.
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26 The European Commission (EC) has set legislative limits for the fusarium
27 mycotoxins including the trichothecene, DON and ZON in cereal grains and cereal-
28 based products intended for human consumption (Anon. 2006a). A combined limit
29 for the trichothecenes HT2 and T2 will be introduced in the near future. The
30 European Commission also set guideline limits in 2006 for fusarium mycotoxins in
31 animal feed (Anon. 2006b). The presence of relationships between mycotoxin
32 concentrations are important as they allow assumptions for one mycotoxin based on
33 the concentration of another. For example, legislative limits have not been set for
34 NIV as it is considered by the EC to be a co-contaminant of DON and as such can be
35 controlled by controlling DON (Anon. 2006a).
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44 There have been limited studies comparing mycotoxin concentrations in
45 organic and conventional oat samples. A study of organic and conventional oats
46 across six locations in Finland identified no difference in the DON concentration
47 between organic and conventional oats (Hietaniemi et al. 2004). In the same study,
48 HT2 and T2 were rarely detected above the limit of quantification (LoQ; 50 $\mu\text{g kg}^{-1}$).
49 A more recent study (Gottschalk 2007), with a more sensitive methodology (LoQ < 1
50 $\mu\text{g kg}^{-1}$) detected a high incidence of HT2 and T2 in 70 German oat and oat products
51 and there was a significantly lower level in organic (n=35) compared to conventional
52 (n=35) samples. However, the combined means (HT2+T2) for organic and
53 conventional samples were both low (8 and 27 $\mu\text{g kg}^{-1}$ respectively).
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3 The aims of the present study were: i) to identify the distribution of fusarium
4 mycotoxins in UK oats, ii) to identify relationships between the concentrations of
5 fusarium mycotoxins detected, iii) to identify how fusarium mycotoxin distributions
6 vary across seasons and regions and iv) to identify if there were differences in the
7 fusarium mycotoxin concentrations of organic and conventional oats in the UK.
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12 13 14 15 16 **Material and methods**

17 18 19 *Sample collection*

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22 Each year, from 2002-2005, about one hundred oat grain samples and related
23 agronomic data were collected by agronomists and growers. Samples were collected
24 at harvest from fields of known agronomy as detailed previously (Edwards, in press)
25 Samples were collected from six geographic regions of the UK; lower numbers were
26 collected from the East of England and Northern Ireland, this was due to the smaller
27 areas of oats in these regions (Table I). Of a total of 458 samples collected,
28 approximately 25% of samples were collected from organic growers (Table II).
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35 On receipt of samples they were processed as described previously (Edwards
36 in press). One laboratory sample was sent to RHM Technology for UKAS accredited
37 mycotoxin analysis. A second sample was held as an archive at -20°C .
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42 **(INSERT Table 1 and 2 here)**

43 44 45 *Mycotoxin analysis*

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48 The GC-MS analysis of trichothecenes was performed by RHM Technology (High
49 Wycombe) according to the published method (Patel 1996). The trichothecenes
50 analysed for were deoxynivalenol (DON), nivalenol (NIV), 3-acetylDON, 15-
51 acetylDON, fusarenone X, T-2 toxin (T2), HT-2 toxin (HT2), diacetoxyscirpenol
52 (DAS), neosolaniol and T-2 triol. The limit of quantification (LoQ) was determined
53 as six times the baseline noise and calculated to be $10\ \mu\text{g kg}^{-1}$.
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Zearalenone was analysed by HPLC as described previously (Scudamore et al.
2007). Results were adjusted according to recovery. The limit of quantification

(LoQ) was determined as six times the baseline noise and calculated to be $3 \mu\text{g kg}^{-1}$. Moniliformin was also analysed by HPLC according to the method of Sharman et al. (1991). Results were adjusted according to recovery and the limit of quantification (LoQ) was calculated to be $10 \mu\text{g kg}^{-1}$.

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 ± 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 $10 \mu\text{g kg}^{-1}$ (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 of HT2+T2 samples below the LoQ were assigned a value of (LoQ)/2, ie $5 \mu\text{g kg}^{-1}$, \log_{10} transformed to stabilise the variance and analysed using Genstat (version 10, Lawes Agricultural Trust).

Results and discussion

Mycotoxin concentration

In the first two years moniliformin was not detected in any samples (n=196) and analysis was discontinued. 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, 296 samples were analysed for zearalenone. Five percent of samples were above the LoQ of 3 $\mu\text{g kg}^{-1}$ and the maximum detected was 29 $\mu\text{g kg}^{-1}$. The legal limit for zearalenone in unprocessed oats intended for human consumption is 100 $\mu\text{g kg}^{-1}$ (Anon. 2006a).

Of the ten trichothecenes analysed, seven were detected, of these DON, NIV, HT2, T2, neosolaniol and T2-triol were detected above 100 $\mu\text{g kg}^{-1}$. 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 32% of samples and the maximum was 282 $\mu\text{g kg}^{-1}$; this is well below the legal limit for DON in unprocessed oats intended for human consumption (1750 $\mu\text{g kg}^{-1}$) (Anon. 2006a). HT-2 and T-2 were detected in 92 and 84% of samples respectively; the concentrations were frequently high. The combined mean, median and maximum concentrations were 570, 213 and 9990 $\mu\text{g kg}^{-1}$ respectively. The distributions were lognormal with a left censor truncated by the LoQ. Comparison of the mean and median values for all other mycotoxins detected indicated they followed a similar lognormal distribution, however the degree of truncation of the distribution at the LoQ varied markedly. NIV was the next most common fusarium mycotoxin detected with a maximum concentration 847 $\mu\text{g kg}^{-1}$.

T-2 triol and neosolaniol were detected in 41% of samples and always as a low concentration, co-contaminant in the presence of a high concentration of the primary contaminants, HT2 and T2. Fusarenone X, diacetoxyscirpenol and 15-acetylDON were not detected in any sample (LoQ = 10 $\mu\text{g kg}^{-1}$).

(Insert Table 3 here)

Regression analysis

The relationships between HT2 and all other detected type A trichothecenes were analysed by regression analysis of logarithmic transformed values (Figures 1-3). The regressions were all highly significant ($p < 0.001$). Such a relationship could be expected as the type A trichothecenes are produced in the same metabolic pathway by the same species (Thrane 2004). HT2 was always detected when other type A trichothecenes were present within a sample.

There were no other positive relationships between the concentrations of other commonly detected fusarium mycotoxins detected in UK oats. In fact both NIV and DON showed signs of mutual exclusion with HT2 and to one another, ie when one mycotoxin was present at high concentration then the other was low (Figures 4-6). This is probably because HT2, DON and NIV in oats are produced by different *Fusarium* species which appear to have different environmental requirements to each other. As a consequence of this relationship, DON concentration cannot be used to predict HT2 or NIV concentration and vice versa.

(Insert Figure 1-6 here)

Year, region and practice

The impact of practice on the combined HT2+T2 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 ($p < 0.001$) between year and region with high concentrations detected in all UK regions (Figure 7). There was also a highly significant difference in the HT2+T2 concentration between organic and conventional samples ($p < 0.001$). Organic samples were approximately five times lower, with predicted means of 50 and 264 $\mu\text{g kg}^{-1}$ HT2+T2 for organic and conventional oats respectively. The summary statistics were determined for conventional and organic samples, and show major differences in the two datasets (Table IV). As the vast majority of oats grown in the UK are produced within conventional agriculture, then the summary statistics for this subset of samples are more representative of oat crops in the UK. A previous limit for discussion set by the

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3 EC for oats intended for human consumption was $500 \mu\text{g kg}^{-1}$ HT2+T2. The
4 percentage of organic and conventional samples exceeding this discussion limit, on an
5 annual basis, fluctuated between zero and 22 and between 18 and 50% for organic and
6 conventional samples respectively between 2002-2005 (Table V).
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15 16 *General discussion*

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19 This research has clearly identified the extent to which UK organic and conventional
20 oats contains fusarium mycotoxins at harvest. Amounts of fusarium mycotoxins in
21 UK oat samples from 2002-2005 were generally low. However, there were high
22 incidences and high mean concentrations of HT2 and T2. Levels of these mycotoxins
23 are higher than those previously reported for any cereal worldwide.
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29 Regression analysis shows that there are strong correlations between the HT2
30 and the other type A trichothecenes (T2, T2 triol and NEO), which would indicate that
31 they are produced by the same *Fusarium* isolates as part of the same synthetic
32 pathway and therefore are co-contaminants. This is of benefit as it would allow an
33 estimate of the content of other type A trichothecenes if the concentration of one of
34 them is known. This is of use as current ELISA analysis kits can detect T2 but cross-
35 react at a known rate with HT2. By knowing the proportion of HT2 to T2 and the
36 cross-reaction ratio then the total content of HT2+T2 can be estimated using ELISA
37 analysis of T2. The regression also indicates that methods to reduce one type A
38 trichothecene should result in overall reduction of all type A trichothecenes.
39 Regression analysis of HT2+T2 against DON and NIV and the regression of DON
40 against NIV suggests some degree of mutual exclusion indicating that the species
41 responsible either compete against one another or prefer different environmental
42 conditions. These results agree with the relationships between trichothecenes
43 determined on wheat (Edwards In press) and reinforces the conclusion that DON and
44 NIV are not co-contaminants and should not be treated as such within European
45 legislation.
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59 Modelling of HT2+T2 concentration of oat samples against year, region and
60 practice identified a highly significant interaction between year and region. This is
probably due to fluctuation in weather between years and regions. There was no trend

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3 from North to South, as seen for DON in wheat (Edwards In press), which would
4 indicate that the temperature difference across the UK is not restrictive to HT2 and T2
5 production on oats. This is different from DON in wheat where there was a lower
6 concentration in the North of Britain. Oat samples with more than 500 ppb HT2+T2
7 were detected in all regions of the UK at similar frequencies. The highest
8 concentration of HT2+T2 occurred in 2005, which was a relatively dry summer. High
9 levels of HT2+T2 were not seen in 2004, when DON and zearalenone were highest on
10 wheat after a wet harvest (Edwards In press). This would indicate that the HT2+T2-
11 producing *Fusarium* species probably have different environmental requirements than
12 *F. culmorum* and *F. graminearum*, which produce DON and prefer wet conditions.
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21 There was a five-fold difference in the predicted mean HT2+T2 content of
22 conventional and organic oat samples with organic samples having a significantly
23 lower HT2 and T2 content compared to conventional oats. Previous studies of
24 organic and conventional oat and oat products have identified lower concentrations of
25 HT2 and T2 in organic samples, but the difference was not as great as reported here
26 (Gottschalk 2007). Organic oats may have a lower HT2 and T2 content due to a
27 number of agronomic factors. These could include previous crop, cultivation and
28 varietal choice. Organic growers tend to have longer rotations which are more diverse
29 and less cereal intensive than conventional growers. This could result in a lower level
30 of the HT2+T2-producing *Fusarium* inoculum within organic production,. A recent
31 UK wheat project identified a significantly lower (ca. two-fold) incidence of HT2+T2
32 in organic compared to conventional samples although the incidence and
33 concentration of these mycotoxins were much lower in wheat (Edwards In press).
34 The same study found no significant difference between DON in organic and
35 conventional wheat (Edwards In press).
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48 UK oats in general had a high content of HT2 and T2. This is the first
49 reported study on mycotoxin content of oats at harvest in the UK. Until legislative
50 limits for HT2+T2 are set, the extent to which these limits will impact on the UK oat
51 industry cannot be determined.
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55 The presence of HT2 and T2, although at a lower concentration than identified
56 in this paper, were first reported in oats in Norway (Langseth and Elen 1996). Results
57 of high HT2 and T2 concentrations have recently been reported in other European
58 countries, particular in Nordic countries and the UK (Pettersson 2007, Scudamore et
59 al. 2007). Of concern is that data from the 1990s to 2006 would indicate that HT2
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3 and T2 concentration in oats appears to be increasing in Sweden in recent years. UK
4 results differ from those in Nordic countries, where high DON can also occur
5 (Langseth and Elen 1996, 1997, Langseth and Rundberget 1999, Scudamore et al.
6 2007). In Canada, DON is routinely detected in oat and oat products, but analysis of
7 HT2 and T2 are not reported (Tekauz et al. 2004) or when analysed are rarely
8 detected (Roscoe et al. 2008). Of concern is that little is known about the *Fusarium*
9 species that produce HT2 and T2 or the factors involved in the presence of HT2 and
10 T2 on cereals and cereal products. Further research on this topic is documented
11 within current European legislation as necessary and of a high priority (Anon. 2006a).
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20 Legislative limits for HT2 and T2 are currently under consideration by the
21 European Commission. A previous limit of 500 $\mu\text{g kg}^{-1}$ HT2+T2 for unprocessed oats
22 was set for discussion. A study of industrial processing of oats has identified that the
23 majority (>90%) of the HT2 and T2 is on the husk which is removed during
24 processing (Scudamore et al. 2007), resulting in much lower levels detected in
25 finished products. During the period of this study, the levels of HT2 and T2 detected
26 during a survey of fusarium mycotoxins in retail oat products were low, and deemed
27 not of concern (Anon. 2004). However, the EC sets legislative limits on unprocessed
28 cereals as well as finished products. Therefore, the concentrations of HT2 and T2 in
29 oats determined in this study could have serious consequences for the UK oat
30 processing industry. How serious, is dependent on the limits set for HT2 and T2 in
31 unprocessed oats intended for human consumption.
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56 advice was provided by Sandro Leidi at Statistical Services Centre, University of
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Table I. Sample distribution by year and region.

Year	Region						Total
	South	East	Midlands	North	Scotland	N.Ireland	
2002	14	12	27	13	11	15	92
2003	22	5	16	22	27	12	104
2004	22	19	27	33	18	9	128
2005	27	17	33	27	21	9	134
Total	85	53	103	95	77	45	458

Table II. Sample distribution by year and practice.

Year	Practice		Total
	Organic	Conventional	
2002	30	62	92
2003	45	59	104
2004	14	114	128
2005	26	108	134
Total	115	343	458

Table III. Concentrations of all trichothecene mycotoxins detected in UK oats in 2002-2005 (458 samples).

	% > 10 $\mu\text{g kg}^{-1}$	Mycotoxin concentration ($\mu\text{g kg}^{-1}$)				
		Mean ^a	Median	90th%	95th%	Max
DON	32	11	0	24	50	282
3AcDON	0.2	<10	<10	<10	<10	26
NIV	72	49	24	120	176	847
Neosolaniol	41	14	<10	38	53	189
T-2 triol	41	17	<10	46	68	263
T-2	84	140	58	389	502	2406
HT-2	92	430	151	1110	1727	7584
HT2+T2	92	570	213	1492	2160	9990

^aBased on a value of 1.667 for all samples below the limit of quantification ($10 \mu\text{g kg}^{-1}$).

Table IV. Concentration of HT2+T2 detected in conventional (n = 343) and organic (n = 115) UK oats in 2002-2005.

	%>10ppb	HT2+T2 concentration (ppb)				
		Mean	Median	90th%	95th%	Max
Conventional	97	687	292	1704	2450	9990
Organic	78	238	49	515	703	8684

Table V. Percentage of UK oat samples (n=458) exceeding 500 $\mu\text{g kg}^{-1}$ HT2+T2.

Year	Conventional	Organic	Overall
2002	18	13	16
2003	41	22	33
2004	31	0	24
2005	50	8	42
Overall	36	11	30

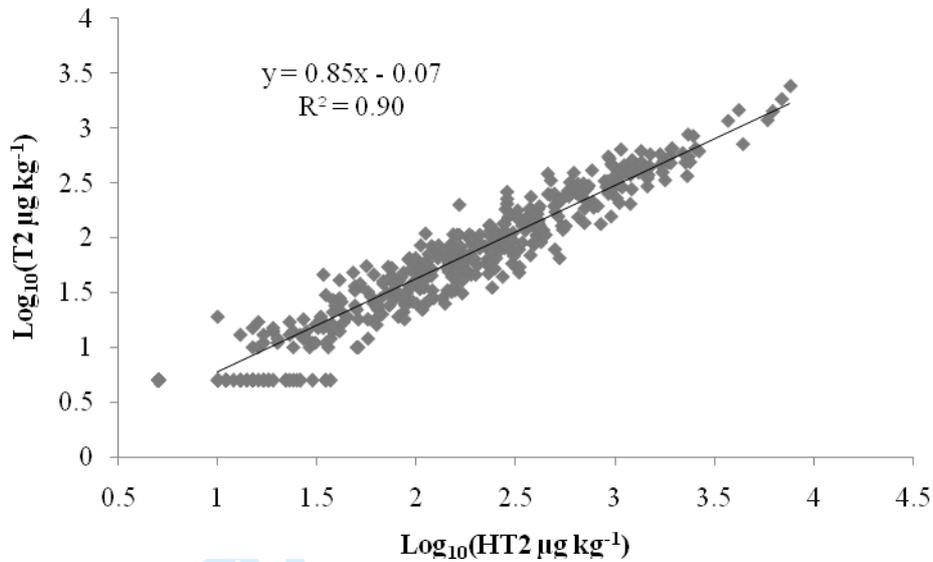


Figure 1. Regression of T2 against HT2 concentration (log log plot) for UK oats from 2002 to 2005 (n = 458). Regressions fitted for all samples above LoQ.

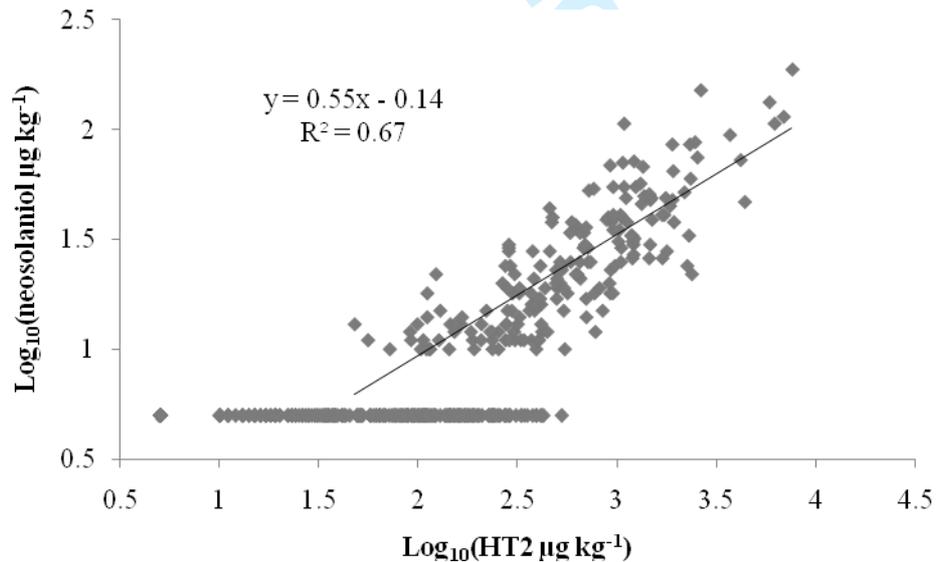
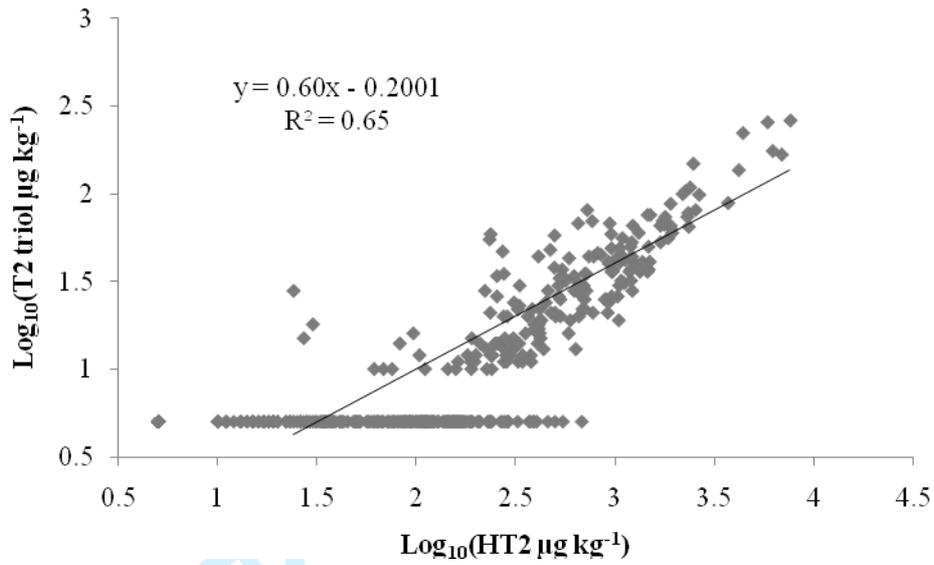
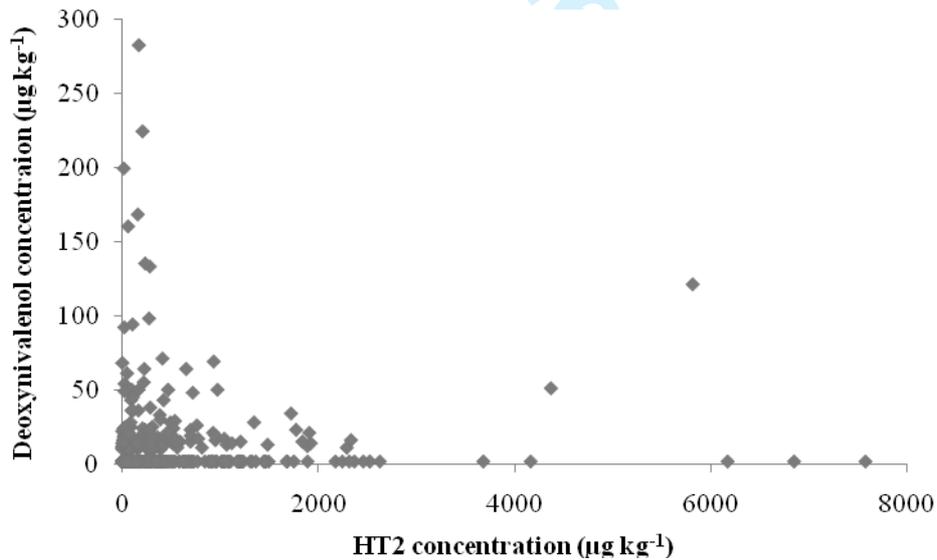


Figure 2. Regressions of neosolaniol against HT2 concentration (log log plot) for UK oats from 2002 to 2005 (n = 458). Regressions fitted for all samples above LoQ.



24 Figure 3. Regressions of T2 triol against HT2 concentration (log log plot) for UK oats
25 from 2002 to 2005 (n = 458). Regressions fitted for all samples above LoQ.
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57 Figure 4. Relationship between HT2 and DON concentration for UK oats 2002-2005
58 (n=458).
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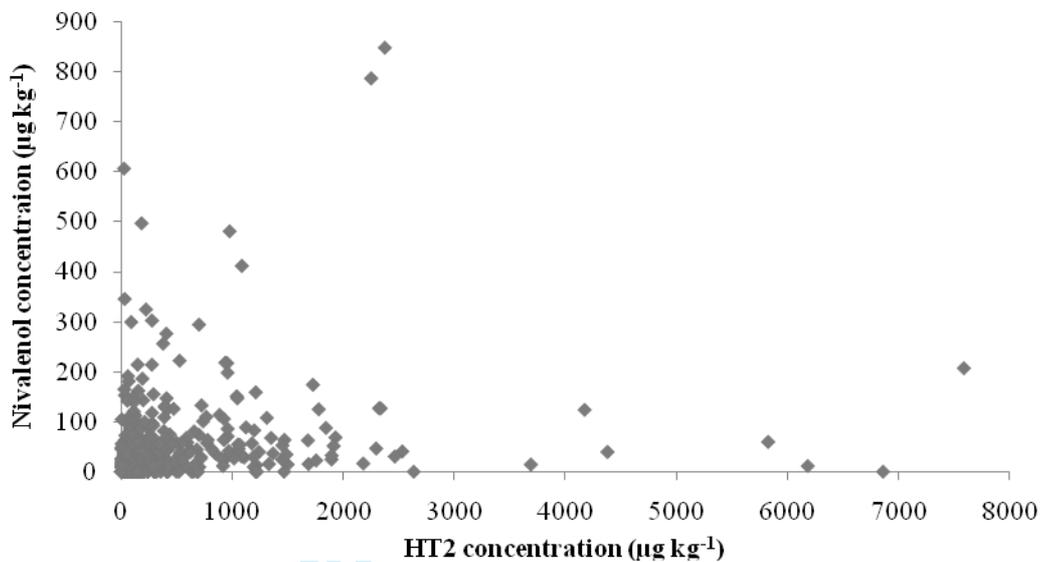


Figure 5. Relationship between HT2 and NIV concentration for UK oats 2002-2005 (n=458).

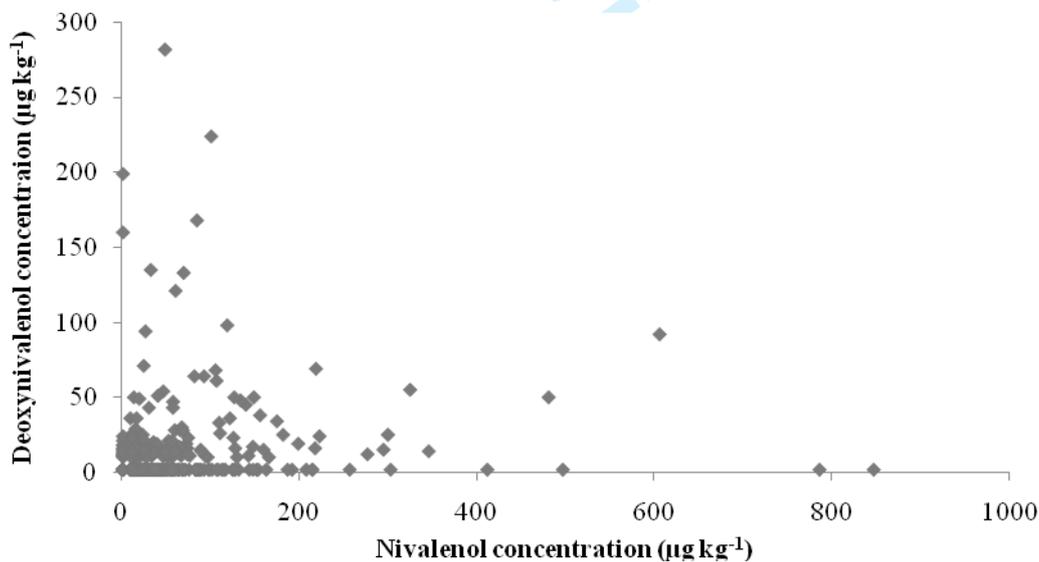


Figure 6. Relationship between NIV and DON concentration for UK oats 2002-2005 (n=458).

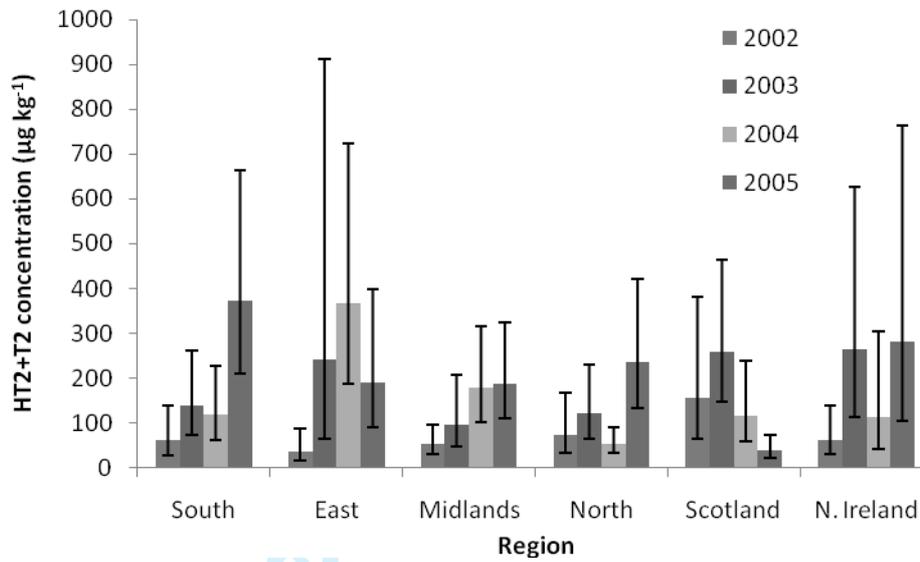


Figure 7. Predicted mean HT2+T2 concentration for UK oats from each region in each year. Bars represent 95% confidence limits for predictions.